Biological function of CYP-produced eicosanoids in the regulation of the pharyngeal activity in the nematode *Caenorhabditis elegans*

Dissertation

Zur Erlangung des akademischen Grades

Doctor rerum naturalium

(Dr. rer. nat.)

eingereicht an der

Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin

von

Yiwen Zhou

Präsident (komm.) der Humboldt-Universität

zu Berlin: Prof. Dr. Peter Frensch

Dekan der Lebenswissenschaftlichen Fakultät: Prof. Dr. Christian Ulrichs

Gutachter

1. PD Dr. Ralph Menzel
2. Dr. Wolf-Hagen Schunck
3. Prof. Dr. Christian Schmitz-Linneweber

Tag der mündlichen Prüfung: 18.03.2022

To my beloved family

献给我挚爱的家人

CONTENTS

LIST OF FIGURES

Appendix

ABBREVIATIONS

× g	Times gravity
5-HT	5-hydroxytryptamine
ALA	Alpha-linolenic acid
ALD	Alcohol-induced liver disease
C. elegans	*Caenorhabditis elegans*
CNS	Central nervous system
COX	Cyclooxygenase
CPR	NADPH- Cytochrome P450 reductase
CYP	Cytochrome P450
DGLA	Dihomo-γ- linolenic acid
DHEQ	Dihydroxyeicosaquatraenoic acid
DHET	Dihydroxyeicosatrienoic acid
DMSO	Dimethyl sulfoxide
dsRNA	Double-strand RNA
EEQ	Epoxyeicosatetraenoic acid
EET	Epoxyeicosatrienoic acid
ELO	Elongase
ECs	Endocannabinoids
EPA	Eicosapentaenoic acid
ETYA	5,8,11,14-eicosatetraynoic acid
G	Guanine nucleotide-binding
GLA	γ-linolenic acid
GPCR	G protein-coupled receptors
GPR	GPCR orphan receptors
h	Hour

HEPE	Hydroxyeicosapentaenoic acid
HETE	Hydroxeicosatetraenoic acid
IPTG	Isopropyl β- d-1-thiogalactopyranoside
JuA	Juniperonic acid
LA	Linoleic acid
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LC-PUFAs	Long-chain polyunsaturated fatty acids
LOX	Lipoxygenase
LXA4	Lipoxin A4
NGM	Nematode growth media
NSMs	Neurosecretory motor neurons
O3AA	n-3 arachidonic acid
OA	Oleic acid
OD	Optical density
PLA2	Phospholipases A2
PUFA	Polyunsaturated fatty acid
R/S	*Rectus/ Sinister*
RNAi	RNA interference
SDA	Stearidonic acid
sEH	Soluble epoxide hydrolase
S/R	*Sinister /Rectus*

Abstract

This thesis investigated the effects of cytochrome P450 (CYP)-produced eicosanoids on the pharyngeal pumping in the nematode *Caenorhabditis elegans* and their cross-link with neurohormones. Within the framework of exploring CYP-eicosanoid signaling in *C. elegans* this work mainly focused on identifying and characterizing key components involved in worm's pharyngeal activity, in particular regio- and stereoisomeric metabolites of EPA (eicosapentaenoic acid, 20:5 n-3) and AA (arachidonic acid, 20:4 n-6), neurohormones, (serotonin and octopamine) as well as the involvement of relevant GPCR (G protein-coupled receptors).

Firstly, the impact of short-term treatment with CYP-eicosanoids and neurohormones on CYP-eicosanoid formation in wildtype and different mutant strains was analyzed. The results showed that 17,18-EEQ (17,18-epoxyeicosatetraenoic acid) mimics the stimulatory effect of serotonin while 20-HETE (20-hydroxeicosatetraenoic acid) overlapped with the inhibitory effect of octopamine. Moreover, serotonin increased free 17,18- EEQ levels, whereas octopamine selectively induced the synthesis of hydroxy-metabolites.

Secondly, stereo-discrimination of 17,18-EEQ was tested to identify the effective enantiomer in the stimulatory function on pharyngeal muscle cells. Only 17(R),18(S)-EEQ was found to rescue impairments of pharyngeal pumping. Moreover, three mutant strains defective in different serotonin GPCRs, *ser-1(ok345)*, *ser-7(tm1325)* and *ser-7(tm1325) ser-1(ok345)*, were selected to decipher which receptor might be involved in the CYP-eicosanoid signaling transduction pathway. In fact, SER-7 seems to be required for the 17-18-EEQ effects on regulation of pharyngeal pumping activity in *C. elegans.*

Third, the identification of a GPCR of the proposed signal transduction pathway down-stream of CYP-eicosanoid production was started. NMUR-2 was selected as a potential candidate GPCR gene.

Keywords:

cytochrome P450, fatty acids, lipidomics, pharynx, 17,18-epoxyeicosatetraenoic acid, stereoisomers, chiral phase, GPCR, *Caenorhabditis elegans*

Zusammenfassung

Diese Arbeit untersucht die Auswirkungen von Cytochrom P450 (CYP)-produzierten Eicosanoiden auf das Pumpen des Pharynx im Nematoden *Caenorhabditis elegans* und deren funktionellen Vernetzung mit dem Wirken von Neurohormonen. Bei der Erforschung der CYP-Eicosanoid-Signalübertragung konzentrierte sich diese Arbeit hauptsächlich auf die Identifizierung und Charakterisierung von Schlüsselkomponenten, welche an der Regulation der pharyngalen Aktivität des Wurms beteiligt sind, insbesondere Regio- und Stereometaboliten von EPA und AA, Neurohormone (Serotonin und Octopamin) sowie relevante GPCR. Zunächst wurde der Einfluss einer Kurzzeitbehandlung mit CYP-Eicosanoiden und Neurohormonen auf die Bildung von freien CYP-Eicosanoiden im Wildtyp und in verschiedenen Mutantenstämmen analysiert. Die Ergebnisse zeigten, dass 17,18-EEQ die stimulierende Wirkung von Serotonin zeigt, während 20-HETE mit der hemmenden Wirkung von Octopamin überlappt. Darüber hinaus erhöhte Serotonin den freien 17,18-EEQ-Spiegel, während Octopamin selektiv die Synthese von Hydroxymetaboliten induzierte. Zweitens wurde die Stereodiskriminierung von 17,18-EEQ getestet, um das wirksame Enantiomer in der stimulierenden Funktion auf die Pharynxmuskelzellen zu identifizieren. Es wurde festgestellt, dass nur 17(R),18(S)-EEQ Beeinträchtigungen des pharyngalen Pumpens beheben konnte. Darüber hinaus wurden drei Mutantenstämme, die in verschiedenen Serotonin-GPCRs defekt sind, *ser-1(ok345)*, *ser-7(tm1325)* und *ser-7(tm1325)ser-1(ok345)*, ausgewählt, um zu entschlüsseln, welcher Rezeptor am CYP-Eicosanoid-Signaltransduktionsweg beteiligt sein könnte. Tatsächlich scheint SER-7 für die 17-18-EEQ-Effekte auf die Regulierung der pharyngalen Pumpaktivität von *C. elegans* erforderlich zu sein. Drittens wurde mit der Identifizierung eines GPCR des vorgeschlagenen Signaltransduktionsweges stromabwärts der CYP-Eicosanoid-Produktion begonnen. NMUR-2 wurde als potenzielles Kandidaten-GPCR-Gen ausgewählt.

Schlüsselwörter: Cytochrom P450, Fettsäuren, Lipidomics, Pharynx, 17,18-Epoxyeicosa-tetraensäure, Stereoisomere, chirale Phase, GPCR, *Caenorhabditis elegans*

Chapter One: Introduction

1.1 The *C. elegans*: pharynx

1.1.1 General overview of the pharynx

Caenorhabditis elegans pharyngeal pumping is the action of the regulatory pharyngeal neurons and muscles which draws bacteria through the pharynx of the worm, crushes and pushes it to the intestine (Rankin, 2002). It is a repetitive circulation of contraction and relaxation that sucks particles (as preferred bacteria) from the environment, expels the liquid and trapping them to the intestine (Fig.1A). So, simply to say, the pharynx is a neuromuscular pump. It contains a contractile element of 20 muscle cells and 20 neurons which constitute the pharyngeal nervous system: 4 glands cells, 8 epithelial cells and 9 marginal cells which strengthen the integrity of the pharynx (Avery and You, 2012b)(Fig.1B). The pharyngeal muscle is divided into three functional groups: the corpus, the isthmus, and the terminal bulb (Fig.1C).

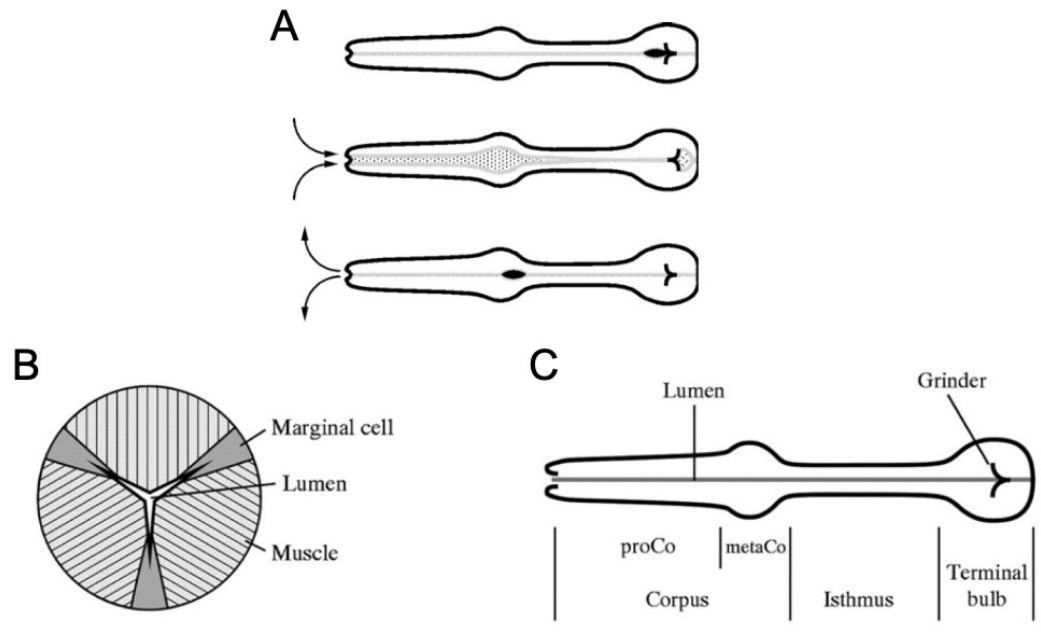


Figure 1: Pharyngeal pumping and anatomical structure.

(**A**) A pharyngeal pump begins with a near-simultaneous contraction of the muscles of the corpus and anterior isthmus which pulls the lumen open. Liquid and suspended particles flow in through the mouth. At nearly the same time the terminal bulb muscles contract, inverting the

plates of the grinder, which grind up bacteria and pass the debris to the intestine. The contraction is followed by a near-simultaneous relaxation that returns the grinder to its resting position and expels liquid from the corpus and isthmus. (**B**) Simplified anatomical structure of the pharynx. Cross-section through the pharynx (**C**) Schematic of a lateral view of the pharynx. Source of pictures: Avery and You (2012b)

The corpus and anterior isthmus contract to open the lumen. In the pharyngeal nervous system, all the 20 neurons are divided into 14 different types with 8 bilaterally symmetric pairs and 6 unpaired neuronal cells, connected to the extrapharyngeal nervous system by a pair of bilaterally symmetric neurons, the RIP neurons (White et al., 1986). Interestingly, after laser beam ablation of RIP, which disconnected the neural connectivity between the pharyngeal and extrapharyngeal nervous systems, the pumping frequency of worms was unaffected either in the presence or absence of food. This observation indicates the endogenous signaling to the pharynx must be important in the regulation of feeding behavior (Dallière et al., 2016). Three types of pharyngeal motor neurons play a vital role to maintain normal pharyngeal pumping. The pharyngeal motor neurons M3 and M4 neurons as well as the MC neuron are sufficient for normal pumping, even if all the other neurons have been laser killed (Avery and Horvitz, 1989a).

1.1.2 *C. elegans* pharynx: Contribution to understanding of the human cardiovascular system

The pharyngeal pumps of the pharynx resemble in some way the heart-beats of vertebrates. In fact, several evidences indicate that *C. elegans* pharynx shares a possible evolutionary orthology to the vertebrate heart. The following similarities exist between the two organs: (1) the anatomical structure, both are composed of lumens using binucleate muscles (Albertson et al., 1976; Kellerman et al., 1992), (2) the electrophysiological assessment, both exhibit motions rely on similar electrical circuitry to control pumping (Avery and Horvitz, 1989a; Bernstein and Morley; Starich et al., 1996; Starich et al., 2003), (3) the responses to neurohormones are transduced by GPCRs, both are regulated by neurohormones (Hobson et al., 2003; Penela et al., 2006; Steger and Avery, 2004), (4) similarity of relying on the channel types, both have contraction via transmembrane 'L-type' voltage-dependent Ca^{2+} and potassium channels (Avery and Horvitz, 1989a; Raizen and Avery, 1994; Salkoff et al., 2005) as

well as (5) the homology and interactions of transcription factors participating in their genesis (Chen and Fishman, 1996; Haun et al., 1998; Okkema and Fire, 1994; Okkema et al., 1997). These observations provide excellent and detailed knowledge of how the pharynx works in *C elegans* and further studying this organ may even contribute to our understanding of the underlying mechanisms of human cardiovascular diseases.

1.2 Serotonin signaling via G protein-coupled receptors (GPCRs) in *C. elegans*

1.2.1 Neurohormones in *C. elegans*

Neurohormones are heterogeneous biochemical substances that pass on information from one nerve cell to another via the synapse. The presence of neurohormones is a prerequisite for the transmission of information in the CNS, which is essential for thoughts and behaviors. Research on these neurohormones in *C. elegans* could help illustrate the molecular and cellular signaling mechanisms which control behavior and provide insights into the underlying mechanisms of human disease. Pharyngeal neurons contain small-molecule neurohormones such as serotonin and octopamine, acting as chemical signals between both neurons and muscles and modulate the frequency of pump. Mutants defective in neurotransmission between pharyngeal muscle and MC neuron exhibit defects in *C. elegans* feeding behavior, as slower pharyngeal pumping (McKay et al., 2004c). Interestingly, in the starved condition, MC activity is low while adding of serotonin triggers MC to be active as in the presence of food, mimicking the food signaling on pharynx (Horvitz et al., 1982a).

Serotonin is a messenger substance that passes on information in the nervous system. Its correct biochemical name is 5-hydroxy-tryptamine, or 5-HT for short. In mammals, since it affects our emotions in addition to many other processes, the popular term serotonin refers to “happiness hormone”. In *C. elegans*, it is synthesized in a pair of neurosecretory motor neurons (NSMs). Except the pumping activity, serotonin also modulates worm’s behavior including egg-laying, mating, foraging, learning and locomotion (Table A1). The pharyngeal motor neurons-MC, M3, M4, and muscle cells are modulated by serotonin released from serotonergic neurons to maintain a

continuous high frequency of pumping in the presence of bacteria (Niacaris and Avery, 2003; Raizen and Avery, 1994; Raizen et al., 1995). *C. elegans* contains at least 9 serotonergic neurons, 2 NSMs, ADFs, and HSNs that synthesize 5-HT directly (Sze et al., 2000). The *tph-1* mutant does not synthesize serotonin and displays a reduction in pumping rate in the presence of food (Sze et al., 2000). In starved condition, serotonin dramatically increases the pumping frequency (Zhou et al., 2015) and stimulates egg laying by GPCR which is located in the muscle cells in *C. elegans* (Carnell et al., 2005). Serotonin treatment also causes fat loss by fat oxidation in *C. elegans* and authors found that serotonergic regulation of fat and feeding are two different molecular pathways (Srinivasan et al., 2008).

1.2.2 Study of serotonin receptors in *C. elegans*

Neurohormones act as ligands for G protein-coupled receptors (GPCRs) to modulate neural and muscle activity. There exist two types of receptors which are activated by neurohormones modulating neural activity. In *C. elegans*, five serotonin receptors have been identified (Table A2 and A3). Four are serotonin-activated G protein-coupled receptors (SER-1, -4, -5 and -7) and one is a serotonin-gated Cl^--channel (MOD-1) (Chase and Koelle, 2007; Hapiak et al., 2009; Hobson et al., 2006a; Tsalik et al., 2003). SER-1, SER-4 and SER-7, which are expressed in pharyngeal neurons or muscles, may participate in the activity of pharyngeal pump (Lee et al., 2017). Stimulation of pumping by food-driven, 5-HT2 ortholog SER-4 and 5-HT1 ortholog SER-1 are involved in pump bursts, whereas exogenous serotonin activates the SER-4 and the 5-HT7 ortholog SER-7 (Lee et al., 2017). SER-7 rather than SER-1 is essential for the serotonin stimulation of both egg laying and pharyngeal pumping (Hobson et al., 2006c). Moreover, *ser-1* defective mutant is unable to turn an essential step of mating, indicating the expression of SER-1 is responsible for the male mating behaviors (Carnell et al., 2005). The 5-HT gated chloride channel, MOD-1, along with SER-1, may appear to be necessary for controlling the locomotion in *C. elegans* (Dernovici et al., 2007). Loss of the SER-5 receptor in *tph-1* mutants results in the failure to increase the response to exogenous serotonin of pharyngeal activity, suggesting SER-5 from ADF neuron cell is required for serotonergic regulation of feeding (Cunningham et al., 2012).

1.3 CYP-produced eicosanoids in *C. elegans*

1.3.1 CYP enzymes and eicosanoids signaling

The third branch of eicosanoid pathway performed by cytochrome P450 was first characterized by Capdevila, Falck and others; it catalyzes arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA) to a group of hydroxy- and epoxy-metabolites (Chacos et al., 1983). Various members of the cytochrome P450 (CYP) superfamily act as epoxygenases and/or hydroxylases of PUFA (Capdevila and Falck, 2002; Konkel and Schunck, 2011a); the metabolized products are collectively termed CYP-eicosanoids (Fig. 2). The biological significance of these eicosanoids in mammals and human have been researched, they play important roles in the regulation of vascular tone, inflammation and autophagy in insulin-sensitive tissues (Lopez-Vicario et al., 2015), infarction injury and the functional recovery of the heart after ischemia/reperfusion (Konkel and Schunck, 2011b), inhibition of the calcium response of cardiomyocytes and relax on human lung arterial and airway smooth muscles (Arnold et al., 2010b; Falck et al., 2011a; Morin et al., 2009). Different regioisomeric epoxyeicosatrienoic acids (EETs) and epoxyeicosatetraenoic acid (EEQ) are derived from AA and EPA, respectively. EETs and EEQs can be further converted to corresponding dihydroxyeicosatrienoic acid (DHET) and dihydroxyeicosatetraenoic acid (DHEQ) by soluble epoxide hydrolases (sEH), respectively (Fig. 2). The CYP hydroxygenase pathway of AA and EPA metabolism generates hydroxyeicosatetraenoic acid (HETE) and hydroxyeicosapentaenoic acids (HEPE) (Fig. 2). In mammals, CYP-eicosanoids have also been suggested to be second messengers of hormones, growth factors and cytokines. Especially the CYP-metabolites of AA, EPA and DHA can exert vascular smooth muscle hyperpolarization and relaxation function (Adkins and Kelley, 2010; Hirafuji et al., 2003; Limbu et al., 2018; Roman, 2002). Previous studies also demonstrated that CYP-produced metabolites may play a potential role on the physiological/pathophysiological significance in rats kidney (Oyekan et al., 1998), in the vasculature (Muthalif et al., 1998), in astrocytes (Harder et al., 1998), as well as in the renal (Wang et al., 1997). 17,18-epoxyeicosatetraenoic (17,18-EEQ) significantly delayed thrombocytes aggregation after 60 minutes of incubation, explaining the possible effective component of resulting in the anti-thrombotic effects of n-3 fatty acid

supplementation (Jung et al., 2012b). Imbalances in CYP-eicosanoid formation are linked to the development of cardiovascular disease, inflammatory disorders, and cancer (Panigrahy et al., 2012; Roman, 2002; Wu et al., 2014). CYP2J2, the human CYP2J sub-family enzyme catalyzes EPA to 17,18-EEQ (Arnold et al., 2010a). 17(R),18(S)-EEQ was a predominant metabolite which oxidized by Human cytochrome P450 1A1 (CYP1A1) (Schwarz et al., 2004). In mammalian system, 17,18-EEQ displays the vasodilation, anti-inflammatory, cardioprotective, antisteatotic and antiangiogenic biological activities (Schunck et al., 2018). Morin (Morin et al., 2010) found 17,18-EEQ exerted anti-inflammatory effects and reduced hyperresponsiveness in human organ-cultured bronchi. Also, invertebrates, such as the nematode *C. elegans* (Maupas, 1900), produces AA and EPA derived CYP-eicosanoids (Kulas et al., 2008b) (Fig. 3). EPA, the predominant n-3 PUFA of *C. elegans*, can be metabolized by Cytochrome P450 enzymes to several classes of epoxygenase and hydroxylase metabolites (Kulas et al., 2008a). CYP-33E2, CYP-29A3, and EMB-8, the worm's NADPH-CYP reductase (CPR), have been identified as contributing enzymes and CYPs are mainly expressed in the pharynx (Kulas et al., 2008b). CYP-33E2 prefers EPA as the substrate over AA and converts it to 17,18-EEQ as dominant metabolite (Kosel et al., 2011b). CYP2J2, a major epoxygenase in the heart, is the most closely related human homolog of CYP-33E2. Previous data suggested that CYP2J2-produced metabolites, EETs, are cardioprotective after ischemia by the activation of mitoKATP and p42/p44 MAPK (Seubert et al., 2004), also a protective role in ischemia-reperfusion injury is presented (Nithipatikom et al., 2006). CYP-29A3 of *C. elegans* presumably contributes to hydroxy-metabolite derivation and is homologous to mammalian CYP4 family members that generate 20-hydroxyeicosatetraenoic acid (20-HETE) as main AA-derived metabolite (Kulas et al., 2008b).

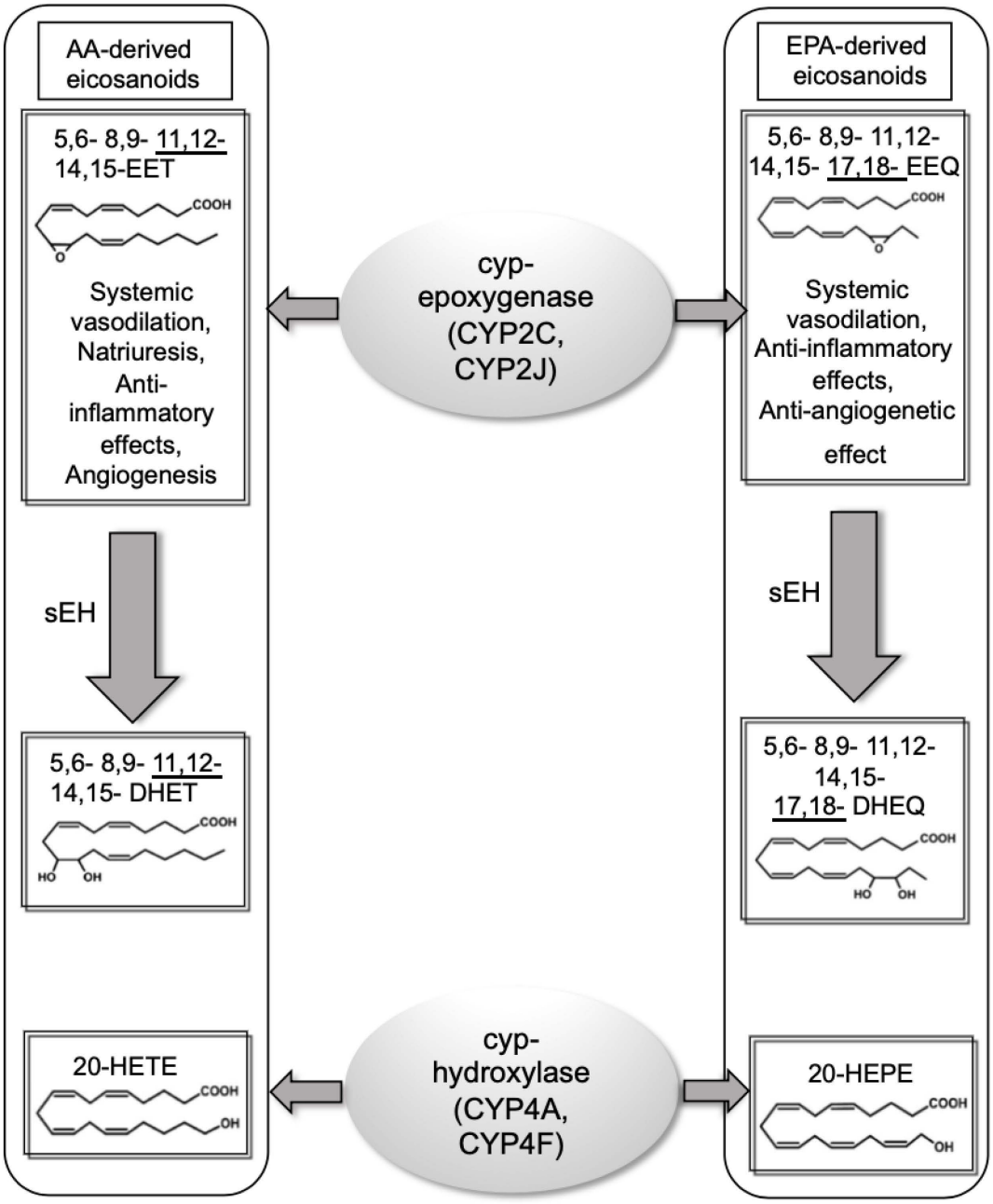


Figure 2: Main AA and EPA metabolites via CYP/sEH and their principal actions.

Different enantiomers are created during the production of physiologically active AA- and EPA-derived CYP-eicosanoids. The illustrated chemical structure refers to the underlined isomer.

AA: Arachidonic acid; EPA: Eicosapentaenoic acid; EETs: Epoxyeicosatrienoic acids; 20-HETE: hydroxyeicosatetraenoic acid; EEQs: epoxyeicosatetraenoic acids; 20-HEPE: 20-hydroxyeicosapentae-noic acids; DHET: dihydroxyeicosatrienoic acid. Modified according to Bonafini and Fava (2017).

1.3.2 Polyunsaturated fatty acid in *C. elegans*

A wide range spectrum of PUFA species makes *C. elegans* a highly attractive platform for genetic dissection of various PUFAs and their metabolites functions, with an advantage that does not exist in mammals (Watts and Browse, 2002). Mammals have dietary fatty acid requirements from the diet (18:2 n-6 and 18:3 n-3), whereas *C. elegans* can endogenously produce both PUFA classes because all the necessary enzymes, fatty acid desaturases and elongases, are present for *de novo* synthesis. Several mutations, described below, are particularly helpful in altering the fatty acid and CYP-eicosanoids compositions (Zhou et al., 2015). For achieving an overview, LC-PUFA/CYP-eicosanoid synthesis pathway in *C. elegans* was summarized in Fig. 3.

In the laboratory, *C. elegans* is exclusively fed with *Escherichia coli* (Migula 1895) OP50, a bacterium that mainly contains palmitic (16:0), palmitoleic (16:1 n-7), and vaccenic (18:1 n-7), but no PUFAs (Satouchi et al., 1993; Tanaka et al., 1996). The biosynthetic pathway of PUFAs in *C. elegans* is displayed with the start from oleic acid (OA, 18:1n-9) (Fig. 3). Key genes expressed in *C. elegans* (but not in mammals) are *fat-2* encoding a Δ-12 fatty acyl desaturase and *fat-1* encoding a n-3 fatty acyl desaturase. In concert with further desaturases and elongases, they enable worms to establish a biosynthetic pathway from OA via linoleic (LA, 18:2 n-6) and alpha-linolenic acid (ALA, 18:3 n-3) to finally AA (20:4 n-6) and EPA (20:5 n-3) (Hutzell and Krusberg, 1982; Watts and Browse, 2002). 18:1n-9 is desaturated by FAT-2 to produce 18:2n6. FAT-1 desaturase can use 18:2n-6 to generate 18:3n-3. The desaturase enzymatic activity of *fat-3*, which encodes a single Δ6-desaturase, can convert 18:2n-6 and 18:3n-3 to stearidonic acid (SDA, 18:4n-3) and γ-linolenic acid (GLA, 18:3n-6), respectively. Then, the elongation of both C18 PUFAs are catalyzed by ELO-1 and ELO-2 to produce n-3 arachidonic acid (O3AA, 20:4n-3) and dihomo-γ-linolenic acid (DGLA, 20:3n-6), which are desaturated via the Δ5 desaturases *fat-4* to produce EPA and AA, respectively. Several CYPs together with the NADPH-CYP-reductase EMB-8 metabolized these C20 PUFA to specific sets of regioisomeric epoxy- and hydroxy-derivatives. *ceeh-1* and *ceeh-2*, encoding soluble epoxide hydrolase, were identified to mediate the enzymatic activity in hydrolysis of epoxides which results in the corresponding diols (Harris et al., 2008a), called DHEQ and DHET, respectively. Mutations in *fat-2* or *fat-3* cause C20-PUFA deficiency, which display defects in

movement, defecation cycle, pharyngeal pumping activity, basal innate immunity and growth. The *fat-3* strain for instance, showed slower development and pumping activity, smaller broods, less movement and defecation cycles, and more dumpy body shape compared with the wild-type (Watts et al., 2003). Almost all of these impairments can be rescued by supplementation with AA and/or EPA to the mutant worms (Lesa et al., 2003; Nandakumar and Tan, 2008; Raabe et al., 2014a; Watts and Browse, 2002). Recently, an alternative PUFA synthesis pathway was revealed in *fat-3(wa22)* mutant resulting in small amounts of juniperonic acid (JuA, 20:4n-3) (Guha et al., 2020). Moreover, exogenous supplementation with JuA can partially rescue the deficiency of *fat-3(wa22)*, indicating that JuA may share biological functions with EPA and/or AA - a circumstance that could explain the astonishing survivability of this mutant, assumed to be C20-PUFA depleted.

