To decipher the role of neuropeptides to regulate reversal frequency in the global search behaviour of *C. elegans*

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Certificate of Examination

This is to certify that the dissertation titled **"To decipher the role of neuropeptides in the global search behaviour of *C. elegans*"** submitted by **Mr. Mohan Lal** (Reg. No. MS12094) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Rajesh Ramachandran Dr.Shravan Kumar Mishra Dr. Kavita Babu (Supervisor)

Dated: April 20, 2017

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Kavita Babu at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma,or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

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Dated: April 20, 2017

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Kavita Babu

(Supervisor)

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Finally, thanks to my parents for all the moral support and the amazing chances they've given me over the years.

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Abstract

In *C. elegans*, during exploratory behavior the switch from local to global search for food is an important for survival and being mediated by the neuropeptides. Previous studies have shown that the FLP-18 neuropeptide is regulating the reversal frequency in global search behavior through NPR-4, one of its G protein coupled receptor . Here we are looking for receptors of FLP-18 and other probable neuropeptides that play role in neuromodulation of exploratory behavior. In our studies, we found FLP-1 and FLP-21 neuropeptides along with FLP-18 regulates reversal frequency during both local and global search of exploratory behavior. Furthermore, FLP-18 also functions through NPR-1 receptor along with NPR-4 receptor. The implication of these studies is that information flow through *C. elegans* circuits depends on neuromodulatory states.

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Chapter 1

Introduction

1.1 *C. elegans* as a model organism

Caenorhabditis elegans is a non-parasitic soil nematode, about 1mm in length and transparent and has been used widely in research laboratories. It has a short life cycle of about three days and lifespan of two to three weeks. *C. elegans* has two sexes that are hermaphrodites and males. Hermaphrodites can self-fertilize or mate with males. Wild-type worms have 959 cells, and the number and position of cells are constant. It is easy to track cells and follow cell lineages during the development from embryo to adult hermaphrodites.

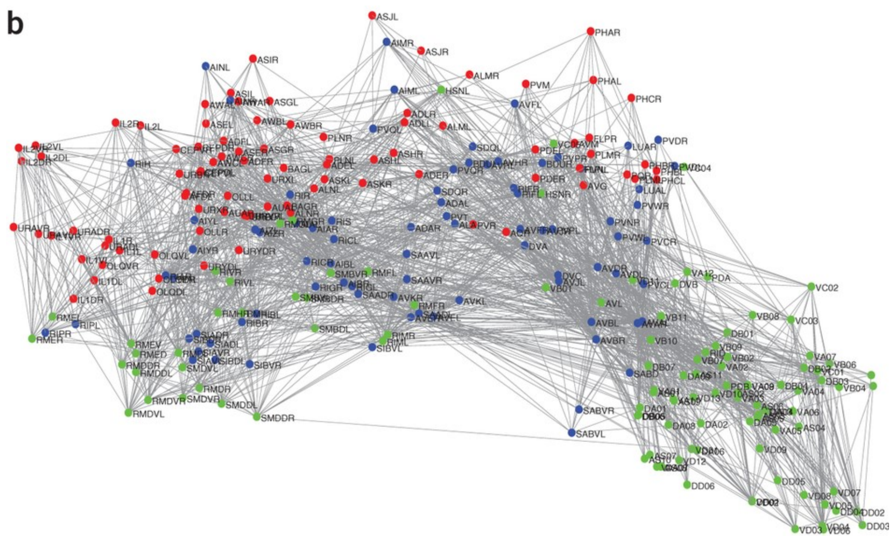

Figure 1.1: The *Caenorhabditis elegans* connectome (Bargmann Marder 2013).

C. elegans has a small nervous system consist of 302 neurons, whose neurons position and the connection between all neurons are known (see figure 1.1). *C. elegans* is an attractive system to study the neural and genetic basis of behavior. *C. elegans* exhibits a plethora of complex behaviors, all of which involve basic locomotion. During locomotion, worms initiate the backward movement to change direction spontaneously or in response to sensory cues. However, the neuromodulation underlying neural circuits responsible for complex behavior are not well defined.

