TYRAMINE DERIVED MOLECULAR PATHWAYS INVOLVED IN DEVELOPMENT OF COMPLEX BEHAVIOUR

AMAL WILSON VARGHESE

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A THESIS SUBMITTED BY

AMAL WILSON VARGHESE

TO

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

2025

DECLARATION

CERTIFICATE

I, Amal Wilson Varghese hereby certify that I had personally carried out the work depicted in the thesis titled, **"Tyramine derived molecular pathways involved in development of complex behaviour"**

No part of this thesis has been submitted for the award of any other degree or diploma prior to this date.

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Amal Wilson Varghese

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The thesis entitled, "**Tyramine derived molecular pathways involved in development of complex behaviour**" was carried out under my direct supervision. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

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LIST OF ABBREVIATIONS

S No	Abbreviation	Expansion
1	^{1}H NMR	Proton nuclear magnetic resonance
2	5-HT	Serotonin
3	5HT3Rs	Serotonin type 3 receptors
4	AADC	L-amino acid decarboxylase
5	ADHD	Attention deficit hyperactivity disorder
6	AgRP	Agouti-related protein
7	Benz	Benzaldehyde
8	BLA	Basolateral amygdala
9	BMA	Basomedial amygdala
10	But	Butanone
11	*C.elegans*	*Caenorhabditis elegans*
12	cAMP	Cyclic AMP
13	CDs	Cyclodextrins
14	CD-TA	Cyclodextrin-tyramine
15	CEA	Central amygdala
16	CH	Cluster headache
17	CI	Chemotaxis index
18	CNS	Central Nervous system
19	CO_2	Carbon dioxide
20	CS-US	Conditional stimulus-Unconditional stimulus
21	DA	Dopamine
22	DRG	Dorsal root ganglia
23	FT-IR	Fourier transform infrared
24	GPCR	G protein coupled receptors
25	IA	Isoamyl alcohol
26	kg	Kilogram
27	LA	Lateral amygdala
28	LB	Luria-Bertani medium
29	L-DOPA	L-3,4-dihydroxyphenylalanine
30	lgc-55	Ligand gated chloride channel-55
31	LGIC	Ligand gated ion channel
32	LTAM	Long term associative memory
33	LTP	Long-term potentiation
34	MAO	Monoamine oxidase

35	MAO-B/A	Monoamine oxidase-B/A
36	MAOI/MAOIs	Monoamine oxidase Inhbitor/s
37	MEA	Medial amygdala
38	mM	Milli molar
39	nAChRs	Nicotinic acetylcholine receptors
40	NaCl	Sodium chloride
41	NGM	Nematode growth media
42	STAM	Short term associative memory
43	NMDAR	NMDA receptor
44	O.D	Optical density
45	OARs	Octopamine receptors
46	OCT/OA	Octopamine
47	OP50	E.coli starin OP50
48	PCR	Polymerase chain reaction
49	PD	Parkinson's disease
50	PEA	Phenethylamine
51	RNA	Ribonucleic acid
52	RNAi	RNA interference
53	ROS	Reactive oxygen species
54	RT-PCR	Reverse transcription-polymerase chain reaction
55	SBP	Systolic blood pressure
56	SEM	Standard error of mean
57	STAM	Short term associative memory
58	TA	Trace amine
59	TAAR	Trace amine-associated receptor
60	TA’s	Trace amines
61	TBH	Tyramine β-hydroxylase
62	tbh-1	Tyramine beta hydroxylase-1
63	TCP	Tranylcypromine
64	tdc-1	Tyrosine decarboxylase-1
65	TDH	Tetrahydropapaverolinet
66	TYR	Tyramine
67	TYRs	Tyramine receptors
68	UV	Ultra violet
69	VGCC	Voltage-gated calcium channel
70	Vis	Visible
71	α-CD	A-Cyclodextrin
72	β-CD	B- Cyclodextrin

SYNOPSIS

The nematode *Caenohabditis elegans* perceive information about its environment by receiving a variety of external stimuli, allowing the animal to distinguish between cues such as the presence of predators, food, temperature, and so on. These stimuli can cause important behavioural changes in the animal. *C. elegans'* chemosensory network, which is made up of different sensory neurons, detects conflicting signals of repellents or attractants and initiates a behavioural response. *C. elegans* frequently have to deal with changing environmental conditions over long timescales, and a sudden change in its surroundings, such as a threat or unfavourable conditions, necessitates a subtle change in their activity and locomotion. An important foraging decision is frequently a choice between the exploitation of existing resources and the exploration of alternative options that may provide new resources. Chemosensory neurons in the local search circuit control acute reorientations by activating excitatory and inhibitory ionotropic receptors on downstream interneurons.

In animals, monoamines mediate a wide range of physiological and homeostatic functions. The most recent findings show that biogenic amines bind to G-protein coupled receptors and ligand-specific channels directly, thereby inhibiting or activating downstream cascades. The receptors that are specific to biologically active trace amine interactions are called Trace amine-associated receptors (TAARs). The processes through which trace amines activate TAARs are still not clearly determined. The endogenous trace amine concentration found in tissue and/or bodily fluids of vertebrates is at concentrations of 500 nM, and at these levels, it can interact specifically with one or more TAARs. Tyramine (TA), one of the precursors for monoamines like dopamine and serotonin, has been shown to act as a neurotransmitter

and interact with TAARs. TA can be found in alcoholic beverages (wine, beer, etc.) and fermented foods. Changes in the concentrations of monoamine neurotransmitters within the brain have been linked to changes in mental processes, movement disorders, and certain neuropsychiatric diseases. Tyramine has been linked to catecholamine release. The molecular mechanisms underlying the tyramine pathway-derived behavioural change, however, remain unknown.

The neuronal pathways that translate sensory information into specific behaviours are not well understood. In *C.elegans*, the monoamine TA is thought to mediate escape response. Escape behaviours improve the chances of prey surviving a predator encounter and have been extensively studied from ethological, neurophysiological, and behavioural genetic standpoints. Many animal species use escape responses to avoid predation, which consists of rapid movements in response to threats. Tyramine is secreted by RIM neurons in *C. elegans*. Tyramine regulates the initial reversal phase of the escape response in *C. elegans* by activating a fast-acting ion channel like LGC-55, which is thought to inhibit or arrest the head muscles, and the later turning phase is facilitated by activating a G-protein coupled receptor (GPCR) like SER-2. Tyraminergic neurons expressing SER-2 GPCR receptors in *C. elegans* could act as a neuromodulator, facilitating the omega-turn-associated escape response. Other tyramine receptors, TYRA-2 and TYRA-3 may act to inhibit aversive behaviour in risk-reward situations. This mechanism might be essential in establishing behavioural modifications and short-lived neuronal alterations in the animals.

HYPOTHESIS

Complex behaviours of organisms are thought to be the result of dynamic sensory input. Adrenaline, like monoamines, can play an active role in altering

complex behaviours. We hypothesized that neurotransmitters such as tyramine and octopamine may influence the organism's adaptive memory and exploration behaviour. Tyramine receptors located near sensory neurons may play an active role in recognizing and responding to a threat signal, thereby influencing behaviour.

RESULTS

The environment around the animal which supports its growth is often stable over the timescale of its development and reproductive life (days), subtle changes in the environment force the animal to adapt to the environment immediately. Biogenic amines like tyramine and octopamine are known to play an active role in the same. This study explores the role of tyramine in controlling the escape response and migration of the organism. Our findings suggest that monoaminergic molecular modulation in RIM and related sensory neurons facilitate changes in short-term memory formations, and that tyramine-assisted multisensory decision in ASH and AVB can alter preferences based on learning and memory in worms.

We evaluated the behaviour of the worm in growth plates, and it was observed that the worms take normal omega turns with a predictable frequency in their natural environment. In the touch response assay, wildtype N2 took a proper omega turn as threat response behaviour.SER-2 is an essential GPCR receptor for tyramine in *C. elegans*. SER-2 receptors are activated through the extrasynaptic release of tyramine from the RIM neurons. On binding to SER-2 receptors in the VD motor neuron, a GABAnergic neuron, results in the hypercontraction of body wall muscles and thereby facilitating the omega turn. This event may naturally happen in its habitat for exploration and movement of the organism. The SER-2 mutant strain (RB1690 ser-

2(ok2103)), was defective in taking proper omega bends during touch response. It is previously known that the SER-2 mutant had a significantly different escape response where the head-to-tail touch during an omega turn does not occur. Similarly, the TDC-1 enzyme mutant TDC-1 (MT10549 [tdc-1(n3421) II]) worms were observed in normal culture plates and it shows a significantly increased escape angle of (79.8 ±24º) compared to N2 with an escape angle of (18.7 ±5.5º). This confirmed that mutant worms generate defective omega turns and results in an altered trajectory during an escape response (without a head-to-tail touch).

LGC-55 is yet another important Ligand-gated chloride channel found primarily in the anterior region of the worm in close proximity to the nerve ring. LGC-55 is a tyramine-gated chloride channel inhibitory in nature and is found to aid in the head twitching behaviour during an escape response. The omega turn response of the LGC-55 mutant (MT14680 [lgc-55(n4331) V]) was similar to that of the wild type. To further test the role of tyramine in escape response behaviour, the mutant strain lacking tyramine decarboxylase-1 enzyme (TDC-1), an essential part of the tyramine synthesis pathway responsible for the conversion of tyrosine to tyramine in neurons was used. TDC-1(MT10549 [tdc-1(n3421) II]) mutant strain, showed incomplete omega bends without the characteristic head-to-tail touch confirming that tyrosine has a critical role in this behavioural response. Since the TDC-1 mutant strain also lacks octopamine in addition to tyramine, we used a control strain lacking the tyrosine besta hydroxylase-1 (TBH-1), which will have the octopamine synthesis blocked but will have normal tyramine synthesis. The TBH-1 enzyme mutant (MT9455 [tbh-1(n3247) X]) showed proper omega turns with head-to-tail touch and angle of escape being (48±5º) when compared to the wild-type worms (18.7 ±5.5º). The tyramine receptor

mutants TYRA-2 (QW42: tyra-2(tm1815)) and TYRA-3 (VC125: tyra-3(ok325)) did not show the omega turn angle defect in the worms. The double mutant AT255 lacking LGC-55 and SER-2 receptors had an incomplete omega turn and an altered angle of escape in comparison with the wild type.

To elucidate whether tyramine receptor mutation affects exploratory behaviour we designed a quantitative exploratory behaviour assay. The principle of the assay is described by Charnov's marginal value theorem, which proposes that the optimal time for an animal to leave a foraging ground occurs when local resource levels fall below the average level in the entire habitat. We employed the exploration strategy of worms in different zones where a minimum amount of food was placed at equidistant spots so that worms had to migrate and explore taking turns and also will have to rely on olfactory cues to discover the food source. The results showed that the wildtype N2 worms and SER-2 mutant worms explored till the farthest zone 3. Surprisingly the LGC-55 mutant worms did not cross past zone 2; this raised the notion that LGC-55 have a defect in olfactory perception during the chemotaxis.

To verify the olfactory defects, LGC-55 and SER-2 receptor mutant strains were then tested for attraction towards different solvents (Benzaldehyde 1/200, Butanone 1/10, Butanone 1/1000, Isoamyl alcohol 1/300, Isoamyl alcohol 1/10). It was observed that the LGC-55 mutant strain showed higher attraction towards attractant (1/200 Benzaldehyde) and neutral solvents (Butanone 1/10). SER-2 mutant strains showed attraction towards Benzaldehyde 1/200, Butanone 1/10, Butanone 1/1000, Isoamyl alcohol 1/300, and Isoamyl alcohol 1/10 similar to N2 wild-type worms except for the lower attraction for 1/10 butanone. These results suggested that in the

absence of the LGC-55 receptor, there is an impairment in distinguishing solvents and associated behaviour. The double mutant of LGC-55 and SER-2 receptor strain AT255 showed a lower attraction towards all the solvents tested irrespective of them being attractive or repellent, suggesting tyramine receptors have an essential role in altering the solvent attraction and chemotaxis of the worm. The plausible reason for the differential behaviour of SER-2 and LGC-55 receptors could be due to their positioning in the worms. SER-2 receptors are present close to the body wall musculature and aid in the turning behaviour. LGC-55, on the other hand, is present in the head neurons of the worm. The proximity of LGC-55 to the olfactory neurons might have a significant influence.To test the impact of exogenous tyramine (TA) in adaptative olfactory behaviour we first evaluated its impact on the worm's movement. TA above 30mM concentration caused complete immobilization in wildtype N2 strains, while the SER2 and LGC 55 mutant strains displayed resistance to immobilization. We used the tyramine cyclodextrin inclusion complex for the slow release of TA. As expected, the tyrosine cyclodextrin lowered the immobilization risk compared to the direct exposure to TA. On direct exposure of TA, the highest degree of resistance to immobilization was seen in SER-2 than in the LGC-55 mutant strains at 30mM concentration. TYRA-2 and TYRA-3 behaved almost like the wild-type N2. Based on the mobilization pattern of the N2 worms we arrived at the concentration (5 mM) that doesn't affect the migration ability of the worm.

The impact of TA exposure on long-term and short-term adaptive memory was tested. For learning and memory assay a Pavlovian model classical conditioning paradigm was adapted using 1/10 Butanone as the conditional stimulus and food as the unconditional stimulus. In N2 wild type, the presence of 5 mM tyramine doesn't alter

the long-term adaptive memory. When the N2 wild-type strains were subjected to short-term training in the presence of TA, an increased retention of memory at 3rd hour was observed. SER-2 receptor mutant worms, however, showed normal short-term adaptive memory in the presence of TA. The naïve LGC-55 mutant strain showed higher chemotaxis towards 1/10 butanone. and the exogenous addition of TA did not normalize the behaviour similar to the N2 strain suggesting a critical role of LGC-55 in chemotaxis behaviour. TDC-1 and TBH-1 mutant strains showed significant defects in short-term memory formation. The naïve TDC-1 mutant strain, which lacks tyrosine decarboxylase-1 enzyme, showed significantly low attraction towards butanone in the chemotaxis assay($P \leq 0.0001$). In the presence of exogenous TA, there was a recovery in chemotaxis behaviour. The TBH-1 enzyme mutants also showed a similar behaviour pattern to that of TDC-1 with significantly lower attraction towards butanone ($P \leq 0.0001$). These data suggest that the combinatorial effect of both biosynthetic enzymes, TDC-1 and TBH-1, can affect the short-term associative memory in the worms. The recovery in behaviour observed post-addition of exogenous TA substantiate this observation.

The role of the LGC-55 receptor was further elucidated using the mutant strain QW224 (Pmyo-3::LGC-55 anion (zfEx31). LGC55 is an anionic channel allowing chloride ions to pass. In the W224 lgc-55 cationic mutant strain, the M1–M2 loop of the lgc-55 tyramine receptor was replaced; thus ion selectivity of the receptor alters from anions to monovalent cations. Thus modified LGC55 becomes an excitatory channel. Contrary to our expectation, this modified LGC 55 channel mutant strain showed a normal short-term associative memory similar to that of the N2 wild-type strain with normal chemotaxis. One possible explanation could be the downstream

pathways initiated by anionic channels plays a critical role in the olfactory behaviour. To test the effect of tyramine on avoidance response in *C elegans*, a fear response paradigm was made that would mimic the real-world scenario. It is known that a calcium-based necrotic wave is propagated in worms that undergo death by anthranilate fluorescence. We subjected the healthy worms to heat stress to induce the "death fluorescence" and extracted the leachates from these worms. This extract was used as the test the avoidance behaviour of the worms. As a control, normal worm extract without heat stress was used in the assay. The wild-type N2 worms showed immediate reversal on presenting the "death-induced" worm extract. But these sudden reversals were absent in the LGC-55 receptor mutants. SER-2 mutant strains, however, showed a normal reversal pattern similar to N2 wildtype. The double mutant AT255 lacking both LGC55 and SER2 also showed reduced reversal behaviour similar to that of the LGC55 receptor mutant. We then tested the choice-based exploration assay having the "death induced" worm extract on one half and the other half without the worm extract. The worms stayed in the region having no worm extract. These data suggested that the worms preferentially avoided the "death-induced" worm extract and this behaviour is LGC-55 receptor-dependent. These results reiterate that LGC-55 plays an active role in threat recognition.

One of the major components in "death fluorescence" is anthranilic acid. We then wanted to test whether anthranilic acid could induce similar behaviour in the worms. We also found that anthranilic acid at 1% concentration can generate a strong repulsion behaviour in worms. In the migration assay, the worms showed a preferred behaviour of exploring the areas without the anthranilic acid suggesting the avoidance response of the worms towards this cue. To check if LGC-55 is essential for the

recognition of anthranilic acid we tested the mutant strains. LGC-55 mutant worms lacked the ability to recognize the anthranilic acid and did not show repulsive behaviour. However, normal repulsion was observed in the of SER-2 mutant strains, similar to that of the N2 wild type. LGC-55-SER2 double mutant AT255 showed reduced reversal response to the anthranilic acid suggesting that anthranilic acid is the active component in the "death induced" worm extract that was causing the avoidance behaviour. The avoidance response is triggered by TA and its binding to the LGC55 receptor - essential cues to shut down the forward locomotion and triggers the reversal response through fast-acting ligand-gated ion channels.

To further elucidate the role of LGC-55 and SER-2 in the threat response and immediate decision-making, we made use of the fructose ring assay. Fructose barrier crossing could result in desiccating the worms, and usually, worms avoid crossing the barrier if it is well-fed. Starvation could result in risk-taking behaviour in worms. In the assay plate, 1-hour starved worms were placed in the middle of the fructose ring and an attractive solvent was placed outside the ring. Well-fed worms were used as the control.. This assay allows to measure the risk-taking behaviour depending on the internal state of the worm. The wild-type well-fed N2 worms stayed inside the fructose barrier in the presence of an attractive solvent outside the fructose ring. LGC-55 and AT255 mutant strains explored and crossed the fructose ring even under a well-fed stage. SER-2 and TDC-1 and TBH-1 mutant strains, under well-fed state, did not cross the barrier. Multisensory decision-making is modulated by top-down extrasynaptic aminergic signals. And food deprivation suppresses the aminergic feedback pathway to increase threat tolerance, the absence of LGC-55 could affect the risk-reward pathway mediated by LGC-55. TDC-1 and TBH-1 not crossing the barrier might be

due to the influence of octopamine which is absent in the system when the enzyme mutation is in place and they seem to have migratory defects in other assays due to the mutation.

Significance of this study

Understanding how the nervous system facilitates the survival of an organism in the natural environment is fascinating. This study shows that the Tyraminergic neurons are involved in risk-taking behaviour. With the aid of GPCR and fast-acting Ligand-gated adrenaline-like channels release the neurotransmitter tyramine from the master control neuron RIM.

Our results indicate that TA which was initially thought as the precursor for dopamine in the system could act as a neurotransmitter with critical function in lower-order organisms. The TAergic neurons are critical in learning and memory and the connectome associated with these neurons is critical in memory recall. LGC-55 is a ligand-gated channel of tyramine, which is a fast-acting channel for the inhibition of neuronal activity. LGC-55 and SER-2 activation can influence and reconfigure the output of neural circuits and orchestrate complex behaviours we show that the LGC-55 receptor is important for animals for initiating a complex behaviour. LGC-55 was also found to be essential in adaptive memory formation and foraging in the worms. Any irregularities in LGC-55 receptor signalling can influence decision-making, olfaction and adaptive memory formation in animals. Several theoretical studies have suggested that the sinusoidal body movements underlying nematode locomotion might not require a central pattern generator circuit but instead, be generated exclusively by motor neuron sensory feedback. Based on our data on LGC-55 and SER-2, it is evident that TA influences the learning and migration in C. *elegans*. This assists the overall

survival of the organism. It is also evident that the LGC-55 ligand-gated chloride channel acts through inhibition of the neurons in the head region to influence decision-making and migration. The absence of tyramine by enzymatic mutants has an immediate impact on the learning pathway and migration. Our study using various mutations and chemotaxis approaches in *C. elegans* shows the role of TA in learning and memory. Thus, indicates that TA neurons play a critical role in the effective processing of cognitive function. Further understanding of these connectomes and their active processing of information will hopefully enrich our understanding of the intrinsic relations between memory, and decision-making at cellular levels.

1. INTRODUCTION

Complex behaviours refer to the capability of an organism to integrate the information and to function in a coordinated manner in a crucial situation that demands the survival of the organism (Pessoa et al., 2021). General animal behaviour includes the ways the organism interacts with its immediate physical environment. A subtle change in the behaviour of an organism can be attributed to a response to a stimulus, an external or internal cue or a combination of cues. Complex behaviours in animals include courtship displays, feeding patterns, nest building, parental care, singing, territoriality and aggression, construction of webs by spiders, migration, foraging, communication, living and hunting in groups, tool use, and nest parasitism (Pessoa et al., 2021). There is a good understanding of how neuronal connectomes and their associated molecular mechanisms influence an organism's behaviour.

Among animals, some behaviours are innate, or genetically hardwired, while some behaviours are learned, or developed through experience. Complex escape response behaviours are intricate and involve assessing the environment, being alert, and avoiding risks, as they require the coordination of neural circuits for movement and exploration. Exposure to stress can lead to behavioural disorders. Homeostatic mechanisms are present in organisms ranging from simple invertebrates to humans. These mechanisms help to keep the functioning of neural circuits within a specific range, regulating the excitability of neurons and synapses (Pirri et al., 2015a). Evolutionary biologists explain the structure and function of the present-day organism

as the product of evolution. For example, in birds feathers are thought to be evolved first as thermal insulation and later on aiding in flight (Ostrom, 1974). The adapted forelimbs in tree animals helped in leaping from branches. Similarly, behaviours are studied in the context of survival value, where a behaviour might have evolved to aid the survival features of an organism(Fujiwara et al., 2002a; Homberger et al., 2021).

Aggression and escape response are the two major factors that contribute to the defensive behaviour in organisms. When an organism perceives danger, it responds with specific behaviours and physiological changes to protect itself. The neural pathways responsible for these quick defensive reactions during stress have been extensively researched (Maguire et al., 2011)**.** These behaviours are often regarded as behavioural adaptations that help in survival and mating. Defensive behaviours are extensively studied to comprehend how an organism focuses its attention on threats or external signals that might cause harm to the organism itself or its group. The threat comprehension, succeeding information processing and preparedness for escape are common responses that require a complex setup of decision-making and execution. Relatively few natural behavioural variations have been mapped to the single-gene level in any animal (Bendesky et al., 2011). Studies focused mainly on the impact of heritable genetic factors on behaviour, excluding the number or complexity of the genes involved or the influence of environmental factors.