The *fat-1(wa9)* mutant strain lacks n-3 PUFAs including EPA, whereas *fat-2(wa17)* as well as *fat-3(wa22)* served as strains to analyze the consequence of both EPA and AA deficiency (see Table A4 for the detailed fatty acid profiles of the different strains). In the response to the lack of EPA, *fat-1(wa9)* mutant does not develop acute functional tolerance (AFT) to ethanol rather than N2 wild-type (Raabe et al., 2014b). Interestingly, transgenic *fat-1* mice were protected from the acute alcohol-induced liver disease (ALD) by increasing endogenous n-3 PUFA, suggesting a preventive function of these PUFA (Huang et al., 2015).

Finally, the *emb-8(hc69)* strain, expressing a temperature-sensitive CPR, was used in this work It allows at the restrictive temperature a conditional knock-down of all CYP monooxygenase activities in *C. elegans*, because they then lack the necessary CYP reductase activity.

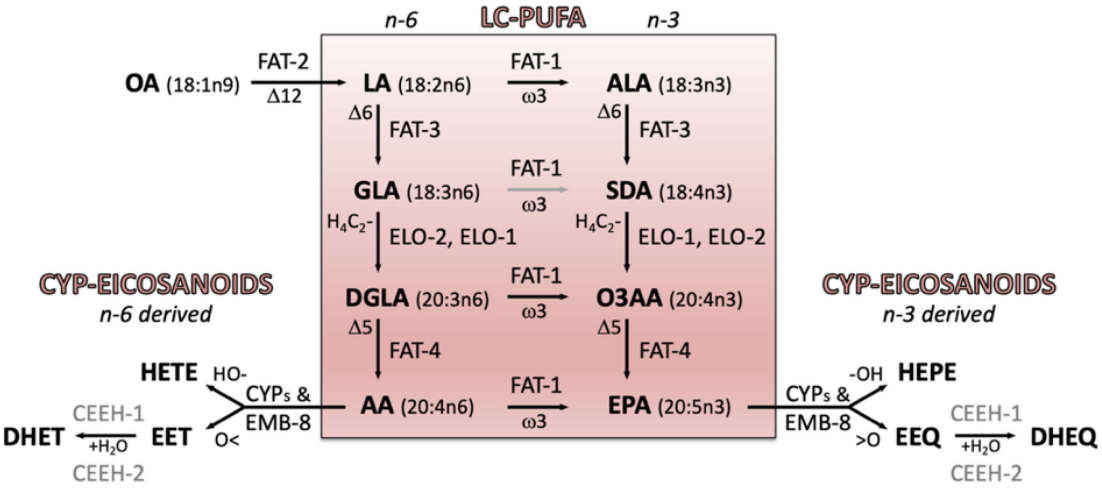


Figure 3: Pathway of PUFA and eicosanoid synthesis in *C. elegans*.

The red box shows PUFA-biosynthesis. AA (20:4n-6); ALA (18:3n-3); DGLA, dihomo-γ-linolenic acid (20:3n-6); EPA (20:5n-3); GLA, γ-linolenic acid; LA (18:2n-6); OA, oleic acid (C18:1n-9); O3AA, n-3 arachidonic acid; SDA, stearidonic acid (18:4n-3). Outside the red box: Synthesis pathway of n-3 and n-6 PUFA derived eicosanoids mediated by CYPs, soluble epoxide hydrolases (sEH, CEEH-1 and -2) catalyzes the hydrolysis of epoxides (Harris et al., 2008a). DHEQ, dihydroxyeicosaquatraenoic acid; DHET, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; HEPE, hydroxyeicosapentaenoic acid. Picture taken from (Zhou et al., 2015).

1.3.3 CYP-eicosanoids, stereoisomers and signaling in the pharynx of *C. elegans*

Interested in potential roles of CYP-eicosanoids in *C. elegans*, gene silencing and pharmacological CYP inhibition were performed and showed that pharyngeal pumping frequencies are reduced (Kulas et al., 2008a). Meanwhile, the localization of *cyp-33E2* gene expression was revealed in the marginal cells of the pharynx (Kosel et al., 2011b). The pumping impairment phenotype also appears in *fat-2* and *fat-3* mutants, the hypothesis that AA- and/or EPA-derived CYP-eicosanoids modulate the pharyngeal activity was born. Pharyngeal pumping is controlled by endogenous and environmental cues and requires muscle-neuron interactions (Song and Avery, 2013), whereby neurohormones, such as serotonin (stimulatory effect) or octopamine (inhibitory effect) play a major role (Horvitz et al., 1982b; Luedtke et al., 2010; Rogers et al., 2001b).

The genome *of C. elegans* encodes 80 CYPs (Laing et al., 2015) and shows homology to mammalian CYP isoforms essential to the production of PUFA metabolites. CYP33E2-produced eicosanoids derived from EPA (Kosel et al., 2011a; Kulas et al.,

2008a) consist of a series of stereoisomeric epoxyeicosatetraenoic acids (EEQs) as well as hydroxyeicosa-pentaenoic acids (HEPEs). The enzymatic nature of the epoxidation reaction of EPA was proven by the unequal ratio of 72:28 of the two stereoisomeric components found, R,S- and S,R-enantiomers of 17,18-EEQ, indicating the presence of a stereo-selective EPA epoxygenase in *C. elegans* (Kulas et al., 2008a). Based on our previous experiments with 17,18-EEQ (Zhou et al., 2015), the mutant *fat-3(wa22)*, failing to synthesize LC-PUFAs and corresponding eicosanoids, exerted a higher increase of pumping activity effects in response to 17,18-EEQ treatment than wild-type. Most of the vertebrate's behavioral effects of serotonin were proven to be mediated by G-protein-coupled receptors (GPCRs). A growing body of evidences suggest CYP-derived eicosanoids may serve as second messengers in the regulation of pharyngeal pumping in *C. elegans*.

Many efforts have been made to elucidate the mechanisms of bioactivation of other PUFA branch-derived eicosanoids, such as the AA lipoxygenase metabolite LXA4, which was identified as the highly stereospecific ligand of the G protein-coupled receptor (GPCR) ALX and acts on specific cellular targets in a stereoselective manner with cell type-specific signaling pathways (Chiang et al., 2006b).

1.4 CYP eicosanoid and G protein-coupled receptors (GPCRs)

The involvement of CYP-eicosanoids in the modulation of ion channels or other physiological processes in *C. elegans* is unknown. It has been shown, however, that many ion channel-forming proteins of the worm have mammalian orthologous. About 70 *C. elegans* genes are coding for K^+-channels, which regulate the excitability of neurons and muscle cells (Wei et al., 1996); there exist also Na^+- (Le and Saier, 1996), Ca^{2+} (Jeziorski et al., 2000) and TRP-channels (Harteneck et al., 2000) comparable to mammalian forms. As an example, impairments of a mutated TRP-channel [*osm-9(ky10)*] could be rescued by transgenic expression of the rat TRPV4 gene in *C. elegans* (Kahn-Kirby et al., 2004). Interestingly, TRP-channels of *C. elegans* can be regulated by lipid derived signals of still unknown identity (Kahn-Kirby and Bargmann, 2006). In mammals, TRPV4 is activated by EETs (Vriens et al., 2005; Watanabe et al., 2003).

Moreover, *C. elegans* harbors a huge number of GPCRs, probably products of gene duplications, loss of introns and subsequent diverse development (Robertson, 1998).

Eicosanoids have been elucidated to activate a variety of intracellular signaling pathways and mediate their functions often through specific cell surface G-protein-coupled receptors. However, the study of GPCRs activated by CYP-produced eicosanoid is still poorly characterized. A highly specific responsiveness to regioisomers and even stereoisomers indicates a specific binding site for the initiation of an CYP-eicosanoid response and previous study assumed that receptors may be involved. So, 14(*R*),15(*S*)-EET rather than 14(*S*),15(*R*)-EET, has a high-affinity binding site in guinea pig mononuclear cell membranes (Wong et al., 1993). Guanine nucleotide-binding protein (Gs) activation plays a vital role in EET functions and modulates G protein-coupled receptors. For example, ADP-ribosylation was stimulated by 14,15-EET in the presence of GTP in rat liver cytosol (Seki et al., 1992). 20-iodo-14,15-epoxyeicosa-8(Z)-enoic acid (20-I-14,15-EE8ZE), a radiolabeled EET agonist, stimulates U937 cell cAMP accumulation, which is a second messenger of GPCR-Gs-adenylyl cyclase signaling cascade, suggesting a specific EET Gs-coupled receptor in U937 cells (Yang et al., 2008). In the last few years, there have been substantially more studies dealing with the specific analysis of receptors of CYP-eicosanoids. The existence of low-affinity 14,15-EET GPCRs were confirmed but none high affinity Gs-coupled receptors were identified (Liu et al., 2017). Garcia (Garcia et al., 2017) revealed the G-protein receptor 75 (GPR75) as the 20-HETE receptor activating a signaling cascade via Gαq/11/PLC/PKC and c-Src/EGFR pathways. This was the first identification of a CYP-eicosanoid receptor. By using the bioinformatic analysis, GPR132 is expected to require for EET-induced hematopoietic stem cell specification in the zebrafish (Lahvic et al., 2018). GPR40 is a low-affinity receptor of 5,6-, 8,9-, 11,12-, and 14,15-EET in vascular cells and arteries. (Park et al., 2018).

1.5 Aims of this work

The nematode *Caenorhabditis elegans* harbors several CYP (cytochrome P450) genes that are homologous with mammalian CYP isoforms important to the production of

physiologically active metabolites of LC-PUFAs, the CYP-eicosanoids. Obviously, mammals and *C. elegans* share similar basic mechanisms of CYP-dependent eicosanoid formation and action. The main objective of the work was to decipher the functional role of CYP-eicosanoids in the regulation of the pharyngeal activity of *C. elegans*. The biosynthesis of eicosanoids in the marginal cells is predicted to occur in response to the release of specific neurohormones of neuronal cells nearby. The CYP-eicosanoids may activate as paracrine regulators of nearby cells via GPCRs and trigger this response immediately to pharyngeal muscles that execute pumping.

The major unresolved problem concerning CYP-eicosanoids, both in mammals and in *C. elegans*, is the identity of their primary molecular targets. From an evolutionary viewpoint this model can offer an excellent early example for the development of CYP-eicosanoid dependent signal transduction to regulate the activity of muscle cells. In detail, this thesis addresses and processes the following questions:

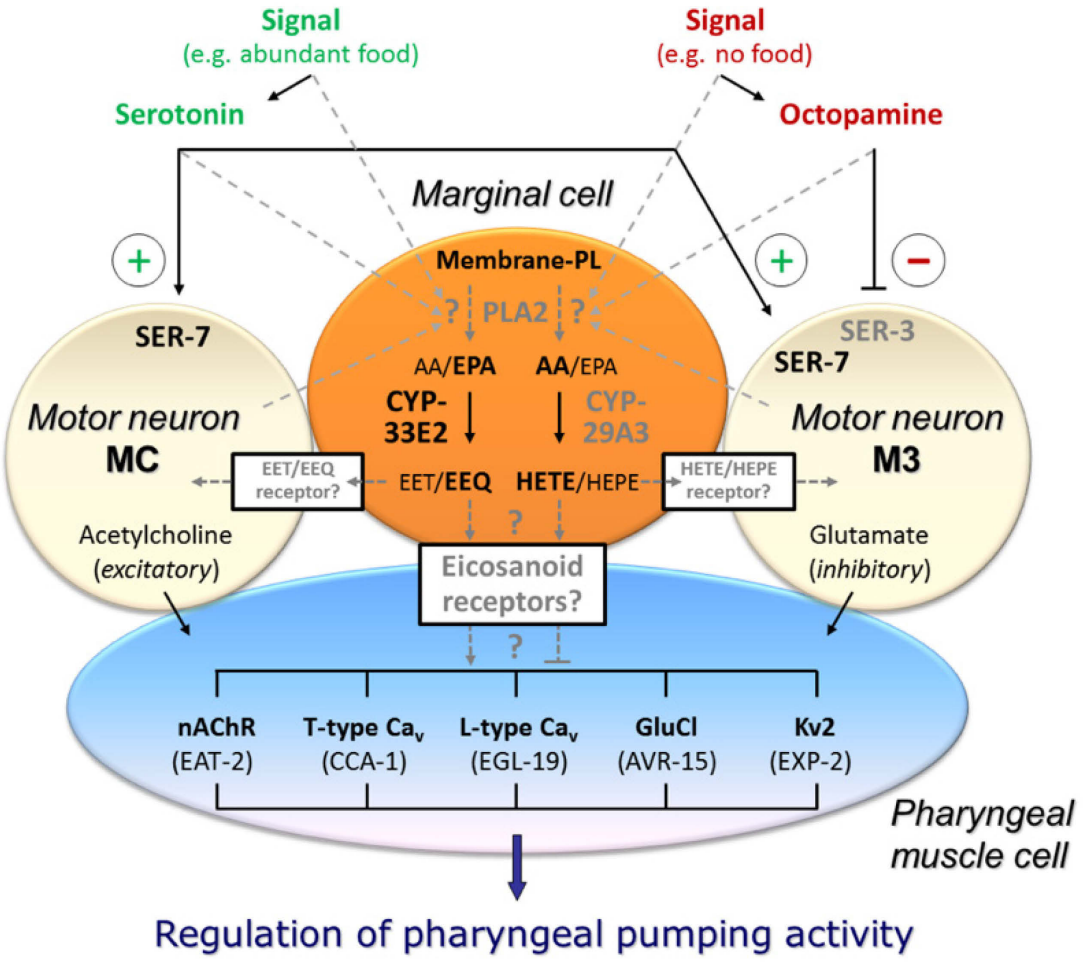
(**I**): Do CYP-eicosanoids and neurohormones share similar function to regulate pumping activity? In turn, how neurohormones affect the formation of CYP-eicosanoids?

(**II**): Does the CYP-eicosanoid 17,18-EEQ work stereospecific when activating the pharyngeal pumping in *C. elegans*? If yes, which enantiomer is active?

(**III**): Does a serotonin as well as a serotonin receptor participate in the regulation pathway to activate 17,18-EEQ biosynthesis?

(IV): Which G-protein coupled receptors do mediate the 17,18-EEQ effects?

This study intents to understand what are the fundamental mechanisms underlying the effects of CYP-eicosanoids in *C. elegans*. The primary molecular targets of CYP-eicosanoids are assumed to be evolutionary conserved. Revealing the identity of these receptors is a key issue and still open question of all the research in the field of CYP-eicosanoids. The use of *C. elegans* as model organism may provide a novel approach to answer this question. Fig. 4 places the posed questions in a cellular context.



<u>Figure 4:</u> Model for the effects of CYP-eicosanoids on muscle cells in *C. elegans*.

(*This scheme is a gift from Dr. Ralph Menzel, not yet published*)

The eicosanoid synthesis is initiated by the release of AA/EPA from membrane phospholipids by a so far unidentified phospholipase A2 (PLA2). Also, the origin of the inducing signal is unknown. Then, CYPs hydroxylate and/or epoxygenate accessible AA/EPA to corresponding eicosanoids. These signaling molecules act via a still unknown signal transduction which probably activates GPCR(s), transmitting the signal either directly to muscle cells or nearby neurons. CYP-eicosanoids act in this respect as ligands for GPCRs to mediate the intended behavioral response, or alternatively, eicosanoids might modulate GPCRs indirectly via additional signaling mediators, such as neuropeptides or endocannabinoids. Also, an activation/inhibition of pharyngeal muscle cells via gap-junctions is conceivable. Final effectors might be one or more of the main ion channels responsible for efficient depolarization/re-polarization of muscle cells. The reciprocal regulation of feeding behavior by serotonin and octopamine, probably providing a mechanism for adapting to the presence and absence of food, respectively, should be mirrored by the contrasting effects caused by different eicosanoids, as 17,18-EEQ and 20-HETE. Solid lines stand for known components and connections, grey broken lines represent presumed parts of the CYP-eicosanoid pathway.

Chapter Two: Materials and Methods

2.1 Strains and cultivation

The *C. elegans* wild-type strain used in this study was Bristol N2. Mutant strains are BX24, *fat-1(wa9)*; BX26, *fat-2(wa17)*; BX30, *fat-3(wa22)*; MJ69, *emb-8(hc69)*; VC40814, *cyp-29A3(gk827495)*; RB2321, *ceeh-1(ok3153)*; DA1814, *ser-1(ok345)*; DA2100, *ser-7(tm1325)*; DA2109, *ser-7(tm1325) ser-1(ok345)*; DA464, *eat-5(ad464)*; DA541, *gpb-2(ad541)*; DA1084, *egl-30(ad806)*; MT363, *goa-1(n363)*; NL594, *gpa-12(pk322)*; *npr-34(tm1665)*; RB1288, *nmur-1(ok1387)*; RB1325, *npr-10(ok1442)*; XA3702, *npr-2(ok419)*; RB1405, *npr-22(ok1598)*; RB761, *npr-7(ok527)*; VC2421, *npr-24(ok3192)*; RB1632, *npr-25(ok2008)*; RB2526 *nmur-2(ok3502)*; DA465 *eat-2(ad465)*; VC39 *cca-1(gk30)*; DA695 *egl-19(ad695)*; DA1051 *avr-15(ad1051)* and JT5132 *exp-2(sa26)*. All strains were provided by the Caenorhabditis Genetics Center (CGC). The million-mutation project derived VC40814 was five-times outcrossed to wild-type before analysis. Selection of *cyp-29A3(gk827495)* genotype took advantage of a missing *Mst*I restriction site in the mutant allele background.

Well-fed animals were maintained on nematode growth media (NGM) plates seeded with *E. coli* OP50 as food source and incubated at 20°C except *emb-8(hc69)*, which was maintained at 15°C and shifted to restrictive 25°C during the assay; for details see (Kulas et al., 2008b). Only hermaphrodite individuals were assayed in all experiments. Unless otherwise stated, bacteria for pumping experiments were UV-killed by 1 h exposure to 5.6 mw/cm^2 UV-light on a transilluminator (Fluo-Link FL-20-M, Bachofer, Reutlingen, Germany). UV treatment was approved as effective when no *E. coli* cells were able to grow after spreading bacterial suspension onto a LB agar plate and incubated overnight at 37°C.

2.2 RNAi by feeding

The bacterial feeding strains were obtained from the RNAi feeding strain stock collection (Gene Service, UK). The RNAi by feeding assay (Hull and Timmons, 2004) was performed on NGM agar plates supplemented with additional antibiotics (50 µg/ml ampicillin, 12.5 µg/ml tetracycline) and 0.8 mM IPTG. *E. coli* HT115 bacteria containing RNAi vectors expressing dsRNA of the genes *cyp-29A3* and *cyp-33E2*, respectively, were pre-cultured overnight, induced with IPTG. The *E. coli* HT115 main culture was incubated for additional 4 h at 37 °C and IPTG was added to a final concentration of 0.4 mM. The cells were harvested by centrifugation, if multiple RNAi feeding strains were utilized equal amounts of cell suspension were collected. The bacteria were resuspended in fresh LB medium, spiked with 50 µg/ml ampicillin, 12.5 µg/ml tetracycline and IPTG (to a concentration of 0.8 mM). Finally, the cells were added to NGM agar plates, containing the same concentration of antibiotics and IPTG as described above. From day three on additional freshly prepared dsRNA producing cells were used to feed the worms daily.

2.3 Chemicals

IPTG and the antibiotics were purchased from Roth (Karlsruhe, Germany), octopamine, serotonin, and EPA from Sigma-Aldrich (St. Louis, MO, USA), and AA, eicosatetraynoic acid (ETYA) as well as all used CYP-eicosanoids from Cayman Chemicals (Ann Arbor, MI, USA). Both quantity and purity of prepared eicosanoid stock solutions were confirmed by LC-MS/MS measurements (data not shown). The compound used as 17,18-EEQ agonist was synthesized as described previously (Falck et al., 2011b). The enantiomers of 17(*R*),18(*S*)-EEQ and 17(*S*),18(*R*)-EEQ were enzymatically produced and purified as described previously (Blum et al., 2019; Schwarz et al., 2004). To prevent autoxidation, all stock solutions, except for neurohormones, were prepared in an oxygen evacuated nitrogen chamber. Dimethyl sulfoxide (DMSO), purchased from Sigma-Aldrich, was used as solvent, only neurohormones were dissolved in deionized water.

2.4 Preparation of assay plates and treatment

For 3d long-term incubation, PUFA stocks were mixed with living OP50 bacteria and seeded on NGM plates at final concentration of 80 µM in the bacterial lawn; a concentration following the work of Watts & Browse (Watts et al., 2003). Plates were dried in the dark. Worms from a mixed culture were chunked to assay plates to ensure that next generation was fed their whole life with dietary PUFA. Then, synchronized L1 progeny was incubated for three days prior use in the pumping assay. Synchronization was achieved by rinsing worms from NGM plates with M9 buffer, filtering through a 10 µm gauze membrane retaining all but first-stage juveniles and incubating them for a further three days on fresh NGM/OP50 agar plates. Control experiments were included by mixing only solvent, 0.3% (v/v) DMSO, with the bacteria. All chemicals used for short-term (40 min) incubation were spread onto the NGM plates together with either UV-killed OP50 or HT115 (RNAi) bacteria. Octopamine was added to a final concentration of 50 mM (Horvitz et al., 1982b). The final concentration for eicosanoid (and EPA) for short-term treatment was 10 µM.

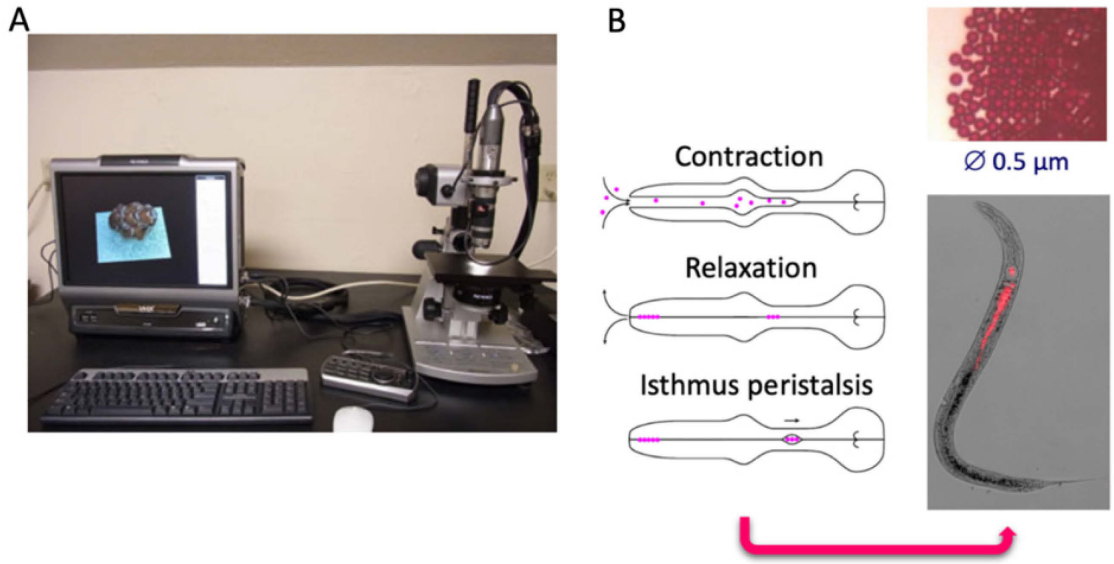
To test the impact of octopamine on pumping in the presence of food, about 4×8 synchronized young adults were set on separated small bacterial lawns for about 10 min to let them adapt. For the corresponding control, bacteria without supplementation were applied. In the eicosanoid assay, the same procedure was carried out except that the incubation time was 40 min. In this case, DMSO containing plates served as vehicle control. The different incubation times of neurohormone and eicosanoid assays required, in case of a combined experiment, two separate assay plates. Here, worms were set first on an eicosanoid containing plate for 30 min, then picked to a neurohormone plus eicosanoid containing plate and stayed for further 10 min.

For testing the impact of serotonin in the absence of food, 4×8 synchronized young adults were set on a M9-agar plate, washed two-times with small M9 drops, re-picked to a second M9-agar plate and incubated for 80 min. This strict procedure prevented a notable carry-over of bacteria and let worms in fact starve, clearly indicated by a sharp drop of pumping frequency in the course of incubation time. Finally, worms were transferred to agar pads supplemented with 2 mM serotonin (Avery and Horvitz, 1990b) for 10 min to let them adapt. In the eicosanoid assay, the procedure was altered in the following way. After deprivation of food for 50 min, a small drop of M9 buffer mixed

with 17,18-EEQ was dropped on an unseeded NGM plate. Then, the starved worms were picked into this drop and incubated for 40 min; the worms were not able to leave. To prevent evaporation, M9-infiltrated filter paper was paved inside the lid and covered up. For the serotonin/17,18-EEQ combined experiment, again two separate assay plates were included and carried out as mentioned above for the octopamine assay. For the corresponding control, plates without supplementation were applied.

2.5 Pharyngeal pumping assay

The feeding assay was valuable for screening the pumping activities in *C. elegans*, not only for measuring the pharyngeal contractions of different kinds of strains, but also testing the effects of signals and stimuli from the environment and chemicals on pumping frequency. In a traditional way, researchers usually tracked and counted pumping rates under the microscope in real-time monitoring with a manual clicker counter. But at some point, it is hard to catch the muscle contractions of pharynx precisely just with eyes monitor if the strains pump too fast. This might result in mistakes when carry out the data analysis. So, in our lab, a VHX-600 digital microscope (Keyence Corporation, Osaka, Japan) was purchased (Fig. 5A) to make a 1 min film of each worm at 500 × magnifications and played back the original video slowly, so that each individual pump can be counted exactly. At least 8 animals were tested per each trial; all experiments were performed at least in triplicate.



<u>Figure 5:</u> Quantification of pharyngeal pumping.

Counting by using a VHX-600 digital microscope (**A**), lab photo. Feeding assay with red fluorescent polystyrene beads (B). (Avery and Horvitz, 1989a), modified.

2.6 Feeding assay

In order to bring high-throughput screening automated analysis and sort to *C. elegans*, more quantitative methods were developed. So, worms were fed iron particles or fluorescent microspheres (Fig. 5B) to count the number of particles in worms' intestine or measure the density of fluorescence within the worms (Avery and Horvitz, 1990a; Boyd et al., 2007; Fang-Yen et al., 2009; Kiyama et al., 2012b; Pulak, 2006; Rohlfing et al., 2011; Smith et al., 2008). Microsphere particles uptake by *C. elegans* worms has been a successful mean to evaluate toxicity and several previous studies have already indicated that the uptake of particles was indeed related to pharyngeal pumping rate and responded to changes in nutritional status (Avery and Horvitz, 1990b; Boyd et al., 2007; Kiyama et al., 2012a; Klass, 1983). Here, a semi-automated and quantitative method was chosen to measure *C. elegans* uptake activity by using an Infinite F200 Pro fluorescence reader (Tecan) that quantitatively measures feeding by worms' incorporated fluorescence after exposure to fluorescence beads.

FluoSphere® carboxylate modified microspheres (red fluorescent; 0.5µm) from Life Technologies (Carlsbad, CA, USA) were used in this assay. The original microspheres were diluted 1:50 in M9 buffer. For well-fed conditions, 25 µl of particle suspension

was mixed with 175 µl of OP50 bacterial suspension (OD_{600}=4.5) containing chemicals at the concentrations as described before and pipetted to a 6 cm NGM agar plate. These exposure plates were dried in the dark. For beads accumulation in the presence of food, 50 age-synchronized young adult hermaphrodites were picked onto a 20-HETE containing or control plate for 30 min pre-exposure. Then, nematodes were transferred to an exposure plate to allow them to ingest of microspheres for 10 min. After that worms were anesthetized using 50 µl of sodium azide (1 M). For testing octopamine, worms were picked directly to exposure plates containing only 50 mM octopamine and incubated for 10 min. For combined exposure, 20-HETE pre-incubated worms were transferred to an exposure plate containing both octopamine and 20-HETE. For starved condition, worms were cultivated in the absence of food prior the test as described above: for 80 min in case of subsequent serotonin exposure and for 50 min in case of individual 17,18-EEQ and subsequent joint exposure. Besides that, M9 buffer was used instead of bacterial suspension, all other steps were identical as in case of well-fed condition.

Before measuring the density of fluorescence, 35 worms were picked on an unseeded part of the used NGM plate to wash them several times with M9 buffer. Then, 5 × 7 worms were transferred to a 96-well V-bottom plate filled with 100 µl of pure ethanol and measured by using an Infinite F200 Pro (Tecan; Männedorf, Switzerland) fluorescence reader (560 nm/612 nm) for three times with a 5 min interval in between. The usage of ethanol prevented sticking of worms on the sidewall of wells and ensured the complete localization on the bottom of plates. The entire test was repeated two-times. All chemicals plates were prepared for the experimental trials according to the two-phase assay procedures. Phase one was for pre-culture containing only eicosanoids, and the phase two was for beads digestion which used both eicosanoids and microspheres. Fig. 6 illustrated the schematic flow of pumping rate and microsphere beads uptake assay. All the plates were prepared fresh for the experiments on the same day. Microscopic images were acquired with an Eclipse E200 from Nikon, (Chiyoda, Tokyo, Japan) coupled to the VHX-600 digital camera (Keyence Corporation, Osaka, Japan).