1.2 Neuropeptides and its function

Neuropeptides are small peptides released by the neurons that function directly or indirectly to modulate synaptic activity. The number of neuropeptides predicted in *C. elegans* is over hundred. These neuropeptides are derived from large neuropeptide precursor molecule which undergo posttranslational modification to produce mature neuropeptide. In endoplasmic reticulum, the processing of neuropeptide precursor molecules is initiated by removal of the signal peptide, and further processing continues in the Golgi complex (Strand, 1999). From Golgi complex, the processed intermediate neuropeptide packed inside the dense core vesicle. Inside the dense core vesicle, the intermediated neuropeptide processed into fully mature neuropeptide and next these dense core vesicle containing neuropeptides transported to the nerve terminal (Strand, 1999).

Neuropeptides act by binding to its receptor that is seven transmembrane domain G protein-coupled receptor (GPCR). These receptors are more than hundred and found heterogeneously throughout the nervous system of *C. elegans*. Some of peptides activate multiple receptors expressed by target neurons and also one receptor may have multiple neuropeptide ligands. There are three main families of neuropeptides present in the *C. elegans* which are Insulin like peptides, FMRF related peptides (FLP) and Neuropeptide like peptides (NLP). Insulin like peptides play important role decision to enter dauer state in absence of food (Thacker and Rose 2000). FMRF related peptides is known to effect locomotion (Cohen et al. 2009), egglaying (Waggoner et al. 2000). The Neuropeptide like peptide is known to have anti-microbial functions (Couillault et al. 2004) and modulating acetylcholine signaling.

1.3 Neural circuits in *C. elegans*

Neural circuits generate behaviour. Locomotion, in particular, represents a general feature of behavior across the animal kingdom. One of the fundamental questions underlie how the nervous system controls movement system? How are locomotor patterns fine-tuned by neuromodulatory states at the level of sensory neurons and interneurons?

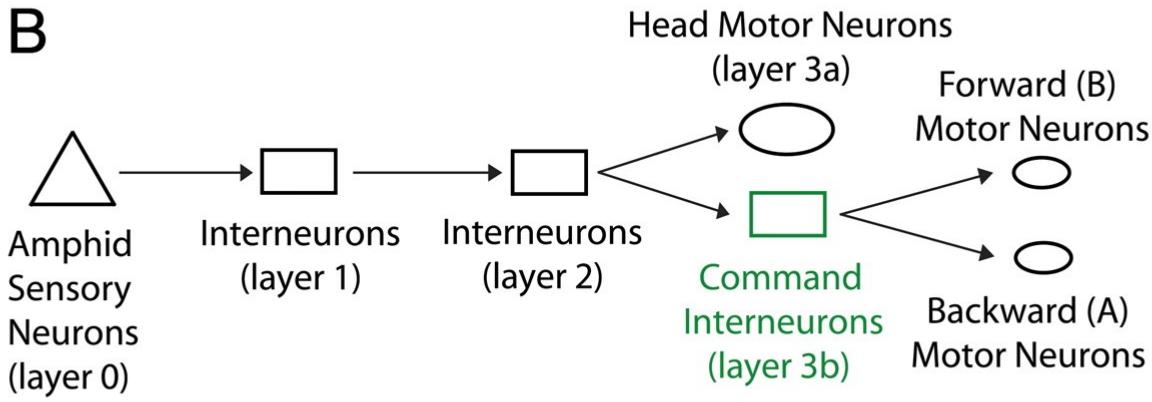

Figure 1.2: Information flow from sensory neurons to motor neurons (Gray, Hill, and Bargmann 2005).

Sensory neurons sense environmental cues through different receptors. Sensory neurons send information to interneurons. Multiple sensory information is integrated and processed in two separate layers of interneurons. Next these interneurons layers relay processed information to command interneurons which finally send the signal to motor neurons.