Notably, defensive behaviours could be created and manipulated in model systems by probing the organism and studying the molecular basis of these behaviours. Behaviour is shaped by natural selection and many behaviours are found to directly increase an organism's fitness, thereby helping in its survival and reproduction. The

molecular basis of this ubiquitous behavioural feature is poorly understood. All organisms have innate behavioural and physiological mechanisms that promote survival. For these mechanisms to be most effective, they must be adaptable and appropriately flexible to account for changes in the environment (Albeg et al., 2011; Füzesi et al., 2016). Escape responses as a mechanism to evade threats can be observed in many animal species (Eaton, 1984). Sequential motor patterns of specific behaviours have been described under these conditions. Studies suggest that there exists cross-species conservation, not only of individual defensive behaviours but also for the patterning of defensive behaviours in response to relevant combinations of threat and expediting stimuli. These behavioural analyses suggest there is an overlapping similarities between nonhuman mammals and humans (Blanchard et al., 2011).

Caenorhabditis elegans is an excellent model system in the field of neurobiology because many characteristics of neuronal function, such as sensory transduction, synaptic plasticity, and G-protein-mediated neuromodulation, are conserved and comparable to vertebrates. Thus the study in model organisms like *C.elegans* will help researchers elucidate the linkages that exist between the large-scale neural circuits which span the complex, naturalistic behaviours and the dynamic changes neuronal circuits could lead to the temporal evolution of behaviours (Pessoa et al., 2021). Recent advancements in neuroimaging and RNA profiling, have made it possible to achieve a better understanding of *C.elegans* behaviours at the molecular and cellular levels. The ability to link behavioural changes to the activity of specific neurons will allow for the use of novel genetic analysis techniques in *C.elegans* to ascertain new components and understand their functions at the molecular level. The

C.elegans nervous system shares many functions with the human brain and is composed of similar neurotransmitters. It is possible that the computational logic used by the *C.elegans* nervous system in sensory integration, decision-making, and learning also has similarities with more complex decision-making in the mammalian brain (Laurent et al., 2015; Sasakura et al., 2013; Schafer, 2005). Elucidating the molecular basis of behaviours in organisms is critical to understanding the basis of complex behaviours.

2. REVIEW OF LITERATURE

2.1 Complex Behaviours

Complex behaviour in animals refers to a comprehensive range of actions that are more advanced than simple reflexes or fixed action patterns. Some examples of complex behaviours in animals include courtship displays, feeding patterns, nest building, parental care, singing, territoriality, aggression etc(Pessoa et al., 2021). Evidence of complex behaviours was traced from free-living ciliates (Figure 2.1) to humans. Complex behaviours are governed by intricate interactions between numerous neural circuits functioning collectively. In humans, complex behaviours are difficult to study due to the complexity of the nervous system.

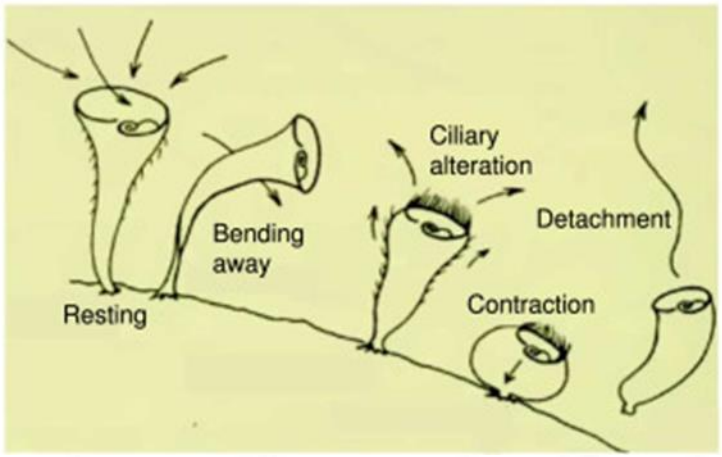


Figure 2.1: Sketch depicting the avoidance behaviour exhibited in *S. roeseli* *(Dexter et al., 2019; Tartar, 1961)*

Complex behaviours in animals require a range of cognitive abilities, including the ability to recognize individuals and learn through observation (Fernald, 2017). For these social systems to develop, these skills must be passed down either genetically or culturally and supported by the evolution of the brain and brain structures. Since animals have diverse skill sets, it is more appropriate to describe their cognitive

behaviours as a social calculus that can change and adapt with experience (Fernald, 2017). A thorough examination of the vertebrate brain reveals that its structure facilitates a high degree of signal communication and integration, particularly in birds and mammals. The central nervous system has been shaped by evolutionary forces to enhance survival (Pessoa et al., 2021). In humans, disruptions in the ability to engage in prosocial interactions can lead to uncoordinated brain activity and are a common characteristic of several disorders, including autism spectrum disorder, depression, and addiction which are earmarked to exhibit complex behaviours in humans (Walsh et al., 2021).

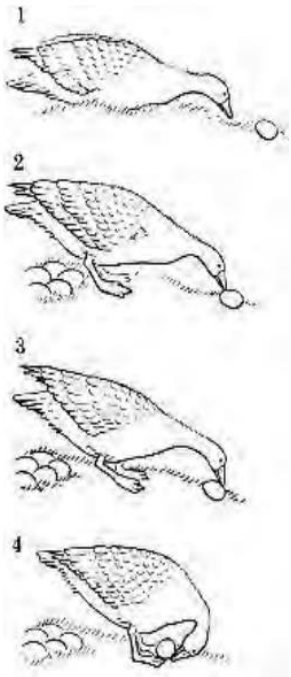


Figure *2*.2: The grey lag goose displaying the egg retrieval behaviour (Prevett and Prevett, 1973)

The existence and survival of higher organisms depend on complex brain functions such as perception, cognition, emotion, attention, memory, and decision-making. The vertebrate brain's architecture facilitates extensive signal communication and integration. The structure of the vertebrate brain provides computational

flexibility, enabling animals to adapt to complex and ever-changing environments (Pessoa et al., 2021). The earlier accounts of complex behaviour were sketched by Jenning in *S. roeseli*. In all organisms, the perceived threat is known to trigger very specific behavioural and physiological responses that ensure overall survival (Füzesi et al., 2016).

The complex behaviours are accounted for in birds in the classical observation known as the "grey lag goose egg retrieval study" where the grey lag goose is explored for their egg retrieval behaviour(Prevett and Prevett, 1973). The mother greylag goose is found to perform a specific sequence of actions to retrieve an egg that has rolled out of her nest (Figure 2.2). This characteristic action follows a fixed action pattern. The sight of the lost egg is known to trigger the behaviour, causing the mother goose to extend the neck towards the egg, stabilise it with the bill, and then pull it back towards the nest. Once this behaviour has been initiated, the mother goose will complete the entire sequence even if the egg is removed or has lost her grip during the process (Prevett and Prevett, 1973; Yao et al., 2019). This behaviour can be attributed to the motor pattern behaviours that are conserved among the species.

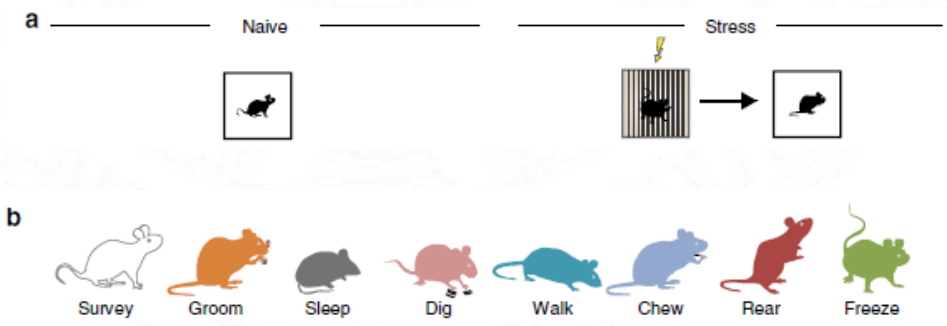


Figure 2.3: Pictorial representation of distinct behavioural patterns exhibited in mice following stress*(Füzesi et al., 2016)*

In mice, a group of cells called PVN CRH cells are known to be involved in a circuit that helps the animal adapt its behaviour to the environmental context after experiencing stress (Figure 2.3). If this network becomes overactive when there is no stress present, it may lead to the animal exhibiting behaviours context-inappropriate behavioural strategies(Füzesi et al., 2016). On observations conducted by monitoring the neurons present in the Dorsal root ganglia (DRG) of live mice, it was found to have coupled firing of DRG neurons in response to sensory neurons when triggered by pain, which was induced by pinching of their paw or injecting a drug that could inflict inflammation like responses, or exposing the animals to heat than their normal ambient temperatures this characteristic firing is defined as called coupled activation.

Figure 2.4: Timeline of events during the escape behaviour of fish and mice respectively *(Evans et al., 2019)*

Fish have two Mauthner cells, or M-cells, in their spinal cords that act as command neurons, triggering a startle reflex on the opposite side of the body in response to unexpected stimuli or to avoid predator attacks (Figure 2.4). These cells are organized like miniature brains, with all the basic mechanisms of synaptic

transmission and functional decision-making (Korn, 1988). This raises the question of whether the orchestrated behaviour towards escape could be less sophisticated in all organisms.

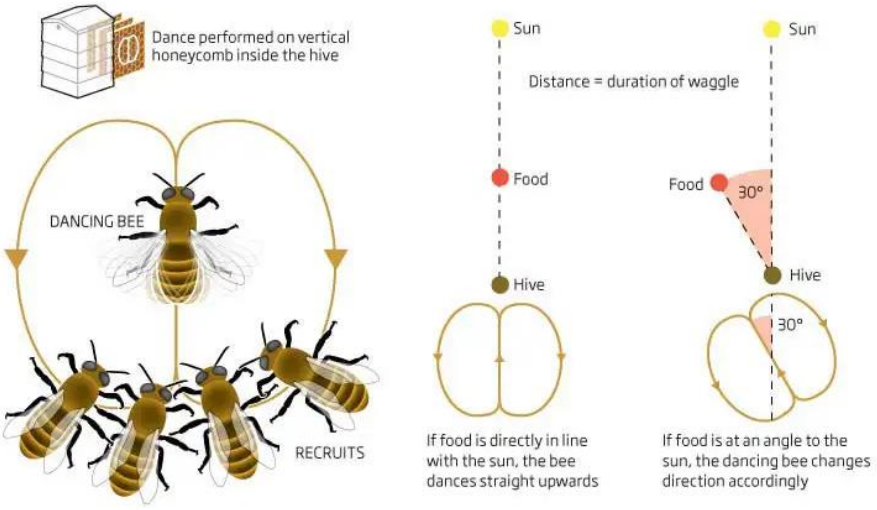


Figure 2.5: Dance display in honey bees namely waggle dance and round dance behaviours*(Frisch, 2014)*

In the honey bee's system of navigation, communication and social organization are extensively studied(Scheiner et al., 2013). The earlier accounts of complex behavioural studies include the classical studies on honey bees because the bees mainly rely on the sun as a reference point for navigation. A complex behavioural display in the form of dance is exhibited to present the direction of food sources concerning the sun. This special type of dance in the hive allows other bees to locate the position of the food. The dance is identified as a waggle and a round dance, respectively (Figure 2.5). The direction of the flight concerning the sun, considering the flow of wind, is critical for navigation and return, which aids the survival of the colony. Dr Karl von Frisch won the Nobel Prize in Physiology or Medicine in the year 1973 for elucidating this complex behaviour of honey bees(Frisch, 2014).

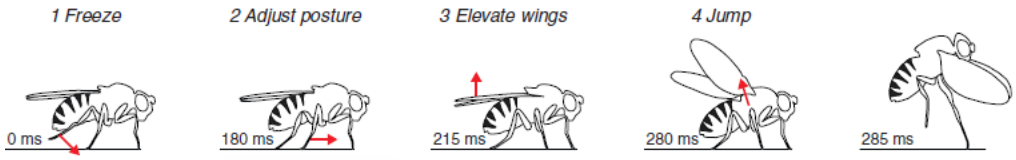


Figure 2.6: Stages of escape behaviour in *Drosophila melanogaster* *(Card, 2012)*

The Drosophila model has been studied to analyse the complex behaviours because insect flights are one of the fastest, most intense and most energy-demanding motor behaviours (Brembs et al., 2007). Despite their brief duration, insect escape behaviours are more complex than previously thought(Figure 2.6). Before jumping to escape a threat, flies perform sophisticated preparatory leg movements that depend on their posture and the stimulus, allowing them to jump away from danger. This flexibility raises questions about how the nervous system can induce a quick and adaptable behavioural response (Card, 2012). Insect flight is modulated on multiple levels by the biogenic amine octopamine. Within the nervous system, octopamine acts directly on the flight central pattern generator, and it affects motivational states (De Rosa et al., 2019). Examining insect escape behaviours in a natural context has exposed more behavioural and neural complexity than previously appreciated in insect escape circuits. While the sequential motor patterns related to certain behaviours have been thoroughly documented, the neural mechanisms responsible for orchestrating a complete behavioural sequence in an organism are not as well understood (Donnelly et al., 2013a). The need to switch between exploratory and defensive behaviour is hardwired in organisms for their survival. However, how this transition is achieved through the complexity of interacting neuronal circuits is not known (Anglada-Figueroa, 2005). The genetic and experimental analyses associated with these

behaviours could offer the real possibility of gaining a cellular and molecular-based understanding of complex behaviour (Bono and Maricq, 2005).

2.1.1 Fight or Flight response

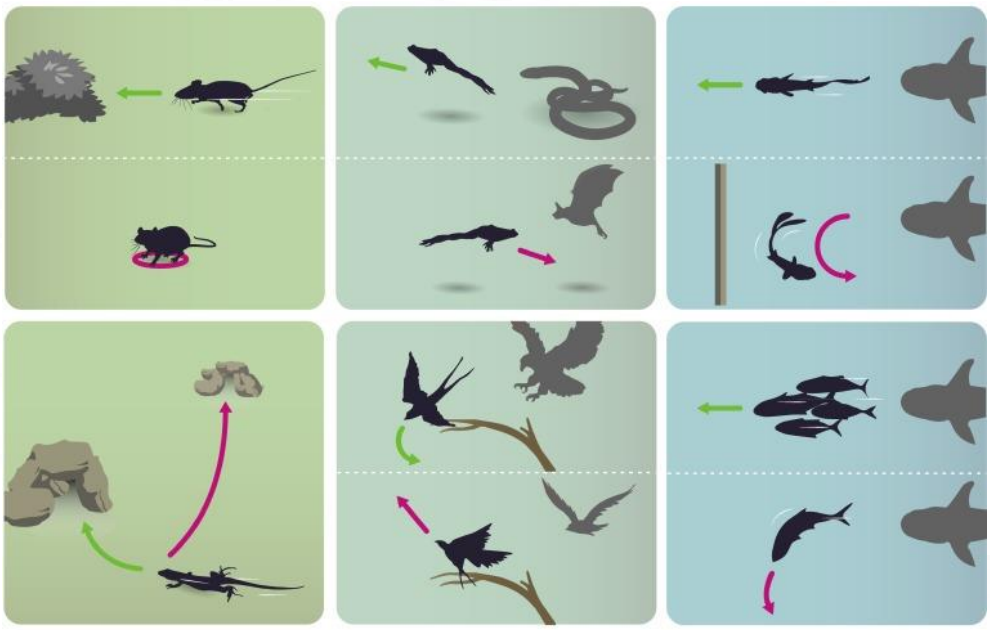


Figure 2.7: Classical escape behaviours displayed across species *(Evans et al., 2019)*

In higher organisms like mammals, displacement activities are common in stressful or conflicting situations where multiple behaviours are simultaneously expressed (Troisi, 2002). To successfully propagate and survive, animals must resolve environmental problems, including dealing with threats such as predators (Fanselow and Lester, 1988). Risk assessment is an important process that helps animals detect and analyze threats and choose the best defence strategy (Blanchard et al., 2011). This process accounts for various factors such as the type and locality of threat and characteristics of the situation, including the presence of escape routes or hiding places. By considering these factors, animals are known to predict which specific defences are effective in minimizing the danger posed by the threat. The directions of escape opted by prey are extensively studied across species (Domenici et al., 2009).

There are studies deducing the escape circuits for fishes, flies, reptiles, vertebrates and nematodes (Figure 2.7) (Gray et al., 2005).

Generally, the natural defensive processing can be broken down into three main stages: Primary being the pre-encounter, followed by post-encounter, and finally the circa-strike. Initially, during the pre-encounter stage, the animal evaluates the likelihood of encountering a predator and adjusts its behaviour based on this assessment. During the post-encounter stage, the animal's behaviour often changes significantly as it assesses the situation. In the circa-strike stage, the animal may choose to either flee or fight, depending on what is most appropriate for the situation(Fanselow and Lester, 1988).

The principal neuronal circuitry which is accountable for the quick defensive behaviours during stress encounters has been studied in different organisms. However, there are very little is known about the behaviours exhibited after the onset of any stressful event (Füzesi et al., 2016). It is understood that fear circuits are regulated at various levels, including structural and functional changes related to learning experiences (Luchkina and Bolshakov, 2019). The capacity to remember cues associated with danger and respond to them by exhibiting escape or avoidance behaviours is crucial and often acts as a life-saving mechanism that has the potential to be studied experimentally (Luchkina and Bolshakov, 2019). When a threat is detected, animals may respond with escape behaviours. The response can depend on various internal and external factors such as the level of risk, hunger, fatigue, bodily health, sexual arousal, distance, and predator behaviour. The animal may continue its ongoing activity while maintaining a safe distance from the predator if the risk is low.

If the risk is higher, the animal may respond with escape behaviours that are appropriate for the situation. The escape process involves determining an adequate route and selecting an appropriate refuge. In summary, an animal's response to a threat is complex and depends on various factors. (Pessoa et al., 2021). The threat-associated cues are studied extensively due to the ease of generating such conditions in experimental organisms.

Interestingly, the model organism *Caenorhabditis elegans* is found to have inducible escape response behaviours, which fall under the complex behaviours in animals(Donnelly et al., 2013a). This considerably throws light on conserved defence mechanisms against pathogens along the developmental evolution(Mallo et al., 2002). As worms are always prone to natural predation and an unfavourable change in their habitat which demands rapid responses of fight or flight.

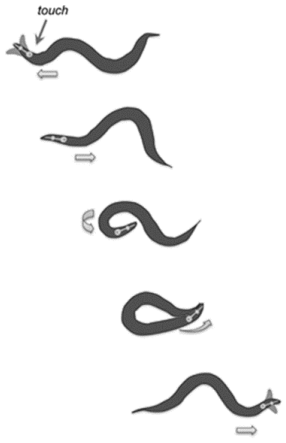


Figure 2.8: The escape response behaviour displayed by *C.elegans* *(Donnelly et al., 2013a)*

C.elegans is known to take an omega turn primarily when the anterior end of *C.elegans*' body is gently touched or when receiving any repulsive signals. These signals trigger an escape response where the worm quickly moves backwards and stops exploratory head movements followed by an omega turn (Figure 2.8) (Clark, 2014;

Pirri and Alkema, 2012a). The sharp omega turn during which the worms's body creates a peculiar shape which resembles the Greek letter Ω so the peculiar turn is termed the omega turn (Gray et al., 2005). There is limited research available on the predators of *C.elegans*. Based on observations in the wild and experiments conducted in the laboratory, potential natural predators of *C.elegans* may include small arthropods like mites or springtails, other nematodes such as Pristionchus spp., and nematode-trapping fungi (Frezal and Felix, 2015). However, it is to be noted that the relationship between the nematode and its prey is not one-sided: as *C.elegans* adults age, they can become food for the same microorganisms on which they fed and lived (Frezal and Felix, 2015).

2.2 C.elegans as a model

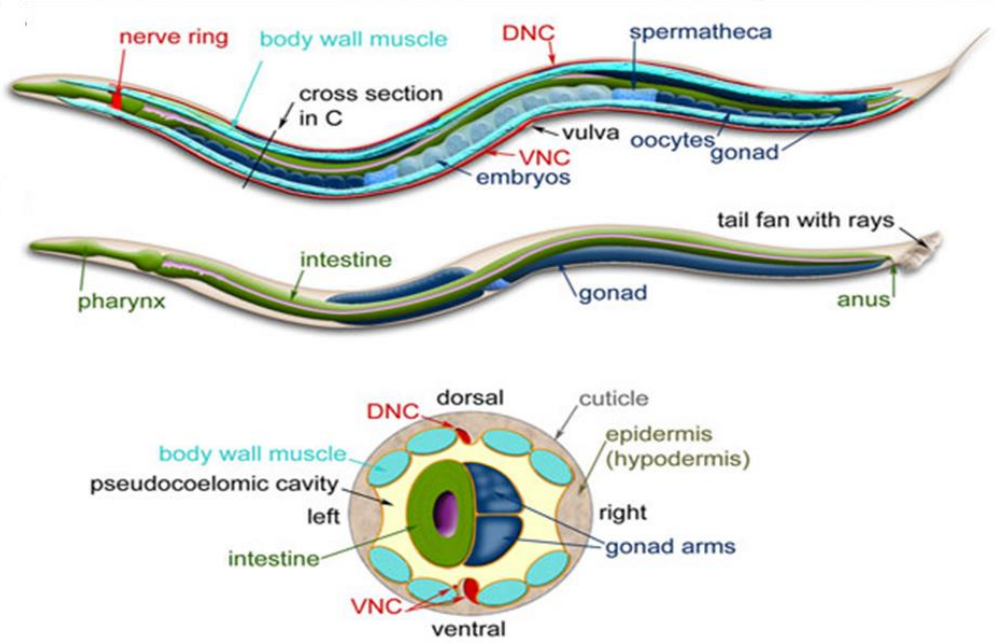


Figure 2.9: *C.elegans* hermaphrodite and male, along with the body wall cross-section of the worm *(Goodman, 2006)*

C.elegans is a soil-dwelling small nematode found globally, mostly in humid temperate regions (Frezal and Felix, 2015).*C.elegans* was first described in the

20th century as *Rhabditis elegans* by biologist Emile Maupas near the city of Algier (Maupas, 1900). Honda et.al isolated and identified the hermaphrodites in *C.elegans* and identified the existence of 6 pairs of chromosomes in the nematode (Honda, 1925). The nematode was later studied around the 1940s and improved upon by Victor Nigon and Ellsworth Dougherty(Fatt and Dougherty, 1963) by studying the culturing methods necessary to rear the worms in a controlled environment for the laboratory (Frezal and Felix, 2015). Eric Kandel and Seymour popularised the usage of invertebrates in neurobiology (Castellucci et al., 1970; Quinn et al., 1974). Notable progress in *C.elegans* biology came after Sydney Brenner's work was published in the 1970's. The *C.elegans* was slowly established as a model system. *C.elegans* is widely used in research due to its many benefits (Brenner, 1974). These nematodes are known to feed on bacteria and other microbes (Bono and Maricq, 2005). *C.elegans* has two distinct sexes: males and self-fertilizing hermaphrodites (Figure 2.9). Most behavioural research has focused on the hermaphrodite, except studies on mating (Bono and Maricq, 2005). This nematode can be easily grown in large populations, with several hundred worms able to live on a single agar plate containing a suitable growth medium and OP50 as a food source. OP50 strain is a uracil auxotroph organism that requires uracil, and its absence on the plate prevents bacterial overgrowth that would otherwise obscure the worms and enables the ease of observation (Brenner, 1974)

A

T	Time (hours after hatch)				
	L1/L2 molt	L2/L3 molt	L3/L4 molt	L4/Adult molt	Egg-laying
16ºC	24	38	55	74	94
20ºC	15	24	34	48	58
25ºC	11	18	26	35	45

B

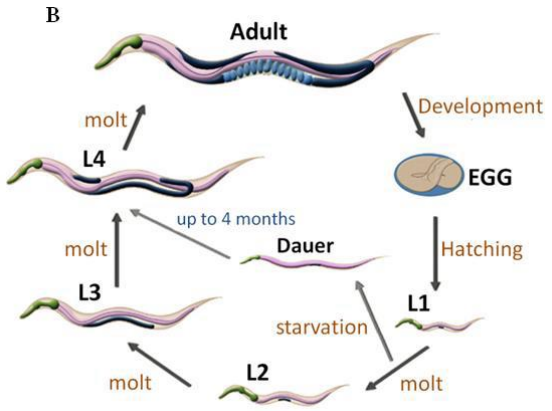


Figure 2.10: *C.elegans* life cycle and time for development at various temperatures after hatch

C.elegans has a unique reproductive mode called androdioecy, in which it can reproduce either through self-fertilizing hermaphrodites (XX) or through mating between hermaphrodites and males (XY). Hermaphrodites develop anatomy and produce a limited number of sperm cells early in adulthood. Males can develop due to the non-disjunction of X chromosomes during meiosis or from the offspring of male-hermaphrodite crosses. The frequency of X chromosome non-disjunction is low and varies with genotype and environment(Frezal and Felix, 2015). *C.elegans'* life cycle consists of four larval stages before reaching the adult stage, and temperature can have a significant effect on the growth of the nematode (Figure 2.10). Hermaphrodites of *C.elegans* have 959 somatic nuclei, while males have 1031. In hermaphrodites, the nervous system is made up of 302 neurons and 56 glial and support cells, while in males, there are 381 neurons and 92 glial and support cells. Approximately half of the neuronal cell bodies are located in the head, encircling a central neuropil known as the nerve ring, with the remainder distributed along the ventral cord and in the tail ganglia. Male-specific neurons are primarily found in the copulatory tail. In both sexes, each neuron can be distinguished by its unique position and morphology (Bono and Maricq, 2005).