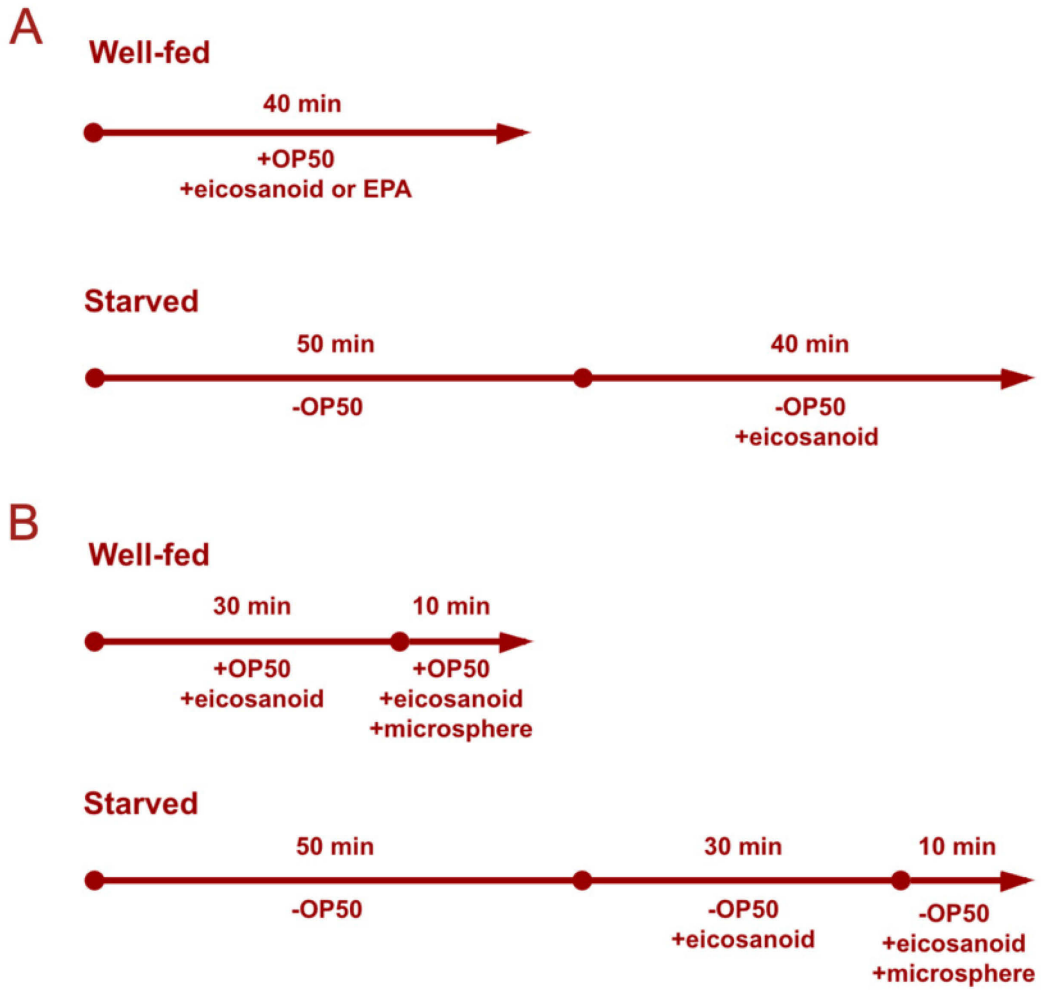


Figure 6: Timeline of experimental design for pumping (A) and microsphere uptake assay (B).

2.7 Analysis of endogenous fatty acid and eicosanoid pattern

Synchronized three days old adult worms were carefully washed off from NGM agar plates with a few ml of M9 buffer by slightly canting the plates back and forth. This procedure removed almost all adult worms but hardly any laid eggs from the plate. All steps were performed in the cold at 4°C. Already hatched small larvae were separated by filtering through a 10 µm gauze membrane. The resulting worm filter cake with the adult worms was rinsed from the membrane and washed two times with M9 buffer to remove adhering bacteria. Then, the worms (about 50 mg fresh weight) were transferred

to a 1.5 mL reaction tube and spun down at 2,000 × g for 1 min; the pellet was frozen at -80°C.

In case of treatment with octopamine, harvested adult worms were immediately set on freshly prepared NGM plates containing 50 mM octopamine in the bacterial lawn of *E. coli* OP50. The corresponding controls without octopamine (or serotonin) were handled in the same way. The incubation time of this bulk culture was 15 min; afterwards, the harvest was performed as described above.

To test the impact of serotonin on free eicosanoid profiles in starved condition, the stock solution of serotonin was diluted fresh in M9 buffer and spread onto M9 agar plates at a final concentration of 2 mM. Plates were allowed to dry at room temperature in the dark. Synchronized young adult worms were carefully washed off from seeded NGM agar plates with a few milliliters of M9 buffer. Three more times rinsing was performed to remove the bacteria of nematodes' cuticles thoroughly. Then, worms were set on M9 agar plates and incubated for 80 mins. The incubation time of this bulk culture on the serotonin assay plates was 15 min; DMSO was paralleled in the same way.

The CYP-eicosanoid and fatty acid profiles of the harvested worms were determined using LC-MS/MS by Lipidomix as described previously (Kulas et al., 2008b). To differentiate between esterified and free CYP-eicosanoids, the homogenates were extracted with or without prior alkaline hydrolysis.

2.8 Statistical analyzes

Pumping assay and eicosanoid pattern datasets were analyzed by t-test or one-way ANOVA to test for significant differences between treatments followed by the Bonferroni test to identify treatments that were significantly different from the control. All statistical tests were performed using Sigma Stat 3.5 (Systat Software Inc., San Jose, CA, USA).

Chapter Three: Results

To characterize the fatty acid and CYP-eicosanoid pattern as well as the pumping activity in *C. elegans*, strains differing in their genetic backgrounds were analyzed. The work of the pumping frequency as well as PUFA and endogenous eicosanoids profiling do partially overlap with previous research. For instance, fatty acid composition of N2 wild-type, *fat-1*, *fat-2* and *fat-3* was detected by Watts group and Menzel group (Kulas et al., 2008a; Watts and Browse, 2002). Endogenous metabolites of AA and EPA in *C. elegans* (N2 wild-type, *fat-1* and *fat-3*) as well as the pumping frequency with PUFA and RNAi by feeding (N2 wild-type and *fat-3*) were analyzed by Menzel group (Kosel et al., 2011a; Kulas et al., 2008a). However, a more precise pumping counting method was used in this work (See 2.5). All experiments should also be carried out under the same conditions and not only earlier data should be used in an uncontrolled manner. Therefore, the pumping activity of N2 and *fat-3* was analyzed via new counting method. Also, all PUFA and eicosanoids pattern were tested freshly in this work.

3.1 Effects of genetic modifications on pharyngeal pumping and the endogenous CYP-eicosanoid profile

Fig. 7 compares the pumping activities of strains characterized by a different fatty acid profile background. The pharyngeal pumping on bacteria was assayed using young adult. In wild-type animals, pumping was consistent with a frequency of 285.7 ± 15.5 (mean±SD) contractions/min (Fig. 7).

In contrast, pumping frequencies on bacteria were dramatically decreased in *fat-2(wa17)* and *fat-3(wa22)* mutant strains. Actually, these AA- and EPA-deficient worms pumped with about 171.55 ± 15.88 [*fat-2(wa17)*] and 200 ± 19.78 [*fat-3(wa22)*] contractions/min, in about 40% and 30% reduction compared to the wild-type activity respectively, (Fig. 7). Supplemental Video S2 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4617398/bin/supp_56_11_2110__index.html) shows a *fat-3(wa22)* worm pumping 207 times per minutes. The inability to maintain a wild-type like quick

pharyngeal activity was more pronounced in *fat-2* than in *fat-3* (Fig. 7). Interestingly, the pumping frequency of *fat-1(wa9)* worms was almost identical to the wild-type, indicating that n-3 PUFA-deficiency alone is not sufficient to cause the impairment of pharyngeal activity as shown in *fat-3* or even *fat-2* worms. Gene inactivation of *emb-8* exhibits a moderate but significant decrease to about 90% of wild-type activity.

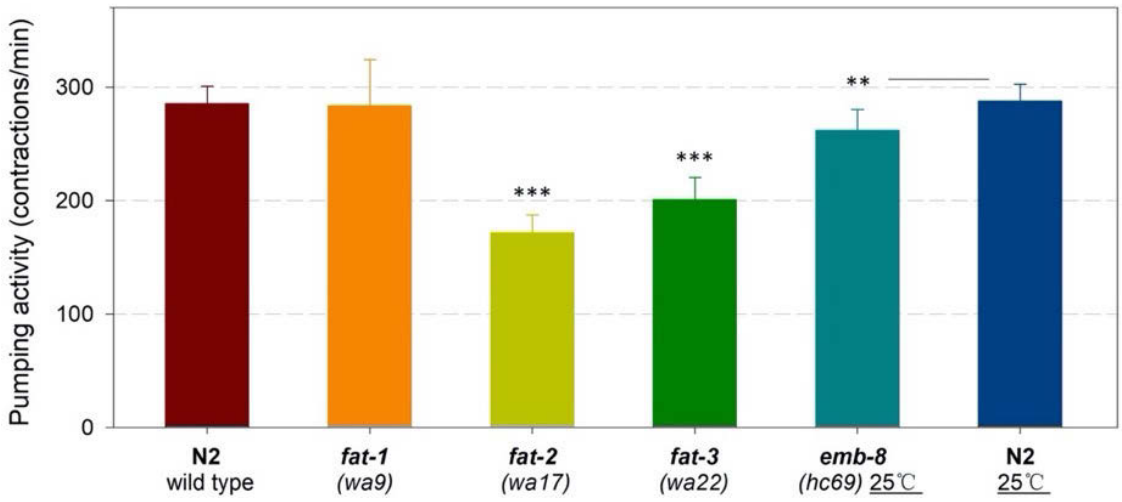


Figure 7: Pumping frequencies in different *C. elegans* strains.

Eicosanoid deficiencies in mutant strains were associated with pumping frequency impairments compared to the N2 wild-type. Data shown as mean ± SD (three trial with n = 8-12 per trial); statistical comparisons were made using one-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$ (Zhou et al., 2015).

The pattern of endogenous CYP-eicosanoids was analyzed both in wild-type nematodes and in pumping activity deficient mutant strains. Eicosanoids isolated from the harvested worms were analyzed by LC/MS/MS and normalized to the protein content of the sample. Fatty acids and multiple eicosanoids of the cytochrome P450 metabolism pathway has been monitored. Total content of 11 FAs and 22 eicosanoids were quantified, respectively (see Tables A4 and A5).

From the stacked column chart (Fig. 8), the eicosanoid pattern of N2 wild-type worms revealed that EPA, the most abundant LC-PUFA in *C. elegans*, was the preferred substrate for CYP enzymes to synthesize corresponding eicosanoids. Stacking all hydroxy- and epoxy-metabolites as well as the corresponding diols compositions, the total content of EPA-derived metabolites was about 10-fold higher compared to their AA-derived counterparts (Fig. 8). 17,18-EEQ and its hydrolysis product 17,18-DHEQ were the prominent CYP-eicosanoids in the wild-type animals, present at a ten-fold

higher concentration than the other metabolites (Table A5). At the restrictive temperature, the thermo-sensitive *emb-8(hc69)* strain exhibited a marked decrease in the level of all EPA- and AA-derived CYP-eicosanoids (Fig. 8 and Table A5). These data support the notion that at the elevated temperature of 25°C, CYP-eicosanoid *de novo* synthesis terminates after the prior period at permissive temperature (15°C). Both AA- and EPA-derived CYP-eicosanoids were only hardly detectable in *fat-2(wa17)* and *fat-3(wa22)* strains. In *fat-1(wa9)*, a strain with almost no detectable EPA-derived metabolites, produced a 7-folder higher amount of AA-derived CYP-eicosanoids compared to the wild-type.

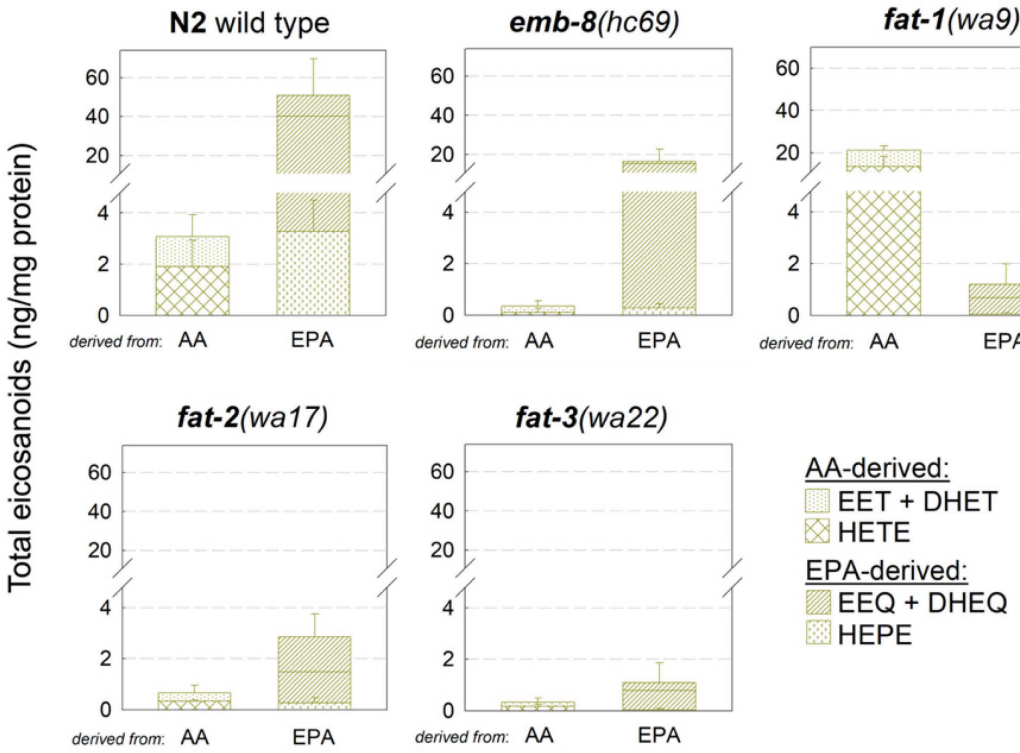


Figure 8: Distribution of PUFAs and eicosanoids in the wild-type and designated mutant nematodes analyzed by LC-MS/MS.
The stacked columns show the total amounts of AA- or EPA-derived metabolites and represent the sum of the corresponding n- and (n-1)-hydroxylase products (HETE/HEPE) as well as the sum of epoxy- and dihydroxy-metabolites (EET+DHET/EEQ+DHEQ). Results are represented by means +S.D. from three independent experiments performed for each strain. For the detailed metabolite profiles see Table A5 (Zhou et al., 2015).

3.2 The impact of treatment with PUFA, eicosanoids and neurohormones on the pharyngeal activities in different mutant strains

3.2.1 Rescue of pharyngeal activity impairments by C20-PUFAs

CYP-eicosanoids, EPA, AA, and ETYA, a non-metabolizable AA analogue (Fig. 9), were compared for their capacities to restore the pharyngeal pumping deficiency of the *fat-3(wa22)* and *fat-2(wa17)* mutant strains. ETYA harbors chemically more stable triple bonds instead of double bonds compared to AA. It is not a substrate for P450 enzymes and was used to provide evidence that CYP-eicosanoid formation is required to restore the pharyngeal activity rather than C20 PUFA themselves. Long-term complementing either EPA or AA significantly restored the impaired pharyngeal activity of both the mutant strains (Fig. 10B,C). However, pharyngeal pumping activity of both *fat-3(wa22)* and *fat-2(wa17)* mutant strains did not reveal this rescue by ETYA (Fig. 10B, C).

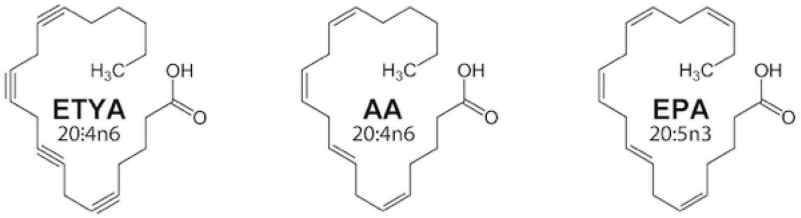
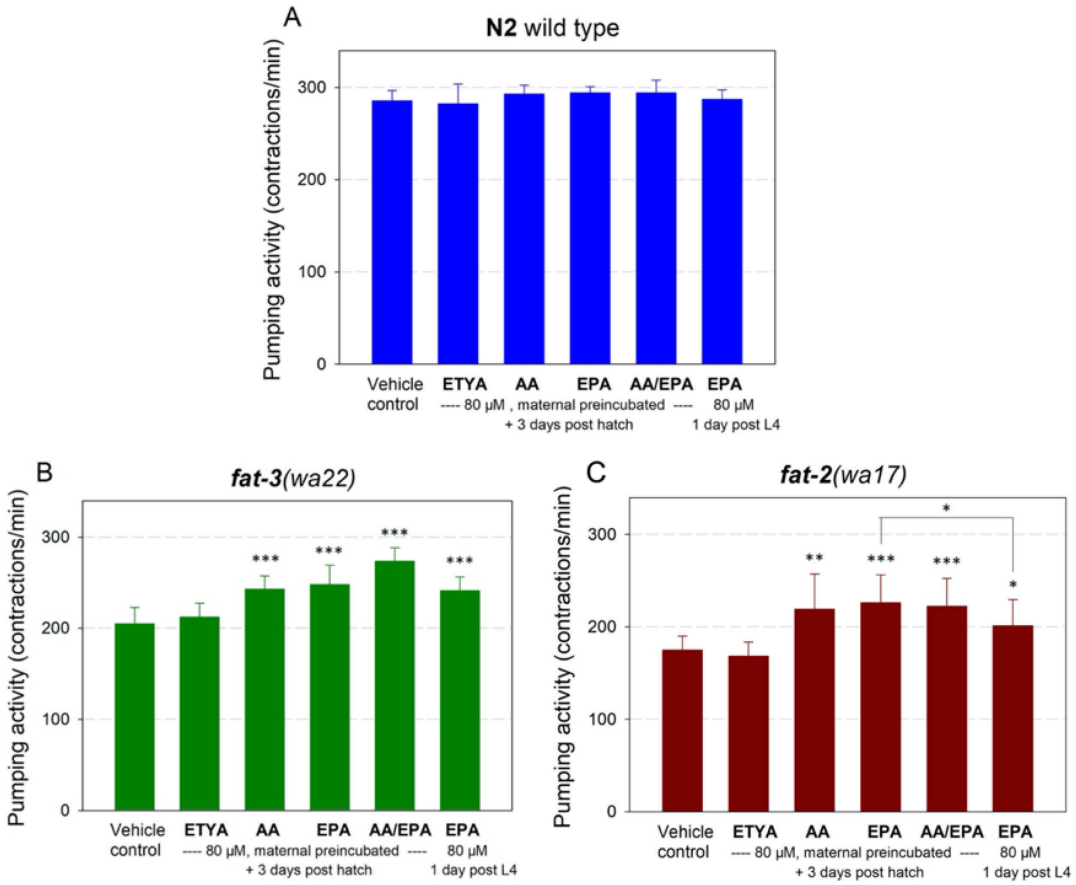


Figure 9: Structural formulas of used PUFAs. ETYA (20:4n-6), AA (20:4n-6) and EPA (20:5n-3).



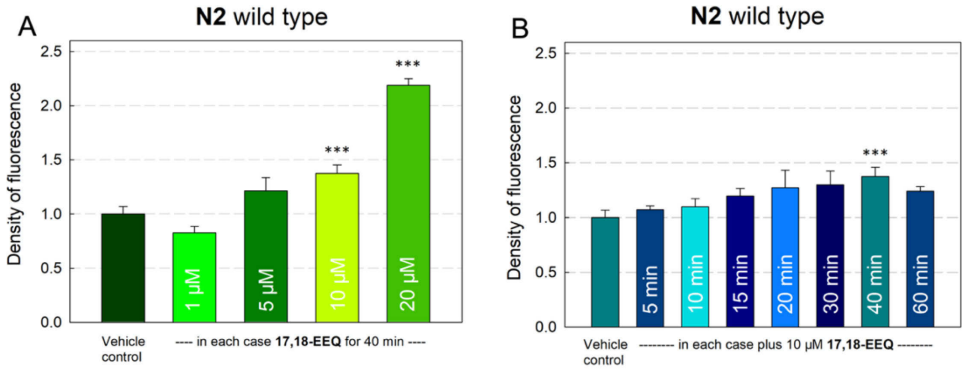
<u>Figure 10:</u> Rescue of *fat-3(wa22)* mutant worms from pumping impairment.

Three days feeding with EPA and AA, but not ETYA, a non-metabolizable AA-analogue, rescued the impaired pumping rate of the *fat-3(wa22)* (**B**) and *fat-2(wa17)* (**C**) strains, but had no effect on pharyngeal activity in the N2 wild-type. Shown are the contractions/min (three trials with n = at least 8 per trial); Results are represented by means +S.D., comparisons were made using one-way ANOVA, $^{**}P$ <0.01, $^{***}P$ < 0.001. (Zhou et al., 2015).

In order to distinguish between developmental or acute requirements for C20-PUFAs in unimpaired pharyngeal pumping, EPA was supplemented to *fat-3(wa22)* and *fat-2(wa17)* mutant strains only after the last larval stage (L4), when major organ and tissue development and differentiation was completed. Biochemical complementation with EPA for 24 h was found sufficient to rescue from impairments in pumping activity in both *fat-3(wa22)* (Fig. 10B) and *fat-2(wa17)* worms (Fig. 10C). While in *fat-3(wa22)* the level of rescue was identical to that in the long-term feeding, *fat-2(wa17)* worms, supplemented with EPA only 24 h post L4, pumped at a significantly lower rate when compared to long-term supplementary worms; its rescue level was only half as high as when EPA was present during the complete development (Fig. 10C).

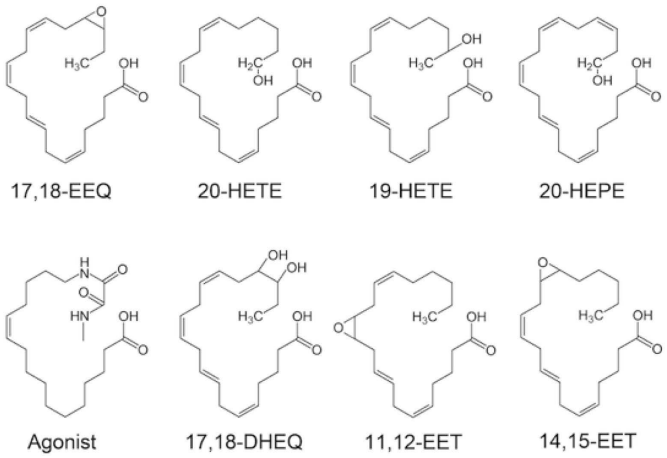
3.2.2 Rescue of pharyngeal activity impairments by CYP-eicosanoids

By searching for the effective metabolites altering the pharyngeal activities, next several EPA- and AA-derived CYP-eicosanoids were tested in wild-type and mutant strains. Based on the LC-MS/MS experiment according to the major EPA-derived metabolite, 17,18-EEQ was selected as a promised CYP-eicosanoid candidate. In order to optimize the experimental condition for the usage of eicosanoids, a preliminary concentration- and time-gradient assay was performed with 17,18-EEQ by using the fluorescence uptake assay. From the results (Fig. 11), the condition of 10 µM effective concentration and 40 min pre-incubation time were chosen for comparing the effects of further compounds (Fig. 12) on pumping frequency.



<u>Figure 11:</u> Concentration- and time-dependent effects of 17,18-EEQ treatment on the food uptake.

Normalized effect of 17,18-EEQ on the accumulation of 0.5 µm microsphere fluorescent beads. The accumulation assay was tested in the presence of bacteria and worms pre-incubated for 40 min with different concentrations of 17,18-EEQ (**A**) or pre-incubated with 10 µM 17,18-EEQ for different time periods (**B**). Data are presented as means + SEM; n=15. P-values were obtained from one way ANOVA, $^{***}P$ <0.001; vehicle: 0.3% DMSO (Zhou et al., 2015).



<u>Figure 12:</u> Structural formulas of used CYP-eicosanoids.
17,18-EEQ, 20-HETE, 19-HETE, 20-HEPE, Agonist, 17,18-DHEQ, 11,12-EET, 14,15-EET.

Under these short-term exposure conditions, EPA had no effect on the pumping activity (Fig. 13), opposite to the long-term rescue experiments described above. However, short-term treatment with 17,18-EEQ accelerated pharyngeal pumping even above frequency of wild-type just on bacteria without any supplementary chemical substances (Fig. 13A), and it rescued the *fat-3(wa22)* mutant strain to the same range as long-term EPA supplementation (compare Fig. 13B and Fig. 10B as well as Supplemental Videos 2 and 3), presenting a *fat-3(wa22)* in well-fed condition with 207 pumps/min and a 17,18-EEQ-treated *fat-3(wa22)* worm pumping with 237 pumps/min (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4617398/bin/supp_56_11_2110__index.html).
Moreover, even if the CPR gene was inactivated *(emb-8)* or RNA interference (RNAi)-mediated inhibition of *cyp-29A3/cyp-33E2* expression, 17,18-EEQ exerted a significant acceleration of pumping frequencies (Fig. 13D) or (Fig. 13F). In case of *fat-2(wa17)* worms (Fig. 13C), only the 2-fold concentration of 20 µM 17,18-EEQ (compare Fig. 10) was effective and sufficient to significantly alter the pharyngeal pumping activity. Notably, a stable and specific synthetic 17,18-EEQ analogue (agonist) was used to confirm the effect of short-range natural 17,18-EEQ by sharing the same capability in elevating the pumping frequencies (Fig. 13F, G). To further confirm the high specificity of the 17,18-EEQ effect, 17,18-DHEQ, the hydrolysis metabolite, 20-HETE, 19-HETE, 20-HEPE as well as 11,12-EET and 14,15-EET, as different regioisomers metabolites of AA were tested, respectively (for structural formulas see Fig. 12). Neither its hydrolysis product, the corresponding diol (17,18-DHEQ) nor the AA-derived

metabolite 14,15-epoxyeicosatrienoic acid (EET) did increase the pumping frequencies (Fig. 13F, G). Only the AA-metabolite 11,12-EET was observed to have a similar effect as 17,18-EEQ (Fig. 13F, G).

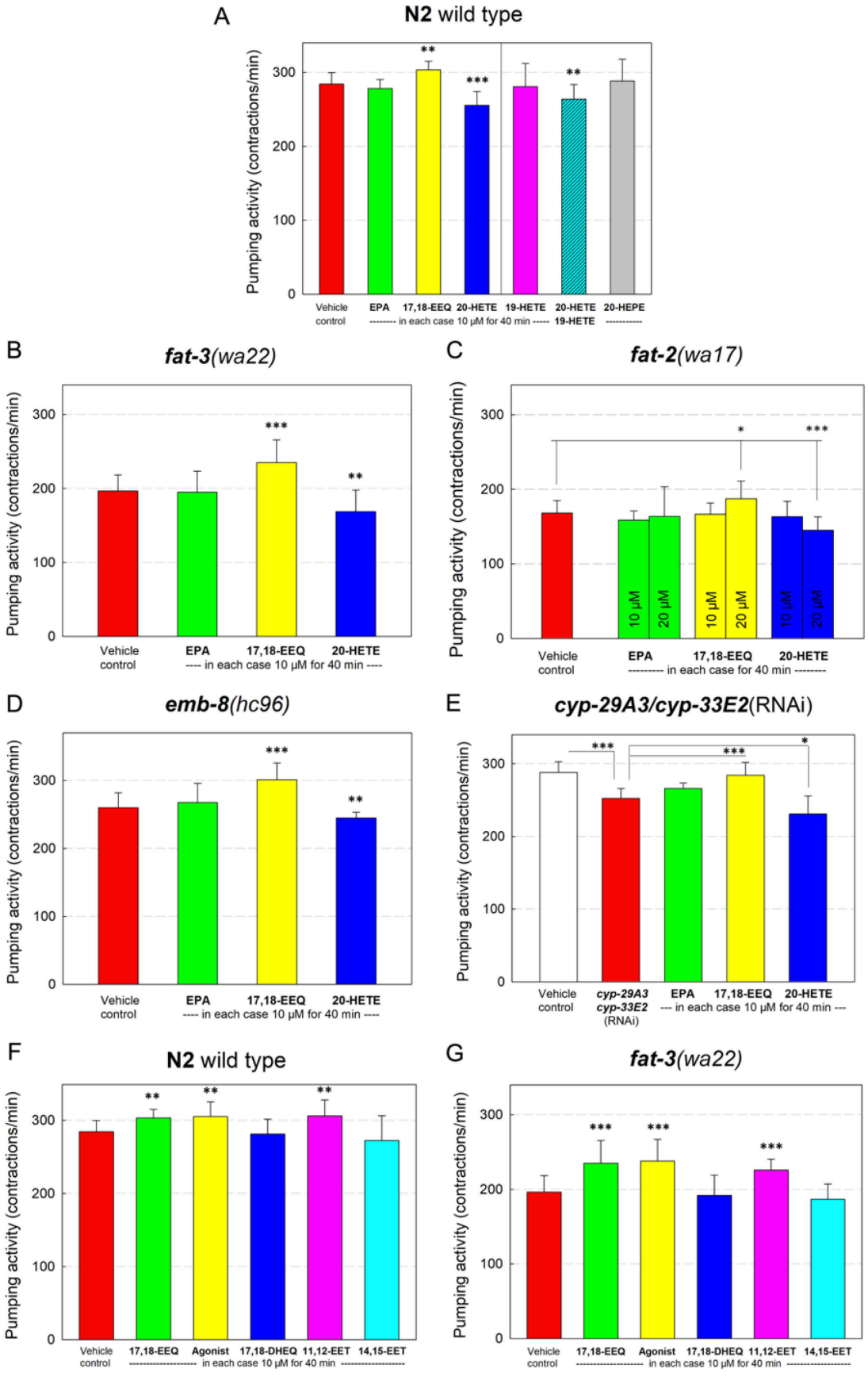


Figure 13: Contrasting effects of different CYP-eicosanoids on pumping activity.

(**A-D**) Reverse effects of CYP-eicosanoids on the pumping frequency in wild-type as well as in the genetically modified strains *fat-3(wa22)*, *fat-2(wa17), emb-8(hc69)* and *cyp-29A3/cyp-33E2*(RNAi). (**F**, **G**) The 17,18-EEQ effect was mimicked by a synthetic agonist but not by 17,18-DHEQ or 14,15-EET. Shown are in each case the contractions/min (three trial with n ≥ 8 per trial); Data are presented as means ± SD, comparisons were made using one-way ANOVA, $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001; vehicle: 0.3% DMSO (Zhou et al., 2015).

Most interestingly, 20-HETE, an AA-derived hydroxide significantly decreased pharyngeal pumping frequencies in the wild-type animals as well as in all genetically modified strains (Fig. 13A-E). The one-minute film of Supplemental Video S4 shows a 20-HETE treated N2 wild-type worm pumping 257 times/min (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4617398/bin/supp _56_11_2110__index.html). In wild-type worms, both its regioisomer 19-HETE as well as the EPA-derived analog 20-hydroxyeicosapentaenoic acid (20-HEPE) had no effect on pumping frequency (Fig. 13A). Moreover, supplementation of both 20-HETE and 19-HETE together did not change any more than 20-HETE alone (Fig. 13A).