1.4 Exploratory Behaviour

Exploratory behavior refers to behavior in *C. elegans* that enables them to orientate to their novel environment in search for food. Exploratory behavior is consist of two behavior states local search and global search. Upon removal from food, *C. elegans* initiates the local search in which it moves rapidly and frequently reverses to explore nearby areas (see figure 1.3; Gray et al., 2005; Hills et al., 2004; Wakabayashi et al., 2004). Over the next one hour, the worm goes for global search behavior in which forward crawling are extended, and a decrease in reversals movements.

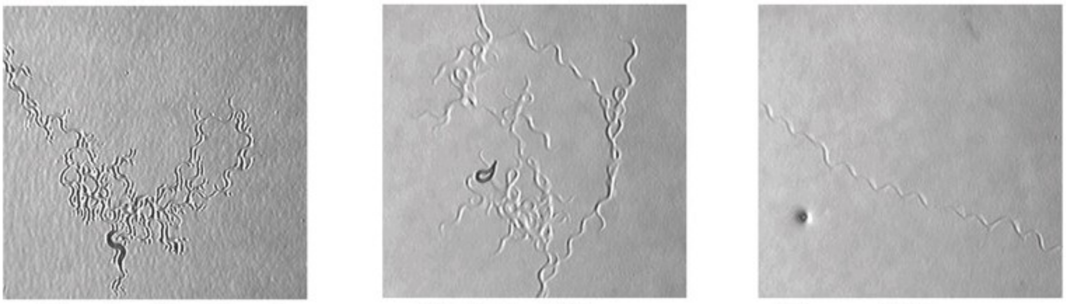

Figure 1.3: Exploratory behaviour: Local search(0min) and Global Search (60min) (Gray, Hill, and Bargmann 2005).

Cell ablation experiments in the past had identified various neurons of sensory, inter and motor layers that regulate local search and global search behavior (Gray, Hill, and Bargmann 2005; Hills 2004; Wakabayashi, Kitagawa, and Shingai 2004). AWC, ASK, ASE, ASI and ADL sensory neurons have a role in controlling local search behavior (Gray, Hill, and Bargmann 2005). Whereas ASI, ASE, ADF and ASH sensory neurons are likely to be involved in the transition to global search behavior (Gray, Hill, and Bargmann 2005). At interneuron layer, AIB interneuron which receives inputs from many sensory neurons gives input to downstream AVA command interneuron to execute reversals in local search, and AIY interneuron suppressed reversals during distant search behavior (Gray, Hill, and Bargmann 2005; Wakabayashi, Kitagawa, and Shingai 2004).

1.5 Research problem

The main objective of this project is to investigate neuromodulation due to neuropeptides that act within the context of neuronal circuitry to control the exploratory behavior of *C. elegans*. Nervous system comprises of neuronal circuits/networks that handle various behavior. These neural networks are subjected to neuromodulators i.e. neuropeptides and bio-amines, which allow circuits to alter their properties rapidly, dynamically and reversibly during their active behaviors (Bargmann 2012). Most of the neuromodulatory inputs that has been involved in neuromodulation are extrasynaptic and difficult to find in the first level of connectome analysis. Thus neuropeptides, which are neuromodulators provide the useful entry points to study how the nervous system controls behavior (Taghert and Nitabach 2012).

First Aim: To decipher the role of NPR-1 and NPR-11 receptors of FLP-18 neuropeptide, during global search behavior of *C. elegans*.

Exploratory behavior is performed in two phase's local search and global search.The switch from local search behavioral state to global search behavioral state is happening through neuromodulation. One of the neuropeptide, FLP-18 is reported to regulate reversal frequency in global search behavior through NPR-4 receptor and not by NPR-5 receptor (see figure 1.4; Cohen et al. 2009). In their study, they have not fully explored this neuromodulation of exploratory behavior.