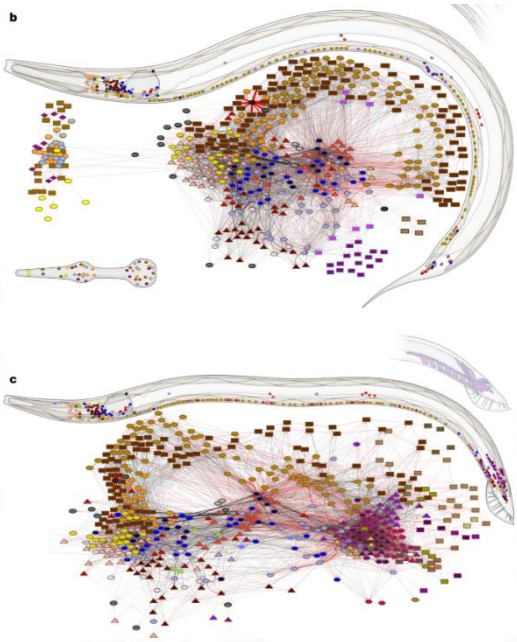


Figure 2.11: Figure depicting the connectome of *C.elegans* in hermaphrodite and male respectively.*(Cook et al., 2019)*

C.elegans genome, is credited as the first genome of a multicellular organism to be sequenced. And this paved the way for other discoveries and was a scientific aid for future progress (Equence et al., 1998). Several proteins are essential for the nervous system were first discovered in *C.elegans*, including the axonal guidance molecule netrin(Guthrie, 1997) and its receptor, the acetylcholine vesicle transporter(Alfonso et al., 1993) (Sandoval et al., 2006), and the first olfactory receptor with a defined odorant specificity(Sengupta et al., 1996). Mutants with mutations in these proteins have been valuable for studying their function in a living organism and as models for human diseases. *C.elegans* has also been used to study the mechanisms of action of psychotropic drugs through genetic pharmacology. *C.elegans* has even been used as a model for epilepsy studies (Wong et al., 2018). *C.elegans* can be subjected to genetic manipulation, where RNAi will act as a powerful tool in the functional genomics study. The genetically transformed bacteria expressing the double-stranded RNA of interest complementary to the sequence of the gene that the researcher desires to

disable can be soaked (Tabara et al., 1999, 1998), fed (Timmons and Fire, 1998) or injected (Kamath and Ahringer, 2003) to transform the worms with specific target gene silencing. Additionally, *C.elegans* can be frozen for long-term storage (Brenner, 1974).

The nematode moves by propagating bends along its body, similar to a snake, and navigates its environment using mechanical, thermal, and chemical cues. *C.elegans* shares its microhabitat with arthropods, bacteria, fungi, and other invertebrates, including other nematodes such as *Oscheius, Pristionchus, Panagrellus*, and other *Caenorhabditis* species such as *Caenorhabditis briggsae*. Notably, *C.elegans* also shares its habitat with *Drosophila melanogaster* and *Saccharomyces cerevisiae*, allowing the cross-species comparison of certain behaviours (Frezal and Felix, 2015).

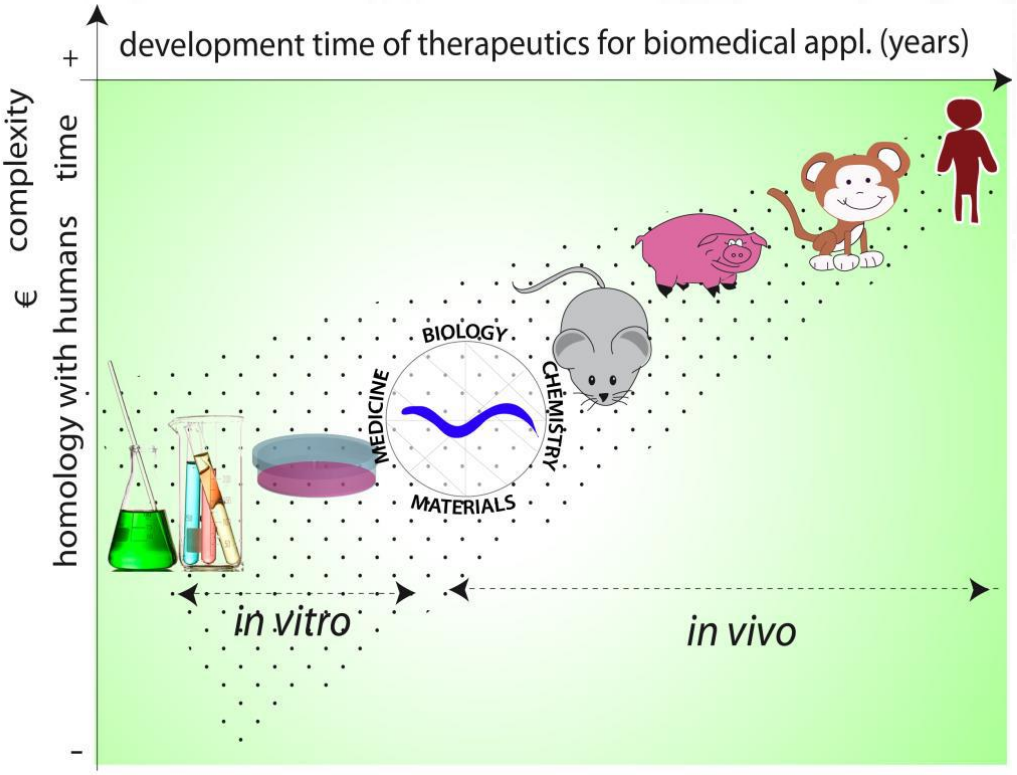


Figure 2.12:Comparative chart depicting the position of *C.elegans* among animal models
(Gonzalez-Moragas et al., 2015)

C.elegans has a simple nervous system and its regular neural connections are known as the "connectome," which has been fully mapped (Figure 2.11) (M. Stain and T. Murphy, 2010a; Markers, 2019). The high-resolution electron microscopic images helped identify all the synapses, map all the connections and work out the entire neuronal circuit. In the hermaphrodite, this system comprises 302 neurons (Kosinski and Zaremba, 2007). There is a *C.elegans* online interactive network which allows the in silico observation and understanding of *C.elegans* neural system and anatomical system (Taylor et al., 2021). In the nematode *C.elegans*, it is possible to analyze circuit function at the single-cell resolution due to its simple nervous system. The functions of many individual *C.elegans* neurons have been inferred from cell ablation studies (Bargmann and Avery, 1995), chronic activation, or recording of neuronal activity (Chalasani et al., 2008).

The behavioural repertoire of *C.elegans* is surprisingly rich, even though it has a simple body structure. The behaviours in these worms can be easily quantified. It is possible to identify and classify behaviours using genetic mutants (Bono and Maricq, 2005). In addition, *C.elegans* is an excellent model, compared to various other models, for studying the molecular and cellular basis of behaviour due to its short generation time, fully sequenced genome, and ability to undergo germline transformation (Figure 2.12). *C.elegans* studies have developed many strong behavioural tests (Bendesky et al., 2011; Chao et al., 2004; Kauffman et al., 2011), making it possible to analyze behavioural mutants. This also makes it relatively simple to identify genes in *C.elegans* and confirm their role in a specific process at the molecular level (Bono and Maricq,

2005). An adult *C.elegans* hermaphrodite has exactly mapped neurons, making up about a third of its total somatic cells. Each neuron has a specific identity that can usually be determined by an experienced observer based on the position of the cell's nucleus in the animal(Schafer, 2005). The nematode with its fully mapped and largely consistent nervous system, along with measurable behaviours, and having a range of genetic and imaging tools, offers a unique opportunity to study basic functioning at cellular levels and directly link them to behaviour (Ouellette et al., 2018).

2.2.1 Escape response in *C.elegans*

C.elegans frequently interacts with a variety of parasites, both obligate and non-obligate, including fungi, microsporidia, bacteria, and viruses (Frezal and Felix, 2015). Little is known about the predators of *C.elegans*. Possible natural predators, based on co-occurrence in the wild and laboratory experiments, include small arthropods such as mites or springtails, and other nematodes like *Pristionchus spp.* (Dieterich, 2006)and trapping nematophagous fungi(Maguire et al., 2011). . In *C.elegans,* a complete synaptic wiring diagram from all neurons was used to identify the escape circuit.

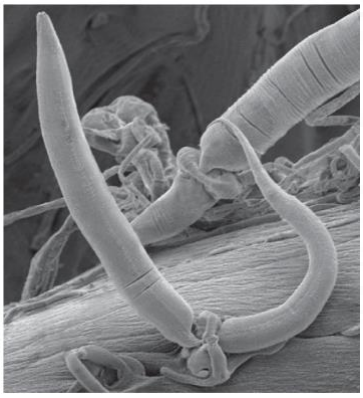


Figure 2.13: *C.elegans* caught by a constricting ring of a nematophagous fungus *Drechslerella doedycoides**(Pirri and Alkema, 2012a)***

C.elegans has specific mechanisms for avoiding toxic or harmful bacteria and fungi (Figure 2.13). The mechanism involves detecting the repellent cues produced by other microbes, which trigger avoidance behaviour upon initial contact. (Bargmann et al., 1993). It has been shown that *C.elegans* can switch between attractive and aversive responses to carbon dioxide (CO_2) based on their recent CO_2 environment. Both responses are controlled by a single pathway of interneurons, which can be either attractive or repulsive depending on the concentration of the chemosensory signal (Guillermin et al., 2017). *C.elegans* also sense mechanical stimuli to avoid predation or nociception (Gray et al., 2005). The mechanosensory stimuli are sensed with the help of six mechanosensory neurons (Goodman, 2006) that detect harmful stimuli. They subsequently make synaptic connections towards four pairs of interneurons known as forward and backwards command neurons. These command neurons synapse onto motor neurons responsible for forward and backwards movement, resulting in a rapid withdrawal from the stimulus. At least 26 neurons are involved in sensing mechanical stimuli around the worm's nose, including the bilaterally symmetric ASH neurons and polymodal nociceptors that detect aversive chemical, osmotic, and mechanical stimuli (Schafer, 2015a). The identification of the escape circuit has allowed for the analysis of its development, regulation, and modification by experience (Gray et al., 2005). A significant change in direction is achieved through a sharp omega-turn, during which the worms's body forms a distinctive shape resembling the Greek letter Ω (Gray et al., 2005). The interneuronal circuitry responsible for generating escape responses to both gentle and harsh touch has been extensively studied, primarily through cell ablation experiments (Schafer, 2015a). In

worms, the PVDs and FLPs are found to be acting as polymodal nociceptors that can respond to both aversive thermal and mechanical stimuli (Schafer, 2015a).

Tyramine is known to regulate the initial reversal phase of the escape response by activating a fast-acting ion channel and the later turning phase by activating a slow-acting G-protein-coupled receptor SER-2 (Donnelly et al., 2013a). The function of mechanosensory neurons in the nose was initially studied through cell ablation experiments(Schafer, 2015a). When worms collide head-on with an object while moving forward, they normally initiate a reversal crawl, moving away from the stimulus, exhibiting an escape behaviour similar to that triggered by touch to the anterior body. (Schafer, 2015a). The ASH and FLP neurons are mainly responsible for this touch-evoked escape response; removing either pair of neurons alone significantly reduces nose touch-evoked reversals, and removing both neurons is found to nearly eliminate the escape responses (Kaplan and Horvitz, 1993). This sufficiently explains the need for an anterior mechanism in *C.elegans* for escape from predation. The RIM neuron has connections with the parallel circuitry responsible for backward movement through a gap junction with the AVA command neuron. When RIM neurons are activated using optogenetics, there is an increase in intracellular calcium in the AVA, indicating that AVA and RIM are coactivated during a backing response (Guo et al., 2009). Food deprivation is hypothesized to suppress the RIM activity, and thus there is an evident RIM-ASH positive feedback circuit that might be working. (Ghosh et al., 2016).

The aversive responses controlled by the two ASH sensory neurons are significantly affected by the nutritional state of *C.elegans*. When a hair dipped in dilute (30%) 1-

octanol is placed in front of a forward-moving worm, backward movement will be initiated within 10 seconds (Hapiak et al., 2013). Precise 5-HT levels are observed to be critical for modulation of response to Octanol (Chao et al., 2004). It has been shown that for *C.elegans* the presence or absence of food, and consequently, 5-HT levels, rapidly and reversibly determine sensitivity to the chemical repellent octanol. Worms respond to diluted octanol more rapidly on-food than off-food. When worms are on-food, ASH neurons are both necessary and sufficient to mediate the response to both diluted and 100% octanol. The presence of food probably results in high 5-HT levels, and increased 5-HT signalling allows worms to respond more robustly to diluted octanol (Chao et al., 2004). The internal physiological state modulates the decision-making circuit and thereby reshapes the behavioural output (Ghosh et al., 2016). Authors found that after 1 hr of food deprivation, worms are more likely to exit the hyperosmotic barrier, indicating that the internal physiological state modulates this decision (Ghosh et al., 2016). Mutants such as eat-4 RNAi have been used to reduce expression and alter behaviour in the ASH, ADL, and AWC sensory neurons. However, it was proved that glutamatergic signalling was not involved in TA inhibition (Hapiak et al., 2013). The threat is identified by the means of touch (Maguire et al., 2011; O'Hagan et al., 2005), taste, temperature(Goodman and Sengupta, 2019) and olfaction(Cho et al., 2016). When the anterior part of a worm's body is touched, three mechanoreceptor neurons (ALML, ALMR, and AVM) become depolarized. These neurons connect with the AVA and AVD command interneurons, which promote backward movement, through gap junctions and form chemical synapses with the AVB and PVC interneurons, which promote forward movement. As a result, touching the anterior body triggers a reversal response reflexively. In contrast, the

PLM mechanoreceptors that detect touch to the posterior body form excitatory gap junctions with the forward command interneurons and chemical synapses with the backward command interneurons (Kindt et al., 2007).

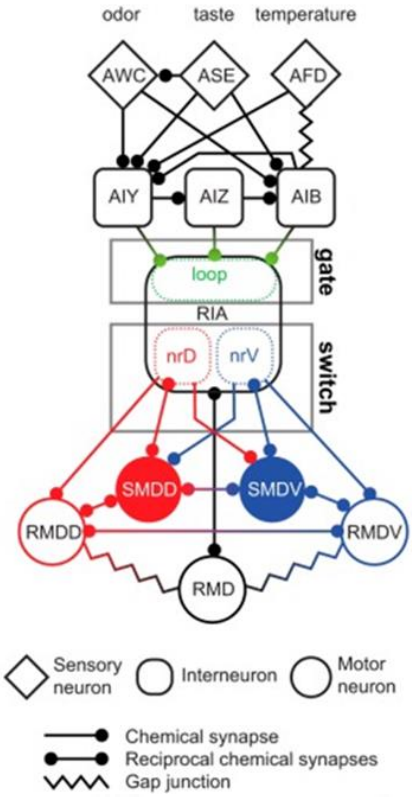


Figure 2.14: RIA-centred head orientation pathway in *C.elegans* where the sensory neuronal pathways and head motor neurons are involved. *(Ouellette et al., 2018)*

AWC neurons play an active role in information processing, aided by the interneurons (AIB and AIY), mainly sensing the food and other odours (Figure 2.14), and inducing behaviours (Chalasani et al., 2008). However, at the most basic level, a threat can either be identified or not identified. When a threat is not detected, the worm may avoid locations where predators have been encountered before and adjust its vigilance levels relying on associable cues. When an animal encounters areas of increased danger, it may opt to avoid the area altogether. However, this decision may be overridden by factors such as extreme thirst or hunger(Pessoa et al., 2021).

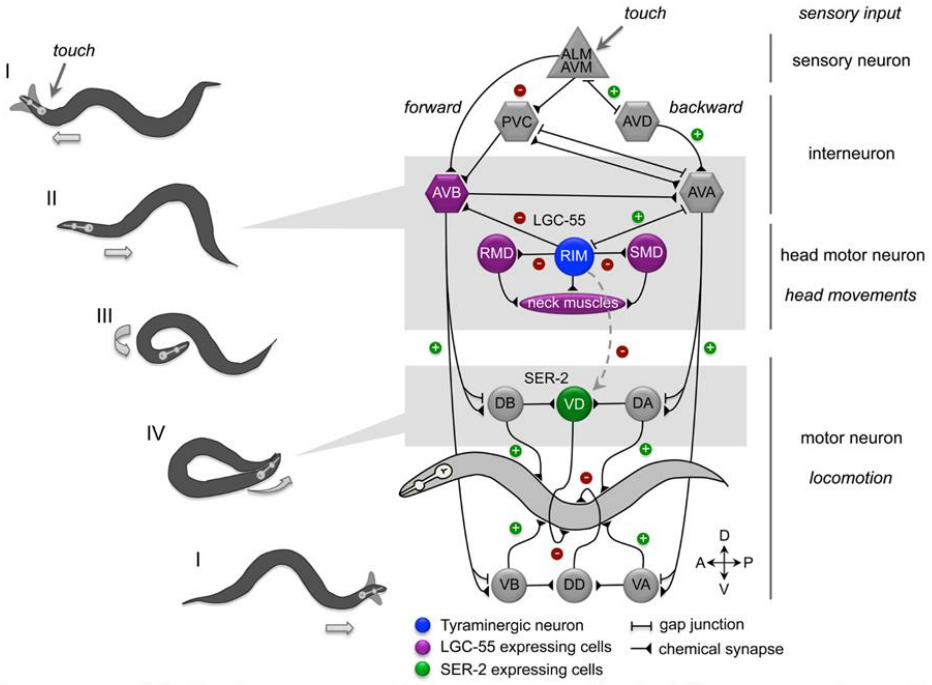


Figure 2.15: The neuronal connectome of *C.elegans* escape*(Donnelly et al., 2013a)*

The sinusoidal movement of *C.elegans* is achieved by the alternate contraction and relaxation of opposing muscles on the ventral and dorsal sides of its body. The neurons contributing to the movement are controlled by cholinergic motor neurons (DB and VB for forward movement, DA and VA for backward movement) and GABAergic motor neurons (VD and DD). When the anterior part of the worm is touched, which generates a nociceptive signal, tyramine is released from the RIM motor neurons (Maguire et al., 2011), which activate LGC-55 in neurons and muscles through synaptic connections (Figure 2.15). This hinders the forward movement and suppresses head movement in the initial phase of the escape response (Pirri et al., 2015b). SER-2 in the GABAergic VD motor neurons is also activated through extrasynaptic connections. This activation reduces the release of GABA on the ventral side of the worm, allowing for hypercontraction of the ventral muscles (Figure 2.15).

As a result, the worm performs a ventral omega turn (Donnelly et al., 2013a). VD motor neurons have input from DB motor neurons, which are cholinergic, and they release GABA to the body wall muscles of the ventral side of the body. DD motor neurons have input from VB motor neurons, which are cholinergic and release GABA to the body wall muscles of the dorsal side of the body (Donnelly et al., 2013a). Collectively, these observations support the idea that impairments in monoamine-associated receptor signalling can have a profound impact on the behaviour of animals.

2.2.2 Movement and Migration in *C.elegans*

C.elegans can chemotaxis toward numerous odorants by using neurons distinct from those that direct chemotaxis to water-soluble compounds. *C.elegans* crawls in a chemoattractant gradient, it gradually steers to orient itself in the preferred direction, a behaviour known as klinotaxis. This behaviour occurs as long as the worm is at an angle relative to the gradient, which allows for sensory sampling across head sweeps.(Ouellette et al., 2018) There are two opposing views on the nature of locomotory patterns in *C.elegans*. One view is that crawling and swimming are at opposite ends of a single gait and represent the output of a single neural circuit. The other view is that crawling and swimming are different gaits produced by functionally distinct neural circuits (Vidal-Gadea et al., 2011). Unlike the temporal integration of the biased random walk, steering behaviour relies on asymmetric head bends that propagate through the normal locomotor body wave, resulting in curved forward movement in nematodes (Ouellette et al., 2018).

C.elegans is a genetically tractable animal whose 302 neurons and ≈8,000 known synapses make it a promising model system for the study of transitions between

motor patterns. On an agar substrate and in water, *C.elegans* moves forward with a dorsoventral bending (Vidal-Gadea et al., 2011). Usually in worms, forward propulsion is achieved through the backward propagation of a flexural wave generated by muscular contraction. *C.elegans* had different moving states. The worm's locomotion could be classified into two behavioural states in the presence of food. The dwelling was defined by low-speed/high-turning, whereas roaming was defined by high-speed/low-turning (Fujiwara et al., 2002b). Crawling is a sinusoidal pattern of forward locomotion characterized by backward propagation of body bends in *C.elegans* (Albrecht and Bargmann, 2011). The average speed of worm speed was 0.2 mm s^{-1} which was found from the experimental average on NGM plates (Albrecht and Bargmann, 2011). In *C.elegans* control of body movements is designated to specialized body wall muscles, meanwhile, the head movements are precisely controlled by the action of head and neck muscles(Branicky and Schafer, 2009). It is known from recent studies that several neuropeptides and G protein-coupled neuropeptide receptors can influence the local search behaviour of the worms (López-Cruz et al., 2019).