3.2.3 Comparison of CYP-eicosanoid and neurohormone effects on pumping frequency

From the data sets above, this is the first demonstration of the effects of CYP-eicosanoids on pharyngeal activity of *C. elegans.* However, it has been well established that the pumping frequency is regulated by neurohormones, such as the biogenic amines serotonin and octopamine (Fig. 14) (Horvitz et al., 1982b; Luedtke et al., 2010; Rogers et al., 2001b). Accordingly, the following experiments were designed to gain insight into potential links between neurohormones and CYP-eicosanoid. Considering that serotonin elicits its stimulatory effect most strongly after starvation and mimics worm's response to re-feeding, worms were now completely deprived of food for 90 min. During the starvation period, the pharyngeal pumping frequency of the wild-type animals decreased from about 285.7 ± 15.5 (compare Fig. 7) to 82.2 ± 11.0 contractions/min and became highly responsive to exogenously added serotonin (Fig. 15). In contrast, well-fed worms were used to study the effect of octopamine since this neurohormone is thought to reduce pharyngeal pumping in the case of satiety.

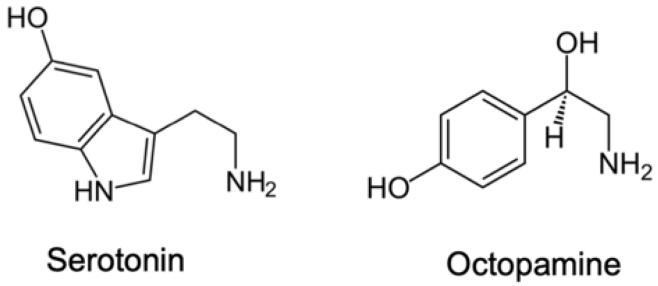


Figure 14: Structural formulas of used neurohormones: serotonin and octopamine.

As shown in Fig. 15, the pharyngeal pumping of starved wild-type worms was dramatically upregulated in response to exogenously administered serotonin. 17,18-EEQ shared the same range of effect in accelerating the pharyngeal activity. The remarkable capacity of 17,18-EEQ in the upregulation of pumping in the absence of food was detectable not only in wild-type, but also in the *emb-8(hc69)*, *fat-3(wa22)* and *fat-2(wa17)* mutant strains, respectively. An even more pronounced stimulating effect was observed by combined administration of serotonin and 17,18-EEQ; significant difference between the groups of single and combination chemicals was observed, however, only in case *emb-8(hc69)* and *fat-2(wa17)* (Fig. 15).

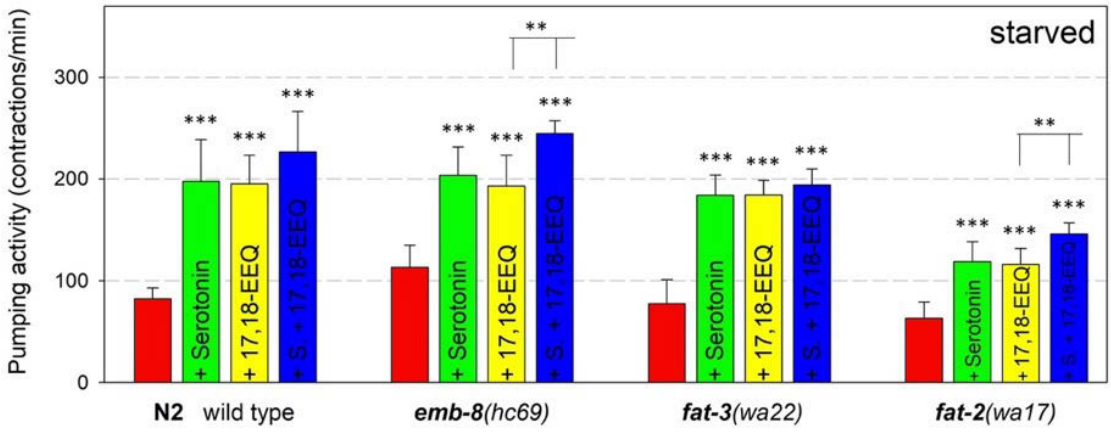


Figure 15: Comparison of CYP-eicosanoid and neurohormone-induced effects on pharyngeal pumping frequencies in starved condition.

17,18-EEQ mimicked the strong stimulating effect of serotonin in starved wild-type or *fat-3(wa22)*, *fat-2(wa17)* and *emb-8(hc69)* mutant strains. Shown are the contractions/min (three trials with n = at least 8 per trial); Data are presented as means ± SD, comparisons were made using one-way ANOVA, $^{**}P<0.01$, $^{***}P<0.001$; S. – serotonin. Unless otherwise specified, the concentration of serotonin was 2 mM and of 17,18-EEQ 10 µM (Zhou et al., 2015).

As shown in Fig. 16, octopamine with the concentration of 50 mM showed its expected inhibitory effect by reducing the pumping frequency in the presence of food in wild-type and

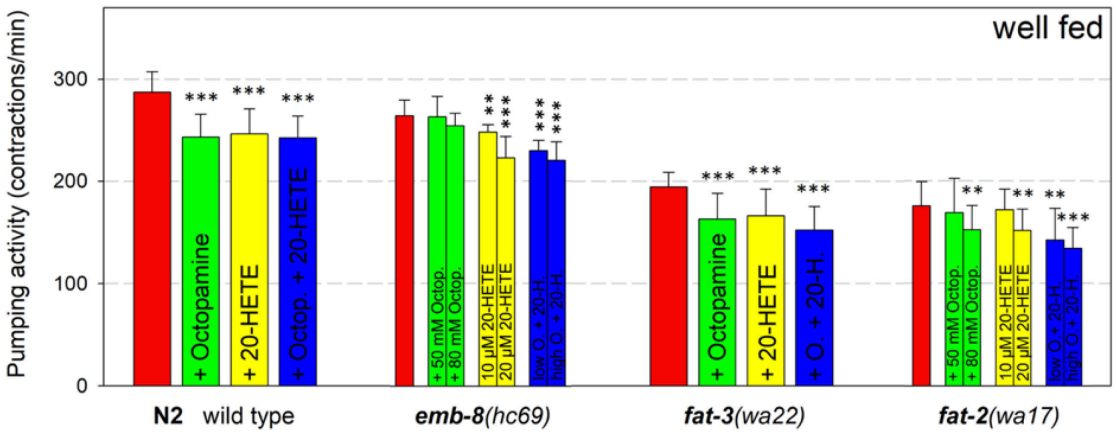


Figure 16: Comparison of CYP-eicosanoid and neurohormone-induced effects on pharyngeal pumping frequencies in well-fed condition.

Exogenous administration of 20-HETE mimicked octopamine by down-regulating the pumping frequency in well-fed wild-type worms and the *fat-3(wa22)* mutant. The *fat-2(wa17)* and *emb-8(hc69)* mutant worms were partially 'octopamine-resistant' but responded to 20-HETE with decreased pumping activity. Shown are the contractions/min (three trials with n = at least 8 per trial); data are presented as means ± SD, comparisons were made using one-way ANOVA, $^{**}P$ <0.01, $^{***}P$ <0.001; O./Octop. – octopamine; 20-H. – 20-HETE. Unless otherwise specified, the concentration of octopamine was 50 mM, and 10 µM for 20-HETE (Zhou et al., 2015).

fat-3(wa22) worms. The same concentration of octopamine had little effect on pharyngeal pumping in either *emb-8(hc69)*, cultivated at restrictive temperature or PUFA-deficient *fat-2* mutants. Raising the octopamine concentration to 80 mM restored the inhibitory effect to some extent, significantly in case *fat-2(wa17)*. Meanwhile, 20-HETE was clearly effective, also when added on top of octopamine (Fig. 16).

Based on this notion, the possible involvement of CYP-29A3 and/or CYP-33E2 in providing 20-HETE was tested next for the response to octopamine (Fig. 17). RNAi-mediated silencing of both genes indeed abolished the response of wild-type worms to octopamine (50 mM). Similarly, no octopamine response was detected in the *cyp-29A3(gk827495)* strain, that carries a loss-of-function mutation in the *cyp-29A3* gene. However, the *cyp-29A3(gk827495)* strain still clearly responded to exogenous 20-

HETE (Fig. 17), resembling the results obtained with *emb-8(hc69)* and *fat-2(wa17)*, the two other octopamine 'resistant' strains (compare Fig. 16). RNAi-mediated silencing of *cyp-33E2* alone caused already a significant deceleration of pumping frequency in well-fed worms. Treatment with octopamine together trended to result in a further decrease of pumping activity; however, this effect was only significant compared to the RNAi plasmid control (Fig. 17).

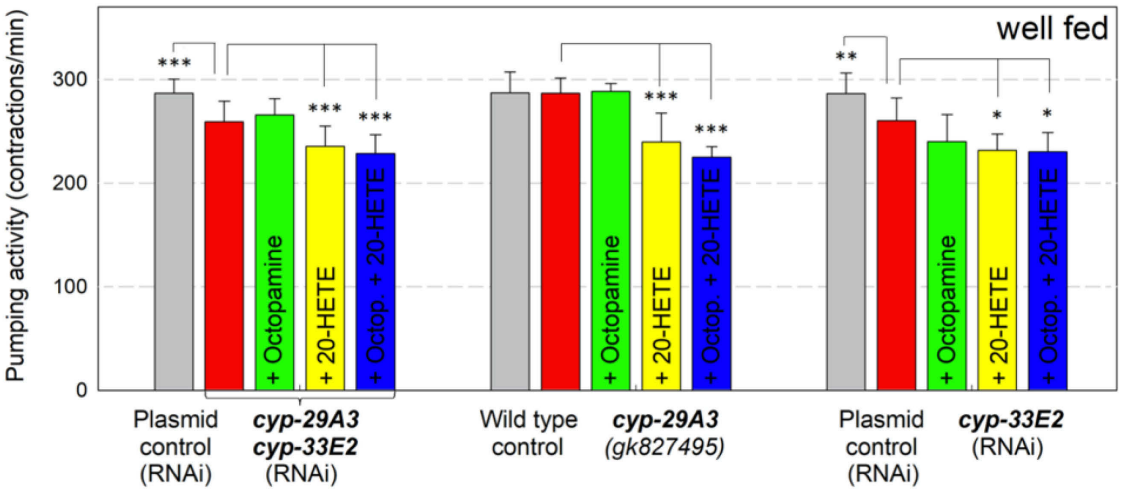


Figure 17: Comparison of CYP-eicosanoid and neurohormone-induced effects on pharyngeal pumping frequencies of CYP-deficient strains.

CYP-29A3 deficient strains did not respond to 50 mM octopamine but still to 20-HETE. Shown are the contractions/min (three trials with n = at least 8 per trial); data are presented as means ± SD, comparisons were made using one-way ANOVA, $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001; plasmid control: empty RNAi feeding control. O./Octop. – octopamine; 20-H. – 20-HETE. Unless otherwise specified, the concentration of octopamine was 50 mM and 10 µM for 20-HETE (Zhou et al., 2015).

3.2.4 Effect of neurohormones on endogenous CYP-eicosanoid formation

To explore the cross-link between the neurohormones and the formation of CYP-eicosanoids in *C. elegans* (Fig. 18), starved and well-fed worms were used for testing the responses to serotonin and octopamine, respectively. Without any neurohormone treatment, wild-type worms showed clear differences in the endogenous levels of free CYP-eicosanoids in the absence and presence of bacteria (compare the left panels in

Figs 18A and B). In particular, the 17,18-EEQ levels were significantly (almost 3-fold) lower in starved than well-fed worms.

Incubation with serotonin for 15 min resulted in a significant, almost 2-fold, increase of free 17,18-EEQ levels in starved wild-type worms (Fig. 18A). Simultaneously, the level of free hydroxy-metabolites decreased after serotonin treatment, which was significant for 19-HEPE but not for 19-/20-HETE (Fig. 18A). Compared with the starved wild-type animals, the CPR- deficient *emb-8(hc69)* strain displayed largely reduced levels of free CYP-eicosanoids profile and was unable to increase 17,18-EEQ formation in response to serotonin (Fig. 18C).

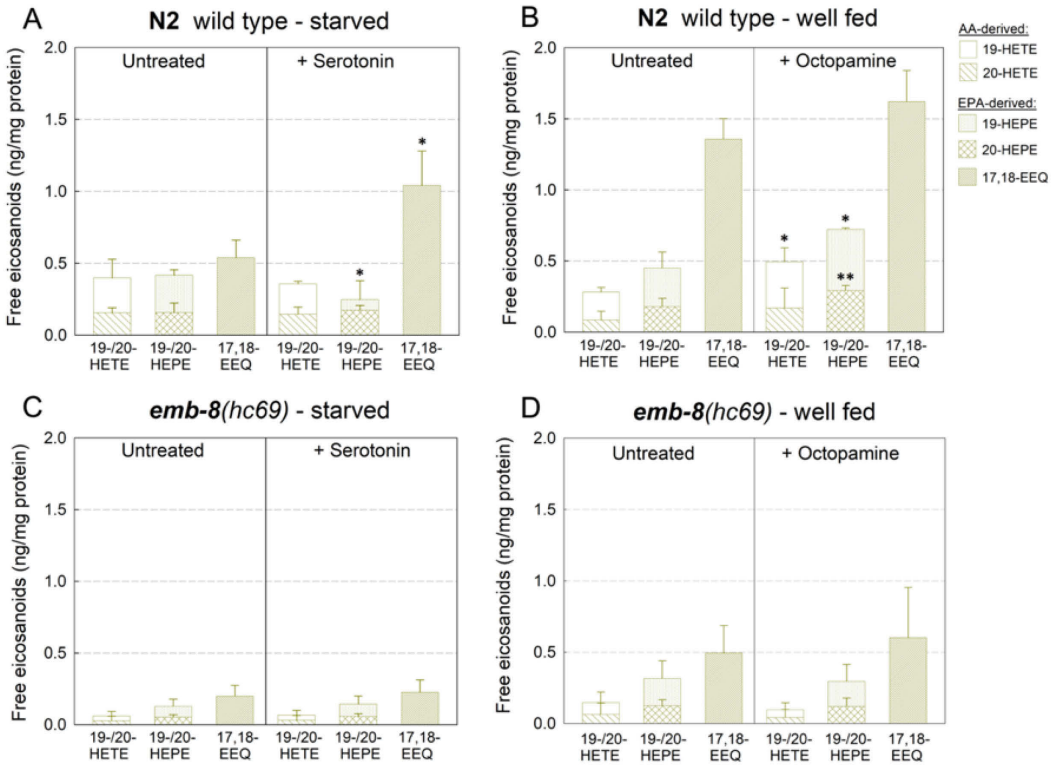


Figure 18: Effect of neurohormones on CYP-eicosanoid formation.

(**A**) Incubation with serotonin in starved wild-type worms increased endogenous levels of free 17,18-EEQ but not of hydroxy-metabolites. (**B**) Octopamine treatment of wild-type worms in the presence of food selectively induced the formation of free AA- and EPA-derived hydroxy metabolites, including 19-HETE, 20-HETE and 19-HEPE, and 20-HEPE. (**C, D**) *emb-8(hc69)* worms incubated at the restrictive temperature featured strongly contained less CYP-eicosanoid levels compared to the corresponding wild-type controls. Moreover, these CPR-deficient worms neither responded to serotonin nor to octopamine with changes in CYP-eicosanoid formation. Results are means + SD from three independent experiments performed for each strain. Note that in case of divided boxes the upper part presents the 19-hydroxy metabolite and the lower part the 20-hydroxy metabolite. Comparisons were made using t-test. $^{*}P$ <0.05, $^{**}P$ <0.01 (Zhou et al., 2015).

In contrast to serotonin, octopamine primarily induced the formation of hydroxy-metabolites (Fig. 18B). Actually, the free levels of 19-/20-HETE as well as of 19-/20-HEPE were almost doubled after treating well-fed wild-type worms for 15 min with octopamine. However, octopamine had no significant effect on the formation of 17,18-EEQ concentration that only slightly increased. Compared to the wild-type, the *emb-8(hc69)* strain showed again significantly lower levels of the CYP-eicosanoids in each stack (Fig. 18D). Importantly, the well-fed CPR-deficient worms also lost the ability to increase hydroxy-metabolite formation in response to octopamine, indicating that *de novo* CYP-eicosanoid synthesis was required for the octopamine-induced changes in the free hydroxy-metabolite levels observed in the wild-type strain (compare Figs. 18B and D).

3.2.5 Effects of CYP-eicosanoids and neurohormones on food uptake

Finally, to study the efficiency of food uptake, accumulation of fluorescent microspheres of 0.5 µm diameter was examined in *C. elegans* worms. This assay provides information not only on pumping, typically counted as cycle of contraction and relaxation of the terminal bulb, but also on peristalsis as an essential second feeding motion generating a moving wave of muscle contractions of the posterior isthmus that carries food from the corpus to the terminal bulb (Avery and You, 2012a). In fact, terminal bulb contractions which are not timed properly with those in the corpus can prevent transport of food (or beads) into the intestine. This assay was also used to demonstrate concentration- and time-dependent effects of 17,18-EEQ treatment on pharyngeal activity in the preliminary assays (compare Fig. 11).

The feeding assay was performed in both well-fed and starved conditions, exclusively incubated with beads. For representative pictures of incubated worms see Fig. 19; the labelling is visible in the terminal bulb of the pharynx and the whole intestinal lumen.

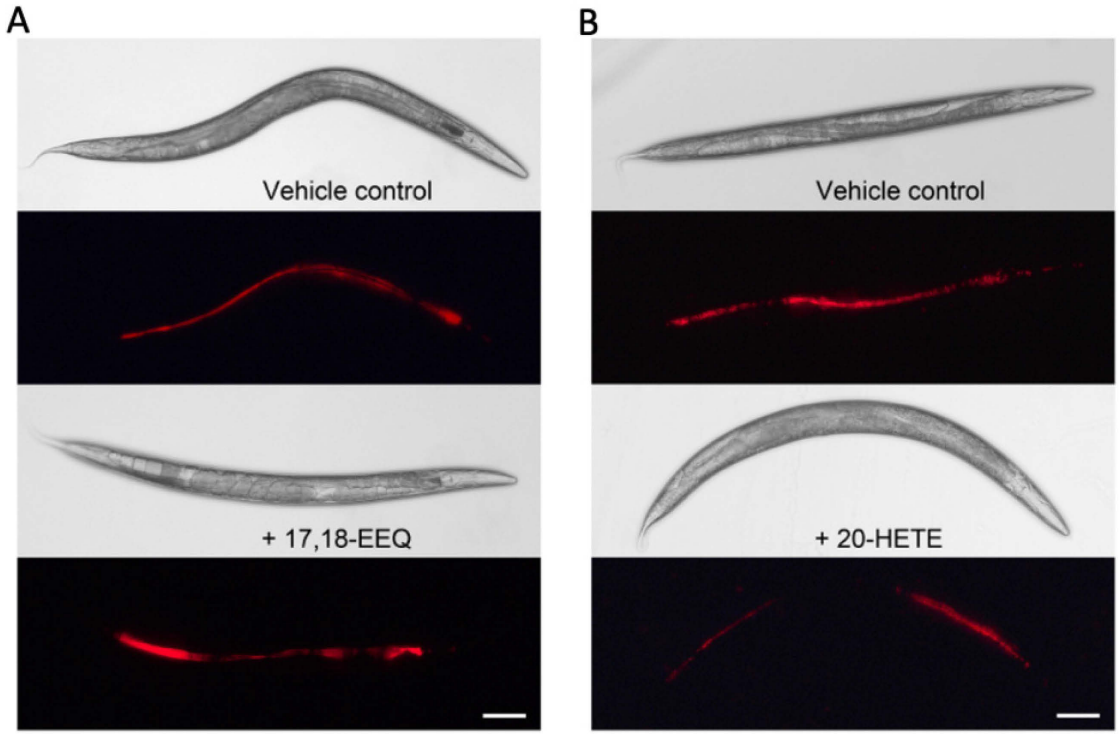


Figure 19: The accumulation of 0.5 µm fluorescent beads.

(**A**) starved and (**B**) well-fed adult worms allowed to uptake the red fluorescent beads for 10 min at 20°C. The photographs were taken at ×100 magnification, using an exposure time of 1/30 s for the fluorescence images in **A** and of 1/5 s for **B**; white bars correspond to 100 µm. (Zhou et al., 2015).

Significantly, more beads were accumulated in starved N2 worms when treated with serotonin (Fig. 20); 17,18-EEQ successfully mimicked this effect and exposure of both chemicals together was slightly more effective than serotonin or 17,18-EEQ (Fig. 20A, compare Fig. 19). The level of beads accumulation in well-fed worms was reduced when treated with octopamine, an effect which was also elicited by 20-HETE with weaker fluorescence intensity than in the control group (Fig. 20B, compare Fig. 19). A combined incubation resulted in an additive effect, which was decreased 3-fold as compared to the control group (Fig. 20B).

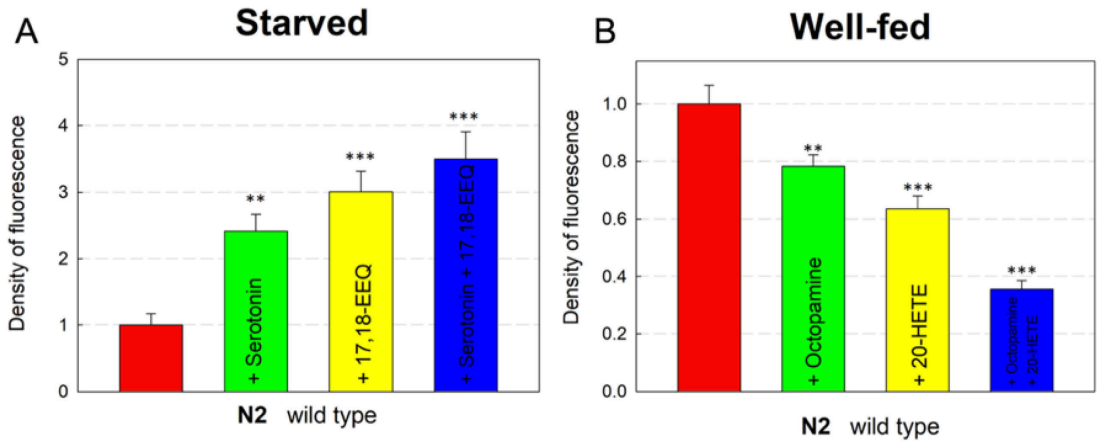


Figure 20: Contrasting effects of 17,18-EEQ and 20-HETE on the food uptake.

Normalized effect of neurohormones and CYP-eicosanoids on the accumulation of 0.5 µm fluorescent beads. The accumulation was assayed with (**A**) starved worms, incubated in the absence of *E. coli* cells, and (**B**) well-fed worms, incubated in the presence of *E. coli* cells. Data are means + SEM; n=15. *P*-values were obtained from one way ANOVA, $^{**}P$ <0.01, $^{***}P$ <0.001; vehicle: 0.3% DMSO. (Zhou et al., 2015).

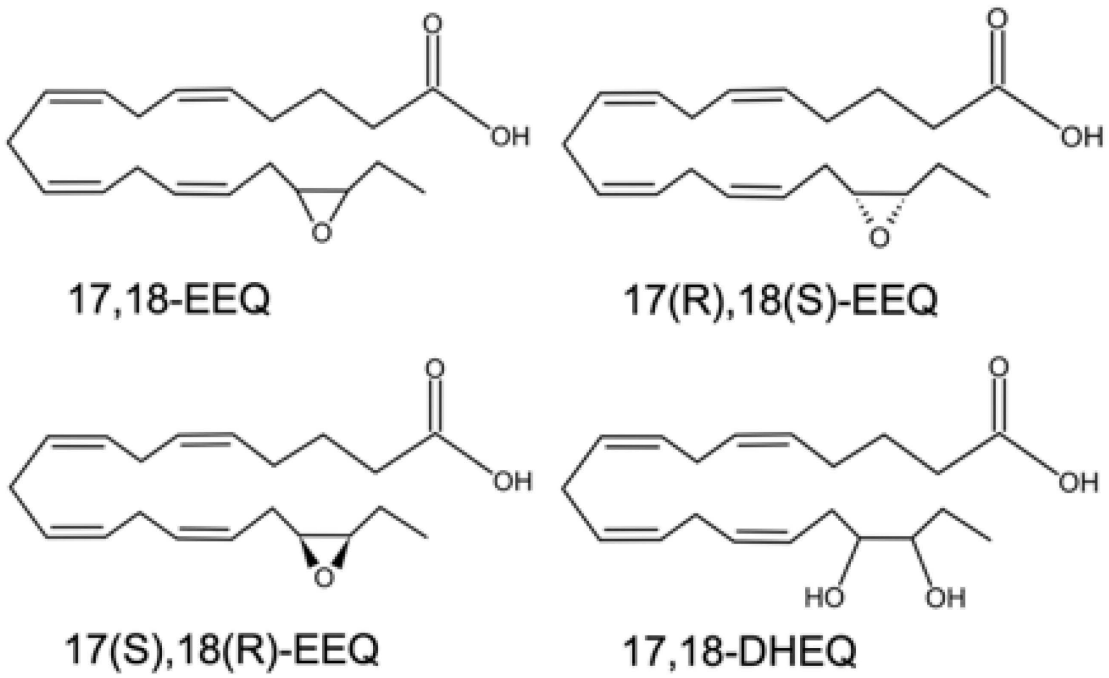
3.3 Stereospecific discrimination of the eicosanoid 17,18-epoxyeicosatetraenoic acid on the pharyngeal pumping

3.3.1 Stereoselectivity of 17,18-EEQ effects on pumping frequency

To prove a possible stereospecific effect of 17,18-EEQ, both enantiomers, 17(*R*),18(*S*)- and 17(*S*),18(*R*)-EEQ (Fig. 21), were used for the following examinations. Under well-fed exposure condition, shown in Fig. 22A-B, 17(*R*),18(*S*)-EEQ, but not by 17(*S*),18(*R*)-EEQ did accelerate pharyngeal pumping above basal frequency, both in the wild-type and *fat-3(wa22)* worms. In the case of *ceeh-1(ok3153)* (Fig. 22C), R,S-isomer even exceeded the effect of 17,18-EEQ racemic mixture, whereas the S,R-isomer had no significant effect on the pumping frequency. (with 297±22 pumps/min for the racemate versus with 313±15 pumps/min for the R,S-isomer, Fig. 22A).

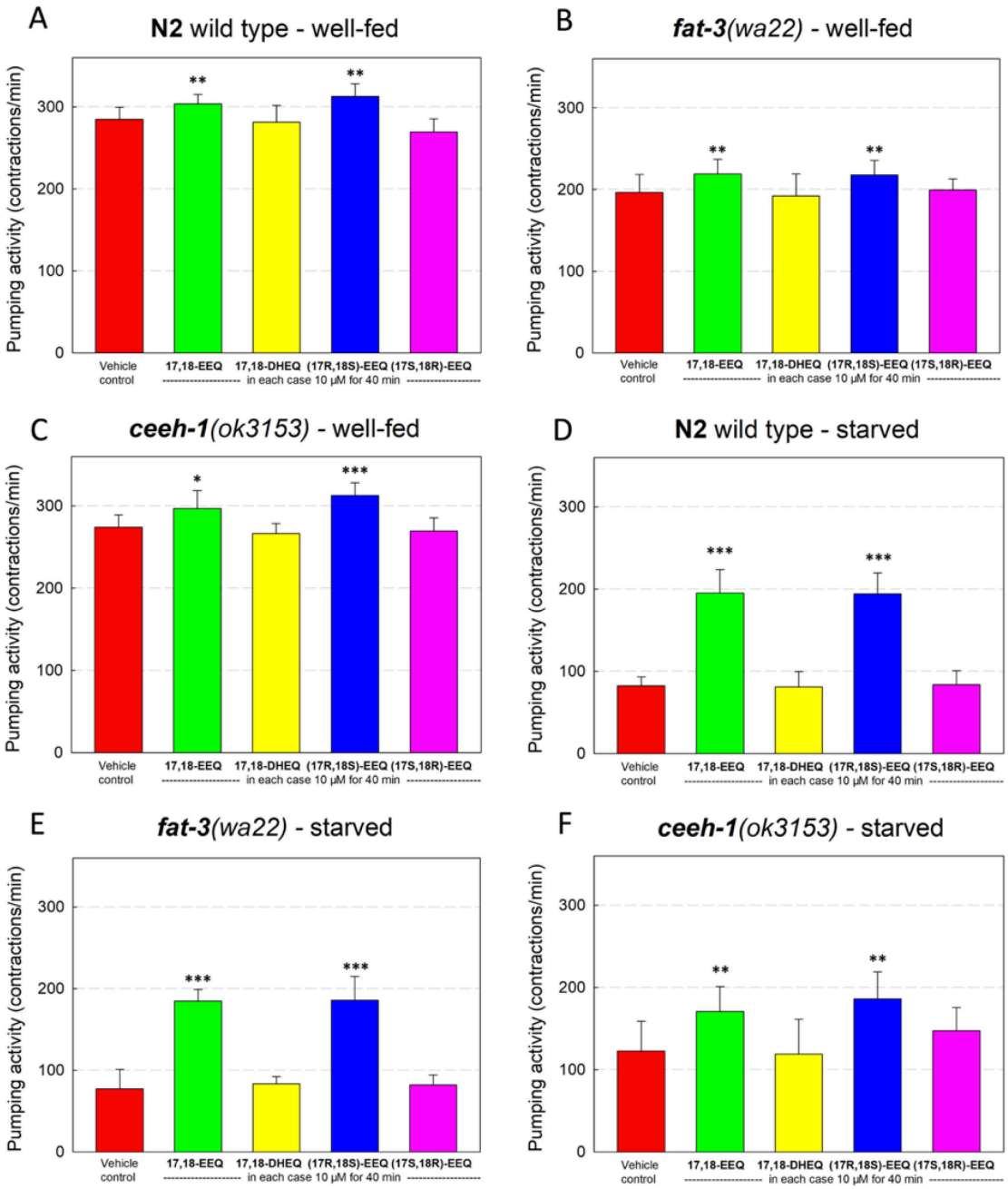
The effect of 17,18-EEQ stereoisomers on pumping frequency was also tested in the absence of food. During the 90 min starvation period, the pumping rate of wild-type strain declined from 284±15 to 82±11 contractions/min (Fig. 22D). Under starved exposure condition, N2 wild-type and *fat-3(wa22)* strains showed a similar response tendency to the 17,18-EEQ racemate and 17(*R*),18(*S*)-EEQ (Fig. 22D, F) (1.4-fold

increase in pharyngeal activity versus vehicle control), whereas just a 0.5-fold increase in pharyngeal activity in *ceeh-1(ok3153)* was detected. In order to confirm the specificity of the 17,18-EEQ effect, the pumping activity of worms was tested with its corresponding hydrolysis product (17,18-DHEQ) again (Fig. 22C, F). Similar to the S,R-isomer, no pronounced pharyngeal response was observed in animals exposed to 17,18-DHEQ. So, only the R,S-isomer had a vital excitatory role both on and off food.