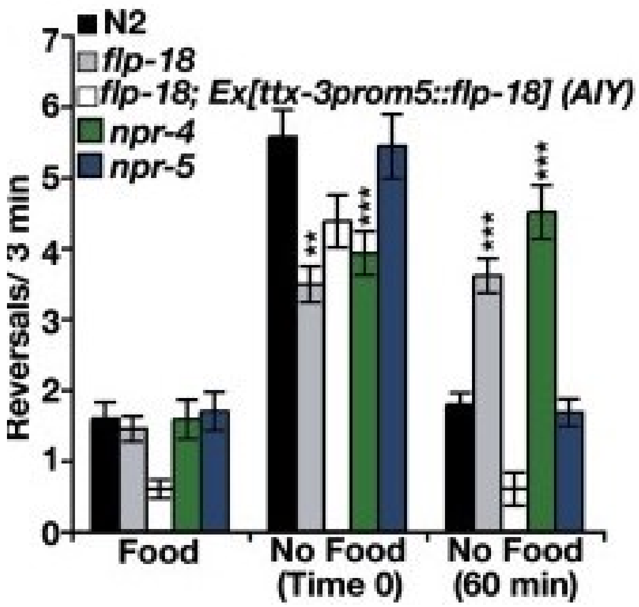

Figure 1.4: FLP-18 regulates reversal frequency in global search behavior (Cohen et al. 2009).

Second Aim: To elucidate the role of FLP-21 neuropeptide whose expression are in many chemosensory sensory neurons during global search behaviour.

Chemosensory neurons are involved in the transition of locomotory states from local search to global search behavior (Wakabayashi, Kitagawa, and Shingai 2004). The *che-2* mutant which is defective in its sensory cilium structure, continued to execute frequent short reversals after one hour of starvation and suggest that the ciliated sensory neurons regulate the locomotory patterns during global search behavior. One such neuropeptide, FLP-21 expresses in the chemosensory neurons and its receptors (NPR-1 and NPR-5) are also presented in the sensory neurons (Peymen et al. 2014).

FLP Neuropeptides	Receptors
FLP-1	NPR-4, NPR-5, NPR-11
FLP-18	NPR-1, NPR-4, NPR-5, NPR-11
FLP-21	NPR-1, NPR-5

Table 1.1: FLP neuropeptides and their receptors (Peymen et al. 2014).

Chapter 2

Material and Methods

2.1 Strains and maintenance

Strain Name	Genotype
N2	Wild Type
RB2126	*flp-1*
VC2016	*flp-18*
RB982	*flp-21*
RB1330	*npr-1*
tm1782	*npr-4*
BAB1501	*npr-1 npr-4*
RB799	*npr-11*

Table 2.1: Strains list.

Strains were obtained from Caenorhabditis Genetics Center. All these strains have been maintained at 20 degree Celsius and cultured as per standard protocol by Brenner (1974).

2.2 Reversal assay

Worms were picked from food plate, transferred to nonfood plate and let it crawl for one minute to remove food from its body. Worms were again moved to nonfood plate and recorded the reversal frequency for first five minutes. Worms were remained on the same nonfood plate for one hour and transferred to another nonfood plate and recorded the reversal frequency for next five minutes.

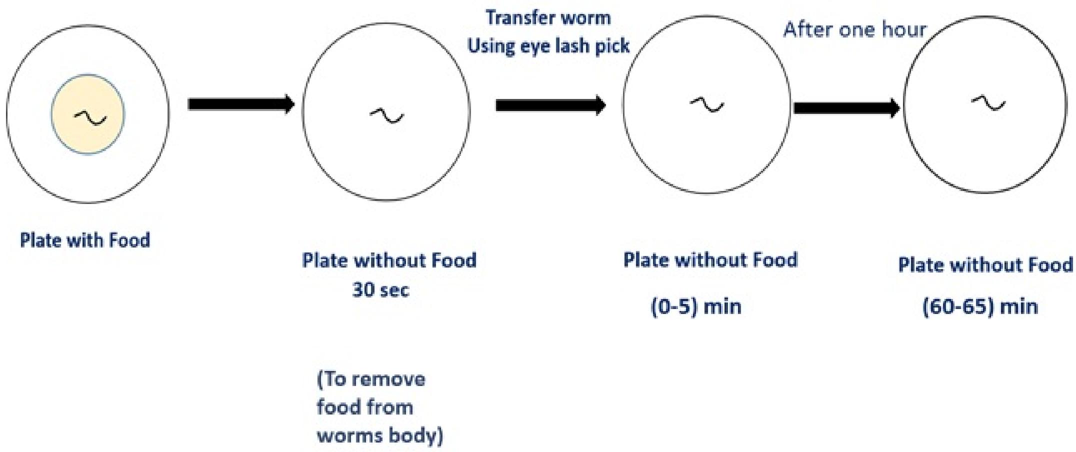

Figure 2.1: Exploratory behaviour: Local search(0min) and Global Search (60min).