2.2.3 Foraging in *C.elegans*

Behavioural adaptations to changing environmental conditions emphasise the significance of an animal's natural history. *C.elegans*' natural habitat, which is characterized by fluctuating temperature, humidity, osmotic pressure, and the presence of other microbes, presents numerous behavioural challenges. The worm must search for bacteria and slime moulds, which make up the majority of its diet (Pirri and Alkema, 2012a). An essential foraging decision is a choice between exploiting existing

resources and exploring other options that may provide new resources. This decision can be described by Charnov's marginal value theorem, which proposes that the optimal time for an animal to leave a foraging ground occurs when local resource levels fall below the average level in the entire habitat (Charnov, 1976). The parallel processing of sensory and nutritional cues is characteristic of mammalian feeding and satiety circuits (López-Cruz et al., 2019). The locomotory behaviour of *C.elegans* in the presence of food is characterized by alternating behavioural states. The worms traverses widely separated regions of the plate (roaming), and in the other state, the worms restrict their activity to a confined region (dwelling). The relative time spent roaming versus dwelling may be regulated by sensory perception (Fujiwara et al., 2002b). In the local search circuit, chemosensory neurons regulate acute reorientations by activating excitatory and inhibitory ionotropic glutamate receptors on downstream interneurons (López-Cruz et al., 2019). Theoretical models have shown that local search is the most effective strategy when animals have information about food availability, such as a recent food encounter, while a global search is most effective when animals need to locate random food sources without prior information (López-Cruz et al., 2019).

2.2.4 Learning and memory in *C.elegans*

C.elegans often encounter environmental conditions that vary over timescales (Pradhan et al., 2018) but the sudden change in the environment demands adaptation to the new environment. In a changing environment, two essential fundamental biological properties aid the survival of an organism: learning and memory. Learning is the acquisition of knowledge or skills (utilising study, experience or teaching), and memory serves as the facility which facilitates the storage and retrieval of learnt

information from the brain. To study learning and memory processes, *C.elegans* has been used as a model system where both its associative and non-associative memories have been characterised. The nematode possesses neural circuits that respond to environmental cues, and the internal state allows execution of both genetically encoded and experience-based strategies (Ghosh et al., 2016; Pradhan et al., 2018). *C.elegans*' known to learn while conditioned with both associative and non-associative memories that could last up to 24 hours (Stain and Murphy, 2010b). An earlier account of memory and learning in nematode *C.elegans* is associative learning, as worms tend to return to their cultivation temperature (Hedgecock and Russell, 1975). The activity of neurotransmitter receptors triggers molecular pathways in neurons, which are believed to activate proteins in synapses that aid in the formation and retention of memories. In higher organisms, kinases and proteins that help cells stick together at synapses have been found to regulate short and intermediate-term memory. However, the targets and pathways that work together with these regulators are not yet well understood (Stain and Murphy, 2010b). The fundamental component of adaptive learning is associative learning which is the ability of living organisms to perceive or learn the correlation between two or more events (Jozefowiez et al., 2012). *C.elegans* could be trained according to the classical Pavlovian fear conditioning (Law, 2014; M. Stain and T. Murphy, 2010b) In *C.elegans*, short-term associative memory requires the activation of cAMP and calcium signalling pathways. Protein translation is also necessary at two different stages: first, during massed associative memory training to extend memory beyond 30 minutes, and second, after memory training to ensure proper decay of the associative memory or forgetting(M. Stain and T. Murphy, 2010b). A protocol that pairs a relatively neutral odour (butanone at a specific concentration) with food creates

a positive association, resulting in a strong attraction to the odour (Stain and Murphy, 2010b). Both associative and non-associative learning contribute to the behavioural responses seen following conditioning in a CS-US pairing-specific paradigm (Morrison et al., 1999). long-term memory after spaced training lasts more than 16 hours. The worms are observed to display an increased response to the odour after training (Lindemann and Hoener, 2005). Neurotransmitters like glr-1, AMPA, NMDA are identified as having a critical role in memory (Ardiel and Rankin, 2010). The connectome of the organism for all motor patterns is seen converging to master control neuron RIM for locomotion-related learning and behaviour(Ardiel and Rankin, 2010; Cho et al., 2016). However, the molecular understanding of how a specific memory is generated is still unknown.

2.2.5 Sensory perception in *C.elegans*

C.elegans employs a well-defined chemosensory network to detect various repellents or attractants. The organism exhibits distinct mechanisms for avoiding toxic or harmful organisms by detecting repellent chemical cues produced by microorganisms, which in turn trigger avoidance behaviour (Bargmann et al., 1993). Another mechanism involves the ingestion of harmful bacteria, leading to associative learning and the ability to avoid bacterial-related cues on subsequent encounters (Hart, 2006). ASH neurons and polymodal nociceptors aid in detecting aversive chemical, osmotic, and mechanical stimuli in the worms (Schafer, 2015b).

The anterior sensory structures in *C.elegans* are classically divided into two types, papillary and amphidial, and are assumed to be responsible for mechano- and chemo-reception, respectively (Ware et al., 1975). The nerve ring receives sensory

input from the anterior tip of the worm utilizing six nerve bundles, all nerve fibres of which have centrally located cell bodies (Ware et al., 1975). The sensory network is based on 16 pairs of sensory neurons that penetrate the cuticle to expose their sensory cilia to the environment (Ballestriero et al., 2016). *C.elegans* is known to sense volatile molecules by using three pairs of chemosensory neurons, AWA, AWB, and AWC, found in the bilaterally symmetric amphid chemosensory organ in the worm's head(Morrison et al., 1999). As. The chemoattractants were used to characterize RIA physiology, and steering has been demonstrated in IAA gradients. RIA-defective worms exhibit normal Ca^{2+} responses, and RIA-ablated worms are capable of olfactory chemotaxis, indicating that they are not sensory impaired (Ouellette et al., 2018). It is identified that the AWC olfactory neurons are responsible to detect volatile odours corresponding to bacteria, ASK neurons are responsible for the identification of amino acids(López-Cruz et al., 2019).

At least 26 neurons are involved in sensing mechanical stimuli around the worm's nose, including the bilaterally symmetric ASH neurons and polymodal nociceptors that detect aversive chemical, osmotic, and mechanical stimuli. (Schafer, 2015a). RIM-ASH Positive Feedback Underlies the Modulation of the Threat-Reward Decision (Ghosh et al., 2016). In *C.elegans*, in addition to neurons that detect external mechanical stimuli, there is an unknown but significant number of neurons that function as proprioceptors, sensing the body's posture. Like other animals, *C.elegans* is believed to use proprioceptive information as sensory feedback to control its locomotion pattern (Schafer, 2015a).

Laser ablation studies reported that AFD was the primary thermosensory neuron, and the AIY interneuron is identified to facilitate the thermophilic movement,

AIZ interneuron intermediates cryophilic movement, and RIA interneuron integrates the thermo and cryophilic drives (Mori and Ohshima, 1995). The AIY interneurons play a role in at least four behaviours: dispersal, thermotaxis, regulation of swimming behaviour in response to chemical and thermal cues, and behavioural plasticity in scenarios where starvation is paired with a thermal or chemical cue (Gray et al., 2005). The roles of AIY in thermotaxis and swimming regulation are rapid and can be attributed to direct inputs from sensory neurons onto AIY. However, some of AIY's effects on plasticity could be indirect, resulting from its general function in suppressing turns and reversals. The frequent reversals in worms lacking AIY could alter locomotion and navigation under various conditions (Gray et al., 2005)

The presence of food can alter the pattern of *C.elegans* locomotion (Gray et al., 2005) It has been understood that *C.elegans* is capable of associative learning, as it was discovered that the worms return to their cultivation temperature only if they had fed at that particular temperature (Hedgecock and Russell, 1975). When associative learning involves the detection of chemical cues by olfactory neurons, it is designated olfactory-dependent learning (Ballestriero et al., 2016). It has been shown that *C.elegans* can switch between attractive and aversive responses to carbon dioxide (CO_2) based on their recent CO_2 environment. Both responses are controlled by a single pathway of interneurons, which can be either attractive or repulsive depending on the concentration of the chemosensory signal (Guillermin et al., 2017). The presence of multiple positive and negative pathways regulating adaptation in different time frames may provide flexibility to olfactory responses in complex and changing environments (Schafer, 2005). The AIY interneurons of *C.elegans* are known to

receive several sensory inputs including gustatory, olfactory, and thermal information (Ashida et al., 2019).

2.2.6 Touch sensitivity in *C.elegans*

To understand the importance of the nematode's touch response in avoiding predation, researchers examined the relationship between fungi that use constricting rings to capture prey and *C.elegans*. Most worms are captured at the front half of their body, indicating the importance of anterior sensory decision-making and escape responses(Maguire et al., 2011). The touch response in *C.elegans* is one of the few instances where the entire sensorimotor circuit has been fully identified [27]. Cell ablation experiments have implicated a total of 14 neurons in the detection of harsh body touch(Schafer, 2015a). The trp-4 gene is necessary to independently interact with dopaminergic neurons to regulate food slowing and nose touch avoidance behaviours in *C.elegans* [28].In *C.elegans* the PVD and FLP neurons respond to fast, high-displacement mechanical stimuli applied to the areas covered by their respective dendritic arbours (Schafer, 2015a). The PVD neuron has a receptive field that covers most of the body, while FLP covers the head and neck region (Albeg et al., 2011). In nematodes, the number of mechanosensory neurons is designated to detect and identify food texture(López-Cruz et al., 2019).

At least 26 neurons are involved in sensing mechanical stimuli around the worm's nose, including the bilaterally symmetric FLP nociceptor neurons, in addition to sensing harsh touch to the side of the head, which also plays an important role in sensing aversive nose touch (Kaplan and Horvitz, 1993). The ASK neuron is less able to drive reorientations during the global search. On optogenetic activation of RIM

interneurons, which belong to a coupled network of neurons downstream of AIA that drives reorientation behaviour, were optogenetically activated. RIM activation resulted in equally strong reorientation responses during local and global searches (López-Cruz et al., 2019). The ASK and RIM activation in different studies suggests that, in addition to changes in ASK activity, the reorientation circuit becomes less sensitive to ASK input during the transition from local to global search. Touch reception is mediated by a DEG/ENaC channel complex that can sense forces as small as 100 nN with a latency of less than 5 ms, allowing the worm to quickly respond to even the lightest touch (Pirri and Alkema, 2012a) mec-4 null mutants in *C.elegans* is known to completely lack mechanoreceptor potentials, and missense alleles of mec-4 and mec-10 are studied to exhibit altered reversal potential of the mechano-transduction current (O'Hagan et al., 2005).

Activation of the anterior touch sensory neurons inhibits the command neurons that drive forward movement (PVC, AVB) and activates those that promote backward movement (AVD, AVA), triggering the worms to avoid the stimulus and move away (Pirri and Alkema, 2012a). It has been found that different classes of head motor neurons have at least four distinct functions: regulating the amplitude of omega turns (SMD), regulating the ventral bias of omega turns (RIV), suppressing reversals (RIM and others), and regulating the amplitude of sinusoidal movements (SMB). The RIV motor neurons may provide a ventral bias to omega turns by synapsing only onto ventral muscles, while most other head motor neuron classes innervate both ventral and dorsal muscles. During a reversal, the worm also suppresses foraging head movements(Gray et al., 2005). The touch sensation is known to be governed by the mechanosensory cells (ALM, AVM, PLM, and PVD) and interneurons (AVA, AVB,

AVD, PVC, and DVA) playing an important role in “tap withdrawal response” (Wicks and Rankin, 1995). The response properties of the mechanosensory neurons could be altered by repeated activations (Suzuki et al., 2003). Another group having a role in regulating touch sensitivity were identified, a K+ channel (shw-3) and an accessory subunit (mps-1) (Cai et al., 2009).

2.3 Trace amines

Amines are low molecular weight organic nitrogenous bases that are produced during the metabolism of living organisms and are commonly found in fermented foods. Trace amines (TAs) are endogenous compounds that are related to biogenic amine neurotransmitters and are present in the mammalian nervous system in trace amounts.(Lindemann and Hoener, 2005). Trace monoamines are so named because they occur in low levels in mammalian tissues, especially when compared to more abundant monoamine neurotransmitters such as epinephrine, norepinephrine, dopamine, and serotonin (Gainetdinov et al., 2018). Trace amines (TAs) are endogenous compounds that are present in the mammalian nervous system in trace amounts. Although with their pronounced pharmacological effects and a tight link to major human disorders(Lindemann and Hoener, 2005), TA’s have also been connected to depression, schizophrenia and other diseases such as migraine, attention deficit hyperactivity disorder(ADHD), substance misuse and eating disorders.(Lindemann and Hoener, 2005). Post-mortem human brains obtained from non-psychiatric patients have shown that trace amines such as phenylethylamine, p-tyramine, m-tyramine, and tryptamine are distributed heterogeneously throughout the brain, with the highest concentrations found in the basal ganglia (Philips et al., 1978). Despite their low levels, animal studies have shown that trace amines have a very rapid turnover rate and their

presence in a brain synaptosomal fraction suggests a possible role in neurotransmission. In rat brains, where phenylethylamine, p-tyramine, and tryptamine have been shown to influence other monoamines (Philips et al., 1978). Although trace amines are known to cause prominent pharmacological effects and have a close connection to major human disorders. The comprehension of their molecular mechanism of action remained incomplete because of the apparent absence of specialised receptors in the human body.

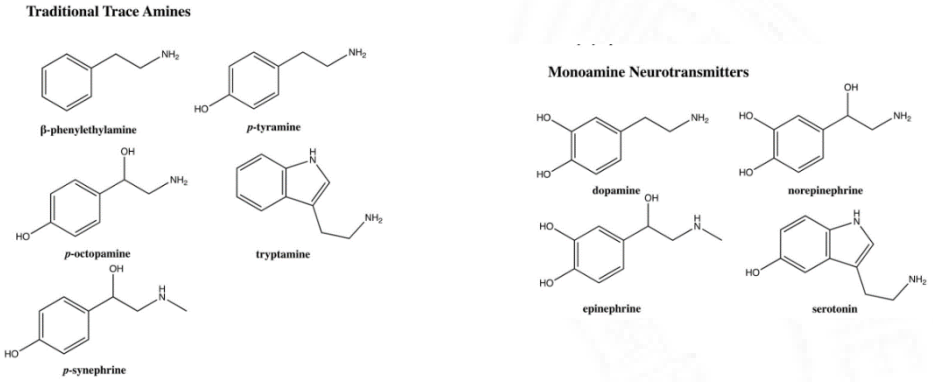


Figure 2.16: Structure of trace amine and monoamines respectively *(Gainetdinov et al., 2018)*

The discovery of a novel family of G-protein-coupled receptors (GPCRs) that are highly specific for trace amines suggests that these compounds may act as neurotransmitters or neuromodulators in vertebrates. Recent research, however, has revealed that the existence of specific receptors, which are specific to trace amine called the trace amine-associated receptors (TAAR) in organisms, may have considerably higher significance than was been thought for many years. This includes their respective physiological role in neuromodulation (Finberg and Gillman, 2011). TAARs are recently targeted for the treatment of various disorders and as sites for environmental chemical interactions that can affect behaviour.

2.3.1 Biosynthesis of amines

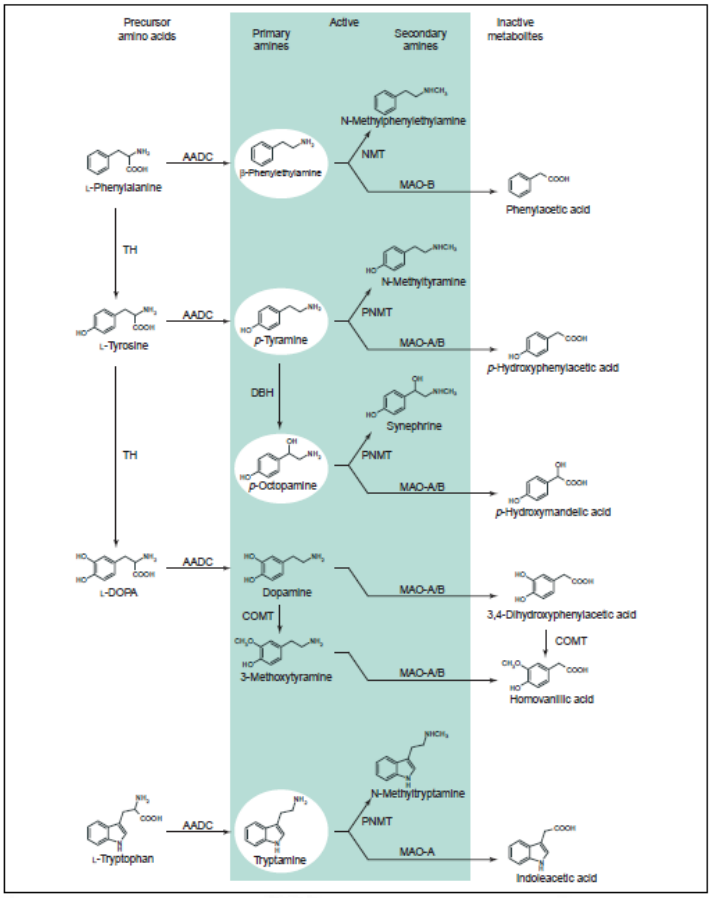


Figure 2.17: The pathway of L-Tyrosine biosynthesis*(Lindemann and Hoener, 2005)*

Biogenic amines are primarily formed through the decarboxylation of amino acids (ten Brink et al., 1990). All monoamines have an amine group separated from an aromatic ring by a two-carbon chain. The major identified monoamines in neurotransmission are Dopamine, norepinephrine, and serotonin are monoamines (Burns and Kidron, 2022). Monoamines, which are also referred to as "biogenic amines," consist of three types of neurotransmitters: Catecholamines (such as Dopamine, norepinephrine, and epinephrine), Indolamines (including Serotonin and Melatonin), and Histamine. DA is an important neurotransmitter in the brain, and a crucial part of it is synthesized in the mesenteric organs (Eisenhofer et al., 1997). The classical pathway for tyrosine biosynthesis takes place in the cytosol of neurons. Tyrosine is considered the precursor

for DA synthesis - phenylalanine is converted into tyrosine through the action of phenylalanine hydroxylase (Figure 2.17) (Fernstrom and Fernstrom, 2007). DA synthesis is initiated by the rate-limiting enzyme tyrosine hydroxylase (Elsworth and Roth, 1997), first producing L-DOPA (dihydroxyphenylalanine) from the precursor tyrosine, and this, in turn, is converted to DA by the action of aromatic amino acid decarboxylase (Fernstrom, 1983).

Changes in monoamine neurotransmitter concentrations in the brain have been linked to changes in mental processes, movement control disorders, and certain neuropsychiatric diseases. Trace amines have also been associated with conditions such as migraines, ADHD, substance abuse, and eating disorders (Lindemann and Hoener, 2005). The biogenic amine tyramine is produced by the decarboxylation of tyrosine by microbes and is therefore found in many fermented, aged, or ripened protein-rich foods (Rafehi et al., 2019). The biosynthesis of octopamine involves two steps: first, tyrosine is converted into tyramine by a tyrosine decarboxylase, and then tyramine is converted into octopamine by a tyramine β-hydroxylase (Alkema et al., 2005).

When combined with monoamine oxidase (MAO) inhibitors, tyramine is observed to cause a hypertensive crisis that can result in symptoms such as headache, migraine, nausea, and vomiting, as well as more serious complications such as end-organ damage, intracerebral haemorrhage, and death (Rafehi et al., 2019). In locusts, tyramine is expressed in all neurons that express octopamine as well as in some cells that do not express either the beta-hydroxylase enzyme or octopamine (Sinakevitch et al., 2017).

In *C.elegans*, a tyrosine decarboxylase gene (tdc-1) and a tyramine β-hydroxylase gene (tbh-1) have been identified. The tdc-1 is required for tyramine biosynthesis, and both tdc-1 and tbh-1 are required for octopamine biosynthesis. It was understood that there are tyraminergic cells that are distinct from octopaminergic cells and that tyramine plays a unique role in *C.elegans* behaviour (Alkema et al., 2005). While trace amine synthesis is often reported as being neuronal due to its similarity to monoamine neurotransmitter synthesis, it is important to note that AADC expression is not restricted to neuronal cells and is present in several other cell types(Gainetdinov et al., 2018).

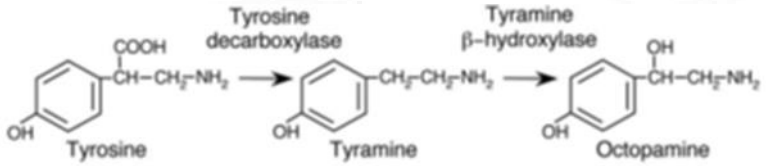


Figure 2.18: The biosynthetic enzymes involved in the conversion of tyrosine to tyramine and octopamine respectively

The *C.elegans* genome sequence comprises the gene, H13N06.6, which encodes a protein with substantial similarity to Drosophila tyramine β-hydroxylase (TBH) (Alkema et al., 2005; Monastirioti et al., 1996). In *C.elegans*, tyramine is produced from tyrosine by a tyrosine decarboxylase (tdc-1), and the conversion of tyramine to octopamine happens with the aid of tyramine beta-hydroxylase (tbh-1) (Figure 2.18). TDC-1 and TBH-1 are co-expressed in a pair of interneurons(Figure 2.19), the RICs, and in the gonadal sheath cells, indicating that these cells are defined with octopaminergic functions (Alkema et al., 2005). Worms that lack tyrosine decarboxylase (tdc-1), the enzyme that produces tyramine, or worms that lack

tyramine are unable to suppress foraging head movements (Pirri and Alkema, 2012a).