<u>Figure 21:</u> Formulas of eicosanoids.

17,18-EEQ, 17(R),18(S)-EEQ, 17(S),18(R)-EEQ,17,18-DHEQ

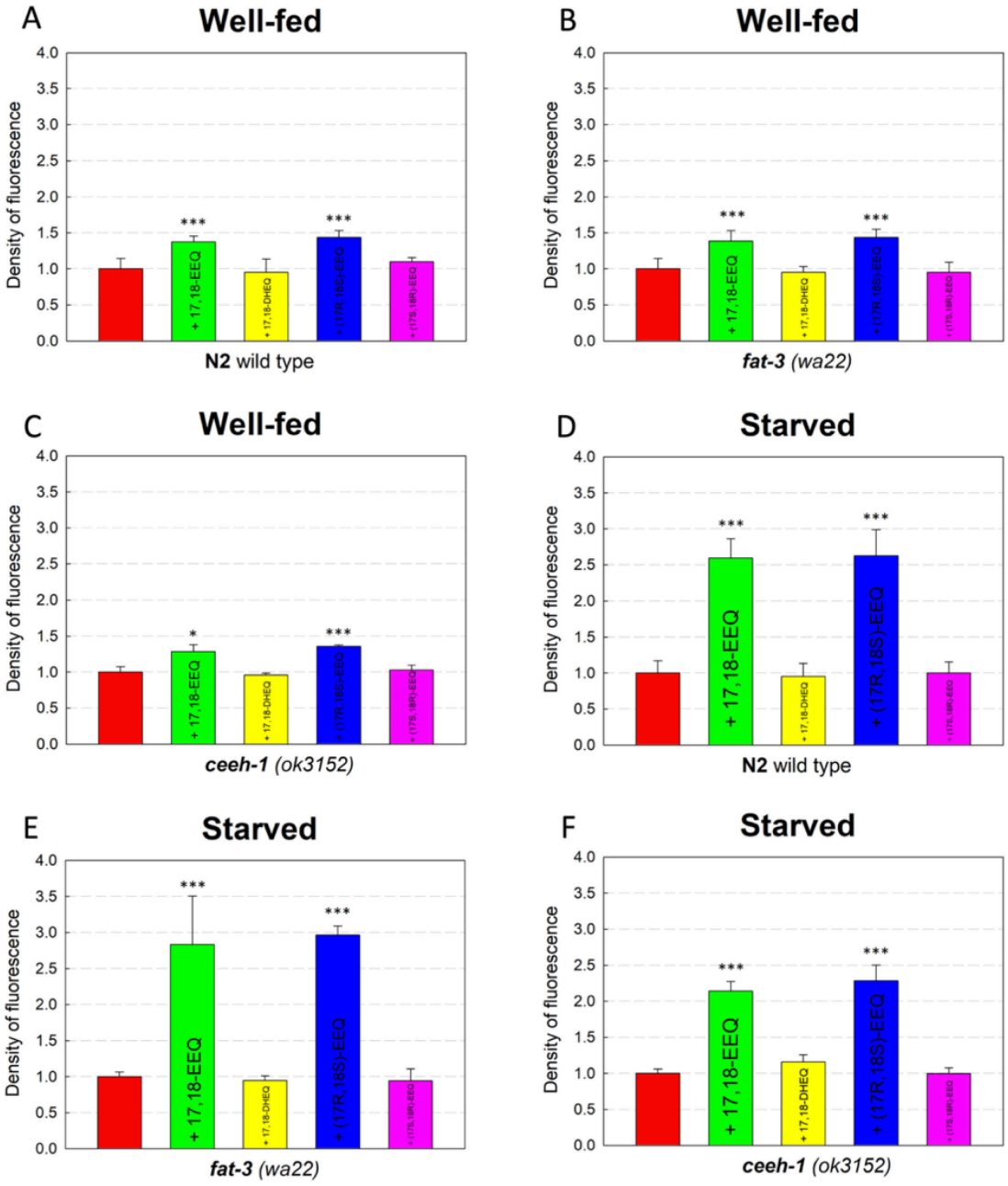


<u>Figure 22:</u> Enantioselectivity of 17,18-EEQ and sEH-catalyzed metabolite on pumping activity.

17,18-EEQ and one of its corresponding enantiomers, 17(*R*),18(*S*)-EEQ increased whereas 17(*S*),18(*R*)-EEQ and 17,18-DHEQ did not change the pumping frequency in wild-type as well as in the genetically null strains *fat-3(wa22)* and *ceeh-1(ok3153)*, with (**A-C**) or without (**D-F**) food. Shown are in each case the contractions/min (three trial with n = at least 8 per trial); error bars represent SD, comparisons were made using one-way ANOVA, $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001; vehicle: 0.3% DMSO.

3.3.2 Effects of CYP eicosanoids on food uptake

The beads incorporation assay was also performed in response to stereoisomer incubation with both well-fed and food-deprived worms, exclusively incubated with 0.5µm microspheres. Fig. 23 presents the results of normalized data across experiments illustrating that nematodes starved for 90 min and exposed to 17,18-EEQ or R,S-isomer accumulated higher levels of fluorescence than on-food nematodes, whereas the diol and S,R-isomer were again ineffective. In the absence of food, all the tested strains showed higher relative accumulation of the beads in the same time of experiment than that in the presence of food.



← Figure 23: Stimulatory effect of 17,18-EEQ and 17(*R*),18(*S*)-EEQ enantiomer on the food uptake. Normalized effect of CYP-eicosanoids on the accumulation of 0.5 µm fluorescent beads. Assays were performed in the presence(**A-C**) or in the absence(**D-F**) of food bacteria. The density of microspheres accumulated by a nematode was measured from the fluorescence intensity inside of intestine. Starved and well-fed adult worms allowed to uptake the red fluorescent beads for 10 min at 20°C. Data are means + SEM; n=15. *P*-values were obtained from one-way ANOVA, $^{*}P$ <0.05, $^{***}P$ <0.001; vehicle: 0.3% DMSO.

3.4 Identification of a serotonin receptor involved in the regulation of pharyngeal activity

Serotonin regulates a variety of behaviors and processes of the worm, including mating, egg-laying, metabolism and pharyngeal activity. The acute effects of serotonin on the formation of free CYP eicosanoids in food-deprived *C. elegans* was revealed as well (*this work*).

As shown in Fig. 24A, B, in wild-type and *ser-1(ok345)* worms, production of 17,18-EEQ and corresponding diol, 17,18-DHEQ, were significantly stimulated by serotonin treatment for 15 min. *ser-7(tm1325)*, however, was unable to generate increasing formation of 17,18-EEQ in response to serotonin (Fig. 24C). In order to investigate the relationship between the genotypes that gave phenotypes in response of serotonin and the epistatic relationship, double mutants (*ser-1(ok345)* and *ser-7(tm1325)*) was analyzed in addition. Serotonin had no significant effect on the formation of 17,18-EEQ and the derived hydroxy-metabolite**,** indicating that SER-7 activity is required for the observed increase in free 17,18-EEQ production as found in the wild-type.

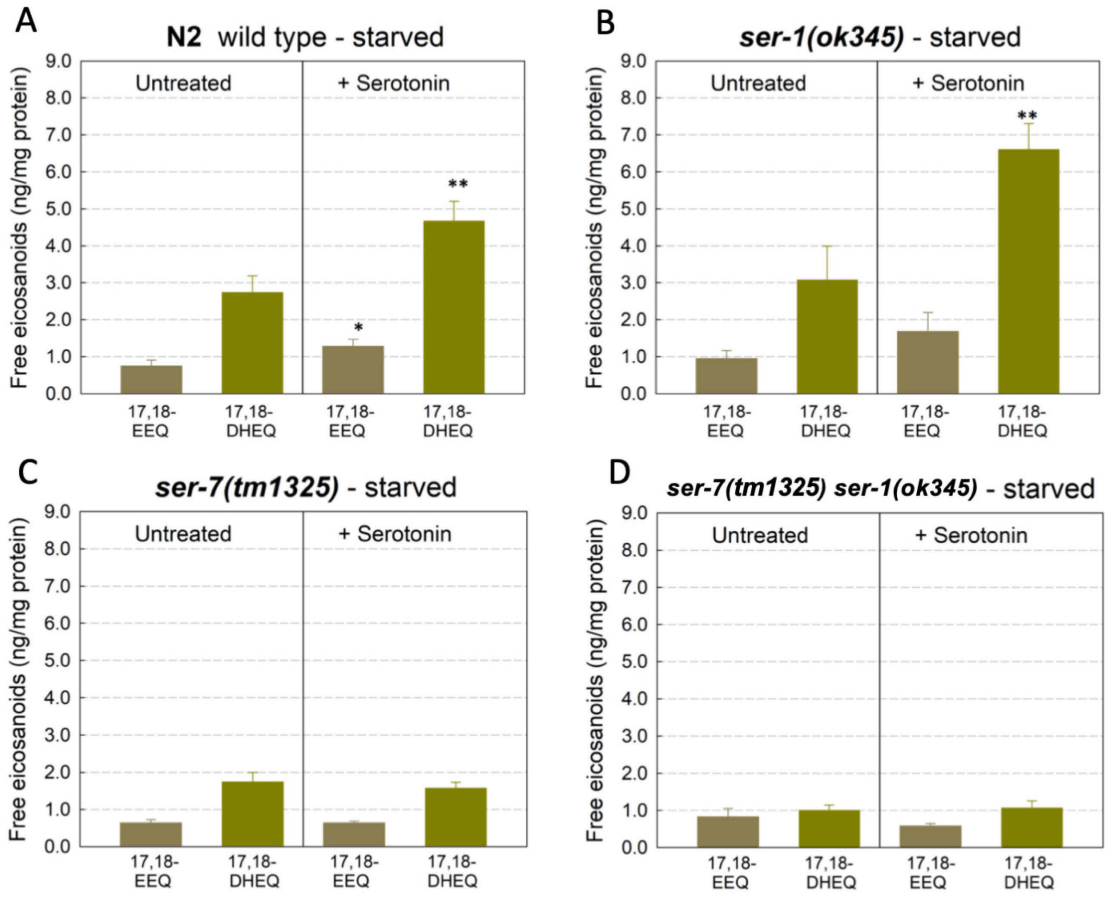


Figure 24: Effect of serotonin on free CYP-eicosanoid formation.

Supplementing serotonin to starved wild-type and the *ser-1(ok345)* null allele worms caused increased endogenous levels of both free 17,18-EEQ and hydroxy-metabolites (**A, B**). Serotonin receptors deficiency strains, *ser-7(tm1325)* and *ser-7(tm1325) ser-1(ok345)* double mutant worms showed no changes in CYP-eicosanoid formation in response to serotonin supplementation (**C, D**). Results are means + SD from three independent experiments performed for each strain. Comparisons were made using t-test. $^{*}P < 0.05$, $^{**}P < 0.01$.

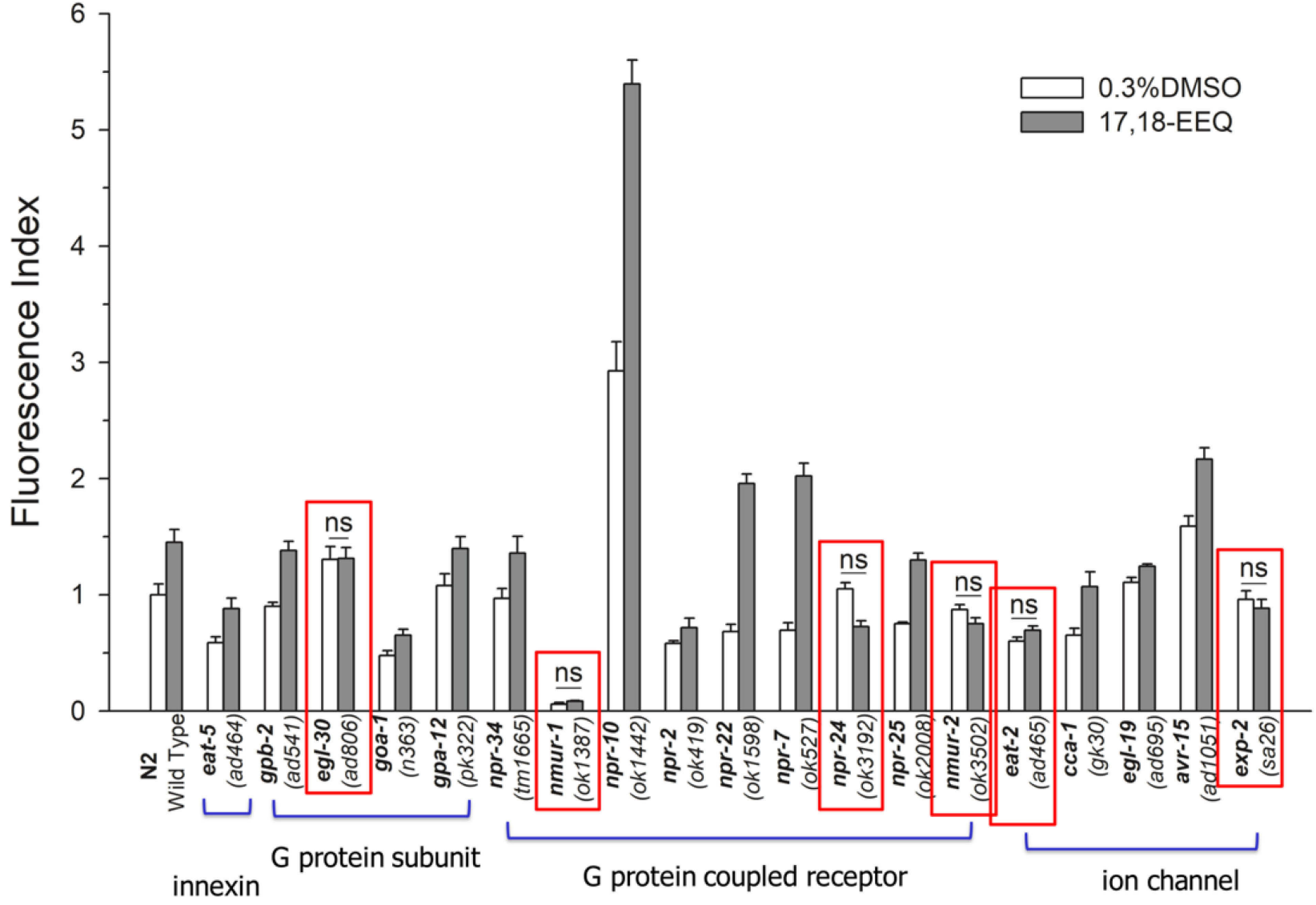
3.5 Identification of G-protein coupled receptors activated by 17,18-EEQ

3.5.1 A fluorescent bead-based feeding assay to screen for GPCR gene-knockout strains

A biased screen selected 19 genes to analyze (Table A6). The selection of these genes based both on sequence similarities to predicted mammalian orthologues and to already known GPCRs binding fatty acids. These strains contain in addition genes encoding G-protein subunits and ion channel subunits. *eat-5* is an innexin which is necessary for

synchronized pharyngeal muscle contractions. *gpb-2*, *egl-30*, *goa-1*, *gpa-12* encode G protein subunits. *npr-34*, *nmur-1*, *npr-10*, *npr-2*, *npr-22*, *npr-7*, *npr-24*, *npr-25* and *nmur-2* encode G protein coupled receptors. Ion channels are encoded by *eat-2, cca-1, egl-19, avr-15* and *exp-2* genes. Specific descriptions of all these *C. elegans* genes are listed in Table A6.

As the starved condition assay had not yet been established at the time of this investigation (more details in the discussion part 4.3), the feeding assay was performed first with well-fed worms in the presence of food, incubated in addition with beads. The density of fluorescent microspheres, in response to 17,18-EEQ treatment, was examined in all mutants and compared to N2 wild-type worms. Fig. 25 shows the results of microsphere fluorescence index in all worm strains. Compared with the non-treated control in the presence of bacteria, a fluorescence level with no significant difference was observed in *nmur-1, nmur-2, npr-24, exp-2, eat-2* and *egl-30* knockout worm strains. These strains failed to respond to the 17,18-EEQ treatment. The other 13 strains showed a significant increase of sphere accumulation compared with those without 17,18-EEQ treatment (Fig. 25).


Fluorescence Index
0
1
2
3
4
5
6
0.3%DMSO
17,18-EEQ
ns
N2 Wild Type
eat-5 (ad464)
gpb-2 (ad541)
egl-30 (ad806)
goa-1 (n363)
gpa-12 (pk322)
npr-34 (tm1665)
nmur-1 (ok1387)
npr-10 (ok1442)
npr-2 (ok419)
npr-22 (ok1598)
npr-7 (ok527)
npr-24 (ok3192)
npr-25 (ok2008)
nmur-2 (ok3502)
eat-2 (ad465)
cca-1 (gk30)
egl-19 (ad695)
avr-15 (ad1051)
exp-2 (sa26)
innexin
G protein subunit
G protein coupled receptor
ion channel

← Figure 25: Criteria of biased screen.

The accumulation of fluorescence beads was assayed in well-fed worms, the subsequent density of fluorescence showed that 17,18-EEQ did not stimulated the uptake of beads in six mutant strains. Young adults, pre-incubation for 40 min with 10 μM 17,18-EEQ, worms allowed to uptake the red fluorescent beads for 10 min at 20°C, n=12 (three trials), ±SEM, ns=not significant, all others are significant different with $p > 0.05$ (t-test).

3.5.2 17,18-EEQ effect on pumping frequency of candidate strains

After successfully finding 6 mutant strains, it was continued to test the 17,18-EEQ effect on pumping frequency for a further confirmation. As show in Fig. 26, the results were consistent with the feeding fluorescent beads assay. *nmur-1, nmur-2, npr-24, exp-2, eat-2* and *egl-30* mutant strains lose their ability to response to 17,18-EEQ treatment.

nmur-1 and *eat-2* worms display an abnormal phenotype in beads uptake and pumping assay, respectively. Both of them showed an impairment of pharyngeal activity. *nmur-1* worms hardly uptake the fluorescence microspheres and *eat-2* displayed largely reduced pumping frequencies with 35±8 contractions/min. Compare with N2 wild-type in the (291±16 contractions/min), the untreated mutant strains *elg-30*, *npr-24* and *exp-2* showed reduced pumping frequencies with 221±31, 230±29 and 269±19 contractions/min, respectively, and had no changes after 17,18-EEQ treatment.

.

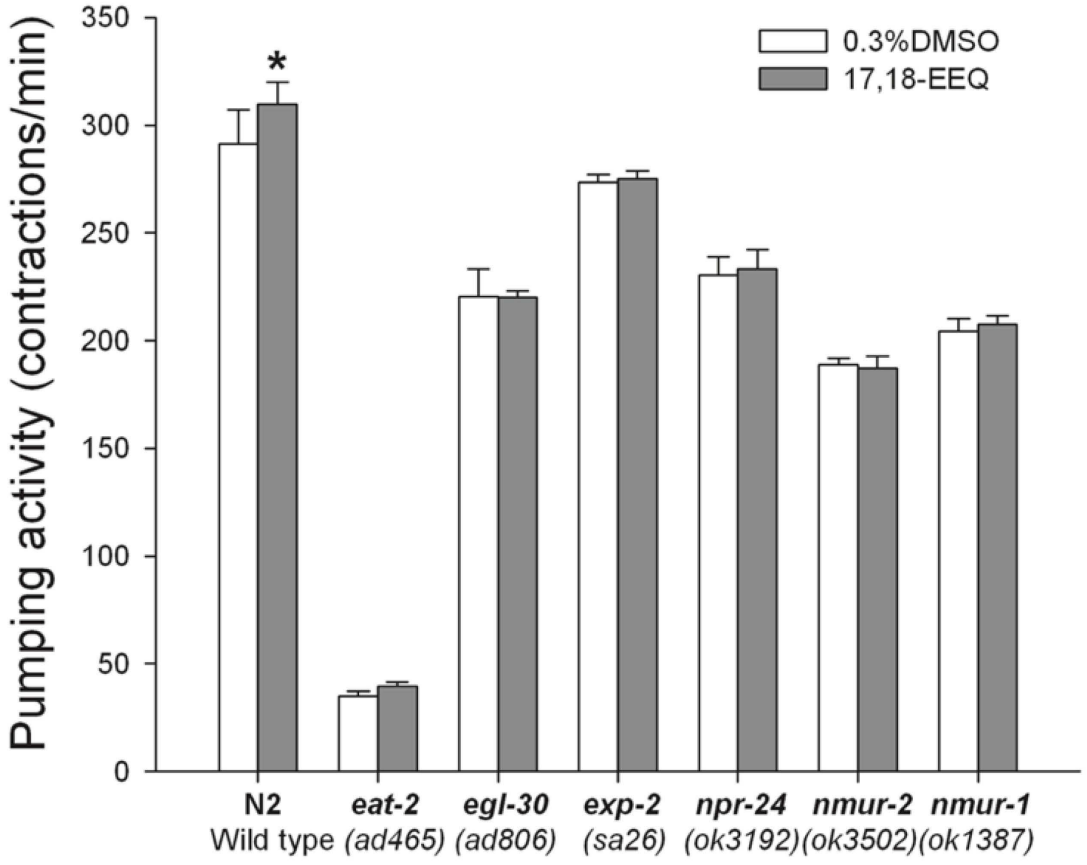


Figure 26: No response to 17,18-EEQ in the pumping frequency assay.

17,18-EEQ had no effect on the pumping frequency (counted) in the genetically modified candidate strains. Young adults, pre-incubation 40 min (17,18-EEQ) in well-fed worms. at 20°C, n=12 (three trials), ±SEM, *P <0.05 (t-test).

Also, the *nmur-2(ok3502)* strain, carrying a 900 bp deletion in the *nmur-2* coding region, was found unable to respond to 17,18-EEQ treatment, tested as uptake of fluorescent particles (Fig. 26) and confirmed by a determination of the pumping frequency in both well-fed (Fig. 26) as well as starved worms (Fig. 27). *nmur-2* accumulated significantly less fluorescence microspheres than N2 wild-type in the well-fed condition. This by about one third reduced pumping frequency resembles a phenotype known from the C20-PUFA deficient *fat-3(wa22)* and *fat-2(wa17)* mutant strains. In both well-fed and starved and condition, the pumping rate of *nmur-2* showed no significant increase after the treatment with 17, 18-EEQ. Selected as a first candidate gene, more details of *nmur-2* are presented in the discussion part 4.

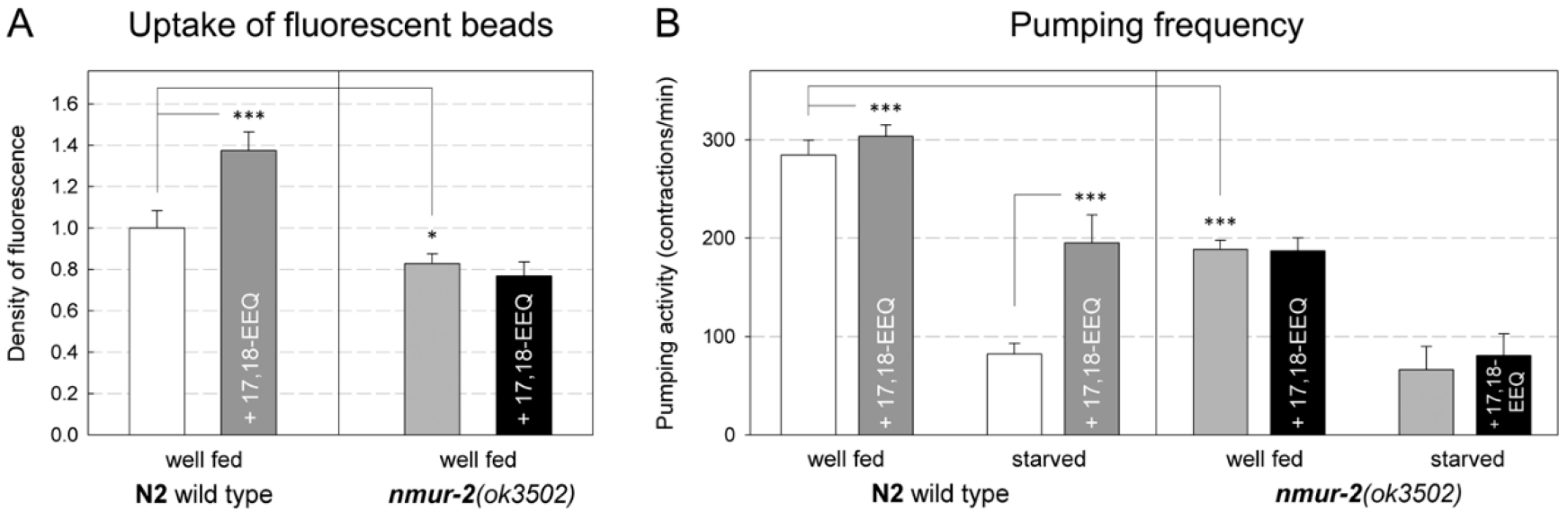


Figure 27: Pharyngeal pumping of nmur-2 knockout worms do not response to 17,18-EEQ treatment.

(**A**) Shown is the relative effect of 17,18-EEQ on the accumulation of 0.5 µm fluorescent beads, assayed with well-fed wild-type or *nmur-2(ok3502)* worms. 5 × 7 worms per condition and trial (n=3), ± SEM (**B**) 17,18-EEQ increases the pumping frequency in well-fed and, even more effective, in starved wild-type worms. In contrast, *nmur-2(ok3502)* worms were found insensitive to this signaling molecule. 10 worms per condition and trial (n=2), ± SEM. Comparisons were performed using one-way ANOVA, *P <0.05, ***P <0.001.

Chapter Four: Discussion

4.1 CYP-produced eicosanoids and neurohormones are involved in the regulation of pharyngeal pumping

The first part of this work provides direct experimental evidence of a role for CYP-eicosanoids in the regulation of pharyngeal pumping as well as the relationship to the particles uptake in the nematode *C. elegans*. In particular, 17,18-EEQ, the most abundant CYP-eicosanoid in *C. elegans,* was found to contribute to a most remarkable restoration of high pumping frequencies in LC-PUFA deficient mutant strains, whereas 20-HETE showed a rather contrasting effect and induced a deceleration of the pumping frequency and food uptake.

4.1.1 The video recording method applied on pumping frequency is more accurate than the traditional way

As mentioned in the result part (see 3.1.1), the pumping rate was consistently with a number of 285.7 ± 15.5 (mean±SD) contractions/min in N2 worms. This value is located at the upper end of the previous data range, of 200-300 pumps/min (Avery and You, 2012a), 250-300 pumps/min (Wilson et al., 2006) and 266.1 ± 3 pumps/min (Song and Avery, 2012) for young adults in the presence of food. Here is important to note that the quantification of pumping frequency by playing back recorded videos at slow speed and counting each individual pump carefully is expected to make the data more accurate. One-minute film of Supplemental Video 1 shows a N2 wild-type worm (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4617398/bin/supp_56_11_2110__index.html) pumping 290 times per minute (Zhou et al., 2015).

4.1.2 A CYP-produced eicosanoid is able to rescue from impairments in pharyngeal activity

Previous studies showed abnormal phenotypes observed in LC-PUFA deficient *fat-2* and *fat-3* mutant strain, which can be restored by the long-term incubation with PUFAs

(Lesa et al., 2003; Watts et al., 2003). Our PUFAs results were consistent with the previous studies. As novel question in this work it was addressed whether the missing C20-PUFAs or corresponding metabolites, i.e. CYP-eicosanoids, are required for maintaining pharyngeal activities. The same question also applies to other behavioral phenotypes associated with C20-PUFA-deficiency and marked lipodystrophy. Dietary supplementation with dihomo-γ-linolenic acid rescued the embryonic lethality and the defective permeability barrier in *seip-1* deletion mutants. *seip-1* encodes a putative adipose-regulatory protein; the mutant strain is expected to show a fatty acid synthesis mis-regulation phenotype (Bai et al., 2020). Interestingly, the touch sensation defect of *fat-3(wa22)* can be rescued by supplementing the worms during complete development with EPA or AA but also with ETYA, an AA-analogue (Vasquez et al., 2014). ETYA has triple instead of double bonds (Tobias and Hamilton, 1979) and has been used as a non-metabolizable analogue of AA and inhibitor of AA-derived eicosanoid formation (Harris et al., 1990; McGiff, 1991; Morisaki et al., 1984). These findings suggested that C20-PUFAs modulate *C. elegans*' touch sensation in while being incorporated into membrane phospholipids and that i.e. CYP-eicosanoid formation is not required to maintain this phenotype (Vasquez et al., 2014). In clear contrast, only EPA and AA were found able to rescue pharyngeal pumping frequencies rather than ETYA. This result combined with the outcome of reduced pumping activities in the CPR- and CYP-33E2-deficient strains clearly indicated that AA- and EPA-derived metabolites rather than the parental C20-PUFAs play the vital role in maintaining high pharyngeal activities. The function of C20-PUFA appears to be in fact independent of developmental roles in pharyngeal activity, as EPA supplementation for 24 h to *fat-3(wa22)* post L4 stage was sufficient for restoration of high pumping frequency in young adults. In contrast, this kind of rescue was not as much as complete in *fat-2(wa17)* worms which produce no PUFA at all. These data indicate that there are at least two separable requirements for EPA (and probably other PUFAs) in normal adult pumping behavior including both acute and developmental requirements. It seems likely that, at least in the *fat-3(wa22)* mutant, the enrichment of C18 LA and ALA (compare Table A4) as well as derived metabolites can partially compensate the absence of C20-PUFA. Most interestingly, a novel alternative synthesis pathway of PUFAs was found to generate JuA in *C. elegans fat-3* mutant strain. Here, ALA was elongated to eicosatrienoic acid (ETE, C20:3n3) by two elongases ELO 1/2 and then desaturated by *fat-4* (Δ5-desaturase) to generate JuA. Moreover, lipid-based signaling molecules,

endocannabinoids (ECs), were found to derive from the JuA in *fat-3* strain (Guha et al., 2020). This alternative pathway in *C. elegans* may present unusual PUFAs and signaling molecules which might compensate for impairments to a certain extent.