2.3 Experimental conditions

For reversal assay, worms were grown at 20 degree Celsius and performed the reversal assay on unseeded (nonfood) 90mm plates at 20 degree Celsius. Reversal assay was recorded using a Zeiss AxioCam MRM camera for first five minutes and after one-hour starvation five minutes. For transferring of worms, eyelash pick was used.

Chapter 3

Results

FLP-18 is regulating the reversals during the global search through the NPR-1 along with NPR-4 receptor but not through the NPR-11 receptor. From the figure 3.1, the reversal frequency of WT, *flp-18* and *npr-4* mutants during initial five minutes and after one hour five minutes is consistent with the past report (Cohen et al. 2009). In our studies, we found *npr-1* mutant shows no significant change in the reversal frequency thus regulating reversal rate during global search behavior. In the case of *npr-11* mutant, they show significant decrease in reversal frequency during global search behavior resembling WT phenotype. Hence FLP-18 is regulating reversals frequency during global search behavior through its NPR-1 and NPR-4 receptor.

Two other neuropeptides FLP-1 and FLP-21, are regulating reversal frequency during global search behavior. From the figure 3.1, the reversal frequency of both *flp-1* and *flp-21* mutants during local and global search remains non-significant. NPR-4 is also a receptor for FLP-1 neuropeptide. Thus the *flp-1;flp18* double mutant, need to be made in future for comparison with *npr-4* mutant for global search behavior. The FLP-21 expression is mostly in the sensory neuron and RMG hub interneuron, and its receptors are NPR-1 and NPR-5. FLP-21 is regulating reversals frequency during global search behavior through its NPR-1 receptor.

The three neuropeptides, FLP-1, FLP-18, and FLP-21 are acting together over the top of the wired neural circuitry via their multiple G protein-coupled receptors present heterogeneously over the whole nervous system. Neurons in the neural circuit responsible for exploratory behavior are subject to neuromodulation by these neuropeptides through their receptors and regulate the reversals during the global search behavior. However, there is also a possibility for neuropeptides other than FLP-1, FLP-18, and FLP-21 to regulate the reversal frequency during global search behavior through their various receptors.