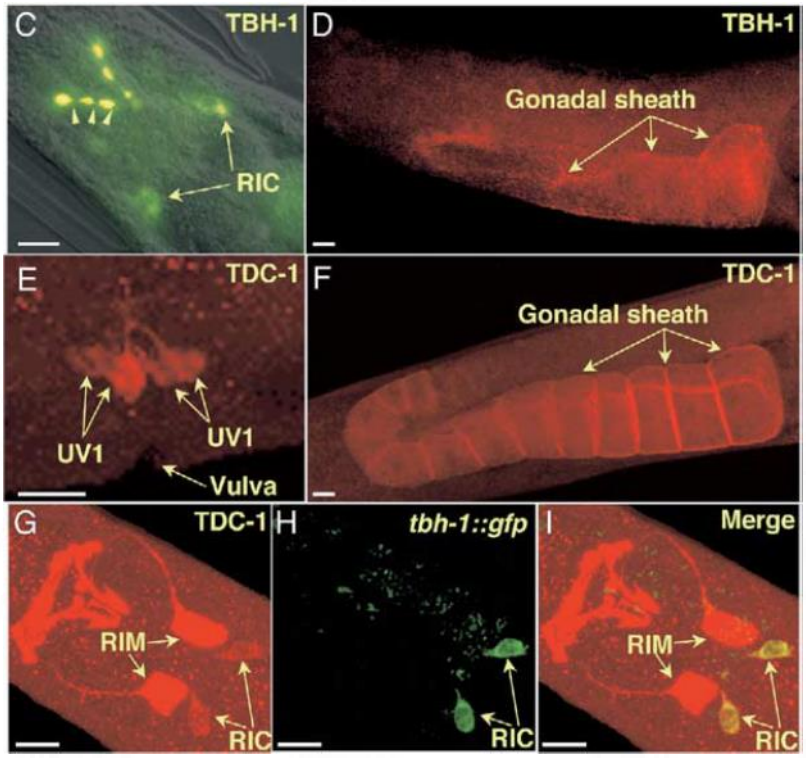


Figure 2.19: The expression pattern of TDC-1 and TBH-1 biosynthetic enzymes in *C.elegans*
(Alkema et al., 2005)

2.4 Tyramine

The origin of the Pharmacology field is linked with the primary demonstration of TYR and PEA. The initial usage of the term ergotamine was to explain tyramine, which was isolated from ergot-contaminated grains. The TYR and PEA were first isolated from the rotting horse meat and contaminated grains, which led to original studies of George S. Walpole, Sir Henry Hallett Dale, George Barger and Alfred J.Clark (Gainetdinov et al., 2018). Tyramine is known to have sympathomimetic effects in vertebrates (Burns and Kidron, 2022). The m- and o-isoforms of tyramine have also been identified, but they have been studied less frequently and are present in even smaller quantities than the studies concerning the p-isoform (Gainetdinov et al., 2018). Tyramine is found in numerous foodstuffs, most noticeably in aged and fermented food products and beverages. Cheeses (notably Parmesan, Cheddar,

Camembert, and Emmental), overripe bananas, avocado, canned figs, peanuts, pickled herring, dried and fermented meat products and alcoholic beverages (wine, beer) are found to contain tyramine (Caballero et al., 2003). Tyramine causes an interaction which is often termed the "cheese reaction". The hypertensive reaction which may occur if patients treated with monoamine oxidase inhibitors (MAOIs) ingest foods or beverages containing tyramine, can be potentially lethal(Anderson et al., 1993). A dose of 10 mg tyramine has been associated with migraine onset; however, levels of 6 mg can cause migraine in patients under treatment with MAO inhibitors (Caballero et al., 2003).Tyramine is a vasoactive amine that promotes blood pressure elevation, resulting in pain. Tyramine leads to cerebral vasoconstriction and subsequent rebound vasodilatation that causes a migraine attack in susceptible persons (Caballero et al., 2003).

Tyramine in the system is made endogenously when not consumed by the decarboxylation of the tyrosine amino acid by the enzyme aromatic L-amino acid decarboxylase (AADC)(Gainetdinov et al., 2018). Tyramine is known to have catecholamine-releasing properties(Gainetdinov et al., 2018) Tyramine was originally known to be the substrate for MAO, which was previously assumed to be tyramine oxidase(Hare, 1928). Until recently, research on tyramine and its receptors in invertebrates has shown that tyramine has sources and functions that are independent of octopamine, rather than simply being a precursor to octopamine. (Sinakevitch et al., 2017).

It is reported that tyramine, octopamine, and synephrine, which are products of tyrosine metabolism, may contribute to the development of cluster headaches and migraines (D'Andrea et al., 2007). Despite their low steady-state levels, some of these amines have high turnover rates (Finberg and Gillman, 2011). MAOI was primarily used to treat the interactions of tyramine, which is clinically termed as cheese effect. The MAOI inhibits the enzymes mainly the tranylcypromine (TCP) or phenelzine. In recent years drugs were developed to give the effect of MAOI which would have selective binding affinity and can even target particular isoforms.

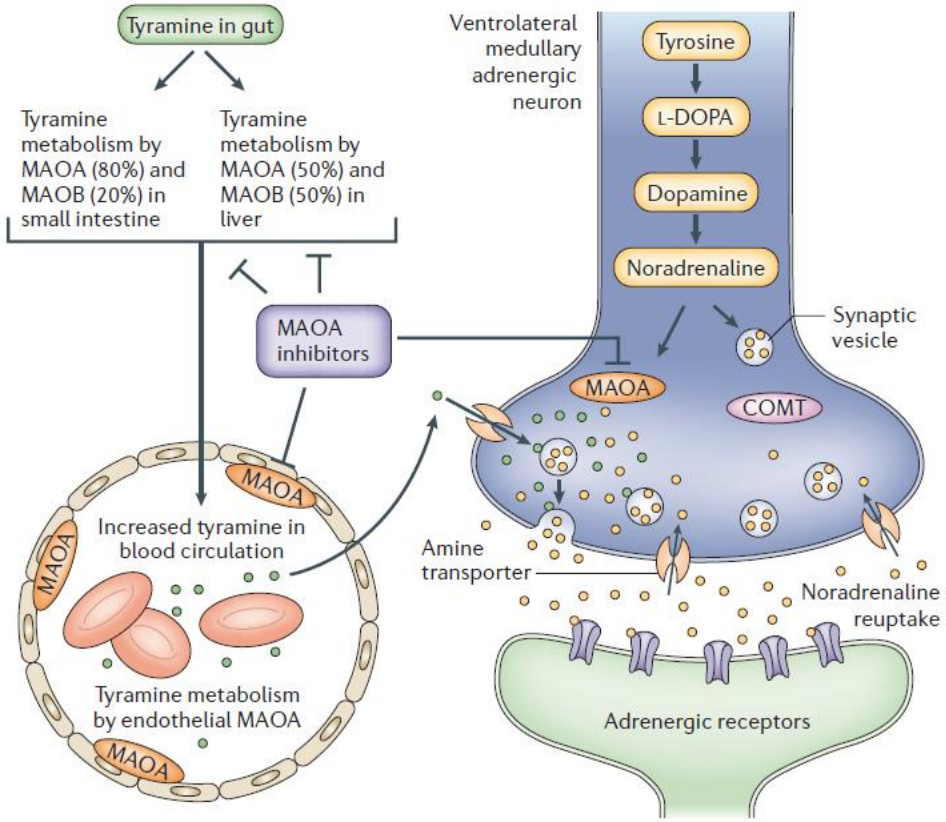


Figure 2.20: Tyramine clearance in the intestinal mucosa of the vertebrate system*(Youdim et al., 2006)*

Tyramine is cleared from the body through the action of MAO-A in the intestinal mucosa, which reduces its oral bioavailability, and through hepatic MAO

(Figure 2.20) (Gillman, 2018). A standard procedure has been developed to measure the oral bioavailability of tyramine in humans by administering increasing doses until systolic blood pressure increases by more than 30 mm Hg, a measurement known as the Tyr30 (Bieck and Antonin, 1989).

Tyramine and octopamine activate intracellular signalling pathways by binding to their respective receptors, octopamine receptors (OARs) or tyramine receptors (TYRs). Most of these receptors are G-protein-coupled receptors (GPCRs) (Sinakevitch et al., 2017). Invertebrates have receptors that are selectively activated by tyramine and octopamine but are distinct from TAARs and more closely related to vertebrate adrenergic receptors (Gainetdinov et al., 2018). Tyramine and octopamine are neuroactive compounds that can influence a variety of invertebrate behaviours, including locomotion, learning and memory, and sensory processing (Sinakevitch et al., 2017). In *Drosophila melanogaster*, tyramine-containing neurons distinct from octopaminergic neurons have been identified in its larval central nervous system (Sinakevitch et al., 2017).

2.4.1 Tyramine receptors involved in *C.elegans* escape response

In higher-order systems like vertebrates, none of the trace amines identified meet all criteria to be identified as neurotransmitters. Thus, those identified amines are regarded as the metabolic by-products of another active compound, which are found to have a physiological relevance (Gainetdinov et al., 2018). Recent findings have unquestionably revealed that a few G-protein-coupled receptors allow the binding of biogenic amines such as tyramine. Trace amine-associated receptors (TAARs) are the

type of receptors that are specific to trace amine interactions and activations(Gainetdinov et al., 2018). The exact processes through which TAs activate TAARs are still not determined (Lindemann and Hoener, 2005). Disorders of TAAR1 activity may be credited to the pathophysiology of some mental disorders, thus, these TAARs have become a promising target for novel psychopharmacological interventions in recent times (Rutigliano et al., 2019). Interestingly,in rodents an endogenously produced amine administration was found to have effects on the CNS which include modification in food intake and sleep pattern composition and yields pro-learning and anti-amnestic effects (Manni et al., 2013).

The *C.elegans* genome holds over 1000 predicted G protein-coupled receptors (GPCRs), many of which are likely to function as chemoreceptors. These receptors belong to large gene families that are highly divergent between *C.elegans* and other species. (Robertson and Thomas, 2006). The majority of known biogenic amine receptors are metabotropic; that is, they exert their effects via slow G protein-mediated responses. Tyramine and octopamine receptors are related to vertebrate adrenergic receptors and are thought to carry out analogous functions. However, the assumption that monoamines function only as neuromodulators may reflect a mammalian/vertebrate bias. GABA and glycine are known to act through ligand-gated anion channels in vertebrates. In *C.elegans*, anion channels gated by serotonin, glutamate, and acetylcholine have been established with the same function (Branicky and Schafer, 2009),(R et al., 2000). Apart from Ligand-gated chloride channels *C.elegans* genome encodes for several tyramine GPCRs related to vertebrate adrenergic receptors, including TYRA-2, SER-2, and TYRA-3 (Ghosh et al., 2016). The ion channel LGC-55 is activated by synaptic kinetics, while the G-protein coupled

receptor SER-2 is activated extrasynaptically. These activations, in a coordinated manner control the escape response. Additionally, the tyramine GPCR TYRA-3 plays a role in pain reflexes. (Donnelly et al., 2013a)

2.4.1.1 LGC-55 receptor

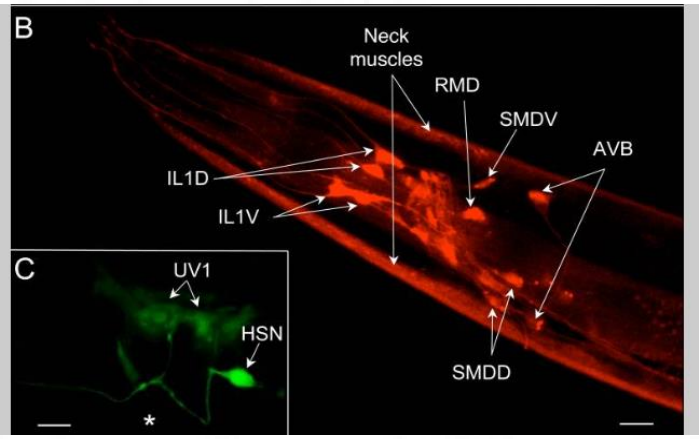


Figure 2.21 : Expression pattern of LGC-55 receptor in the head region of *C.elegans* *(Pirri and Alkema, 2012a)*

LGC-55 is an ionotropic receptor, which is one of the first receptors identified with tyramine as a ligand (Branicky and Schafer, 2009). In *C.elegans*, ligand-gated ion channels (LGICs) are the core signalling mechanisms that mediate fast inhibitory and excitatory neurotransmission (Pirri et al., 2015a). The lgc-55 is a gene that encodes a Cys-loop ligand-gated ion channel (LGIC) (Branicky and Schafer, 2009).. When expressed in Xenopus oocytes, this channel is activated by octopamine, dopamine, and weakly by serotonin, but is most strongly activated by tyramine. This suggests that tyramine may be its endogenous ligand (Branicky and Schafer, 2009).

The lgc-55 gene encodes a tyramine-gated chloride channel that can act as a fast inhibitory neurotransmitter in vivo. Cys-loop LGIC receptors, including nicotinic acetylcholine receptors (nAChRs), serotonin type 3 receptors (5HT3Rs), and anion-

selective GABAA and glycine receptors, form pentameric complexes in the plasma membrane (Figure 2.22) (Pirri et al., 2015a). Wild-type *C.elegans* suppress head oscillations during this backing response, and LGC-55 mutants have comparatively normal locomotion patterns or head oscillations during normal foraging(Chalfie et al., 1985; Pirri et al., 2009).

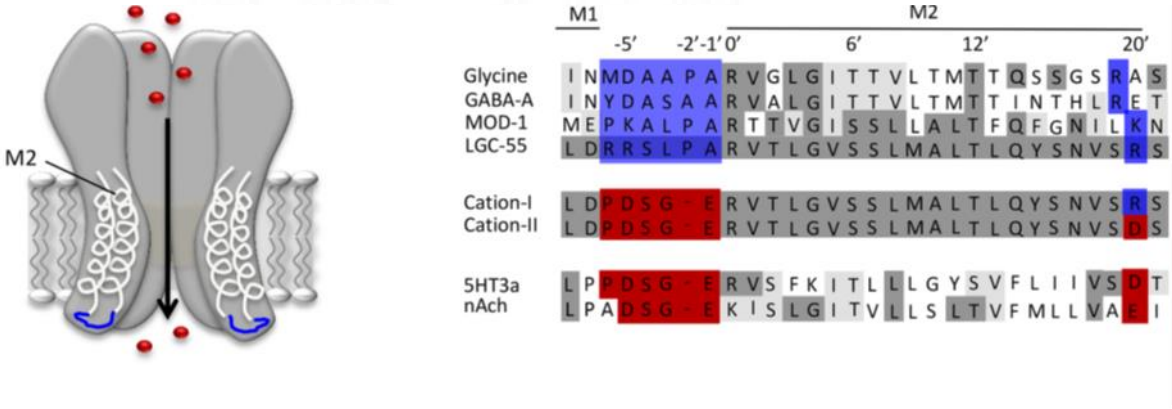


Figure 2.22:Ligand gated ion channel- LGC-55 with M1 and M2 and the sequence comparison between Glycine, GABA, MOD-1 LGC-55 cationic, 5HT3a and nAch receptors respectively
(Pirri et al., 2015b)

Research has demonstrated that tyramine can function as a neurotransmitter by activating the tyramine-gated chloride channel LGC-55, which coordinates backward locomotion and suppresses head movements during an escape response (Pirri et al., 2009).LGC-55 expressions in neurons and muscles which are directly postsynaptic to the tyraminergic neurons. LGC-55 . The LGC-55 receptor, when expressed using the LGC-55 promoter (LGC-55::GFP), fluorescence was observed in neuronal cell bodies and punctate structures in the nerve ring, suggesting that LGC-55 receptors cluster at postsynaptic sites, potentially including neuromuscular synapses between the RIMs and neck muscles(Figure 2.21). The neck muscles, AVB, RMD, SMDD, and SMDV neurons are postsynaptic to the tyraminergic RIM motor neurons(Figure 2.23) (Pirri et al., 2015a, 2009).

C.elegans has both anion- and cation-selective ACh and GABA-gated LGICs. The M2 domain, which lines the pore of the ion channel, determines the ion selectivity of cys-loop LGICs (Pirri et al., 2009). The binding of tyramine to the N-terminal domain induces a conformational change that opens the channel pore. LGC-55 is closely related to this family of ligand-gated ion channels (Pirri et al., 2009). When the M1-M2 loop of the *C.elegans* tyramine receptor LGC-55 is replaced with that of related cation channels, the ion selectivity changes from anions to monovalent cations. Alkema et al. have shown that engineered receptors with switched ion selectivity properly localize to the synapse and are functional in vivo.(Pirri et al., 2015a)

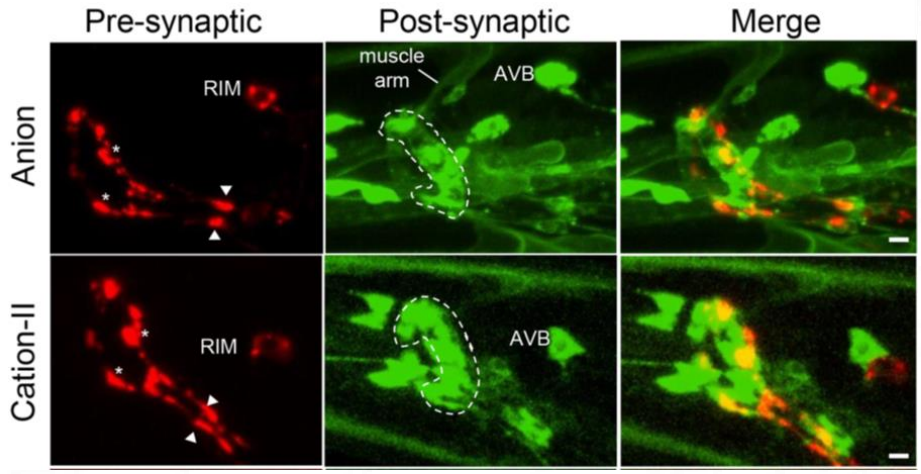


Figure 2.23: The expression of anionic and cationic LGC-55 receptor in *C.elegans* *(Pirri et al., 2015b)*

Worms expressing the LGC-55 anion channel show opposite behavioral outputs compared to those expressing the LGC-55 cation channel. This suggests that the reversal in behavioral outputs is due to the difference in ionic selectivity (Pirri et al., 2015a). *C.elegans* has a single pair of RIMs, which activate the homomeric tyramine-gated chloride channel LGC-55 (Pirri et al., 2015a).LGC-55, being a fast-acting channel and with close proximity to the command neurons like AVB raises the

question that they might be playing a critical role in decision making or sensory perception. There has been evidence that LGC-55 plays an active role in sensing co_2(Riedl et al., 2022)

2.4.1.2 SER-2 receptor

SER-2 is a G protein-coupled receptor for tyramine, activated by the extrasynaptic release of tyramine from RIM on receiving a nociceptive signal (Donnelly et al., 2013a)SER-2 is not expressed in any neurons that are postsynaptic to RIM, suggesting that tyramine acts extrasynaptically on these receptors (Branicky and Schafer, 2009). SER-2 in the escape response is often regarded as the SER-2 serpentine reporter in mid-body regions (Donnelly et al., 2013a). Pser-2::mCherry was highly expressed in a subset of GABAergic motor neurons(Figure 2.25)(Donnelly et al., 2013a).

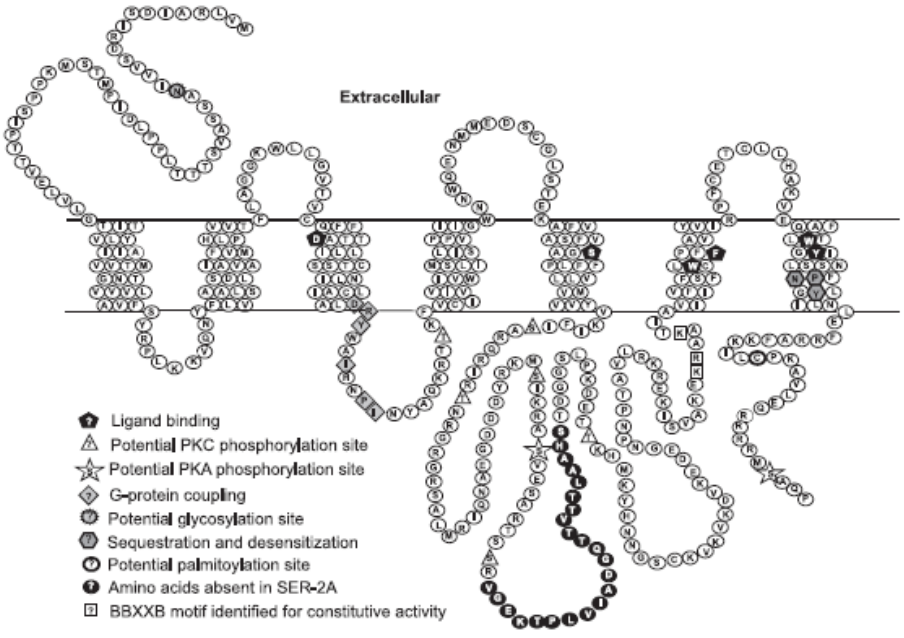


Figure 2.24: Amino acid sequence and trans-membrane models of SER-2 *(Rex and Komuniecki, 2002)*

The sensitivity of ser-2 mutants to exogenous tyramine was restored by the ser-2 genomic rescuing transgene. This indicates that the transgene is expressed in cells that are sensitive to exogenous tyramine (Donnelly et al., 2013a). SER-2 is similar to mammalian alpha(2)-adrenergic receptors, which inhibit neurotransmitter release and cause vasoconstriction during a fight-or-flight response(Figure 2.24) (Brede et al., 2004).SER-2 recptor is primarily found expressing in VD and VB in *C.elegans*.

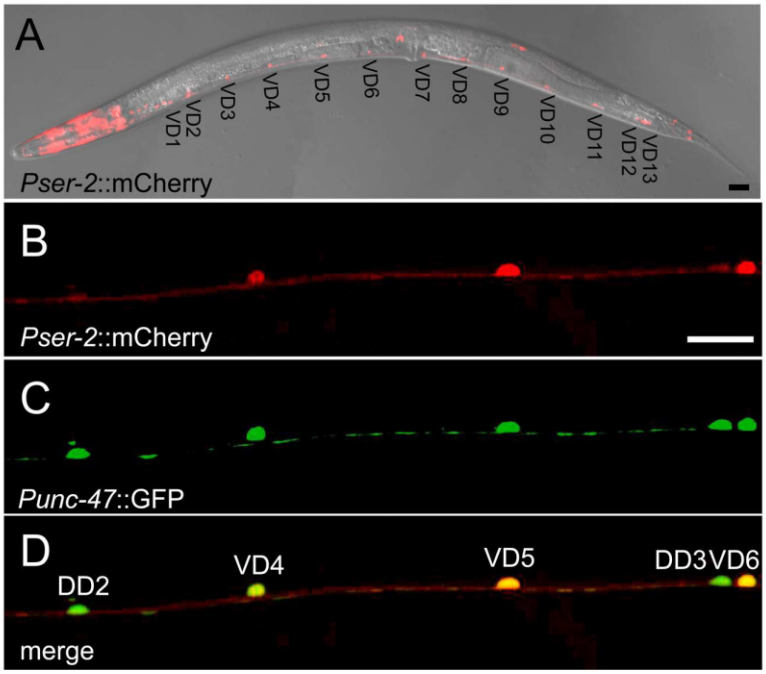


Figure 2.25 : SER-2 receptor expression in *C.elegans* *(Donnelly et al., 2013b)*

2.4.1.3 TYRA-2 and TYRA-3 receptors

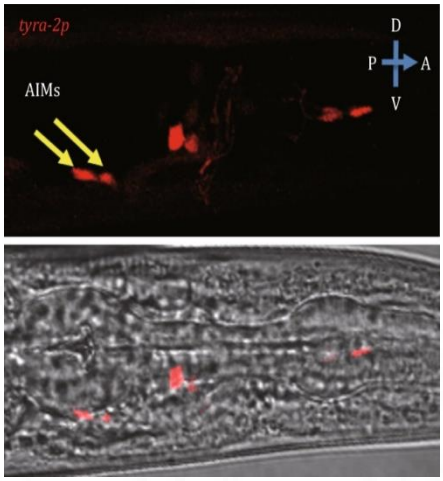


Figure 2.26: Expression pattern of TYRA-2 receptors in *C.elegans* (Fu et al., 2018; Ghosh et al., 2016)

Little is known about tyra-2, and the authors, with their results demonstrate that TYRA-2 is the receptor through which tyramine secreted by RIM controls threat-reward decision-making (Ghosh et al., 2016). TYRA-2 is a Gαi/o-coupled tyramine receptor that acts as a signal target at the peripheral site in the AIM interneurons. It receives signals from RIM/RIC neurons in the central integration circuit to suppress feeding behaviour. In summary, TYRA-2 is a receptor that helps regulate feeding behaviour by receiving signals from other neurons(Fu et al., 2018).