To identify the bioactive metabolites in the regulation of pharyngeal activities, 17,18-EEQ was selected as a candidate, the major CYP-eicosanoid endogenously produced in wild-type worms. In fact, 17,18-EEQ was alone sufficient to rescue impaired pharyngeal pumping in the LC-PUFA- as well as in the CPR- or CYP-33E2-deficient strains. Moreover, the formation and action of 17,18-EEQ was found to be associated with the typical behavioral response of *C. elegans* to re-feeding. Re-feeding induces a signal producing a clear increase in pumping rate after starvation. The levels of both pumping rates and free 17,18-EEQ strongly decreased after deprivation of food (bacteria). Starved worms responded to exogenous 17,18-EEQ with rapid pumping and concomitantly with increased food uptake capacity as visualized by enhanced incorporation of fluorescent beads. However, 17,18-EEQ-treatment exerted also a slight stimulating effect in well-fed worms, suggesting that the endogenous 17,18-EEQ levels did not fully saturate the stimulating mechanisms even in the presence of abundant food.

It might be argued that in isolated mammalian cells, 17-18-EEQ at an even lower concentration was found already effective, as 1 μM in respect to ristocetin-induced thrombocyte aggregation (Jung et al., 2012a) or even 30 nM, which caused anti-arrhythmic effects in isolated neonatal rat cardiomyocytes (Arnold et al., 2010b). Moreover, 17,18-EEQ induced an outward current, consistent with an activation of BK channels at 100 nmol/L (Lauterbach et al., 2002). However, *C. elegans* as living organism and *in vivo* invertebrate model cannot be considered as comparably accessible as *in vitro* experimental model such as cell cultures. This applies even more in the light of an encapsulated pharynx, isolated from the rest of the worm by a specialized basal lamina. Moreover, recent investigations revealed that the more economic approach of spotting compounds on the agar surface achieved lower absorption efficiency in worms when compared with other drug delivering methods, as pouring compounds together with agar (Zheng et al., 2013).

A reaction potentially limiting 17,18-EEQ levels consists in the hydrolysis of this epoxy-metabolite to the corresponding vicinal diol (17,18-DHEQ). Given that the soluble epoxide hydrolases (sEH) metabolize fatty acid epoxides to their corresponding diols, an increase in sEH expression would be expected to decrease EEQ production.

Supporting this notion, the liquid chromatography tandem mass spectrometry (LC-MS/MS) data showed, besides 17,18-EEQ, also high 17,18-DHEQ levels in the wild-type worms. Notable, it was found that 17,18-DHEQ did not share the capacity of 17,18-EEQ to stimulate pharyngeal activity. In mammals, hydrolysis of LC-PUFA-derived epoxy-metabolites is catalyzed by sEH and frequently also results in a loss of their biological activities (Harris and Hammock, 2013). In C. elegans, ceeh-1 and ceeh-2, two genes encoding enzymes orthologous to mammalian sEH, metabolize 17,18-EEQ to the corresponding diol (Harris et al., 2008b).

Our study also indicates that 17,18-EEQ can be partially replaced by other metabolites, as 11,12-EET to elicit the stimulating effect. Strong evidence for this notion comes from the finding that the EPA-deficient *fat-1(wa9)* strain did not show any impairment in pharyngeal pumping activity. This strain produced largely increased amounts of AA-derived metabolites. Accordingly, it is tempting to speculate that one or more of the regioisomeric EETs (i.e. 11.12-EET) are able to functionally replace 17,18-EEQ. 20-HETE decreased pharyngeal activities when added to well-fed worms which reversed the stimulating effect of 17,18-EEQ. 20-HETE thus elicited a response otherwise indicating satiation. This effect was detectable not only in wild-type but also in all of the genetically modified strains tested. The requirement of a doubled concentration of eicosanoids for being effective also in *fat-2(wa17)* gave further evidence that developmental deficits in this strain are not fully compensable by dietary supplementation, the complete absence of PUFAs in mutant's development resulted in impairments that probably interfere with the restoration of high pumping frequencies. 20-HETE was selected as a representative of the various hydroxy-metabolites present in *C. elegans*. An additional testing of 19-HETE and 20-HEPE did not reduce the pumping speed of well-fed young wild-type adults, confirming the specificity of the 20-HETE effect. However, the inhibitory effect of 20-HETE is possibly shared by other AA- and EPA-derived hydroxy-metabolites or further derived metabolites; this has still to be tested in future experiments.

4.1.3 CYP-produced eicosanoids mirror the effects of neurohormones

A central questions of this work asks whether or not CYP-eicosanoids are integrated as second messengers of neurohormones into the complex regulation of pharyngeal activity and food uptake in *C. elegans*. Avery and You (2012a) found that pharyngeal

muscle activities are coordinated by the pharyngeal nervous system allowing efficient contractions and stimulation of fast pumping in response to food and slow pumping in response to starvation or satiation. In the absence of the pharyngeal nervous system, a muscle-intrinsic pathway promotes only very slow pumping (26 pumps/min in the presence of food) (Avery and Horvitz, 1989b). In the unaffected wild-type, both serotonin and acetylcholine stimulate fast pumping, whereas glutamate acts as an inhibitory neurotransmitter (Dent et al., 1997; Horvitz et al., 1982b; McKay et al., 2004a). Also octopamine reduces pharyngeal pumping when added to intact worms (Horvitz et al., 1982b) or isolated pharynx preparation (Rogers et al., 2001b). The cellular origins of this neurohormone are RIC interneurons of the head region and gonadal sheath cells (Alkema et al., 2005); known octopamine receptors, SER-3 and OCTR-1, are expressed in head neurons, too (Sun et al., 2011; Suo et al., 2006). 20-HETE not only mimicked the inhibitory effect of octopamine, but was also increasingly produced in response to octopamine, suggesting that lack of endogenous 20-HETE formation might be responsible for the inability of the *emb-8(hc69)* and *fat-2(wa17)* strains to response to 50 mM octopamine (Fig. 16). Moreover, the CPR- or CYP-29A3-deficient strains failed to respond to the inhibitory neurohormone, but still responded to 20-HETE. On the other hand, C20-PUFA deficient strains *fat-3(wa22)* and *fat-2(wa17)* were able to respond to octopamine treatment in pumping frequency, but the latter only with the doubled concentration of 80 mM. Also, the data of the fluorescent beads uptake experiments with the wild-type in the presence of octopamine and 20-HETE tend to argue for a rather independent (additive) action of both substances. Further work will be necessary to close this knowledge gap, e.g., why a notably high 20-HETE concentration in the *fat-1(wa9)* mutant, producing only n-6 PUFA and derived CYP-eicosanoids, is not sufficient to lower its pumping frequency.

The interpretation of the results obtained with 17,18-EEQ is similarly complex. The data show that 17,18-EEQ is involved in maintaining high pumping and food uptake rates in the presence of bacteria. Moreover, 17,18-EEQ was increasingly produced in response to serotonin and elicited, like this neurohormone, the behavioral response of re-feeding. However, 17,18-EEQ was obviously not essential for mediating the serotonin effect. Supporting this notion, CPR-deficient worms was found to respond to serotonin with increased pumping frequencies, despite their inability for *de novo* 17,18-EEQ synthesis. Furthermore, pharyngeal pumping of *fat-2* mutant worms was

serotonin-responsive, although this strain was completely devoid of any PUFAs and CYP-eicosanoids. These results indicate that serotonin can exert its stimulatory effect both via 17,18-EEQ-dependent and independent pathways. Moreover, it appears possible that 17,18-EEQ is used as a second messenger primarily by other transmitters that act down-stream (e.g. acetylcholine) or independent of serotonin. Mutants defective in the serotonin receptor SER-7 do not respond to serotonin, but still respond to bacterial food, suggesting that serotonin is indeed not the only transmitter relaying the physiological response of re-feeding (Hobson et al., 2006b).

4.1.4 Perspective of CYP-eicosanoids in the regulation of smooth muscle cell activities

Various aspects of the hypothetical pathway remain to be clarified. A first question concerns the identity and cellular localization of the CYP-isoforms involved. CYP-29A3 appears responsible for the formation of 20-HETE and other hydroxy-metabolites as indicated by the lack of clear octopamine response in the CYP-29A3 knock-out strain. However, the expression site of CYP-29A3 remains to be defined considering that the pharyngeal machinery is constituted by various neuronal, marginal, and muscle cells. CYP-33E2 is the leading candidate for producing 17,18-EEQ, based on the activity of the recombinant enzyme, its localization in pharyngeal marginal cells, and the reduced pharyngeal activities observed after down-regulating CYP-33E2 expression (Kosel et al., 2011b). However, pharyngeal marginal cells also express CYP-13A12, a CYP enzyme that is involved in *C. elegans'* response to hypoxia/reoxygenation and presumably shares with CYP-33E2 the capacity of producing 17,18-EEQ (Ma et al., 2013). Another important open question concerns the selective induction of 17,18-EEQ versus 20-HETE formation, respectively. CYP-enzymes require free fatty acids as substrates. This feature ensures coupling of CYP-eicosanoid *de novo* synthesis to the action of extracellular signals that activate phospholipases A2 (PLA2) which in turn release free C20-PUFAs from membrane phospholipids (Capdevila and Falck, 2002; Spector, 2009). Currently, it is unclear whether or not one of the serotonin or octopamine receptors may trigger PLA2 activation and which of the diverse PLA2 enzymes expressed in *C. elegans* are involved. Finally, it may be presumed, but has to be directly shown, that pharyngeal muscle cells contain receptors selectively

recognizing 17,18-EEQ or 20-HETE and in turn trigger signaling pathways accelerating or decelerating pharyngeal pumping.

From a more general perspective, the questions raised above similarity of the formation and action of CYP-eicosanoids in *C. elegans* and mammalian systems. Resembling the findings on the opposite roles of 17,18-EEQ and 20-HETE in *C. elegans*, EETs act as second messengers of vasodilatory hormones, whereas 20-HETE mediates vasoconstriction in the mammalian vasculature (Campbell and Fleming, 2010; McGiff and Quilley, 1999). EETs were first characterized as endothelium-dependent hyperpolarizing factors when analyzing the components mediating the vasorelaxant effects of acetylcholine and bradykinin (Campbell and Falck, 2007). EET-generating CYP enzymes are primarily localized in endothelial cells and 20-HETE formation occurs predominantly in vascular smooth muscle cells. Partially explaining the opposite effects, EETs activate whereas 20-HETE inhibits large conductance Ca^{2+}-activated potassium (BK) channels in vascular smooth muscle cells (Campbell and Fleming, 2010; Zou et al., 1996a). In *C. elegans*, BK channels are involved in regulating muscle Ca^{2+}-transients (Chen et al., 2011a; Chiang et al., 2006a) as well as neurohormone release at neuromuscular junction (Wang et al., 2001). 17,18-EEQ shares and, in several vascular beds, even largely exceeds the vasodilatory (Agbor et al., 2012) and BK channel activating effects of EETs (Lauterbach et al., 2002). Moreover, 17,18-EEQ relaxes airway smooth muscle cells in the human lung (Morin et al., 2009) and potently modulates the contractility of cardiomyocytes (Arnold et al., 2010b). Interestingly, a synthetic compound developed to mimic the effect of 17,18-EEQ on cardiomyocytes (Falck et al., 2011b) was also effective when assayed in *C. elegans* model. In mammalian systems, CYP-eicosanoids probably act via both intracellular targets such as peroxisome-proliferator activated receptors and membrane receptors that remain to be identified (Chen et al., 2011b; Spector, 2009; Yang et al., 2008).

4.2 17(R),18(S)-EEQ contributes to the regulation of pharyngeal pumping activity pathway

As shown and discussed before, 17, 18-EEQ may act via a still unknown signal transduction which probably activates a GPCR, transmitting the signal either directly

to muscle cells or nearby neurons. And it may not be a coincidence that the reciprocal regulation of feeding behavior by serotonin and octopamine (Zhou et al., 2015), probably providing a mechanism for adapting to the presence and absence of food, respectively, is mirrored by the contrasting effects caused by 17,18-EEQ and 20-HETE (Zhou et al., 2015).

The stereospecific discrimination of 17,18-EEQ have not been previously reported in the pharynx of *C. elegans*. In the present study, an important and novel observation has been made. A stereoselectivity of 17,18-EEQ effect was delineated and found that 17(*R*),18(*S*)-EEQ stereoisomer produces exactly the same effect as the racemate rather than 17(*S*),18(*R*)-EEQ. Like a specific ligand, the effect of 17,18-EEQ was highly stereoselective.

Chiral analysis of the rat liver microsomal AA epoxygenase metabolites showed enantioselective formation of 8,9-, 11,12-, and 14,15-EET in an approximately 2:1, 4:1, and 2:1 ratio of antipodes, respectively. Interestingly, treatment with the CYP-inducer phenobarbital caused a concomitant inversion in the absolute configurations of this metabolites (Capdevila et al., 1990). Other examples demonstrated the roles of 5-HETE isomers on cytosolic calcium change and lung inflammatory responses (Bittleman and Casale, 1995; Rossi et al., 1988). Only the 11(S),12(R)-EET enantiomer is efficient to shorten the duration of cardiomyocyte action potentials (Lu et al., 2002), while 11(R),12(S)-EET rather than 11(S),12(R)-EET is potent to activate the BK channels in renal smooth muscle cells (Zou et al., 1996b). Both the racemate and the R,S-enantiomer of 17,18-EEQ reduced the spontaneous beating rate of neonatal rat cardiomyocytes, whereas 17(S),18(R)-EEQ was inactive (Arnold et al., 2010b). In rat cerebral vascular smooth muscle cells, only 17(R),18(S)-, but not 17(S),18(R)-EEQ stimulated BK channel activation (Lauterbach et al., 2002). In clear cut, the activation of pumping activities of *C. elegans* by 17,18-EEQ racemate involves absolute stereospecificity; only the 17(R),18(S)-EEQ enantiomer is active. Comparably, this is further indication for the existence of a high-affinity binding site for 17(R),18(S)-EEQ in the pharynx on a specific receptor of *C. elegans*.

Nevertheless, the stereoselectivity and activeness of 17(*R*),18(*S*)-EEQ could be an indication of some specific interaction between the stereoisomer and an ion channel itself or an interaction of the enantiomer with further signal transduction molecules. So far, very limited studies have been done on the specific binding of EEQs and the

relevant receptor has not yet been identified, neither in *C. elegans* nor in another organism. It is likely that such specificity would bring about an important function.

4.3 Involvement of SER-7 GPCR in the regulation of pharyngeal pumping activity pathway

Table A3 displayed the functions of G protein coupled serotonin receptors in nematodes. Only *ser-1* and *ser-7* have the phenotype of feeding or pharyngeal activity. The results of this work revealed that SER-7might contribute to the regulation of pharyngeal pumping activity pathway in *C. elegans* by a significant stimulation of free 17,18-EEQ formations in response to serotonin.

Three types of pharyngeal neurons are of particular importance for normal feeding. M4 is essential for normal isthmus peristalsis (Avery and Horvitz, 1987). MC and M3 control the starts and stops timing of a pumping contraction, respectively (Avery, 1993; Raizen and Avery, 1994; Raizen et al., 1995). Surprisingly, the other neuron types are unnecessary for functional pumping and development (Avery and Horvitz, 1989a). As already mentioned, the neurohormone serotonin plays a vital role in the regulation of pharyngeal pumping in worms. Evidence suggests a function of serotonin in modulating pharyngeal action potentials through the M3 and MC motor neuron. Two serotonin receptors, SER-7 and SER-1, are expressed in these two types of neurons and nearby pharyngeal muscle cells, respectively (Hobson et al., 2003; Hobson et al., 2006a; Tsalik et al., 2003). As hypothesized, CYP eicosanoids may serve as second messengers of neurohormones in the complex regulation of pharyngeal activity in *C. elegans.* These signaling molecules may transmit the signals through activating GPCR(s) either directly to muscle cells or nearby motor neurons to mediate the intended pharyngeal activities. To facilitate a clear understanding of 17,18-EEQ signaling in nematodes and to identify the key receptors involved, it is important to characterize involvement of serotonin receptors in this pathway. The results confirmed the hypothesis of an involvement of such a receptor in the CYP-eicosanoids pathway effecting on pharyngeal activity in *C. elegans*. The formation of free 17,18-EEQ did not longer response to exogenous serotonin in a mutant defective in the serotonin receptor SER-7, indicating that this receptor may be involved. Thus, this work provides for the first-time

strong evidence for a functional link between serotonin and 17,18-EEQ in the mediation of pumping activity in *C. elegans*.

The stimulatory effect of both the neurohormone and the eicosanoid on the pumping frequency in *C. elegans* indicates their functional overlap. A series of previous studies have shown similar connections. A subtype 2 5-HT receptor (5-HT2) mediated the release of arachidonic acid and subsequent eicosanoid formation in the mouse hippocampus (Felder et al., 1990). In the neuroepithelial 1C11 cells, a COX-produced AA metabolite mediated the function of 5-HT1B/D receptor by the crosstalk of 5-HT2B and 5-HT2A receptors (Tournois et al., 1998). Octopamine and serotonin, which may share a receptor type, mediated cellular immune responses via eicosanoids biosynthesis in *Spodoptera exigua* (Kim et al., 2009). Serotonin-2B receptor (5-HT2BR) signaling plays a vital role in the control of tissue-nonspecific alkaline phosphatase (TNAP) activity which is mediated by prostaglandin and leukotriene synthesis in C1 osteoprogenitor cell line (Baudry et al., 2010). Also a new signaling pathway linking 5-HT2BR and nuclear peroxisome proliferator-activated receptor (PPAR) β/δ via the overproduction of prostacyclin was confirmed in primary cultures of mouse osteoblastic cells (Chabbi-Achengli et al., 2013). All these previous studies as well as the results obtained in this work strengthen the assumption that neurohormones mediate various pathological and physiological processes via eicosanoids to express cellular responses. To the best of our knowledge, this is the first report of establishing a link between serotonin and 17,18-EEQ - via the SER-7 receptor - to trigger the pharyngeal pumping in *C. elegans.*

In conclusion, only 17(R),18(S)-EEQ stereoisomer accelerates the pharyngeal pumping in *C. elegans*. Additionally, the data show that the SER-7 receptor contributes to the signaling pathway of 17,18-EEQ in *C. elegans*' pharyngeal muscle activities. These observations provide a foundation for the refreshing fundamental mechanism of components and connections of CYP-eicosanoid pathway and underlying the cross-talk role of eicosanoids in neurotransmission.

4.4 NMUR-2, a 17,18-EEQ specific GPCR candidate

Most of the biological functions of CYP-eicosanoids are obviously based on the capacity of these metabolites to trigger distinct intracellular signaling pathways. In

analogy to the action of COX- and LOX-eicosanoids, this feature suggests the existence of GPCRs that become activated upon binding the CYP-dependent metabolites. *C. elegans* is considered as particularly suitable model to screen for a 17,18-EEQ receptor and the identification and localization of a CYP-eicosanoid GPCR is the key to the understanding of the whole pathway.

A biased screen of 19 selected genes (Table A6), first revealed 6 candidate genes possibly involved in a CYP-eicosanoid signaling. *eat-2* mutants showed slow but strong and regular pumping activity (Avery, 1993). As a nicotinic acetylcholine receptor subunit, *eat-2* is required for neuron MC to stimulate pharyngeal muscle and for nicotinic neurotransmission in *C. elegans* pharynx (McKay et al., 2004b). *egl-30* encodes G protein α subunit and was expressed in pharyngeal muscle cells (Lackner et al., 1999). *exp-2* gene encodes a potassium channel to repolarize the pharyngeal muscle in *C. elegans* (Davis et al., 1999). *nmur-2*, *npr-24* and *nmur-1* are also candidate genes which have G protein-coupled receptor activity. *nmur-1* is expressed in sensory neurons and interneurons (Maier et al., 2010). This gene regulates lifespan via the sensory system and modulate salt chemotaxis behavior (Maier et al., 2010; Watteyne et al., 2019).

However, *nmur-2* was selected as a target gene in our research finally. First, *nmur-1* and *eat-2* mutant displayed severe deficiencies in feeding and beads uptake as well as reduced pharyngeal pumping. Therefore, these two mutants were excluded as the receptor candidates. Second, compared with the N2 wild-type, only *nmur-2* showed a reduced pumping frequency by one third; a phenotype known also from the C20-PUFA deficient *fat-3* and *fat-2* mutant strains. It is for these two reasons that *nmur-2* was selected as the first 17,18-EEQ GPCR candidate gene.

Neuromedin U (NMU) is a neuropeptide originally isolated from porcine spinal cord (Minamino et al., 1985). In vertebrates, neuromedin U (NMU) plays a fundamental multifunctional role in key physiological processes such as feeding behavior, muscle contraction, ion transport in the gut, and stress responses as well as regulation of intestinal ion transport and blood pressure homeostasis (Brighton et al., 2004a; Ivanov et al., 2002). The first NMU-like receptor in invertebrates was described in the fruit fly *D. melanogaster* (Schoofs et al., 1993). In *C. elegans*, four NMU genes, *nmur-1*, *nmur-2*, *nmur-3* and *nmur-4,* have been identified. *nmur-2* encodes a G protein-coupled receptor that is similar to the insect pyrokinin-1 receptor and the vertebrate neuromedin

U (NMU) receptor (Brighton et al., 2004b). *nmur-2* gene is derived from the *nlp-44* precursor gene. When expressed in HEK293T cells, the product of *nmur-2* is able to bind a synthetic AFFYTPRI-NH2 neuropeptide, whose sequence was derived from the *C. elegans* protein product of *nlp-44*, expressed in the pharyngeal nervous system (Lindemans et al., 2009). Human GPCRs related to *nmur-1* and *nmur-2* show some protein sequence homology to human GPR39. GPR39 has been described as a Zn^{2+}-activated GPCR that regulates pancreatic, gastrointestinal and neuronal functions (Hershfinkel, 2018; Popovics and Stewart, 2011). Recently, Alkayed (Alkayed et al., 2018) reported preliminary data suggesting that GPR39 functions as an eicosanoid receptor in microvascular smooth muscle cells. According to this study, 15-HETE stimulates GPR39 to increase intracellular calcium and augment coronary vascular resistance, while 14,15-EET inhibits these actions.

4.5 NMU signaling and 17,18-EEQ

The relationship of NMU signaling and eicosanoids (for example, 17,18-EEQ) is still unknown. Previous study showed that analgesia effect of EETs was inhibited by β-endorphin neuropeptides which is an antisera targeting substance (Zhang et al., 2014). 17,18-EEQ with the combination of capsaicin caused the release of a proinflammatory neuropeptide and calcitonin-gene related peptide (CGRP) from dorsal root ganglia (DRG) neurons released significantly higher than with the incubation of capsaicin alone (Schäfer et al., 2020). Like the neurohormones, NMUs play an important role as signal molecules as well. NMUs act by binding the relevant NMU receptors to carry out the chemical signaling. Therefore, the following hypothetical modes of action of NMU and 17,18-EEQ's in the pharynx regulation of *C. elegans* are suggested; **i**) 17,18-EEQ might modulate the binding of worm's NMU to NMUR-2, or the receptor NMUR-2 acts with the pharynx neuron system to affect pumping frequency to modulate the 17,18-EEQ effect. **ii**) 17,18-EEQ can activate MUR-2 directly and transmits the signal either directly to muscle cells or nearby neurons. **iii**) 17,18-EEQ might modulate NMUR-2 indirectly via an additional signaling mediator influencing NMU.

References

Adkins, Y., and D.S. Kelley. 2010. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *J. Nutr. Biochem.* 21:781-792.

Agbor, L.N., M.T. Walsh, J.R. Boberg, and M.K. Walker. 2012. Elevated blood pressure in cytochrome P4501A1 knockout mice is associated with reduced vasodilation to omega-3 polyunsaturated fatty acids. *Toxicol. Appl. Pharmacol.* 264:351-360.

Albertson, D.G., J.N. Thompson, and S. Brenner. 1976. The pharynx of *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*. 275:299-325.

Alkayed, N.J., Z. Cao, Z.Y. Qian, S. Nagarajan, X. Liu, J. Nelson, F. Xie, B. Li, W. Fan, L. Liu, M.R. Grafe, X. Xiao, A.P. Barnes, and S. Kaul. 2018. Bidirectional Control of Coronary Vascular Resistance by Eicosanoids via a Novel GPCR. *bioRxiv*:420406.

Alkema, M.J., M. Hunter-Ensor, N. Ringstad, and H.R. Horvitz. 2005. Tyramine functions independently of octopamine in the *Caenorhabditis elegans* nervous system. *Neuron*. 46:247-260.

Arnold, C., M. Markovic, K. Blossey, G. Wallukat, R. Fischer, R. Dechend, A. Konkel, C. von Schacky, F.C. Luft, D.N. Muller, M. Rothe, and W.-H. Schunck. 2010a. Arachidonic Acid-metabolizing Cytochrome P450 Enzymes Are Targets of ω-3 Fatty Acids*. *J. Biol. Chem.* 285:32720-32733.

Arnold, C., M. Markovic, K. Blossey, G. Wallukat, R. Fischer, R. Dechend, A. Konkel, C. von Schacky, F.C. Luft, D.N. Muller, M. Rothe, and W.H. Schunck. 2010b. Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of {omega}-3 fatty acids. *J. Biol. Chem.* 285:32720-32733.

Avery, L. 1993. The genetics of feeding in *Caenorhabditis elegans*. *Genetics*. 133:897-917.

Avery, L., and H.R. Horvitz. 1987. A cell that dies during wild-type *C. elegans* development can function as a neuron in a ced-3 mutant. *Cell*. 51:1071-1078.

Avery, L., and H.R. Horvitz. 1989a. Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. *Neuron*. 3:473-485.

Avery, L., and H.R. Horvitz. 1990a. Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* 253:263-270.

Avery, L., and H.R. Horvitz. 1990b. Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* 253:263-270.

Avery, L., and R. Horvitz. 1989b. Pharyngeal pumping continues after laser killing of the pharyngeal nervous-system of *C. elegans*. *Neuron*. 3:473-485.

Avery, L., and Y.J. You. 2012a. *C. elegans* feeding. *In* WormBook. T.*C.e.*R. Community, editor.

Avery, L., and Y.J. You. 2012b. *C. elegans* feeding. *WormBook*:1-23.

Bai, X., L.-J. Huang, S.-W. Chen, B. Nebenfuehr, B. Wysolmerski, J.-C. Wu, S.K. Olson, A. Golden, and C.-W. Wang. 2020. Dietary supplementation with PUFAs rescues the eggshell defects caused by *seipin* mutations in *C. elegans*. *bioRxiv*:2020.2001.2023.916718.

Baudry, A., J. Bitard, S. Mouillet-Richard, M. Locker, A. Poliard, J.-M. Launay, and O. Kellermann. 2010. Serotonergic 5-HT2B Receptor Controls Tissue-nonspecific Alkaline Phosphatase Activity in Osteoblasts via Eicosanoids and Phosphatidylinositol-specific Phospholipase C. *J. Biol. Chem.* 285:26066-26073.

Bernstein, S., and G. Morley. Gap Junctions and Propagation of the Cardiac Action Potential.

Bittleman, D.B., and T.B. Casale. 1995. 5-Hydroxyeicosatetraenoic acid (HETE)-induced neutrophil transcellular migration is dependent upon enantiomeric structure. *Am. J. Respir. Cell Mol. Biol.* 12:260-267.

Blum, M., I. Dogan, M. Karber, M. Rothe, and W.H. Schunck. 2019. Chiral lipidomics of monoepoxy and monohydroxy metabolites derived from long-chain polyunsaturated fatty acids. *J. Lipid Res.* 60:135-148.

Bonafini, S., and C. Fava. 2017. Omega-3 fatty acids and cytochrome P450-derived eicosanoids in cardiovascular diseases: Which actions and interactions modulate hemodynamics? *Prostaglandins Other Lipid Mediat.* 128-129:34-42.

Boyd, W.A., S.J. McBride, and J.H. Freedman. 2007. Effects of genetic mutations and chemical exposures on Caenorhabditis elegans feeding: evaluation of a novel, high-throughput screening assay. *PLoS One*. 2:e1259.

Brighton, P., P. Szekeres, and G. Willars. 2004a. Neuromedin U and Its Receptors: Structure, Function, and Physiological Roles. *Pharmacol. Rev.* 56:231 - 248.

Brighton, P.J., P.G. Szekeres, and G.B. Willars. 2004b. Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol. Rev.* 56:231-248.

Campbell, W.B., and J.R. Falck. 2007. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension*. 49:590-596.

Campbell, W.B., and I. Fleming. 2010. Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Archiv : European journal of physiology*. 459:881-895.

Capdevila, J.H., and J.R. Falck. 2002. Biochemical and molecular properties of the cytochrome P450 arachidonic acid monooxygenases. *Prostaglandins Other Lipid Mediat.* 68-69:325-344.

Capdevila, J.H., A. Karara, D.J. Waxman, M.V. Martin, J.R. Falck, and F.P. Guengerich. 1990. Cytochrome P-450 enzyme-specific control of the regio- and enantiofacial selectivity of the microsomal arachidonic acid epoxygenase. *J. Biol. Chem.* 265:10865-10871.

Carnell, L., J. Illi, S.W. Hong, and S.L. McIntire. 2005. The G-Protein-Coupled Serotonin Receptor SER-1 Regulates Egg Laying and Male Mating Behaviors in Caenorhabditis elegans. *The Journal of Neuroscience*. 25:10671-10681.

Chabbi-Achengli, Y., J.-M. Launay, L. Maroteaux, M.C. de Vernejoul, and C. Collet. 2013. Serotonin 2B Receptor (5-HT2B R) Signals through Prostacyclin and PPAR-ß/δ in Osteoblasts. *PLOS ONE*. 8:e75783.

Chacos, N., J. Capdevila, J.R. Falck, S. Manna, C. Martin-Wixtrom, S.S. Gill, B.D. Hammock, and R.W. Estabrook. 1983. The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase. *Arch. Biochem. Biophys.* 223:639-648.

Chase, D.L., and M.R. Koelle. 2007. Biogenic amine neurotransmitters in *C. elegans*. *WormBook*:1-15.

Chen, B., P. Liu, H. Zhan, and Z.W. Wang. 2011a. Dystrobrevin controls neurotransmitter release and muscle Ca(2+) transients by localizing BK channels in *Caenorhabditis elegans*. *J. Neurosci.* 31:17338-17347.

Chen, J.N., and M.C. Fishman. 1996. Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. *Development*. 122:3809-3816.

Chen, Y., J.R. Falck, V.L. Manthati, J.L. Jat, and W.B. Campbell. 2011b. 20-Iodo-14,15-epoxyeicosa-8(Z)-enoyl-3-azidophenylsulfonamide: photoaffinity labeling of a 14,15-epoxyeicosatrienoic acid receptor. *Biochemistry*. 50:3840-3848.

Chiang, J.T., M. Steciuk, B. Shtonda, and L. Avery. 2006a. Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. *J. Exp. Biol.* 209:1859-1873.