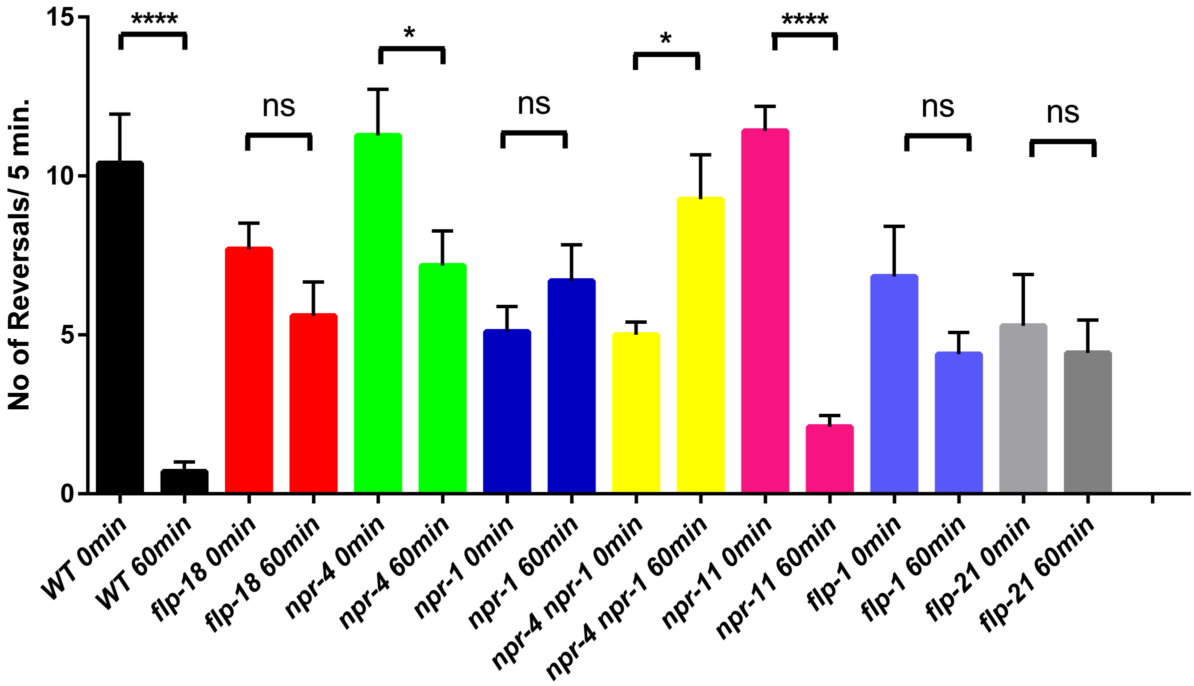

Figure 3.1: Reversal assay for Local search (0min) and Global Search (60min).

Chapter 4

Summary and Conclusions

4.1 Concluding Remarks

The neuropeptides are known for making multilayer network over the top of neural connectome (Bentley et al. 2016). They are changing the output of the neural circuit by neuromodulation. Neuropeptides control the gain of various sensory inputs and thus behavioral features (Taghert and Nitabach 2012). This gain of sensory inputs could be a result of direct activation and inactivation of peptide receptors in sensory neurons themselves but also in interneurons that relay sensory information for further processing. The regulation of behavior by neuropeptides in our studies will explain their relation to sensory inputs and interneuron information processing and motor outputs.

Neuromodulation at the sensory level, regulating reversal frequency during global search behavior. The expression of FLP-21, NPR-1, and NPR-5 are in the sensory layer neurons. FLP-21 is a ligand for both NPR-1 and NPR-5 receptor (Peymen et al. 2014). NPR-1 is inhibitory, and NPR-5 is excitatory kind of GPCR. FLP-21 is silencing the sensory neurons via NPR-1 and activating sensory neurons via NPR-5. As the neuropeptide signaling is wireless, which means the FLP-21 is silencing and activating the neurons more where the FLP-21 is being released and expressing the NPR-1 (negative autoregulatory loop) or NPR-5 (positive autoregulatory loop) receptors. Moreover, NPR-1 is expressing on the hub interneuron named RMG and which is connected via gap junctions to other neurons which are playing an important role in regulating reversal movement (see figure 4.1; Macosko et al. 2009).

Through NPR-1, the RMG neuron is shutting down itself and other neuronal activity through gap junction. The result is downstream AVA command interneuron will not receive sufficient inputs to give reversals.