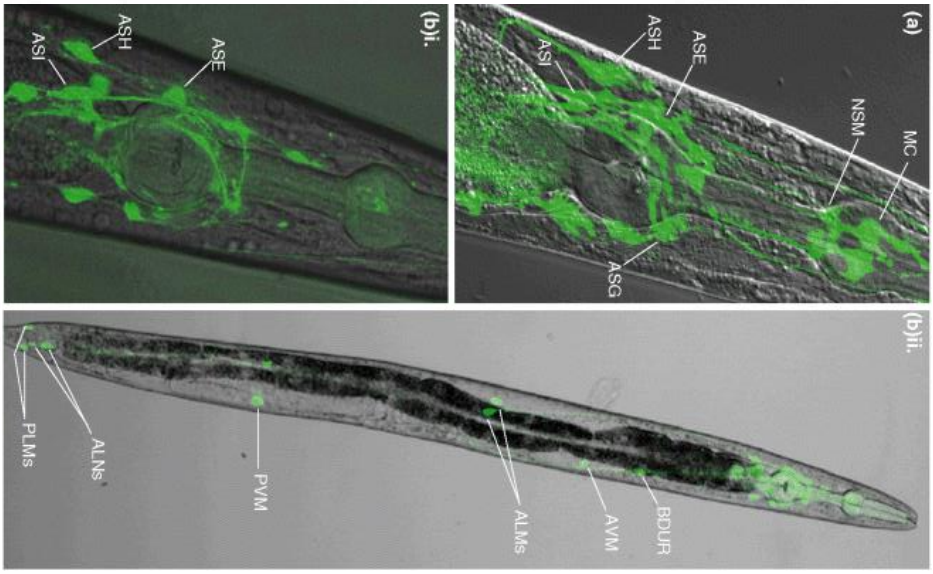


Figure 2.27: Expression pattern of TYRA-3 receptor in *C.elegans* (Hapiak et al., 2013)

TYRA-3 functions in dopaminergic, octopaminergic, and peptidergic neurons to inhibit the 5-HT stimulation of ASH-mediated aversive responses (Hapiak et al., 2013).TYRA-3 stimulates the release of multiple inhibitory peptides from the ASI sensory neurons(Hapiak et al., 2013). There is evidence that demonstrates that TA inhibits 5-HT-stimulated aversive responses through the Gq-coupled TA receptor TYRA-3 and activates a global inhibitory signalling cascade that involves a complex mix of additional monoamines, including OA and dopamine (DA), and neuropeptides (Hapiak et al., 2013).

Complex behaviours of organisms are thought to be the result of dynamic sensory input. Adrenaline, like monoamines, can play an active role in altering complex behaviours. It has been convincingly proved by previous studies that octopamine and tyramine are invertebrate analogues for epinephrine and norepinephrine. However, the exact mechanism by which they influence the behavioural outcome is not yet fully understood. We hypothesize that neurotransmitters such as tyramine and octopamine may influence the organism's adaptive memory and exploration behaviour. Tyramine receptors located near sensory neurons may play an active role in recognizing and responding to a threat signal, thereby influencing behaviour.

3. MATERIALS AND METHODS

3.1 Caenorhabditis elegans Strains

All *C. elegans* strains were grown at room temperature (20±0.5°C) on nematode growth media (NGM) with OP50 Escherichia coli as a food source. The strains used in this study, unless otherwise mentioned, were provided by the Caenorhabditis Genetic Centre (CGC, Minnesota, St. Paul). The following strains were used in this study: The WT strain N2 (Bristol); MT10549 [tdc-1(n3421) II]; MT9455 [tbh-1(n3247) X]; MT14680 [lgc-55(n4331) V]; RB1690 ser-2(ok2103) X; MT15434 [tph-1(mg280) II]; The following strains QW42: tyra-2(tm1815), VC125: tyra- 3(ok325), QW224 Pmyo-3::lgc-55 cation-II(zfEx41) were a gift from Dr Mark J.Alkema UMass, Worcester MA, USA. The strain AT255: lgc-55(n4331); ser-2(ok2103) was generated in laboratory by crossing MT14680 lgc-55(n4331) and RB1690 ser-2(ok2103). Most classes of sensory neurons and interneurons consist of two left/right homologs with similar synaptic connectivity. For simplicity, we indicated only the neuronal class in the text, with the understanding that this shorthand refers to the pair(s) of neurons.

3.2 Worm Cultivation

C. elegans were cultivated at 20°C on Nematode Growth Media (NGM)-3 g -NaC1, 2.5 g peptone, and 17 g agar dissolved in 975 ml distilled water. After autoclaving, 1 ml cholesterol in ethanol (5 mg/ml), 1 ml 1M $CaCl_2$, 1 ml 1M $MgSO_4$, and 25 ml M potassium phosphate buffer (pH 6.0) are added and plated. The plates were then seeded with OP50 E. coli using standard methods (Brenner, 1974). All washing and transfer were carried out using M9 buffer (3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 5 g NaCl, 1 mL $MgSO_4$ (1 M) per L H_2O). Worms were synchronized by hypochlorite

treatment and tested for further assays on Day 1 of adulthood at room temperature (Brenner, 2003)

3.3 Microscopy

For observation, transfer, washing, picking, etc of the nematodes. stereo microscopes (Magnus Analytics, India and Leica S8AP0 microscope) were used. For fluorescence imaging, Olympus IX51 inverted microscope (Olympus Imaging, Center Valley, PA, USA) equipped with the image acquisition software NIS Elements-Advanced Research (NIKON) and Rolera XR monochrome camera (QImaging, Canada) and Leica Microscope Model DMi8 (Leica Microsystems. Wetzlar & Mannheim, Germany) were used.

3.4 Single-Worm PCR

Adult worms were picked from a region with less OP50 and placed on hold plates to remove bacteria and L1 larvae. Worms were then dropped to PCR buffer (10 mM Tris,50 mM KCl,2 mM $MgCl_2$ (pH 8.0) final working solution added with Proteinase K at concentration -500 ng/µl) and the total volume of the extract was 10ul/worm in a PCR tube maintained Tubes were maintained at 4°C and are frozen immediately at -80° C for lysis and then placed at 60° C for 1 hour for the maximum activity of proteinase K. Proteinase K was later deactivated at 95° C for 15 minutes (Bell *et al.*, 2014). The content was centrifuged at 8000 rpm and the supernatant collected is stored at -20ºc for amplification reactions.

3.5 Agarose gel electrophoresis

Tris-acetic acid- EDTA Buffer (50X): Tris-Cl – 2M, Glacial acetic acid – 1M, EDTA disodium salt –50mM was added and the final volume was made up to 500ml with distilled water. The agarose gels were prepared using low met agarose

Molecular Biology grade agarose. The images were acquired using Vilber E-Box, Gel documentation and imaging system.

3.6 Image acquisition

The Dino-Lite EDGE model# AM4115T-GFBW and MBF biosciences worm imaging system with NIKKOR micro AF 60mm lens and Basler 35µM camera were used. The imaging of worms and video analysis was done using the Wormlab imaging system and the locomotion parameters of these mutants were measured by the WormLab 2022 (ver-2022.1.1**)** automated imaging platform (MBF Bioscience, Vermont, SA).

3.7 Image analysis

Using the video captured using a Dinolite Premiere digital microscope with a capture field-of-view of 10.7 X 8.0 mm. The software interface for the capture and behaviour analysis was from WormLab 2022 (MBF Bioscience, Williston, VT, USA). The scale set during image capture was done using the calibration scale provided by MBF Biosciences. Image analysis was carried out using Fiji (an open-source image processing package based on ImageJ). Bending angles and locomotion trajectories were computed using Image J software analysis from stills retrieved from the movies.

3.8 Generating strain AT255 - lacking both LGC 55 and SER 2 receptors

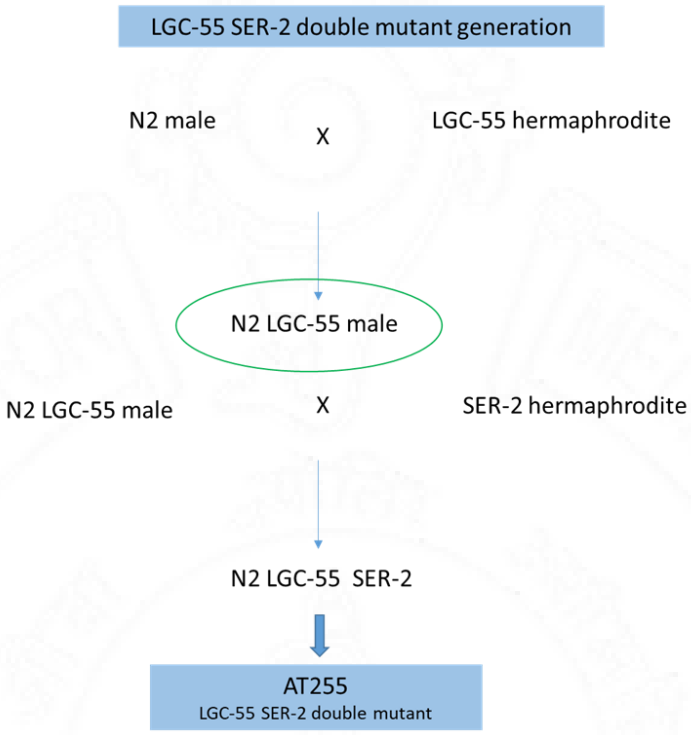


Figure 3.1: Generating double mutant strain lacking LGC-55 and SER-2 receptors
Schematic diagram depicting a cross between SER-2 receptor mutant with LGC-55 receptor mutant to generate AT255 double mutant.

C. elegans has a very distinctive mode of reproduction called androdioecy. It is known to reproduce either by self-fertilizing (selfing) in the case of hermaphrodites or by hermaphrodites (XX) mating with breeding with males (X0). Males occur in nematodes by the non-disjunction of the X chromosomes at meiosis or in the progeny of male-hermaphrodite crosses. The frequency of non-disjunction of X chromosomes is very low frequency ie; 0.1% in the laboratory, and this frequency varies with genotype and environment (Frezal and Felix, 2015). We crossed the N2

male with lgc-55(n4331) hermaphrodite. The F1 generation male was crossed with SER-2 receptor mutant (hermaphrodite) to generate the double mutant.

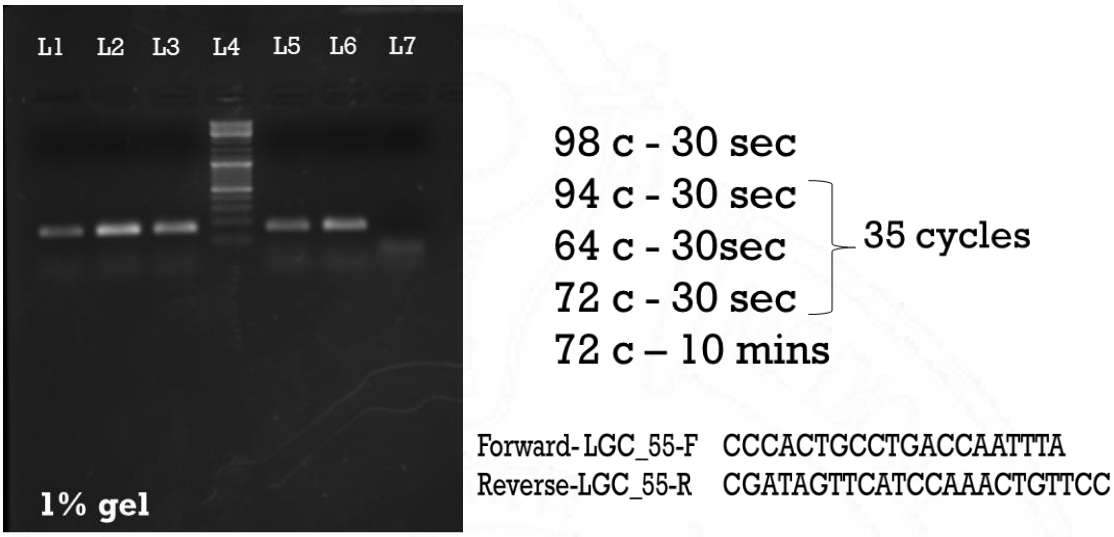


Figure 3.2: Validation of strain AT255 by PCR

a.) PCR amplification of mutated region in lgc-55 receptor (n4331) L1-N2, L2- N2, L3- Suspected worm 1 carrying the double mutation, L4- Ladder, L5- Suspected worm 2 carrying the double mutation, Lane 6- Suspected worm 3 carrying the double mutation, Lane 7- Suspected worm 4 carrying double mutation **b.)** Conditions for PCR amplification of n4331 region of lgc-55 receptor.**c.)**Forward and reverse primers targeted towards n4331 region and primers were designed using NEBuilder software (version 2.3.1).

The AT255 strain generated was isolated from the adult worms by manually scoring them for persistent head twitching while backing. Those worms were grown to generate a healthy population and were further tested to see if the phenotypic characters of both lgc-55(n4331) and ser-2(ok2103) existed in the mutant. The mutants generated were further assessed for their genotypic characters by PCR amplification of the deletion region. The primers were designed for the deletion region of n4331 at the 3rd and 4th intron (annexure I) of lgc-55 (Donnelly *et al.*, 2013). The worms with lgc-55 mutations were further backcrossed with N2 to generate a pure AT255 strain

with both ser-2 and lgc-55 phenotype. The PCR reaction was carried out using Thermo Scientific® Phusion High-Fidelity DNA Polymerase.

3.9 ASSAYS

3.9.1 Short-Term Associative Memory (STAM)

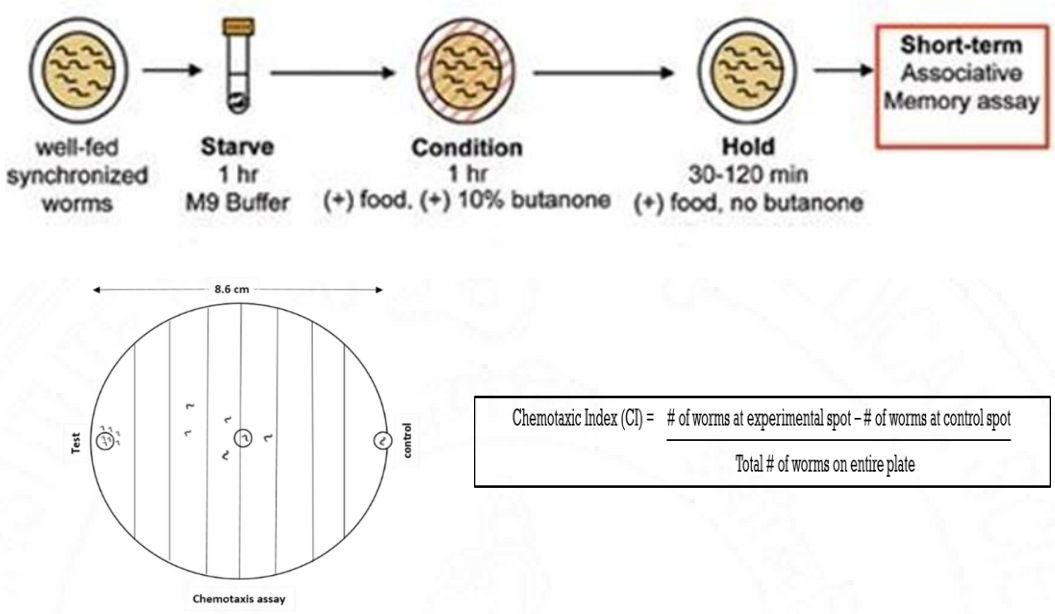


Figure 3.3: Short-term associative memory assay

a.) Illustration of short-term associative memory assay **b.)** Illustration of Chemotaxic plate where worms are placed in the centre. The test solvent is placed at the "test" spot and the diluent is placed at the "control" spot. **c.)** The formula for chemotaxic index (C.I) adapted from (M. Stain and T. Murphy, 2010; Kauffman *et al.*, 2011)

Pavlovian model reward-based associative training with 10% butanone as a conditional stimulus was given along with OP50 for 1 hour. The chemotaxis assay was carried out to assay the response towards the associated solvent. Chemotaxis assay was scored based on comparison with the worms attracted with the non-attracted counterparts to generate Chemotaxic Index (CI) (M. Stain and T. Murphy, 2010). Briefly, 50–100 day 1 adult worms were placed at the origin on a 90mm NGM plate with butanone (3 µL 10% butanone in ethanol + 1 µL NaN_3) and ethanol control + 1 µL NaN_3 equidistant from the origin (Kauffman *et al.*, 2011). The chemotaxis was

calculated as per the formula index =[(n attractant)-(n control)]/[(Total-n origin)](Kauffman *et al.*, 2011).

3.9.2 Long-Term Associative Memory (LTAM)

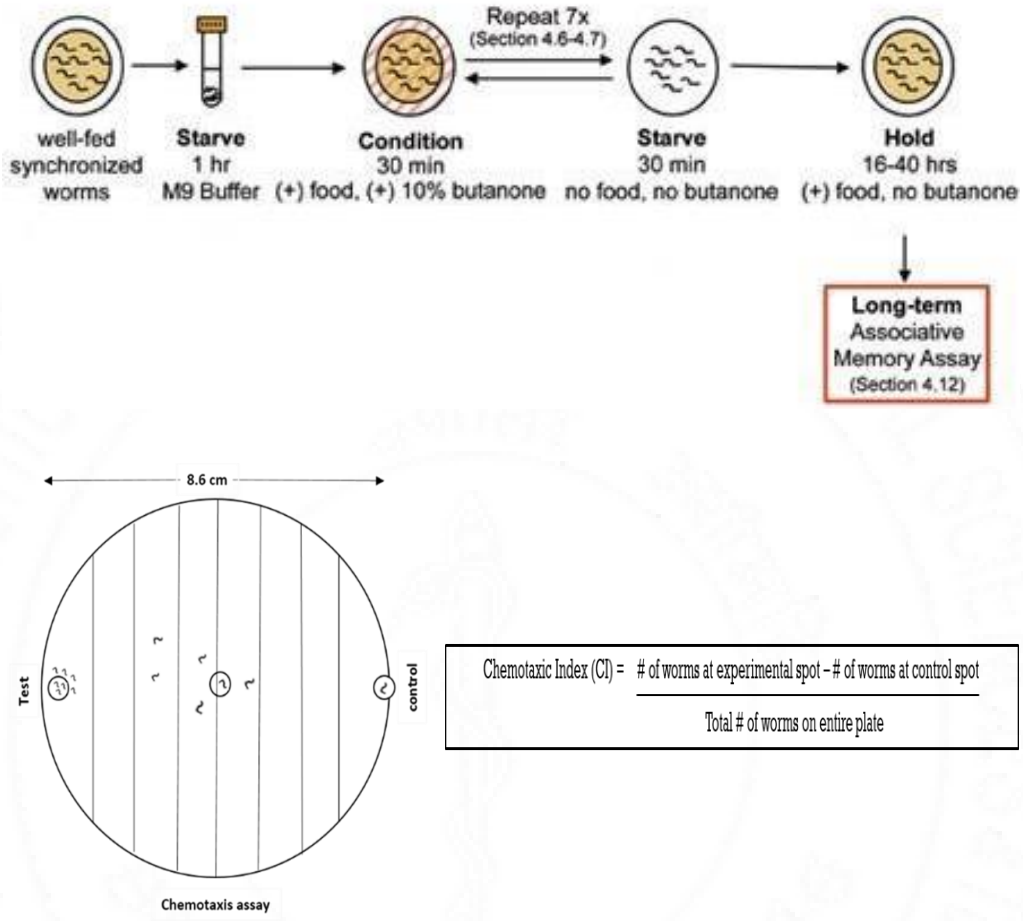


Figure 3.4: Long-term associative memory assay

a.) illustration of Long-term associative memory assay **b.)** Illustration of Chemotaxic plate where worms are placed in the centre. The test solvent is placed at the "test" spot, and the diluent is placed at the "control" spot. **c.)** The formula for chemotaxis index (C.I) adapted from (M. Stain and T. Murphy, 2010; Kauffman *et al.*, 2011)

After 1 h of starvation, worms received seven training blocks of training (30 min on training plates with food and 1/10 butanone, followed by two M9 washes and 30 min on plates without food). Worms were then tested immediately for spaced learning ("0^{th} hr") or transferred to holding plates for 16 h or 24 h chemotaxis assays

(Kauffman *et al.*, 2010). Briefly, 50–150 day 1 adult worms were placed at the origin on a 90mm NGM plate with butanone (1 µL 10% butanone in ethanol + 1 µL NaN3) and ethanol control (+ 1 µL NaN3) equidistant from the origin (Kauffman *et al.*, 2011). For tyramine treatment, the worms were treated with 5 mM TA during the conditioning period in NGM plates seeded with op50. The chemotaxis was calculated as per the formula index =[(n attractant)-(n control)]/[(Total-n origin)](Kauffman *et al.*, 2011).

3.9.3 Immobilization assay

NGM plates were prepared on the morning of the experiment by adding TA (final concentration, 5 mM- 100 mM) to liquid NGM before pouring. For the assay, worms were placed on a transfer plate for 10 min to minimize carryover and then transferred to fresh test plates and incubated for 10 min before assay. Approximately 10 worms were transferred to assay plates and scored for locomotion every minute over a 20-minute period. Following exposure, inactive ones were scored under a dissecting microscope. Worms were scored as immobilized if there was no sustained forward or backward locomotion in a 5-s time period and they did not move even when prodded with platinum wire (Wragg *et al.*, 2007).