Chiang, N., C.N. Serhan, S.E. Dahlen, J.M. Drazen, D.W. Hay, G.E. Rovati, T. Shimizu, T. Yokomizo, and C. Brink. 2006b. The lipoxin receptor ALX: potent ligand-specific and stereoselective actions in vivo. *Pharmacol. Rev.* 58:463-487.

Cho, J.H., J. Bandyopadhyay, J. Lee, C.S. Park, and J. Ahnn. 2000. Two isoforms of sarco/endoplasmic reticulum calcium ATPase (SERCA) are essential in *Caenorhabditis elegans*. *Gene*. 261:211-219.

Cunningham, Katherine A., Z. Hua, S. Srinivasan, J. Liu, Brian H. Lee, Robert H. Edwards, and K. Ashrafi. 2012. AMP-Activated Kinase Links Serotonergic Signaling to Glutamate Release for Regulation of Feeding Behavior in *C. elegans*. *Cell Metab.* 16:113-121.

Dallière, N., N. Bhatla, Z. Luedtke, D.K. Ma, J. Woolman, R.J. Walker, L. Holden-Dye, and V. O'Connor. 2016. Multiple excitatory and inhibitory neural signals converge to fine-tune *Caenorhabditis elegans* feeding to food availability. *The FASEB Journal*. 30:836-848.

Davis, M.W., R. Fleischhauer, J.A. Dent, R.H. Joho, and L. Avery. 1999. A mutation in the *C. elegans* EXP-2 potassium channel that alters feeding behavior. *Science (New York, N.Y.)*. 286:2501-2504.

Dempsey, C.M., S.M. Mackenzie, A. Gargus, G. Blanco, and J.Y. Sze. 2005. Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate *Caenorhabditis elegans* egg-laying behavior. *Genetics*. 169:1425-1436.

Dent, J.A., M.W. Davis, and L. Avery. 1997. *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.* 16:5867-5879.

Dernovici, S., T. Starc, J.A. Dent, and P. Ribeiro. 2007. The serotonin receptor SER-1 (5HT2ce) contributes to the regulation of locomotion in *Caenorhabditis elegans*. *Developmental neurobiology*. 67:189-204.

Donohoe, D.R., R.A. Jarvis, K. Weeks, E.J. Aamodt, and D.S. Dwyer. 2009. Behavioral adaptation in C. elegans produced by antipsychotic drugs requires serotonin and is associated with calcium signaling and calcineurin inhibition. *Neurosci. Res.* 64:280-289.

Falck, J.R., G. Wallukat, N. Puli, M. Goli, C. Arnold, A. Konkel, M. Rothe, R. Fischer, D.N. Muller, and W.H. Schunck. 2011a. 17(R),18(S)-epoxyeicosatetraenoic acid, a potent eicosapentaenoic acid (EPA) derived regulator of cardiomyocyte contraction: structure-activity relationships and stable analogues. *J. Med. Chem.* 54:4109-4118.

Falck, J.R., G. Wallukat, N. Puli, M. Goli, C. Arnold, A. Konkel, M. Rothe, R. Fischer, D.N. Muller, and W.H. Schunck. 2011b. 17(R),18(S)-epoxyeicosatetraenoic acid, a potent eicosapentaenoic acid (EPA) derived regulator of cardiomyocyte contraction: Structure-activity relationships and stable analogues. *J. Med. Chem.* 54:4109-4118.

Fang-Yen, C., L. Avery, and A.D.T. Samuel. 2009. Two size-selective mechanisms specifically trap bacteria-sized food particles in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*. 106:20093-20096.

Felder, C.C., R.Y. Kanterman, A.L. Ma, and J. Axelrod. 1990. Serotonin stimulates phospholipase A2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proceedings of the National Academy of Sciences*. 87:2187-2191.

Garcia, V., A. Gilani, B. Shkolnik, V. Pandey, F.F. Zhang, R. Dakarapu, S.K. Gandham, N.R. Reddy, J.P. Graves, A. Gruzdev, D.C. Zeldin, J.H. Capdevila, J.R. Falck, and M.L. Schwartzman. 2017. 20-HETE Signals Through G-Protein Coupled Receptor GPR75 (Gq) to Affect Vascular Function and Trigger Hypertension. *Circ. Res.* 120:1776-1788.

Gomez-Amaro, R.L., E.R. Valentine, M. Carretero, S.E. LeBoeuf, S. Rangaraju, C.D. Broaddus, G.M. Solis, J.R. Williamson, and M. Petrascheck. 2015. Measuring Food Intake and Nutrient Absorption in *Caenorhabditis elegans*. *Genetics*. 200:443-454.

Guha, S., S. Calarco, M.S. Gachet, and J. Gertsch. 2020. Juniperonic Acid Biosynthesis is Essential in Caenorhabditis Elegans Lacking Delta6 Desaturase (fat-3) and Generates New omega-3 Endocannabinoids. *Cells*. 9.

Guo, M., T.H. Wu, Y.X. Song, M.H. Ge, C.M. Su, W.P. Niu, L.L. Li, Z.J. Xu, C.L. Ge, M.T. Al-Mhanawi, S.P. Wu, and Z.X. Wu. 2015. Reciprocal inhibition between sensory ASH and ASI neurons modulates nociception and avoidance in *Caenorhabditis elegans*. *Nature communications*. 6:5655.

Gürel, G., M.A. Gustafson, J.S. Pepper, H.R. Horvitz, and M.R. Koelle. 2012. Receptors and other signaling proteins required for serotonin control of locomotion in *Caenorhabditis elegans*. *Genetics*. 192:1359-1371.

Hamdan, F.F., M.D. Ungrin, M. Abramovitz, and P. Ribeiro. 1999. Characterization of a novel serotonin receptor from Caenorhabditis elegans: cloning and expression of two splice variants. *J. Neurochem.* 72:1372-1383.

Hapiak, V.M., R.J. Hobson, L. Hughes, K. Smith, G. Harris, C. Condon, P. Komuniecki, and R.W. Komuniecki. 2009. Dual excitatory and inhibitory serotonergic inputs modulate egg laying in *Caenorhabditis elegans*. *Genetics*. 181:153-163.

Harder, D.R., N.J. Alkayed, A.R. Lange, D. Gebremedhin, and R.J. Roman. 1998. Functional Hyperemia in the Brain. *Stroke*. 29:229-234.

Harris, G., A. Korchnak, P. Summers, V. Hapiak, W.J. Law, A.M. Stein, P. Komuniecki, and R. Komuniecki. 2011. Dissecting the serotonergic food signal stimulating sensory-mediated aversive behavior in *C. elegans*. *PLoS One*. 6:e21897.

Harris, G.P., V.M. Hapiak, R.T. Wragg, S.B. Miller, L.J. Hughes, R.J. Hobson, R. Steven, B. Bamber, and R.W. Komuniecki. 2009. Three distinct amine receptors operating at different levels within the locomotory circuit are each essential for the serotonergic modulation of chemosensation in *Caenorhabditis elegans*. *J. Neurosci.* 29:1446-1456.

Harris, R.C., K.A. Munger, K.F. Badr, and K. Takahashi. 1990. Mediation of renal vascular effects of epidermal growth factor by arachidonate metabolites. *FASEB J.* 4:1654-1660.

Harris, T.R., P.A. Aronov, P.D. Jones, H. Tanaka, M. Arand, and B.D. Hammock. 2008a. Identification of two epoxide hydrolases in *Caenorhabditis elegans* that metabolize mammalian lipid signaling molecules. *Arch. Biochem. Biophys.* 472:139-149.

Harris, T.R., P.A. Aronov, P.D. Jones, H. Tanaka, M. Arand, and B.D. Hammock. 2008b. Identification of two epoxide hydrolases in *Caenorhabditis elegans* that metabolize mammalian lipid signaling molecules. *Arch. Biochem. Biophys.* 472:139-149.

Harris, T.R., and B.D. Hammock. 2013. Soluble epoxide hydrolase: gene structure, expression and deletion. *Gene*. 526:61-74.

Harteneck, C., T.D. Plant, and G. Schultz. 2000. From worm to man: three subfamilies of TRP channels. *Trends Neurosci.* 23:159-166.

Haun, C., J. Alexander, D.Y. Stainier, and P.G. Okkema. 1998. Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene. *Proc. Natl. Acad. Sci. U. S. A.* 95:5072-5075.

Hershfinkel, M. 2018. The Zinc Sensing Receptor, ZnR/GPR39, in Health and Disease. *Int J Mol Sci*. 19:439.

Hirafuji, M., T. Machida, N. Hamaue, and M. Minami. 2003. Cardiovascular protective effects of n-3 polyunsaturated fatty acids with special emphasis on docosahexaenoic acid. *J. Pharmacol. Sci.* 92:308-316.

Hobson, R.J., J. Geng, A.D. Gray, and R.W. Komuniecki. 2003. SER-7b, a constitutively active Gαs coupled 5-HT7-like receptor expressed in the *Caenorhabditis elegans* M4 pharyngeal motorneuron. *J. Neurochem.* 87:22-29.

Hobson, R.J., V.M. Hapiak, H. Xiao, K.L. Buehrer, P.R. Komuniecki, and R.W. Komuniecki. 2006a. SER-7, a *Caenorhabditis elegans* 5-HT7 like Receptor, Is Essential for the 5-HT Stimulation of Pharyngeal Pumping and Egg Laying. *Genetics*. 172:159-169.

Hobson, R.J., V.M. Hapiak, H. Xiao, K.L. Buehrer, P.R. Komuniecki, and R.W. Komuniecki. 2006b. SER-7, a *Caenorhabditis elegans* 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics*. 172:159-169.

Hobson, R.J., V.M. Hapiak, H. Xiao, K.L. Buehrer, P.R. Komuniecki, and R.W. Komuniecki. 2006c. SER-7, a *Caenorhabditis elegans* 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics*. 172:159-169.

Horvitz, H., M. Chalfie, C. Trent, J. Sulston, and P. Evans. 1982a. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science*. 216:1012-1014.

Horvitz, H.R., M. Chalfie, C. Trent, J.E. Sulston, and P.D. Evans. 1982b. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science*. 216:1012-1014.

Huang, W., B. Wang, X. Li, and J.X. Kang. 2015. Endogenously elevated n-3 polyunsaturated fatty acids alleviate acute ethanol-induced liver steatosis. *Biofactors*. 41:453-462.

Hull, D., and L. Timmons. 2004. Methods for delivery of double-stranded RNA into *Caenorhabditis elegans*. *Methods Mol. Biol.* 265:23-58.

Hutzell, P.A., and L.R. Krusberg. 1982. Fatty acid compositions of *Caenorhabditis elegans* and *C. briggsae*. *Comp. Biochem. Physiol. B Comp. Biochem.* 73:517-520.

Ivanov, T.R., C.B. Lawrence, P.J. Stanley, and S.M. Luckman. 2002. Evaluation of neuromedin U actions in energy homeostasis and pituitary function. *Endocrinology*. 143:3813-3821.

Jeziorski, M.C., R.M. Greenberg, and P.A. Anderson. 2000. The molecular biology of invertebrate voltage-gated Ca(2+) channels. *J. Exp. Biol.* 203:841-856.

Jung, F., C. Schulz, F. Blaschke, D.N. Muller, C. Mrowietz, R.P. Franke, A. Lendlein, and W.H. Schunck. 2012a. Effect of cytochrome P450-dependent epoxyeicosanoids on Ristocetin-induced thrombocyte aggregation. *Clin. Hemorheol. Microcirc.* 52:403-416.

Jung, F., C. Schulz, F. Blaschke, D.N. Muller, C. Mrowietz, R.P. Franke, A. Lendlein, and W.H. Schunck. 2012b. Effect of cytochrome P450-dependent epoxyeicosanoids on Ristocetin-induced thrombocyte aggregation. *Clin. Hemorheol. Microcirc.* 52:403-416.

Kahn-Kirby, A.H., and C.I. Bargmann. 2006. TRP channels in *C. elegans*. *Annu. Rev. Physiol.* 68:719-736.

Kahn-Kirby, A.H., J.L. Dantzker, A.J. Apicella, W.R. Schafer, J. Browse, C.I. Bargmann, and J.L. Watts. 2004. Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. *Cell*. 119:889-900.

Kellerman, S., J.A. Moore, W. Zierhut, H.G. Zimmer, J. Campbell, and A.M. Gerdes. 1992. Nuclear DNA content and nucleation patterns in rat cardiac myocytes from different models of cardiac hypertrophy. *J. Mol. Cell. Cardiol.* 24:497-505.

Kim, G.S., M. Nalini, Y. Kim, and D.-W. Lee. 2009. Octopamine and 5-hydroxytryptamine mediate hemocytic phagocytosis and nodule formation via eicosanoids in the beet armyworm, Spodoptera exigua. *Arch. Insect Biochem. Physiol.* 70:162-176.

Kiyama, Y., K. Miyahara, and Y. Ohshima. 2012a. Active uptake of artificial particles in the nematode *Caenorhabditis elegans*. *J. Exp. Biol.* 215:1178-1183.

Kiyama, Y., K. Miyahara, and Y. Ohshima. 2012b. Active uptake of artificial particles in the nematode *Caenorhabditis elegans*. *The Journal of Experimental Biology*. 215:1178-1183.

Klass, M.R. 1983. A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mech. Ageing Dev.* 22:279-286.

Konkel, A., and W.H. Schunck. 2011a. Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim. Biophys. Acta*. 1814:210-222.

Konkel, A., and W.H. Schunck. 2011b. Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim. Biophys. Acta*. 1814:210-222.

Kosel, M., W. Wild, A. Bell, M. Rothe, C. Lindschau, C.E. Steinberg, W.H. Schunck, and R. Menzel. 2011a. Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of *Caenorhabditis elegans*. *Biochem. J.* 435:689-700.

Kosel, M., W. Wild, A. Bell, M. Rothe, C. Lindschau, C.E.W. Steinberg, W.H. Schunck, and R. Menzel. 2011b. Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of *Caenorhabditis elegans*. *Biochem. J.* 435:689-700.

Kulas, J., C. Schmidt, M. Rothe, W.-H. Schunck, and R. Menzel. 2008a. Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*. *Arch. Biochem. Biophys.* 472:65-75.

Kulas, J., C. Schmidt, M. Rothe, W.H. Schunck, and R. Menzel. 2008b. Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*. *Arch. Biochem. Biophys.* 472:65-75.

Kullyev, A., C.M. Dempsey, S. Miller, C.J. Kuan, V.M. Hapiak, R.W. Komuniecki, C.T. Griffin, and J.Y. Sze. 2010. A genetic survey of fluoxetine action on synaptic transmission in *Caenorhabditis elegans*. *Genetics*. 186:929-941.

Lackner, M.R., S.J. Nurrish, and J.M. Kaplan. 1999. Facilitation of Synaptic Transmission by EGL-30 Gqα and EGL-8 PLCβ: DAG Binding to UNC-13 Is Required to Stimulate Acetylcholine Release. *Neuron*. 24:335-346.

Lahvic, J.L., M. Ammerman, P. Li, M.C. Blair, E.R. Stillman, E.M. Fast, A.L. Robertson, C. Christodoulou, J.R. Perlin, S. Yang, N. Chiang, P.C. Norris, M.L. Daily, S.E. Redfield, I.T. Chan, M. Chatrizeh, M.E. Chase, O. Weis, Y. Zhou, C.N. Serhan, and L.I. Zon. 2018. Specific oxylipins enhance vertebrate hematopoiesis via the receptor GPR132. *Proceedings of the National Academy of Sciences*. 115:9252-9257.

Laing, R., D.J. Bartley, A.A. Morrison, A. Rezansoff, A. Martinelli, S.T. Laing, and J.S. Gilleard. 2015. The cytochrome P450 family in the parasitic nematode Haemonchus contortus. *Int. J. Parasitol.* 45:243-251.

Lauterbach, B., E. Barbosa-Sicard, M.H. Wang, H. Honeck, E. Kargel, J. Theuer, M.L. Schwartzman, H. Haller, F.C. Luft, M. Gollasch, and W.H. Schunck. 2002. Cytochrome P450-dependent eicosapentaenoic acid metabolites are novel BK channel activators. *Hypertension*. 39:609-613.

Le, T., and M.H. Saier, Jr. 1996. Phylogenetic characterization of the epithelial Na+ channel (ENaC) family. *Mol. Membr. Biol.* 13:149-157.

Lee, K.S., S. Iwanir, R.B. Kopito, M. Scholz, J.A. Calarco, D. Biron, and E. Levine. 2017. Serotonin-dependent kinetics of feeding bursts underlie a graded response to food availability in *C. elegans*. *Nature communications*. 8:14221.

Leiser, S.F., H. Miller, R. Rossner, M. Fletcher, A. Leonard, M. Primitivo, N. Rintala, F.J. Ramos, D.L. Miller, and M. Kaeberlein. 2015. Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span. *Science*. 350:1375-1378.

Lesa, G.M., M. Palfreyman, D.H. Hall, M.T. Clandinin, C. Rudolph, E.M. Jorgensen, and G. Schiavo. 2003. Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans*. *J. Cell Sci.* 116:4965-4975.

Li, Y., Y. Zhao, X. Huang, X. Lin, Y. Guo, D. Wang, C. Li, and D. Wang. 2013. Serotonin control of thermotaxis memory behavior in nematode *Caenorhabditis elegans*. *PLoS One*. 8:e77779.

Limbu, R., G.S. Cottrell, and A.J. McNeish. 2018. Characterisation of the vasodilation effects of DHA and EPA, n-3 PUFAs (fish oils), in rat aorta and mesenteric resistance arteries. *PloS one*. 13:e0192484-e0192484.

Lindemans, M., T. Janssen, S.J. Husson, E. Meelkop, L. Temmerman, E. Clynen, I. Mertens, and L. Schoofs. 2009. A neuromedin-pyrokinin-like neuropeptide signaling system in *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 379:760-764.

Liu, X., Z.-Y. Qian, F. Xie, W. Fan, J.W. Nelson, X. Xiao, S. Kaul, A.P. Barnes, and N.J. Alkayed. 2017. Functional screening for G protein-coupled receptor targets of 14,15-epoxyeicosatrienoic acid. *Prostaglandins Other Lipid Mediat.* 132:31-40.

Loer, C.M., and C.J. Kenyon. 1993. Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. *J. Neurosci.* 13:5407-5417.

Lopez-Vicario, C., J. Alcaraz-Quiles, V. Garcia-Alonso, B. Rius, S.H. Hwang, E. Titos, A. Lopategi, B.D. Hammock, V. Arroyo, and J. Claria. 2015. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. *Proc. Natl. Acad. Sci. U. S. A.* 112:536-541.

Lu, T., M. VanRollins, and H.-C. Lee. 2002. Stereospecific Activation of Cardiac ATP-Sensitive K^+ Channels by Epoxyeicosatrienoic Acids: A Structural Determinant Study. *Mol. Pharmacol.* 62:1076-1083.

Luedtke, S., V. O'Connor, L. Holden-Dye, and R.J. Walker. 2010. The regulation of feeding and metabolism in response to food deprivation in *Caenorhabditis elegans*. *Invertebr. Neurosci.* 10:63-76.

Ma, D.K., M. Rothe, S. Zheng, N. Bhatla, C.L. Pender, R. Menzel, and H.R. Horvitz. 2013. Cytochrome P450 drives a HIF-regulated behavioral response to reoxygenation by *C. elegans*. *Science*. 341:554-558.

Maier, W., B. Adilov, M. Regenass, and J. Alcedo. 2010. A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on *C. elegans* lifespan. *PLoS Biol.* 8:e1000376-e1000376.

McGiff, J.C. 1991. Cytochrome P-450 metabolism of arachidonic acid. *Annu. Rev. Pharmacol. Toxicol.* 31:339-369.

McGiff, J.C., and J. Quilley. 1999. 20-HETE and the kidney: resolution of old problems and new beginnings. *Am. J. Physiol.* 277:R607-623.

McKay, J.P., D.M. Raizen, A. Gottschalk, W.R. Schafer, and L. Avery. 2004a. *eat-2* and *eat-18* are required for nicotinic neurotransmission in the *Caenorhabditis elegans* pharynx. *Genetics*. 166:161-169.

McKay, J.P., D.M. Raizen, A. Gottschalk, W.R. Schafer, and L. Avery. 2004b. *eat-2* and *eat-18* Are Required for Nicotinic Neurotransmission in the *Caenorhabditis elegans* Pharynx. *Genetics*. 166:161-169.

McKay, J.P., D.M. Raizen, A. Gottschalk, W.R. Schafer, and L. Avery. 2004c. *eat-2* and *eat-18* Are Required for Nicotinic Neurotransmission in the *Caenorhabditis elegans* Pharynx. *Genetics*. 166:161-169.

Mendel, J.E., H.C. Korswagen, K.S. Liu, Y.M. Hajdu-Cronin, M.I. Simon, R.H. Plasterk, and P.W. Sternberg. 1995. Participation of the protein Go in multiple aspects of behavior in *C. elegans*. *Science*. 267:1652-1655.

Minamino, N., K. Kangawa, and H. Matsuo. 1985. Neuromedin U-8 and U-25: Novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* 130:1078-1085.

Morin, C., M. Sirois, V. Échavé, R. Albadine, and E. Rousseau. 2010. 17,18-Epoxyeicosatetraenoic Acid Targets PPARγ and p38 Mitogen–Activated Protein Kinase to Mediate Its Anti-inflammatory Effects in the Lung. *Am. J. Respir. Cell Mol. Biol.* 43:564-575.

Morin, C., M. Sirois, V. Echave, E. Rizcallah, and E. Rousseau. 2009. Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 296:L130-139.

Morisaki, N., J.A. Lindsey, J.M. Stitts, H. Zhang, and D.G. Cornwell. 1984. Fatty acid metabolism and cell proliferation. V. Evaluation of pathways for the generation of lipid peroxides. *Lipids*. 19:381-394.

Murakami, H., and S. Murakami. 2007. Serotonin receptors antagonistically modulate *Caenorhabditis elegans* longevity. *Aging Cell*. 6:483-488.

Muthalif, M.M., I.F. Benter, N. Karzoun, S. Fatima, J. Harper, M.R. Uddin, and K.U. Malik. 1998. 20-Hydroxyeicosatetraenoic acid mediates calcium/calmodulin-dependent protein kinase II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *Proceedings of the National Academy of Sciences*. 95:12701-12706.

Nandakumar, M., and M.W. Tan. 2008. Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity. *PLoS Genet.* 4:e1000273.

Niacaris, T., and L. Avery. 2003. Serotonin regulates repolarization of the *C. elegans* pharyngeal muscle. *J. Exp. Biol.* 206:223-231.

Nithipatikom, K., J.M. Moore, M.A. Isbell, J.R. Falck, and G.J. Gross. 2006. Epoxyeicosatrienoic acids in cardioprotection: ischemic versus reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* 291:H537-542.

Nurrish, S., L. Ségalat, and J.M. Kaplan. 1999. Serotonin inhibition of synaptic transmission: Galpha(0) decreases the abundance of UNC-13 at release sites. *Neuron*. 24:231-242.

Nuttley, W.M., K.P. Atkinson-Leadbeater, and D. Van Der Kooy. 2002. Serotonin mediates food-odor associative learning in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 99:12449-12454.

Okkema, P.G., and A. Fire. 1994. The Caenorhabditis elegans NK-2 class homeoprotein CEH-22 is involved in combinatorial activation of gene expression in pharyngeal muscle. *Development*. 120:2175-2186.

Okkema, P.G., E. Ha, C. Haun, W. Chen, and A. Fire. 1997. The *Caenorhabditis elegans* NK-2 homeobox gene ceh-22 activates pharyngeal muscle gene expression in combination with pha-1 and is required for normal pharyngeal development. *Development*. 124:3965-3973.

Olde, B., and W.R. McCombie. 1997. Molecular cloning and functional expression of a serotonin receptor from *Caenorhabditis elegans*. *J. Mol. Neurosci.* 8:53-62.

Oyekan, A.O., K. McAward, and J.C. McGiff. 1998. Renal functional effects of endothelins: dependency on cytochrome P450-derived arachidonate metabolites. *Biol. Res.* 31:209-215.

Panigrahy, D., M.L. Edin, C.R. Lee, S. Huang, D.R. Bielenberg, C.E. Butterfield, C.M. Barnes, A. Mammoto, T. Mammoto, A. Luria, O. Benny, D.M. Chaponis, A.C. Dudley, E.R. Greene, J.A. Vergilio, G. Pietramaggiori, S.S. Scherer-Pietramaggiori, S.M. Short, M. Seth, F.B. Lih, K.B. Tomer, J. Yang, R.A. Schwendener, B.D. Hammock, J.R. Falck, V.L. Manthati, D.E. Ingber, A. Kaipainen, P.A. D'Amore, M.W. Kieran, and D.C. Zeldin. 2012. Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *J. Clin. Investig.* 122:178-191.

Park, S.K., A. Herrnreiter, S.L. Pfister, K.M. Gauthier, B.A. Falck, J.R. Falck, and W.B. Campbell. 2018. GPR40 is a low-affinity epoxyeicosatrienoic acid receptor in vascular cells. *J. Biol. Chem.* 293:10675-10691.

Penela, P., C. Murga, C. Ribas, A.S. Tutor, S. Peregrín, and F. Mayor, Jr. 2006. Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. *Cardiovasc. Res.* 69:46-56.

Pocock, R., and O. Hobert. 2010. Hypoxia activates a latent circuit for processing gustatory information in *C. elegans*. *Nat. Neurosci.* 13:610-614.

Popovics, P., and A.J. Stewart. 2011. GPR39: a Zn(2+)-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. *Cell Mol Life Sci*. 68:85-95.

Pulak, R. 2006. Techniques for Analysis, Sorting, and Dispensing of *C. elegans* on the COPAS™ Flow-Sorting System in *C. elegans*. Vol. 351. K. Strange, editor. Humana Press. 275-286.

Raabe, R.C., L.D. Mathies, A.G. Davies, and J.C. Bettinger. 2014a. The omega-3 fatty acid eicosapentaenoic acid is required for normal alcohol response behaviors in *C. elegans*. *PLoS One*. 9:e105999.

Raabe, R.C., L.D. Mathies, A.G. Davies, and J.C. Bettinger. 2014b. The omega-3 fatty acid eicosapentaenoic acid is required for normal alcohol response behaviors in *C. elegans*. *PloS one*. 9:e105999-e105999.

Raizen, D.M., and L. Avery. 1994. Electrical activity and behavior in the pharynx of *Caenorhabditis elegans*. *Neuron*. 12:483-495.

Raizen, D.M., R.Y.N. Lee, and L. Avery. 1995. Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*. *Genetics*. 141:1365.

Ranganathan, R., S.C. Cannon, and H.R. Horvitz. 2000. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in *C. elegans*. *Nature*. 408:470-475.

Ranganathan, R., E.R. Sawin, C. Trent, and H.R. Horvitz. 2001. Mutations in the Caenorhabditis elegans serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J. Neurosci.* 21:5871-5884.

Rankin, C.H. 2002. From gene to identified neuron to behaviour in *Caenorhabditis elegans*. *Nat. Rev. Genet.* 3:622-630.

Rex, E., S.C. Molitor, V. Hapiak, H. Xiao, M. Henderson, and R. Komuniecki. 2004. Tyramine receptor (SER-2) isoforms are involved in the regulation of pharyngeal pumping and foraging behavior in *Caenorhabditis elegans*. *J. Neurochem.* 91:1104-1115.

Robertson, H.M. 1998. Two large families of chemoreceptor genes in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* reveal extensive gene duplication, diversification, movement, and intron loss. *Genome Res.* 8:449-463.

Rogers, C.M., C.J. Franks, R.J. Walker, J.F. Burke, and L. Holden-Dye. 2001a. Regulation of the pharynx of *Caenorhabditis elegans* by 5-HT, octopamine, and FMRFamide-like neuropeptides. *J. Neurobiol.* 49:235-244.

Rogers, C.M., C.J. Franks, R.J. Walker, J.F. Burke, and L. Holden-Dye. 2001b. Regulation of the pharynx of *Caenorhabditis elegans* by 5-HT, octopamine, and FMRFamide-like neuropeptides. *J. Neurobiol.* 49:235-244.

Rohlfing, A.-K., Y. Miteva, L. Moronetti, L. He, and T. Lamitina. 2011. The *Caenorhabditis elegans* Mucin-Like Protein OSM-8 Negatively Regulates

Osmosensitive Physiology Via the Transmembrane Protein PTR-23. *PLoS Genet*. 7:e1001267.

Roman, R.J. 2002. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.* 82:131-185.

Rossi, A.G., M.J. Thomas, and J.T. O'Flaherty. 1988. Stereospecific bioactions of 5-hydroxyicosatetraenoate. *FEBS Lett.* 240:163-166.

Salkoff, L.B., A. Wei, B. Baban, A. Butler, G.L. Fawcett, G. Ferreira, and C.M. Santi. 2005. Potassium channels in *C. elegans*.

Satouchi, K., K. Hirano, M. Sakaguchi, H. Takehara, and F. Matsuura. 1993. Phospholipids from the free-living nematode *Caenorhabditis elegans*. *Lipids*. 28:837-840.

Sawin, E.R., R. Ranganathan, and H.R. Horvitz. 2000. C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron*. 26:619-631.

Schäfer, S.M.G., M. Sendetski, C. Angioni, R. Nüsing, G. Geisslinger, K. Scholich, and M. Sisignano. 2020. The omega-3 lipid 17,18-EEQ sensitizes TRPV1 and TRPA1 in sensory neurons through the prostacyclin receptor (IP). *Neuropharmacology*. 166:107952.

Schoofs, L., J. Vanden Broeck, and A. De Loof. 1993. The myotropic peptides of Locusta migratoria: structures, distribution, functions and receptors. *Insect Biochem. Mol. Biol.* 23:859-881.

Schunck, W.H., A. Konkel, R. Fischer, and K.H. Weylandt. 2018. Therapeutic potential of omega-3 fatty acid-derived epoxyeicosanoids in cardiovascular and inflammatory diseases. *Pharmacol. Ther.* 183:177-204.

Schwarz, D., P. Kisselev, S.S. Ericksen, G.D. Szklarz, A. Chernogolov, H. Honeck, W.H. Schunck, and I. Roots. 2004. Arachidonic and eicosapentaenoic acid metabolism by human CYP1A1: highly stereoselective formation of 17(R),18(S)-epoxyeicosatetraenoic acid. *Biochem. Pharmacol.* 67:1445-1457.

Segalat, L., D. Elkes, and J. Kaplan. 1995. Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. *Science*. 267:1648-1651.