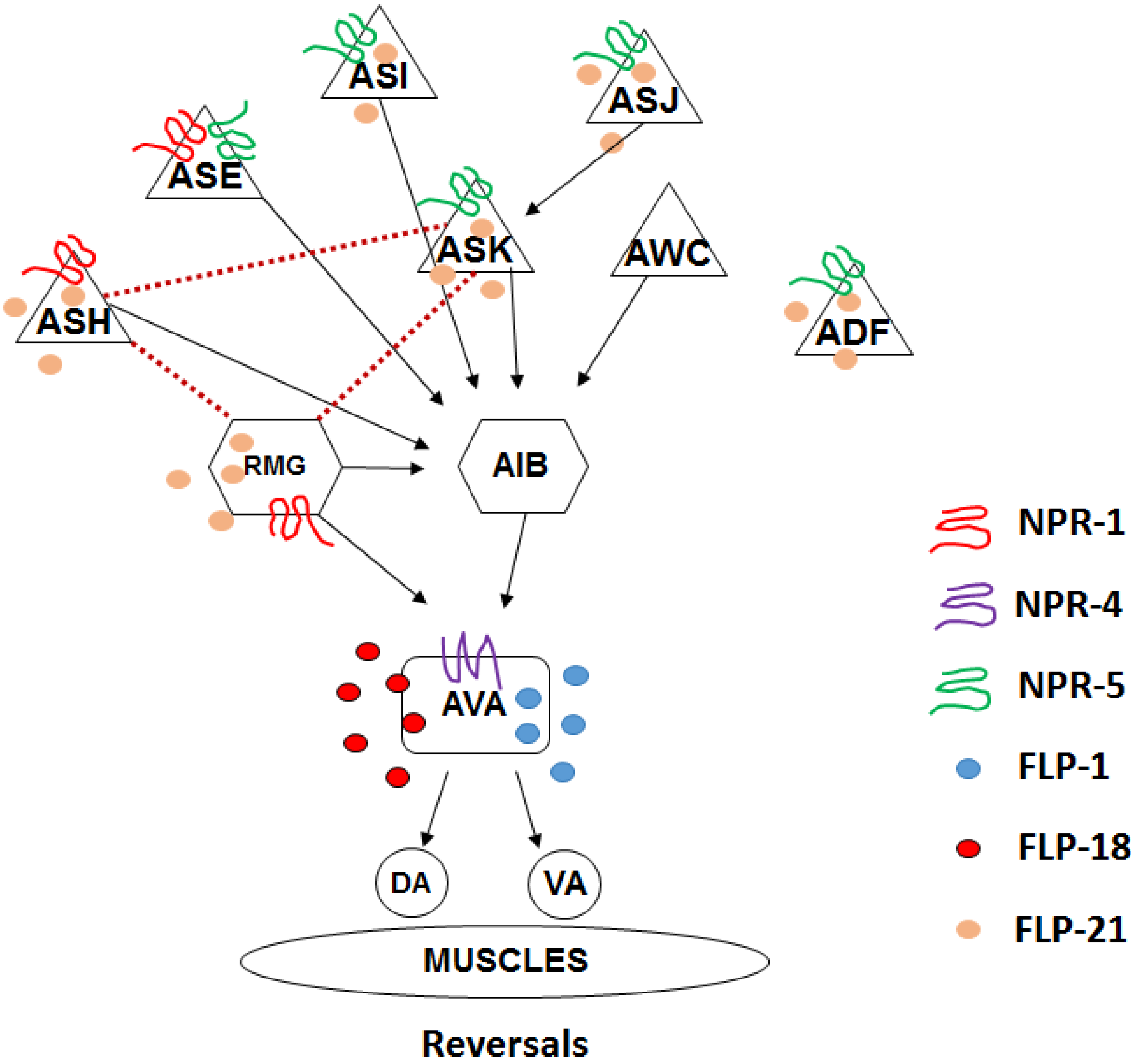

Figure 4.1: Peptidergic Modulation of Exploratory Behavior

Neuromodulation at command interneuron level, regulating reversal frequency during global search behavior. AVA command interneuron releases both FLP-1 and FLP -18 neuropeptides. NPR-4 is an inhibitory kind of GPCR receptor for FLP-1 and FLP-18 neuropeptides, and present on the AVA which is known for controlling the reversal movements in the worms (Frooninckx et al. 2012). Both FLP-1 and FLP-18 are making a negative autoregulatory loop through NPR-4 over

AVA. Thus regulating the reversal frequency by silencing the activity of AVA during global search behavior.

This study has started to give us an idea of the modulation of exploratory behavior through neuropeptides and what are the consequences of such modulation to behavioral features.

4.2 Future outlook

Reversal assay using *flp-18;flp-1* double mutant.

Rescue experiments specifically for NPR-1 receptor in sensory neurons.

1 Psra-6::NPR-1 ASH neuron Specific Rescue.

2 Pgpa-4::NPR-1 ASI neuron Specific Rescue.

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