3.9.4 Worm extract preparation

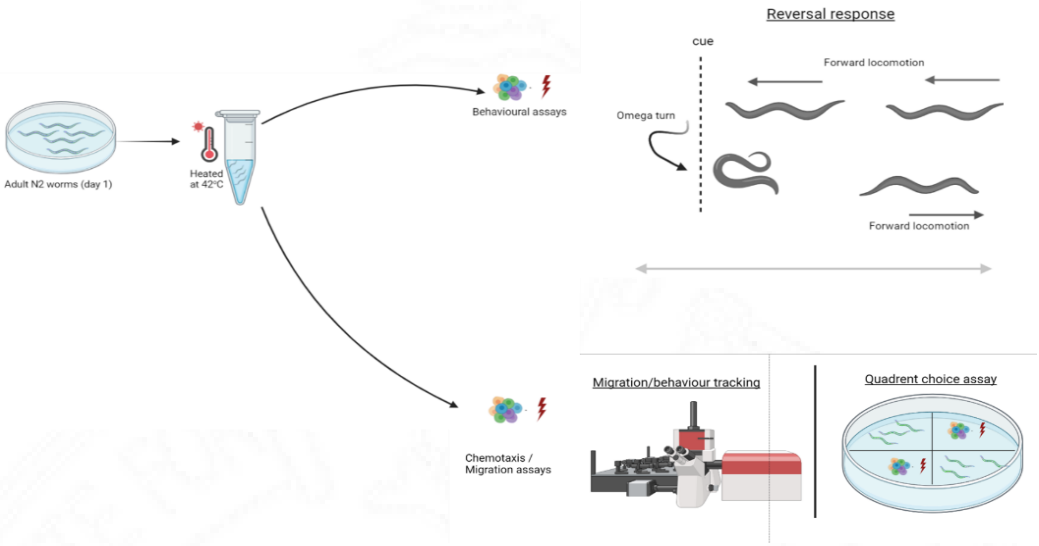


Figure 3.5: Illustration depicting the worm extract workflow

a.) Worm extract is obtained by heating the worms at 42 ºC **b.)** Reversal response of the worms is checked by presenting the worms with the extract **c.)** The worms are imaged to ensure the death fluorescence occurs at the temperature (Ex-280nm Em-480nm) and the exploratory choice assay was done as mentioned in section 3.10.5.

On day 1, adult worms were picked from a synchronized plate from an area without op50 and placed on a holding plate to remove the excess bacteria. The worms were then transferred to centrifuge tubes with distilled water at a ratio of 2 worms per 10µl. The worms were heated using a dry bath for 30 minutes at 42 ºC. It was ensured that the worms undergo death by prodding them in the head region with a platinum pick after transferring the solution along with the worms onto a cover slide after heat exposure. Separate tubes from the same set were taken and retrieved for observation of death fluorescence under the microscope at 10 mins,20 mins and 30 mins respectively. The images were acquired using a fluorescence microscope at Ex-280nm Em-480nm at different time points: 0^{th} min, 5^{th} min, 10^{th} min, 15^{th} min, 20^{th} min,25^{th} min, and 30^{th} min, respectively. For obtaining the worm extract, the tubes were centrifuged at 5000 rpm to settle the contents and the supernatant was carefully collected without disturbing the pelleted worms. The extract was presented to adult

worms as cues by drawing a barrier line in front of the worms as they moved forward. The immediate behaviour of the worms sensing the cue was scored. The reflex of escape response by immediate reversal, followed by omega turn, was scored as repulsive. If the worms moved forward after presenting the cue, then worms were scored as non-repulsive (Figure: 3.6).

3.9.5 Avoidance assay

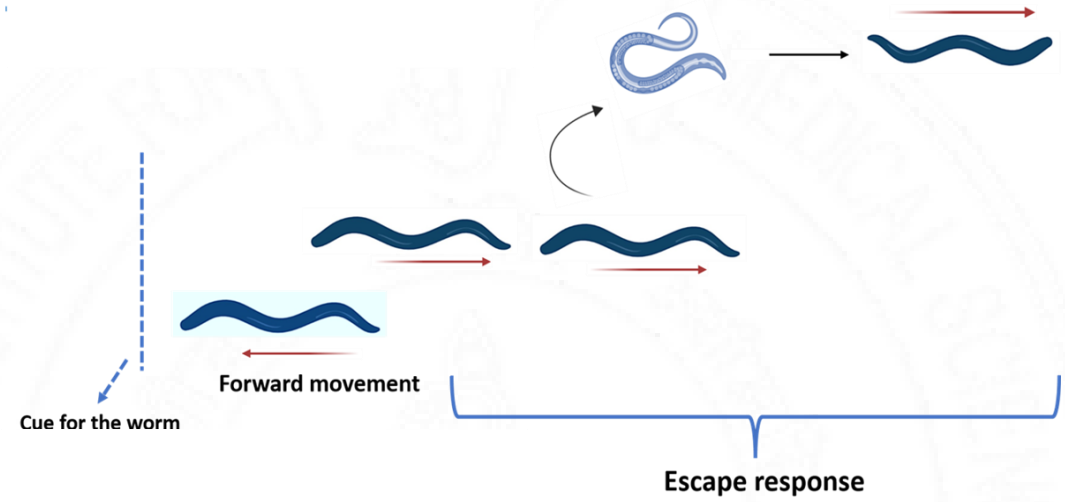


Figure 3.6: Illustration of avoidance assay with worm extract/anthranilic acid

Test solvent as cue is presented in front of the worm moving forward and subsequent repulsion or attractive behaviour of the worm is scored.

On day 1 adult worms were picked from a synchronized plate and placed on a hold plate to remove bacteria. The worms were then transferred to the assay plates without op50, an acclimatization time of 5 minutes is given before the assay. The worms were presented with the test solvent by dipping the eyelash in the solvent and drawing a barrier line in front of the worms moving forward. The worm extract obtained using earlier extraction procedures at 10 mins,20 mins and 30 mins time points was tested similarly. Anthranilic acid was presented at 1% concentration diluted in 50% ethanol. The immediate behaviour of the worms sensing the cue was scored. The reflex of escape response by immediate reversal followed by omega turn

was scored as repulsive. If the worms moved forward after presenting the cue, the worms were scored as non-repulsive. The assay was reproduced with slight modifications from (Wang, Graziano and Bianchi, 2023)

3.9.6 Exploratory choice assay

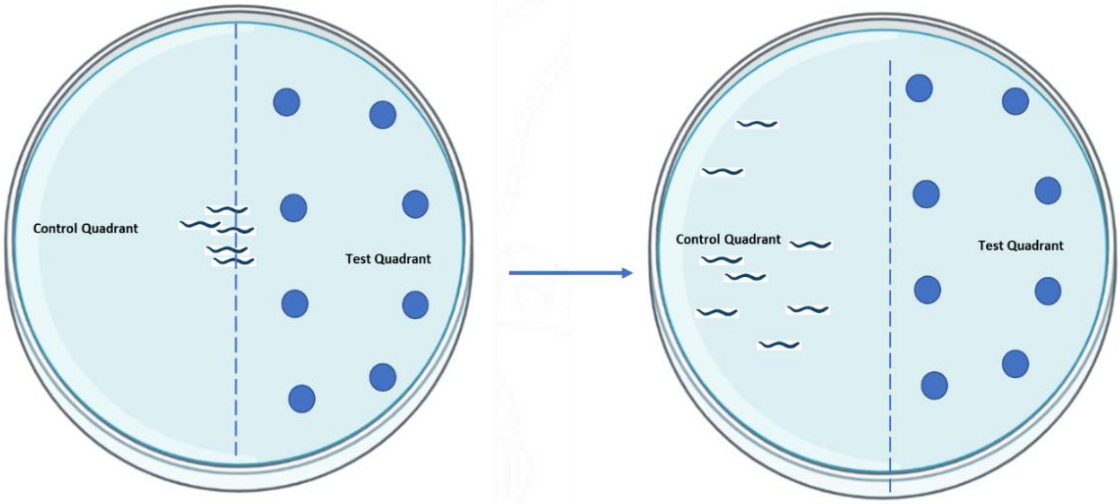


Figure 3.7: Illustration of exploratory choice assay

Test solvent was placed in one half of the plate, and worms were placed in the centre and the exploratory choice was scored after 1 hour in the control quadrant and test quadrant.

A choice-based exploration assay was conducted where day 1 adult worms were placed on a completely OP50-seeded plate in the centre. The test solution (2ul/spot) was placed equidistantly in one half of the plate, and the other half was added with diluent for the solvent. The plate was scored for the total no. of worms in each half. The exploratory choice during exploration was seen depending on the solvent. The percentage of worms was calculated as [(total no. of worms- total no. of worms in control half)X 100]/ total no. of worms. The assay was reproduced with slight modifications from (Abada *et al.*, 2009)

3.9.7 Exploratory behaviour assay/ Food-sensing Behaviour Assay

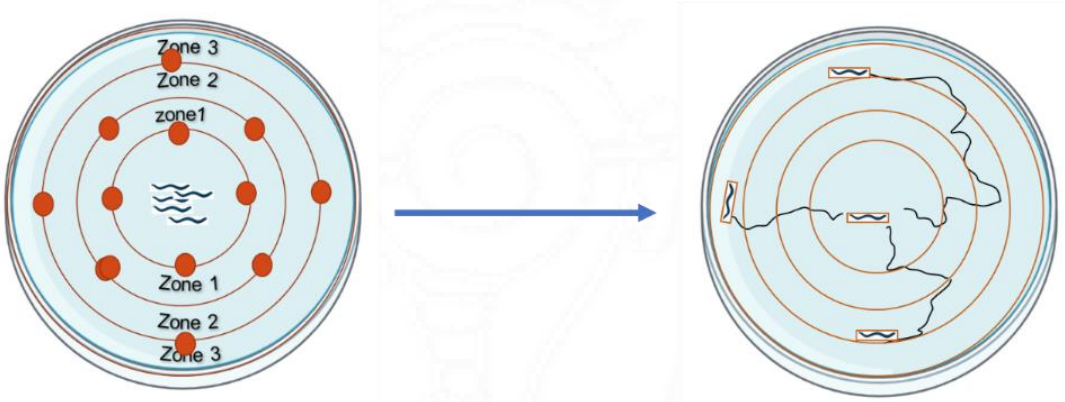


Figure 3.8: Exploratory behaviour assay

The pictorial representation of the assay plate is divided into three zones (zone 1, zone 2 and zone 3 (orange circles) and op50 is placed in different zones (orange dots). The plate is divided into concentric rings to estimate the dispersal of worms by scoring the worms exploring the food patches.

The assay relies on exploiting the exploration tendency of the worms where they had to migrate, explore, take turns and will have to rely on olfactory cues for the discovery of the food source. Adult day 1 worms were subjected to starvation for 1 hour in NGM plates and post starvation the worms were let in NGM plates with OP50 seeded at equidistant spots (diluted in LB to OD600 = 0.2 OP50) at different zones (Figure: 3.4). The plate was divided into zones 1,2 and 3 where the zone 3 being the farthest. The exploration behaviour of worms was scored by looking into trails of worms over the OP50 and an exploration score was given. Assay was reproduced with slight modifications from Pradhan et.al (Pradhan *et al.*, 2018).

3.9.8 Fructose barrier assay

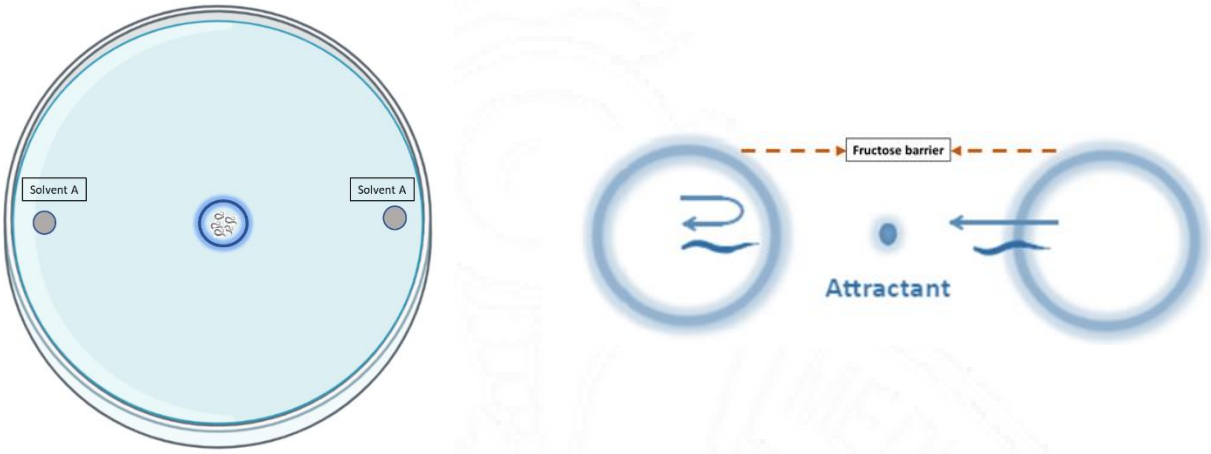


Figure 3.9: Fructose barrier assay

The pictorial representation of the assay plate where worms are placed inside 1.5 cm fructose barrier (4M) and the attractant 1/300 is placed 1cm away from the edge of the plate. The assay is scored by counting the number of worms inside and outside the ring post 60 minutes.

A ring-shaped barrier of 1.5cm diameter was drawn using fructose solution (4M) in the centre of the 90 mm NGM plate. 20-30 adult worms were transferred inside the ring and 3 ul of 1/300 IA was placed on each side of the plate, at least 1 cm from the border. One hour later, the number of worms outside the ring was counted. In the case of food deprivation assays, worms were transferred into the ring from another hold plate without food for the indicated time interval. Assay was reproduced with slight modifications from Ghosh et.al (Ghosh *et al.*, 2016)

3.9.9 Mechanosensation Assay

For every genotype, 10-20 individual worms were scored by touching the worms with a thin hair lash at the anterior end. The worms typically respond by backing away from the touch. The responsiveness of each worm was scored multiple times; for example, seven responses out of 10 touches on the anterior part were recorded as 70% responsiveness. (Hobert *et al.*, 1999)

3.10 TA-Inclusion complex with cyclodextrin

Cyclodextrin inclusion complex for slow drug release of tyramine was done by plating Cyclodextrin-TA inclusion complex (15mM TA :15mM CD) at (1:1) on NGM plates 2-3 hrs before the assay. The immobilization studies were performed as previously mentioned in section (XX). Insertion of any guest TA into the hydrophobic cavity of β-CD consequences in the chemical shift of the guest and b-CD nm. Bruker AVANCE 400 MHz equipment was used to acquire NMR spectra. UV–visible spectra were recorded by (Infinite Mplex, Multimode microplate reader; Tecan, Switzerland) and FT-IR analysis of CDs was carried out by pelleting the sample with KBr (JASCO, Japan).

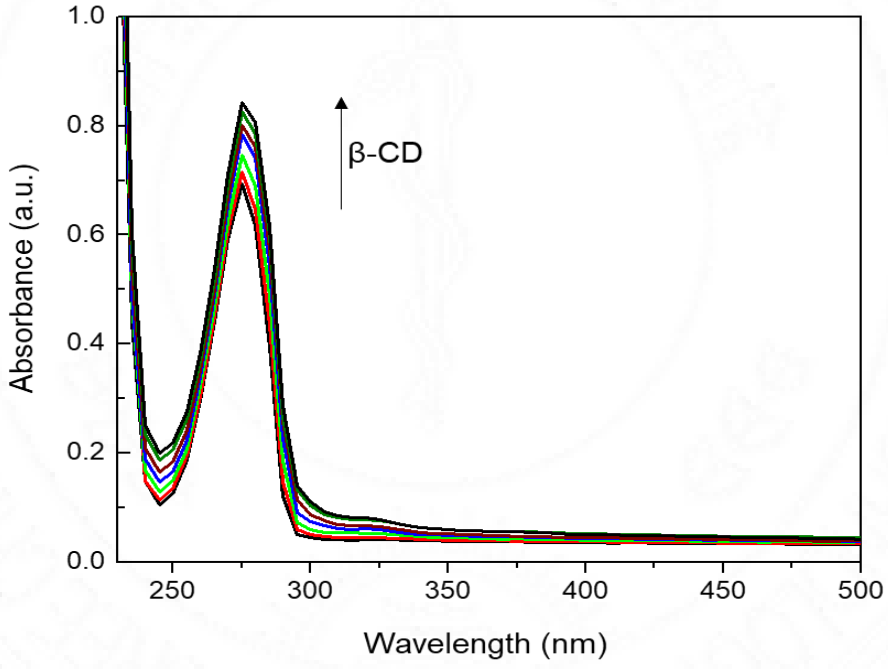


Figure 3.10: Concentration-dependant absorbance spectra of β-CD with absorbance maxima at 275 nm.

As CDs have nearly no characteristic absorption and as a result, the formation of inclusion complex of guest molecules can be studied by monitoring the absorption spectral profile of host molecules. As a result, to study the inclusion complex

formation the absorbance spectral studies of tyramine hydrochloride were carried out in varying concentrations of β-CDs. As shown in Figure 3.10, tyramine exhibits a characteristic absorption maximum at 275 nm owing to the phenyl group (Roy et al., 2016b). It is apparent that with the increase in the concentrations of β-CDs, the absorption intensity of tyramine at 275 nm significantly increased, while the concentration of neurotransmitters remains the same. This suggests that tyramine interacts with β-CDs and resulted in an increase in its solubility. It is to be noted that the increase in absorption intensity of tyramine at 275 nm is consistent with the addition of β-CDs suggesting the initiation of inclusion phenomena between tyramine and β-CDs. It has been reported earlier that CDs are capable of forming both inclusion and non-inclusion complexes. The formation of the inclusion complex is usually associated with the increases in molar absorption of guest molecules at a particular wavelength with respect to the concentration of β-CDs. Thus, in the present case, the increase in absorption intensity of tyramine clearly indicates the inclusion of complex formation with β-CDs. Tyramine with its hydrophobic aromatic ring with phenolic -OH group could readily undergo host-guest interaction with β-CDs. The data obtained from the absorption spectral studies was then utilised to construct the Benesi-Hildebrand plot by using the following equation to determine the binding constant and nature of binding. The calculations were made according to (Roy et al., 2016a)

$$\frac{1}{\Delta A} = \frac{1}{(\Delta\varepsilon)[\text{tyramine}]} + \frac{1}{(\varepsilon_1 - \varepsilon_0)[\text{tyramine}]k_{bin}} \frac{1}{[\beta - \text{CDs}]} \dots$$

where, DA corresponds to the difference in absorption intensities of tyramine in the absence and presence of β-CDs (DA = (A_0-A), A_0 is the absorption intensity of tyramine in the absence of β-CDs and A is the absorption intensity of tyramine at a given concentration of β-CDs, [tyramine] is the initial concentration of tyramine and K_{bin} is the binding constant. As shown in Figure 3.11, it is apparent that a good linear relationship was obtained for the plot 1/ΔA vs 1/[β-CDs]. It has been previously reported that a linear plot is indicative of 1:1 complexation between the tyramine and β-CDs. As a result, from the double reciprocal plot, it can be inferred that the stoichiometry ratio for the formation of inclusion complex between tyramine and β-CDs is 1:1 with a binding constant of Kbin = 10.15 M^{-1}, which is in good agreement with the previously reported works.

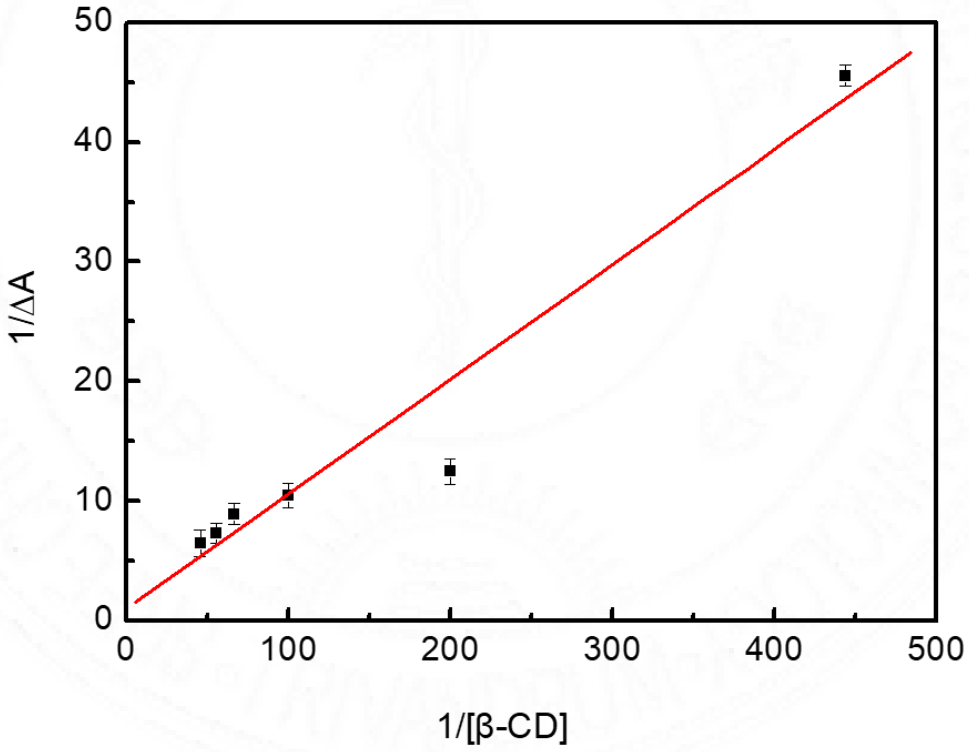


Figure 3.11: Benesi-Hildebrand plot for β-CD inclusion complex formation .

3.10.1 FT-IR and ^{1}H NMR spectral studies

FT-IR and ^{1}H NMR spectral studies of tyramine, β-CDs and tyramine-β-CDs were carried out to ascertain the inclusion of complex formation. Researchers have used FT-IR and ^{1}H NMR to establish the formation of inclusion complexes of various guest molecules and neurotransmitters with α-CDs and β-CDs, respectively. The FT-IR analysis of tyramine, β-CDs and tyramine-β-CDs was carried out to ascertain the inclusion of tyramine within the cavity of β-CDs (Figure 3.12). As shown in Figure 3.12a, the FTIR spectrum of tyramine exhibits a broad band at 3106 cm^{-1} corresponding to the vibration of amine The band at 1598 cm^{-1} is ascribed to the C=C bond of the phenol group. The C–N stretch appears between 1385-1023 cm^{-1}, while the bands between 832-620 cm^{-1} correspond to the N-H wag. The FT-IR profile of β-CDs shown in Figure 3.12b exhibit bands at 3352 and, 2921 cm^{-1} ascribed to the O-H stretching and C–H stretching vibrations, respectively. The asymmetric stretching vibration of C–O–C glycosidic bridge appears at 1154 cm^{-1}. The bands located at 1080, and 1039 cm^{-1} are ascribed to the C–O stretching vibration, and C–C stretching vibration, respectively. It is obvious from Figure 3.12c that the FT-IR spectrum of the inclusion complex of tyramine-β-CDs exhibits bands at 1648, 1417 and 1327, 857 cm^{-1} which can be attributed to the presence of tyramine within the cavity of β-CDs. The slight shift in the band position of tyramine suggests the possible interaction between tyramine and β-CDs. Moreover, the bands corresponding to the O-H, C-H stretching vibrations of β-CDs also exhibited a slight shift to 3344 and 2924 cm^{-1}, respectively. The C–O–C glycosidic and C–C stretching vibrational bands appeared at 1154 and 1032 cm^{-1} without any noticeable shift.