Seki, K., A. Hirai, M. Noda, Y. Tamura, I. Kato, and S. Yoshida. 1992. Epoxyeicosatrienoic acid stimulates ADP-ribosylation of a 52 kDa protein in rat liver cytosol. *Biochem. J.* 281 (Pt 1):185-190.

Seubert, J., B. Yang, J.A. Bradbury, J. Graves, L.M. Degraff, S. Gabel, R. Gooch, J. Foley, J. Newman, L. Mao, H.A. Rockman, B.D. Hammock, E. Murphy, and D.C. Zeldin. 2004. Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K+ channels and p42/p44 MAPK pathway. *Circ. Res.* 95:506-514.

Shyn, S.I., R. Kerr, and W.R. Schafer. 2003. Serotonin and Go modulate functional states of neurons and muscles controlling *C. elegans* egg-laying behavior. *Curr. Biol.* 13:1910-1915.

Smith, E., T. Kaeberlein, B. Lydum, J. Sager, K.L. Welton, B. Kennedy, and M. Kaeberlein. 2008. Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in *Caenorhabditis elegans*. *BMC Dev. Biol.* 8:49.

Song, B.-m., S. Faumont, S. Lockery, and L. Avery. 2013. Recognition of familiar food activates feeding via an endocrine serotonin signal in *Caenorhabditis elegans*. *eLife*. 2:e00329.

Song, B.M., and L. Avery. 2012. Serotonin activates overall feeding by activating two separate neural pathways in *Caenorhabditis elegans*. *J. Neurosci.* 32:1920-1931.

Song, B.M., and L. Avery. 2013. The pharynx of the nematode *C. elegans*: A model system for the study of motor control. *Worm*. 2:e21833.

Spector, A.A. 2009. Arachidonic acid cytochrome P450 epoxygenase pathway. *J. Lipid Res.* 50 Suppl:S52-56.

Srinivasan, S., L. Sadegh, I.C. Elle, A.G. Christensen, N.J. Faergeman, and K. Ashrafi. 2008. Serotonin regulates *C. elegans* fat and feeding through independent molecular mechanisms. *Cell Metab.* 7:533-544.

Starich, T.A., R.Y. Lee, C. Panzarella, L. Avery, and J.E. Shaw. 1996. eat-5 and unc-7 represent a multigene family in Caenorhabditis elegans involved in cell-cell coupling. *J. Cell Biol.* 134:537-548.

Starich, T.A., A. Miller, R.L. Nguyen, D.H. Hall, and J.E. Shaw. 2003. The *Caenorhabditis elegans* innexin INX-3 is localized to gap junctions and is essential for embryonic development. *Dev. Biol.* 256:403-417.

Steger, K.A., and L. Avery. 2004. The GAR-3 Muscarinic Receptor Cooperates With Calcium Signals to Regulate Muscle Contraction in the *Caenorhabditis elegans* Pharynx. *Genetics*. 167:633-643.

Sun, J., V. Singh, R. Kajino-Sakamoto, and A. Aballay. 2011. Neuronal GPCR controls innate immunity by regulating noncanonical unfolded protein response genes. *Science*. 332:729-732.

Suo, S., Y. Kimura, and H.H.M. Van Tol. 2006. Starvation induces cAMP response element-binding protein-dependent gene expression through octopamine-G(q) signaling in *Caenorhabditis elegans*. *J. Neurosci.* 26:10082-10090.

Sze, J.Y., M. Victor, C. Loer, Y. Shi, and G. Ruvkun. 2000. Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature*. 403:560-564.

Tanaka, T., K. Ikita, T. Ashida, Y. Motoyama, Y. Yamaguchi, and K. Satouchi. 1996. Effects of growth temperature on the fatty acid composition of the free-living nematode *Caenorhabditis elegans*. *Lipids*. 31:1173-1178.

Tobias, L.D., and J.G. Hamilton. 1979. The effect of 5,8,11,14-eicosatetraynoic acid on lipid metabolism. *Lipids*. 14:181-193.

Tournois, C., V. Mutel, P. Manivet, J.-M. Launay, and O. Kellermann. 1998. Cross-talk between 5-Hydroxytryptamine Receptors in a Serotonergic Cell Line: INVOLVEMENT OF ARACHIDONIC ACID METABOLISM. *J. Biol. Chem.* 273:17498-17503.

Tsalik, E.L., T. Niacaris, A.S. Wenick, K. Pau, L. Avery, and O. Hobert. 2003. LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the *C. elegans* nervous system. *Dev. Biol.* 263:81-102.

Vasquez, V., M. Krieg, D. Lockhead, and M.B. Goodman. 2014. Phospholipids that contain polyunsaturated fatty acids enhance neuronal cell mechanics and touch sensation. *Cell reports*. 6:70-80.

Vriens, J., G. Owsianik, B. Fisslthaler, M. Suzuki, A. Janssens, T. Voets, C. Morisseau, B.D. Hammock, I. Fleming, R. Busse, and B. Nilius. 2005. Modulation of the Ca2 permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ. Res.* 97:908-915.

Waggoner, L.E., G.T. Zhou, R.W. Schafer, and W.R. Schafer. 1998. Control of alternative behavioral states by serotonin in *Caenorhabditis elegans*. *Neuron*. 21:203-214.

Wang, W., M. Lu, M. Balazy, and S.C. Hebert. 1997. Phospholipase A2 is involved in mediating the effect of extracellular Ca2+ on apical K+ channels in rat TAL. *Am. J. Physiol.* 273:F421-429.

Wang, Y., L. Tang, X. Feng, W. Du, and B.F. Liu. 2011. Ethanol interferes with gustatory plasticity in *Caenorhabditis elegans*. *Neurosci. Res.* 71:341-347.

Wang, Z.W., O. Saifee, M.L. Nonet, and L. Salkoff. 2001. SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron*. 32:867-881.

Watanabe, H., J. Vriens, J. Prenen, G. Droogmans, T. Voets, and B. Nilius. 2003. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature*. 424:434-438.

Watteyne, J., P. Van der Auwera, K. Peymen, C. Borghgraef, E. Vandewyer, I. Rutten, J. Lammertyn, R. Jelier, L. Schoofs, and I. Beets. 2019. Neuromedin U signaling regulates memory retrieval of learned salt avoidance in a *C. elegans* gustatory circuit. *bioRxiv*:683888.

Watts, J.L., and J. Browse. 2002. Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 99:5854-5859.

Watts, J.L., E. Phillips, K.R. Griffing, and J. Browse. 2003. Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *Caenorhabditis elegans fat-3* mutants. *Genetics*. 163:581-589.

Wei, A., T. Jegla, and L. Salkoff. 1996. Eight potassium channel families revealed by the *C. elegans* genome project. *Neuropharmacology*. 35:805-829.

Weinshenker, D., G. Garriga, and J.H. Thomas. 1995. Genetic and pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. *J. Neurosci.* 15:6975-6985.

White, J.G., E. Southgate, J.N. Thomson, and S. Brenner. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 314:1-340.

Wilson, M.A., B. Shukitt-Hale, W. Kalt, D.K. Ingram, J.A. Joseph, and C.A. Wolkow. 2006. Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging Cell*. 5:59-68.

Wong, P.Y., K.T. Lin, Y.T. Yan, D. Ahern, J. Iles, S.Y. Shen, R.K. Bhatt, and J.R. Falck. 1993. 14(R),15(S)-epoxyeicosatrienoic acid (14(R),15(S)-EET) receptor in guinea pig mononuclear cell membranes. *J Lipid Mediat*. 6:199-208.

Wu, C.C., T. Gupta, V. Garcia, Y. Ding, and M.L. Schwartzman. 2014. 20-HETE and blood pressure regulation: clinical implications. *Cardiol. Rev.* 22:1-12.

Xiao, H., V.M. Hapiak, K.A. Smith, L. Lin, R.J. Hobson, J. Plenefisch, and R. Komuniecki. 2006. SER-1, a Caenorhabditis elegans 5-HT2-like receptor, and a multi-PDZ domain containing protein (MPZ-1) interact in vulval muscle to facilitate serotonin-stimulated egg-laying. *Dev. Biol.* 298:379-391.

Yang, W., V.R. Tuniki, S. Anjaiah, J.R. Falck, C.J. Hillard, and W.B. Campbell. 2008. Characterization of epoxyeicosatrienoic acid binding site in U937 membranes using a novel radiolabeled agonist, 20-125i-14,15-epoxyeicosa-8(Z)-enoic acid. *J. Pharmacol. Exp. Ther.* 324:1019-1027.

Zhang, G., S. Kodani, and B.D. Hammock. 2014. Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer. *Prog. Lipid Res.* 53:108-123.

Zheng, S.Q., A.J. Ding, G.P. Li, G.S. Wu, and H.R. Luo. 2013. Drug absorption efficiency in *Caenorhbditis elegans* delivered by different methods. *PLoS One*. 8:e56877.

Zhou, Y., J.R. Falck, M. Rothe, W.-H. Schunck, and R. Menzel. 2015. Role of CYP eicosanoids in the regulation of pharyngeal pumping and food uptake in *Caenorhabditis elegans*. *J. Lipid Res.* 56:2110-2123.

Zou, A.P., J.T. Fleming, J.R. Falck, E.R. Jacobs, D. Gebremedhin, D.R. Harder, and R.J. Roman. 1996a. 20-HETE is an endogenous inhibitor of the large-conductance $Ca^{(2+)}$-activated K^+ channel in renal arterioles. *Am. J. Physiol.* 270:R228-237.

Zou, A.P., J.T. Fleming, J.R. Falck, E.R. Jacobs, D. Gebremedhin, D.R. Harder, and R.J. Roman. 1996b. Stereospecific effects of epoxyeicosatrienoic acids on renal vascular tone and K(+)-channel activity. *American Journal of Physiology-Renal Physiology*. 270:F822-F832.

Appendix

Table A1 Serotonin and its effects on *C. elegans* (Chase and Koelle, 2007)

Drug	**Mammalian / invertebrate targets**	***C. elegans* targets[a]**	**Effects of drugs on wild-type *C. elegans***
Serotonin	Serotonin receptors	SER-1 (Dempsey et al., 2005; Hamdan et al., 1999), SER-2 (Dempsey et al., 2005; Rex et al., 2004), MOD-5(Ranganathan et al., 2001), MOD-1(Ranganathan et al., 2000; Sawin et al., 2000)	Inhibits locomotion (Horvitz et al., 1982a; Sawin et al., 2000; Segalat et al., 1995),Inhibits defecation (Horvitz et al., 1982a; Segalat et al., 1995). Stimulates pharyngeal pumping (Horvitz et al., 1982a; Niacaris and Avery, 2003; Rogers et al., 2001a; Segalat et al., 1995) (in the absence of food. Stimulates egg laying (Horvitz et al., 1982a; Mendel et al., 1995; Waggoner et al., 1998; Weinshenker et al., 1995). Causes male tail curling (Loer and Kenyon, 1993). Causes reduced sensitivity to aldicarb-induced paralysis (Nurrish et al., 1999). Stimulates vulval muscle calcium transients (Shyn et al., 2003). Inhibits HSN calcium transients (Shyn et al., 2003). Inhibits olfactory adaptation (Nuttley et al., 2002)

Table A2 G protein coupling and pharmacological profiles of *C. elegans* G protein coupled serotonin receptors

Serotonin receptors	Experimental evidence for ligand assignment	G protein coupling	References G protein coupling	References binding studies
SER-1	Binding studies, Hetero-logous cell signaling, Mutant resistant to serotonin	Gαq in heterologous cells	(Hamdan et al., 1999)	(Hamdan et al., 1999)
SER-4	Binding studies, heterologous cell signaling, mutant resistant to serotonin	Gαi/o in heterologous cells	(Olde and McCombie, 1997)	(Olde and McCombie, 1997)
SER-5	Mutant resistant to serotonin	Gαs predicted from sequence analysis	(Harris et al., 2009)	
SER-7	Binding studies, heterologous cell signaling, mutant resistant to serotonin	Gαs in heterologous cells, Gαs in MC neurons, Gα12 in M4 neuron	(Hobson et al., 2003; Hobson et al., 2006c; Song and Avery, 2012)	(Hobson et al., 2003; Hobson et al., 2006c)

Table A3 Genetically-established functions and expression patterns of *C. elegans* G protein coupled serotonin receptors

Serotonin receptors	Functions revealed in mutants	References mutant phenotype	Expression pattern	References expression pattern
SER-1	Exogenous serotonin-stimulated egg laying, feeding, and slowing of locomotion, α-methyl-5-HT stimulated egg laying, male ventral tail curling, food-induced slowing, food modulation of aversive response, heat shock response, longevity	(Carnell et al., 2005; Dempsey et al., 2005; Dernovici et al., 2007; Harris et al., 2011; Murakami and Murakami, 2007; Xiao et al., 2006)	Pharyngeal muscle (pm3, pm4, pm5, pm6, pm7, pm8), CEP, RMG, RMH, RMF, RMD, RIA, RIC, URY, additional head neurons, vulval muscle and epithelial cells, tail neurons (PVT, PVQ, possibly DVC) ventral nerve cord, excretory cell, uterine cells, ray sensory neurons in male	(Carnell et al., 2005; Cho et al., 2000; Dempsey et al., 2005; Dernovici et al., 2007; Tsalik et al., 2003; Xiao et al., 2006)
SER-4	Egg laying induced by imipramine but not by fluoxetine or serotonin, inhibition of locomotion by serotonin, effects of	(Cho et al., 2000; Dempsey et al., 2005; Gürel et al., 2012; Li et al., 2013; Murakami and Murakami,	NSM, RIB or AIB, RIS, pharyngeal neuron, pair of sublateral interneurons or motorneurons, retrovesicular ganglion, PVT tail neuron, vm2 vulval muscles,	(Gürel et al., 2012; Tsalik et al., 2003)

	ethanol on gustatory plasticity and locomotion, thermotaxis memory behavior	2007; Srinivasan et al., 2008; Wang et al., 2011)	DVA or DVC tail interneuron	
SER-5	Exogenous serotonin-stimulated egg laying, food and serotonin-dependent increase in sensitivity of ASH neurons to octanol and decrease in sensitivity to Cu2+. Reduced sensitivity to serotonin and fluoxetine induced paralysis.	(Guo et al., 2015; Hapiak et al., 2009; Harris et al., 2011; Kullyev et al., 2010)	Neurons including AWB and ASH, body wall muscles, vulval muscles	(Hapiak et al., 2009)
SER-7	Exogenous serotonin-stimulated egg laying, pharyngeal pumping, and food intake.	(Donohoe et al., 2009; Gomez-Amaro et al., 2015; Hapiak et al., 2009; Hobson et al.,	Pharyngeal neurons MC, M4, I2, I3, M5, M3, I4, I6, and M2, vulval muscles	(Hobson et al., 2006c)

	Regulation of pharyngeal pumping. Egg laying. Hypoxia regulation of gustatory and effects of ethanol on gustatory plasticity, thermotaxis memory behavior	2006c; Leiser et al., 2015; Li et al., 2013; Pocock and Hobert, 2010; Song et al., 2013; Song and Avery, 2012; Wang et al., 2011)		

Table A4 Total fatty acid composition of wild-type and mutant strains.

	Fatty acid composition (mol %)				
Fatty acid	**N2** (wild-type)	***emb-8*** *(hc69)*	***fat-1*** *(wa9)*	***fat-2*** *(wa17)*	***fat-3*** *(wa22)*
	n=5	*n=9*	*n=3*	*n=4*	*n=3*
C16:0[1]	16.3±2.0	6.2±1.0***	10.0±0.9*	17.7±3.3	14.6±1.2
C18:0	9.3±2.3	6.3±1.0	8.2±0.2	8.7±0.6	8.1±0.1
C18:1[2]	29.0±4.8	23.0±2.6	28.4±2.8	66.1±2.3***	31.2±1.6
C18:2 n-6	7.8±1.4	17.1±1.1***	5.5±1.0	3.7±0.1	25.6±1.1***
C18:3 n-6	5.3±0.6	6.0±1.2	13.1±1.0***	n.d.	1.1±0.1***
C18:3 n-3	0.6±0.1	1.1±0.1*	1.0±0.12*	n.d.	14.8±0.1***
C20:1 n-9	0.1±0.1	0.2±0.1	n.d.	n.d.	n.d.
C20:3 n-6	5.7±0.9	6.7±0.5	15.2±1.4**	n.d.	2.0±0.2*
C20:4 n-6	1.4±0.3	1.4±0.3	17.0±1.1***	0.4±0.1*	0.5±0.0*
C20:4 n-3	3.3±0.5	3.6±0.6	n.d.	n.d.	n.d.
C20:5 n-3	21.2±3.0	28.4±1.1*	1.6±0.1**	2.6±0.1***	0.9**±0.1

n.d. – not detectable

[1] C16:0 refers the total mol % of 16:0, C16:iso, and 16:1

[2] C18:1 refers the total mol % of C18:1 n-7 and C18:1 n-9

$^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001, One way ANOVA. (Zhou et al., 2015)

Table A5 CYP-eicosanoid composition of wild-type and mutant strains.

CYP-eicosanoid pattern (ng/mg protein)					
CYP-eicosanoid	**N2** (wild-type)	***emb-8*** *(hc69)*	***fat-1*** *(wa9)*	***fat-2*** *(wa17)*	***fat-3*** *(wa22)*
	n=11	*n=4*	*n=6*	*n=3*	*n=6*
19-HETE	1.1±0.2	n.d.	8.8±0.9***	0.3±0.0*	0.2±0.0***
20-HETE	0.8±0.1	n.d.	4.7±1.1***	n.d.	n.d.
14,15-DHET	0.1±0.0	n.d.	0.6±0.1***	n.d.	n.d.
14,15-EET	0.2±0.0	n.d.	1.9±0.2***	n.d.	n.d.
11,12-DHET	n.d.	n.d.	0.2±0.0***	n.d.	n.d.
11,12-EET	0.2±0.1	n.d.	1.5±0.1***	n.d.	n.d.
8,9-DHET	n.d.	n.d.	n.d.	n.d.	n.d.
8,9-EET	0.2±0.1	n.d.	1.3±0.1***	n.d.	n.d.
5,6-DHET	0.2±0.1	n.d.	0.4±0.1	0.2±0.2	n.d.
5,6-EET	0.1±0.0	n.d.	1.6±0.3***	n.d.	n.d.
19-HEPE	1.9±0.4	0.2±0.1**	n.d.	n.d.	n.d.
20-HEPE	1.4±0.1	0.1±0.0*	n.d.	0.2±0.1***	n.d.
17,18-DHEQ	30.3±0.3	14.5±2.6*	0.4±0.1***	1.0±0.0***	0.6±0.19***

17,18-EEQ	6.7±0.6	0.6±0.2**	0.2±0.0***	0.2±0.1***	0.1±0.0***
14,15-DHEQ	2.2±0.3	0.3±0.1**	0.2±0.0***	0.4±0.1*	n.d.
14,15-EEQ	2.1±0.5	n.d.	n.d.	n.d.	n.d.
11,12-DHEQ	0.7±0.2	0.3±0.1	0.1±0.0*	0.2±0.1	n.d.
11,12-EEQ	1.9±0.2	n.d.	n.d.	0.1±0.0***	n.d.
8,9-DHEQ	0.3±0.1	0.2±0.1	n.d.	0.1±0.0	n.d.
8,9-EEQ	0.9±0.1	0.1±0.0**	n.d.	0.1±0.0***	n.d.
5,6-DHEQ	1.0±0.3	n.d.	n.d.	0.1±0.1*	0.1±0.0***
5,6-EEQ	1.4±0.3	n.d.	n.d.	0.3±0.1*	n.d.

n.d. – not detectable, $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001, One way ANOVA

All DHETs and DHEQs are hydrolysis metabolites of previously corresponding EETs and EEQs, respectively. (Zhou et al., 2015).

Table A6 Orthologous genes in mammals (Database courtesy: https://wormbase.org//#012-34-5 and https://www.genecards.org/)

C. elegans	Mammals
nmur-1: an ortholog of human GPR39 (G protein-coupled receptor 39) and members of the NMUR (Neuromedin U receptors) family	NMUR: G protein-coupled receptor **NMU1R** (FM3), a cognate receptor for neuromedin U, a neuropeptide distributed in the gut and central nervous system, expressed in peripheral tissues
*nm*ur-2: encodes a G protein-coupled receptor that is similar to the insect PK-1 (pyrokinin-1) receptor and Exhibits neuropeptide receptor activity. Is involved in neuropeptide signaling pathway	NMUR: G protein-coupled receptor **NMU2R** (FM4), a cognate receptor for neuromedin, expressed in specific regions of the brain
npr-24: an ortholog of human MCHR1 (melanin-concentrating hormone receptor 1)	MCHR1: a cyclic **peptide** that regulates a variety of functions in the mammalian brain, in particular **feeding behavior**
exp-2: encodes a member of the six-transmembrane voltage-activated (Kv-type) family of potassium channels	KCNB2, KCNB1, KCNF1: **KCNB2** belongs to a family of outwardly rectifying potassium channels, controlling the influx of Ca^{2+} through voltage-gated channels, **KCNB1** delayed rectifier potassium currents in pyramidal neurons of the hippocampus and cortex
eat-2: encodes a ligand-gated ion channel subunit most closely related to the non-alpha-subunits of nicotinic acetylcholine receptors	CHRNA7: (Cholinergic receptor, neuronal nicotinic, alpha polypeptide 7), essential for inhibiting cytokine synthesis by the cholinergic anti-inflammatory pathway
egl-30: encodes an ortholog of the heterotrimeric G protein alpha subunit Gq (Gq/G11 class). involved in cellular response to dopamine; positive regulation of protein import	GNA11, GNAQ: **GNAQ** (Guanine nucleotide-binding protein, Q polypeptide) overexpression of wildtype GNAQ resulted in hypertrophic growth, **GNA11** (Guanine nucleotide-binding protein,

into nucleus; and regulation of secretion. Localizes to several cellular components, including axon; extrinsic component of cytoplasmic side of plasma membrane; and neuronal cell body. Is expressed in several structures, including vulval cell.	alpha-11), required for maintenance of **basal blood pressure** and for development of salt-induced hypertension
eat-5: (EATing: abnormal pharyngeal pumping), predicted to have gap junction hemi-channel activity and expressed in K' cell and pharynx	/
gpb-2: involved in several processes, including regulation of pharyngeal pumping	GNB5 (G protein subunit beta 5): integrate signals between receptors and effector proteins
goa-1: G protein-coupled acetylcholine receptor activity. Is expressed in several structures, including neurons; non-striated muscle; pharynx; spermatheca; and vulva	GNAO1: represents the alpha subunit of the Go heterotrimeric G-protein signal-transducing complex. Defects in this gene are a cause of early-onset epileptic encephalopathy
gpa-12: predicted to have D5 dopamine receptor binding activity; G-protein beta/gamma-subunit complex binding activity; and GTPase activity and expressed in several structures, including excretory cell; intestine; nerve ring; neurons; and pharynx	GNA12 (G protein subunit alpha 12) and GNA13 (G protein subunit alpha 13): Diseases associated with GNA12 include Familial Hyperaldosteronism and Gastrointestinal Defects And Immunodeficiency Syndrome. Among its related pathways are G-protein signaling_RhoA regulation pathway and Signaling by GPCR. Diseases associated with GNA13 include Retinitis Pigmentosa 14 and Burkitt Lymphoma. Among its related pathways are G-protein signaling_RhoA regulation pathway and Signaling by GPCR.

npr-34: predicted to have neuropeptide Y receptor activity	GALR1 (galanin receptor 1): inhibits adenylyl cyclase via a G protein of the Gi/Go family. GALR1 is widely expressed in the brain and spinal cord, as well as in peripheral sites such as the small intestine and heart
npr-10: predicted to have neuropeptide Y receptor activity	PRLHR (prolactin releasing hormone receptor): implicated in hypogonadotropic hypogonadism 3 with or without anosmia and type 2 diabetes mellitus
npr-2: predicted to have neuropeptide Y receptor activity. Is expressed in head neurons and interneuron	MCHR1 (melanin concentrating hormone receptor 1); MTNR1B (melatonin receptor 1B); and NPY1R (neuropeptide Y receptor Y1): implicated in hypogonadotropic hypogonadism 3 with or without anosmia and type 2 diabetes mellitus
npr-22: exhibits neuropeptide receptor activity. Determination of adult lifespan; negative regulation of feeding behavior; and positive regulation of oviposition. Is expressed in body wall musculature; intestine; neurons; and pharyngeal muscle cell	NPFFR2 (neuropeptide FF receptor 2): implicated in hypogonadotropic hypogonadism 3 with or without anosmia and type 2 diabetes mellitus
npr-7: predicted to have neuropeptide Y receptor activity	AC018709.1; MTNR1B (melatonin receptor 1B); and OR11H6 (olfactory receptor family 11 subfamily H member 6): implicated in hypogonadotropic hypogonadism 3 with or without anosmia and type 2 diabetes mellitus

npr-24: predicted to have G protein-coupled receptor activity and peptide binding activity	SSTR5 (somatostatin receptor 5): is used to study Parkinson's disease. Human ortholog(s) of this gene are implicated in hypogonadotropic hypogonadism 8 with or without anosmia
npr-25: predicted to have G protein-coupled receptor activity	CCR2 (C-C motif chemokine receptor 2): a receptor for monocyte chemoattractant protein-1, a chemokine which specifically mediates monocyte chemotaxis. Monocyte chemoattractant protein-1 is involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors
cca-1: predicted to have low voltage-gated calcium channel activity and voltage-gated sodium channel activity. Is involved in membrane depolarization; positive regulation of action potential; and regulation of pharyngeal pumping. Is expressed in several structures, including alimentary muscle; distal tip cell; pharyngeal neurons; serotonergic neurons; and ventral nerve cord	CACNA1I (calcium voltage-gated channel subunit alpha1 I): implicated in several diseases, including epilepsy (multiple); heart conduction disease (multiple); and neuropathy (multiple)
egl-19: Exhibits voltage-gated calcium channel activity. Determination of left/right asymmetry in nervous system; positive regulation of striated muscle contraction; and regulation of pharyngeal pumping. Localizes to plasma membrane. Is expressed in several structures, including	CACNA1C (calcium voltage-gated channel subunit alpha1 C) and CACNA1S (calcium voltage-gated channel subunit alpha1 S): implicated in several diseases, including epilepsy (multiple); heart conduction disease (multiple); and neuropathy (multiple)

alimentary muscle; body wall musculature; neurons; preanal ganglion; and tail	
avr-15: predicted to have chloride channel activity. Is involved in action potential; locomotion involved in locomotory behavior; and regulation of pharyngeal pumping. Is expressed in motor neurons; pharynx; preanal ganglion neurons; somatic nervous system; and in male	GLRA3 (glycine receptor alpha 3); GLRA4 (glycine receptor alpha 4 (pseudogene)); and GLRB (glycine receptor beta): implicated in hyperekplexia 1 and hyperekplexia 2

Publications

Zhou, Y., J.R. Falck, M. Rothe, W.-H. Schunck, and R. Menzel. 2015. Role of CYP eicosanoids in the regulation of pharyngeal pumping and food uptake in *Caenorhabditis elegans*. *J. Lipid Res.* 56:2110-2123.

Zhou, Y., J.R. Falck, M. Rothe, W.-H. Schunck, L. Rueß and R. Menzel. Stereospecific bioactivity of the eicosanoid 17,18-epoxyeicosatetraenoic acid on the pharyngeal pumping of *C. elegans* (submitted).

Abstracts and Posters

Zhou, Y., Ju J, E. Nehk, Yin L., C. E. W. Steinberg and R. Menzel. Biological function of PUFA-derived eicosanoids in *Caenorhabditis elegans*. 19th International *C. elegans* Conference, 2013, California, Los Angeles, USA.

Zhou, Y., W.-H. Schunck and R. Menzel. CYP eicosanoids contribute to the regulation of pharyngeal pumping in *Caenorhabditis elegans*. The 15th International Winter Eicosanoid Conference, 2014, Baltimore, Maryland, USA. **(Travel Award Recipients)**

Zhou, Y., W.-H. Schunck, and R. Menzel. CYP eicosanoids contribute to the regulation of pharyngeal pumping activity in *Caenorhabditis elegans*. Berlin *C. elegans* meeting, 2014, Berlin, Germany.

Zhou, Y. and R. Menzel. Disentangling the CYP-eicosanoids dependent regulation of the pharyngeal pumping in *Caenorhabditis elegans.* European Worm Meeting (EWM), 2016, Berlin, Germany.

Zhou, Y. and R. Menzel. i) Disentangling the CYP-eicosanoids dependent regulation of the pharyngeal pumping in *Caenorhabditis elegans*. ii) Stereospecific bioactivity of the eicosanoid 17,18-epoxyeicosatetraenoic acid on the pharyngeal pumping of *Caenorhabditis elegans*. 21st International *C. elegans* Conference, 2017, California, Los Angeles, USA.

Acknowledgements

I would like to express my gratitude deep appreciation for all the lovely people who helped me during these years of study in Berlin. My deepest gratitude first and foremost goes to my supervisor PD Dr. Ralph Menzel for his careful and patient guidance. His suggestions and comments, from the research to the publications, were always thoughtful and considerate. Meanwhile, I want to express my sincere gratitude to Prof. Dr. Liliane Rueß for offering the opportunity and providing support to let me carry on my PhD study. Without your help, the completion of this thesis would have not been possible.

I would like to express my heartfelt gratitude to Prof. Dr. Christian EW Steinberg, who gave me the helpful guidance and encouragement. My special thanks go to Dr. Wolf-Hagen Schunck. His advices and help, from the research to publications, were always helpful! I am thankful to all the colleagues of the research groups from Prof. Dr. Liliane Rueß and Prof. Dr. Christian EW Steinberg, many of who shared their wonderful experiences with me. I thank Dr. Michael Rothe and his colleagues, for their help in LC-MS/MS measurement.

I also thank Dr. Nadine Saul and Dr. Hazel Ruvimbo Maboreke. Both of you gave me much assistance not only in the research but also in the life.

Last but not the least, a deep sense of gratitude goes to my parents for their endless support for study. I love you!

Thesis declaration

I, Yiwen Zhou, hereby declare that I have completed the thesis independently using only the aids and tools specified. All aids used in this thesis as well as scientific ideas from or based on other sources were cited at the respective point. I have not applied for a doctor's degree in the doctoral subject elsewhere and do not hold a corresponding doctor's degree.

I, have taken due note of the Faculty of Mathematics and Natural Sciences I PhD Regulations, published in the Official Gazette of Humboldt-Universität zu Berlin on 27 June 2012.

.....
Date / signature of the candidate