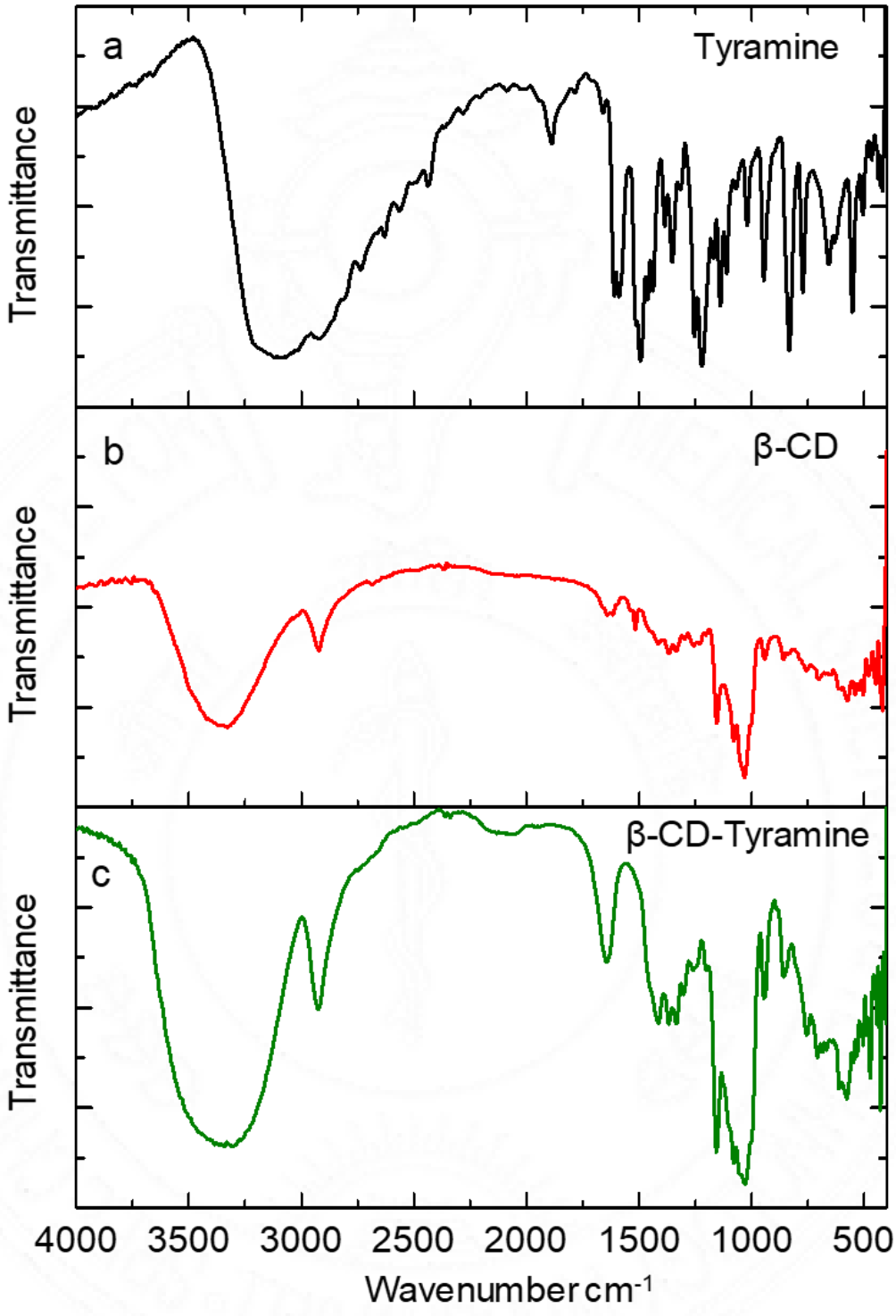


Figure 3.12 - FTIR profile of a) tyramine alone b) β-CD alone c) β-CD- Tyramine inclusion complex respectively

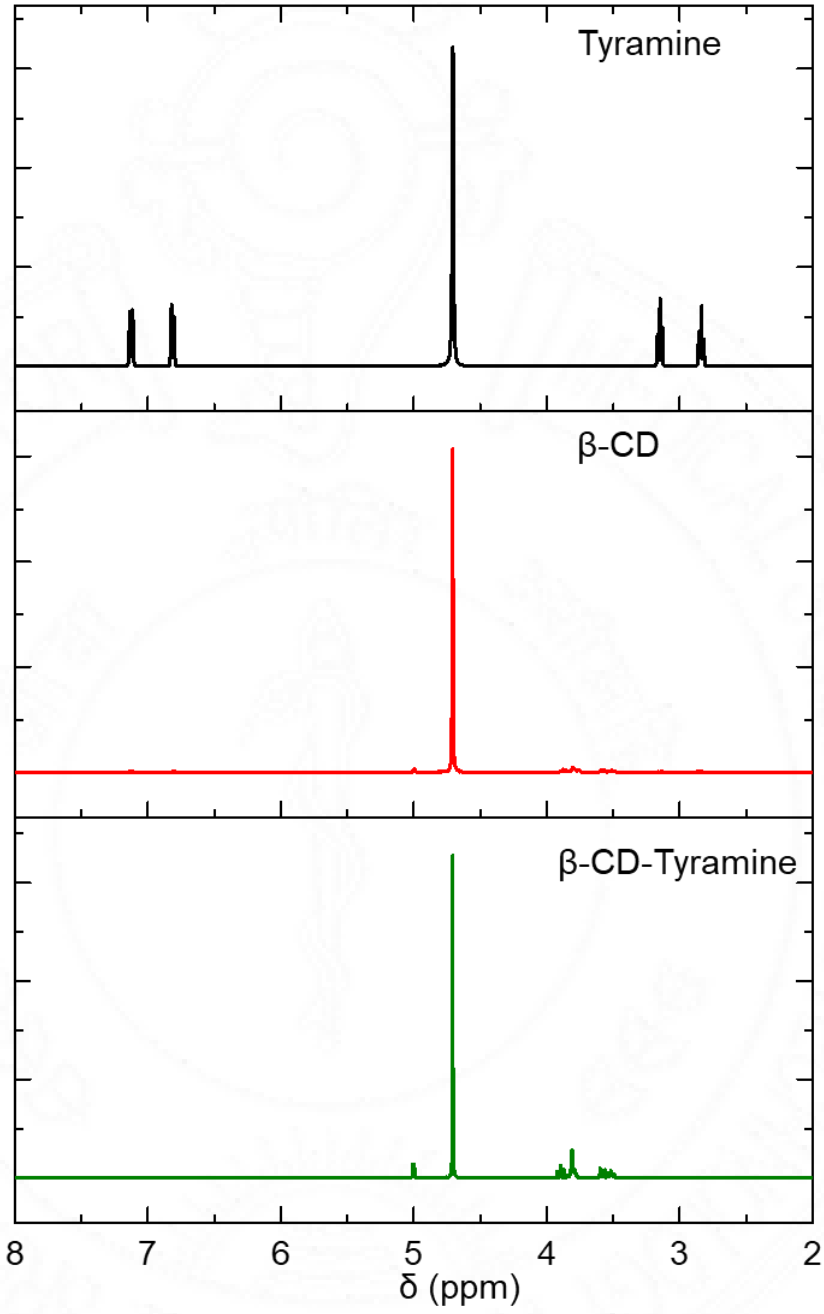


Figure 3.13: NMR profile of a) tyramine alone b) β-CD alone c) β-CD- Tyramine inclusion complex respectively

Figure 3.13 shows the ^{1}H NMR profile of tyramine exhibiting distinctive signals for both pairs of aromatic ring protons namely for “a” pair and “b” pair at 6.9 and 7.2 ppm, respectively(Zhumakova et al., 2022). The chemical shifts at 2.9 and 3.2 ppm are attributed to the aliphatic side chain protons(Zhumakova et al., 2022). The ^{1}H NMR of β-CDs displayed in Figure 3.13 b shows chemical shifts at 3.49, 3.54 ppm corresponding to the protons H-2 and H-4 located in the outer portion of the β-CDs, respectively(Roy et al., 2017; Zhumakova et al., 2022). The shifts at 3.60, 3.79, 3.85 and 3.99 ppm are due to the H-5, H-6 and H-3 protons located in the inner hydrophobic bonding surface of β-CDs, respectively(Roy et al., 2017; Zhou et al., 2012; Zhumakova et al., 2022). The ^{1}H NMR spectrum of tyramine-β-CDs inclusion complex displayed in Figure 3.13 c clearly shows that all the protons corresponding to β-CDs exhibit upfield shift changes(Ali et al., 2007; Maheshwari et al., 2013; Roy et al., 2016b). The observed upfield shift is attributed to the magnetic anisotropy affects within the cavity protons of β-CDs, owing to the inclusion of tyramine(Maheshwari et al., 2013; Roy et al., 2017). Furthermore, the protons of tyramine also showed significant upfield shift changes as compared to that of pure tyramine. The upfield shift of β-CD cavity protons and protons of guest tyramine, is a clear indication of tyramine-β-CDs inclusion complex and it is consistent with the literature reports(Maheshwari et al., 2013; Roy et al., 2016b). Thus, from the FT-IR and ^{1}H NMR analysis, it can be inferred that tyramine has complexed within the hydrophobic cavity of β-CDs to form an inclusion complex.

3.11 Statistical analysis

GraphPad Prism versions 8 and 9 (GraphPad Software Inc.) was used for graphical representation and statistical analysis of the data. The data are presented as

Mean + SEM as indicated. Significance was represented as follows *p<0.05, **p<0.01, ***P<0.001, ****p<0.0001, ns – non-significant.

4. RESULTS

4.1 Tyramine influences bending pattern in C.elegans

The omega turn is the classical escape response behaviour exhibited by the *C.elegans* in case of a threat or when a sharp change in direction is needed. During an omega turn, the largest change in direction is generated, and the worm's body shape resembles the Greek letter omega Ω (Schafer, 2015b) (Figure 4.1a). The nose mechanosensory neurons aid threat avoidance when worms hit head-on with an object during forward locomotion (Donnelly et al., 2013b), when exposed to a stimulus, they typically crawl backwards away from it, exhibiting an escape behaviour similar to that evoked by anterior body touch or a nociceptive signal (Kaplan and Horvitz, 1993). SER-2 receptors expressed in VD and VB in *C.elegans* are necessary for the omega turn, and the lgc-55 ligand-gated ion channels expressed in RMD and SMD neurons are required for the suppression of head twitching in worms(Donnelly et al., 2013b). A healthy wild-type N2 worm, while encountering an anterior threat, immediately ceases forward locomotion and suddenly reverses by suppressing the head twitches. This is followed by an omega-shaped bend, which facilitates the worm to make a complete turn from the initial direction of travel. A classical omega bend has the characteristic head-to-tail touch and a false angle omega turn is characterized by no head-to-tail touch during omega turns (Figure 4.1a). To test if the worms take a closed omega turn, the omega turn was examined, where worms were observed under the microscope after being prodded on the head region. If the worm's nose touches the tail

during the execution of the turn (closed omega turn), it was taken as a proper omega turn and if worm without head-to-tail touch is scored as a false angle turn.

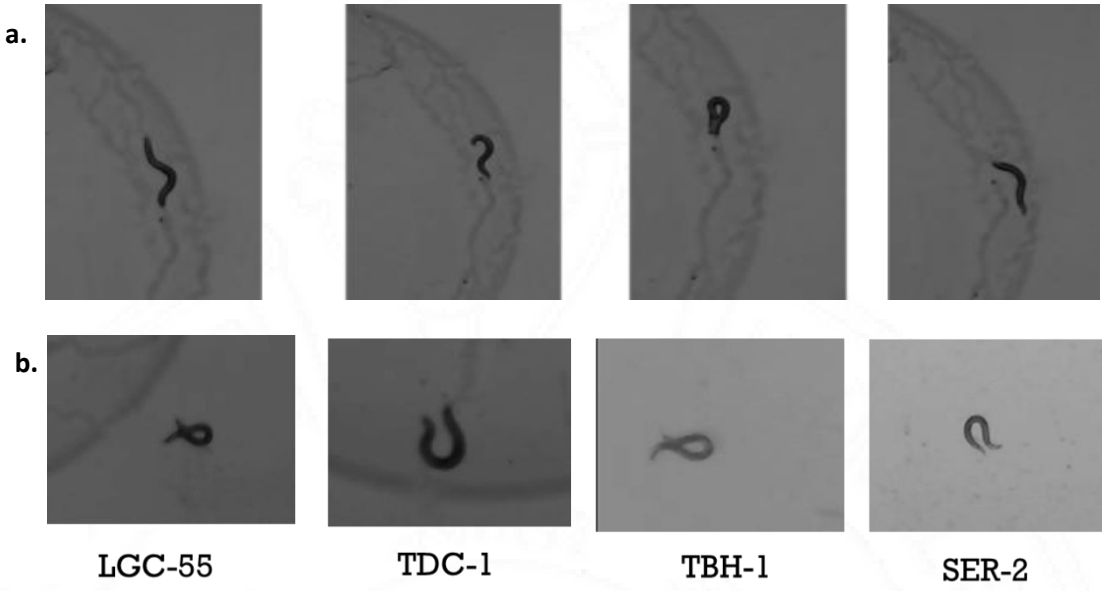


Figure 4.1: **Omega turn response of tyramine mutants**

a.) Omega turn response of tyramine mutants- N2 worms displayed omega turn with head-to-tail touch **b.)** Omega turn display in tyramine biosynthesis mutants (MT10549 [tdc-1(n3421) II] , MT9455 [tbh-1(n3247) X]) and receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X) respectively.

N2 worms exhibited proper omega turns with head-to-tail touch (84.3±1.155%) compared to incomplete omega turns (Figure 1a). LGC-55 receptor mutant (MT14680 [lgc-55(n4331) V] displayed normal omega turns (79.33±4.04%) with head-to-tail touch (Figure 4.1b) which was comparable to N2. However, had persisting head twitching while reversing before omega turns. Notably, SER-2 receptor mutant strain (RB1690 ser-2(ok2103) X) exhibited suppressed head movements during reversals and had an impaired head-to-tail touch (31.9±2.7%) during the omega turns(Figure 4.1b). The omega percent of proper omega turn was significantly lower than wildtype N2($P<0.0001$).Similarly, the double mutant strain of LGC-55 and SER-2 receptor (AT255: lgc-55 (n4331); ser-2(ok2103)) exhibited

incomplete omega bend(25.0±5%) without head-to-tail touch which was significantly lower in comparison with N2 (P<0.0001).

We tested the omega bend characteristics of tyramine-deficient tdc-1 mutant (MT10549 [tdc-1(n3421) II]) worms, which don't possess tyramine and octopamine due to the absence of (tyrosine decarboxylase-1 enzyme). The tdc-1 worms showed incomplete omega bends (26.03±3.57) (Figure 4.2), which is comparable to loss of function mutant ser-2 (ok2103) and N2 (P<0.0001). Meanwhile, the tbh-1 enzyme mutants only lack octopamine and are seen executing complete omega turns (68.3±2.88). Other receptor mutants for tyramine such as TYRA-2 (QW42: tyra-2(tm1815)) and TYRA-3 (VC125: tyra- 3(ok325)) receptor mutant worms were tested. The tyra-2(tm1815) mutant exhibited proper omega turns (70.9±5.23). Similarly, the tyra-3 (ok325) worms exhibited proper omega turns (84.267±7.39) comparable with N2(Figure 4.2).

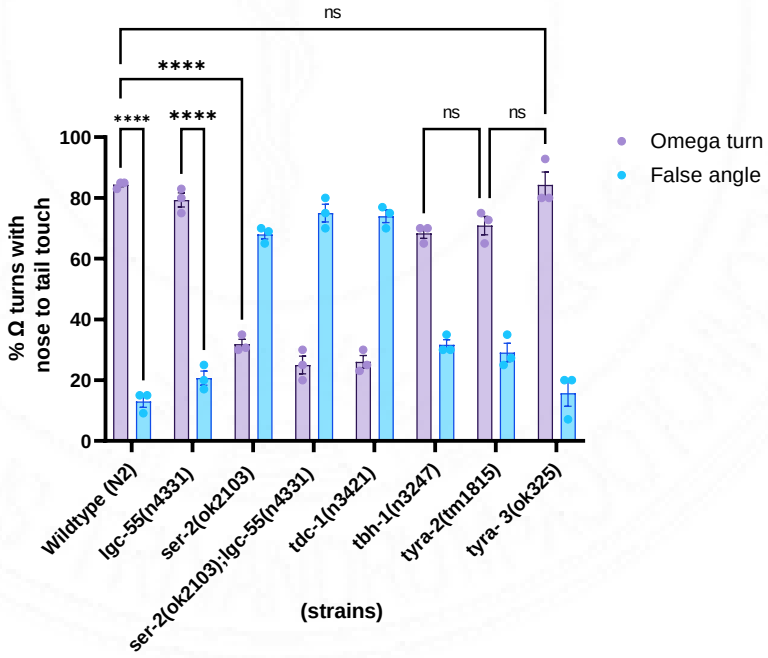


Figure 4.2: **Percentage omega turn response of tyramine mutants**

Precent omega turn display in wildtype N2, receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X, AT255: lgc-55(n4331); ser-2(ok2103), QW42: tyra-2(tm1815) & VC125: tyra-

3(ok325) and tyramine biosynthesis mutants (MT10549 [tdc-1(n3421) II] , MT9455 [tbh-1(n3247) X]) respectively. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; ns-non significant $n \geq 20$ worms 3 trials each

The angle of escape trajectory after an omega turn was measured in the worms which took an omega turn in NGM plates. The wildtype (N2) worms displayed proper omega turns with head-to-tail touch and the angle of escape (18.71±5.54º) (Figure 4.3). However the biosynthesis enzyme (tyrosine decarboxylase-1) mutant tdc-1(n3421) which lacked tyramine and octopamine displayed omega turns devoid of head-to-tail and had an significantly larger angle (79.98 ±24.4º) (Figure 4.3) On the other hand, the TBH-1 (tyramine beta hydroxylase-1) enzyme mutant showed normal omega turns with proper head-to-tail touch and angle of escape being (48.8 ±5.6º) (Figure 4.3) We then tested the escape angle of LGC-55 receptor mutant, which was not statistically different to the escape angle of N2 wild type (28.09±11.49º) (Figure 4.3). However, the SER-2 receptor mutant ser-2(ok2103), which lacked SER-2 receptor, was observed taking an escape angle of (81.9±11.1º) which was significantly higher than N2 with ($p<0.0001$) (Figure 4.3). Similarly, the AT255: lgc-55(n4331); ser-2(ok2103) double mutant for SER-2 and LGC-55 tyramine receptor was observed taking an escape angle of (78.2±19.45º) (Figure 4.3). The influence of tyramine and SER-2 receptor in omega bend angle was previously reported by Donnelly JL et.al.(Donnelly et al., 2013b). The angle of escape in tdc-1(n3421), AT255: lgc-55(n4331); ser-2(ok2103) and ser-2(ok2103) had increased angle of escape and were statistically not different (P- non-significant) from each other. Our results support this observation and also show the influence of SER-2 receptor in migration and escape responses as the worms with mutated receptor for tyramine had a significant change in the trajectory

of their escape, which could possibly affect the migration pattern and escape evading a predator in its natural habitat.

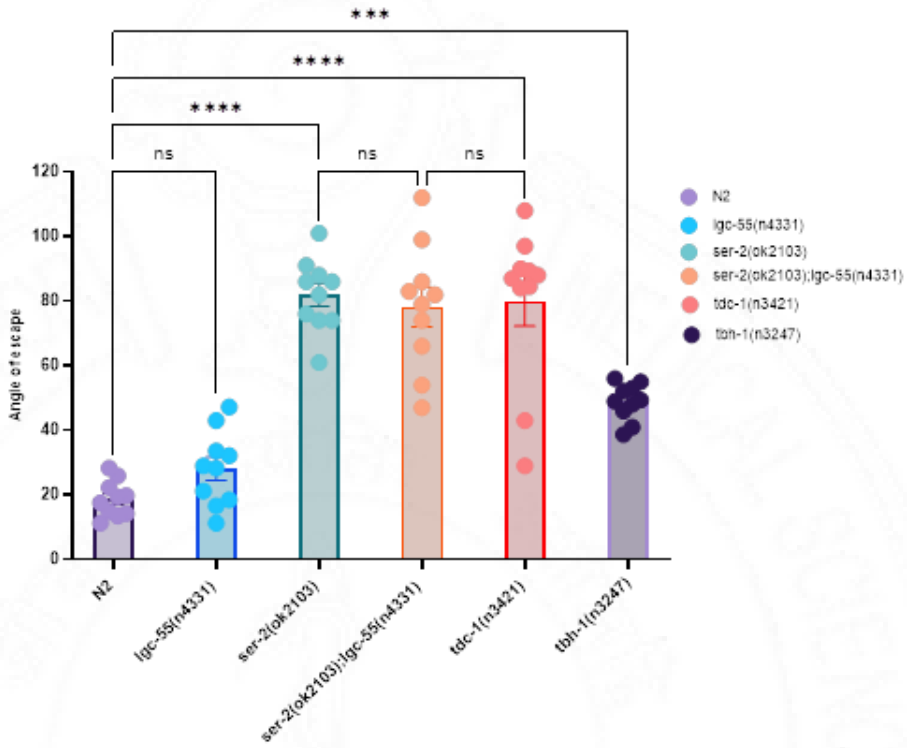


Figure 4.3: **The escape angle of *C.elegans* is influenced by tyramine**
The angle of escape in NGM plates measured for wildtype N2, receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X , AT255: lgc-55(n4331); ser-2(ok2103)) and tyramine biosynthesis mutants (MT10549 [tdc-1(n3421) II] , MT9455 [tbh-1(n3247) X]) respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test and significance represented as ****- p<0.0001; ***- p<0.001, ns-not significant (n≥10 worms)

4.2 Exogenous tyramine activates ionotropic and metabotropic receptors causing immobilization

Exogenous tyramine is known to cause immobilization in worms(Donnelly et al., 2013b) and induces immobilization through the activation of SER-2 and Gαo signalling pathway in the GABAergic neurons(Donnelly et al., 2013b). To study whether exogenous tyramine could interact with tyramine receptors, immobilization assay was performed. At concentration 30mM TA wild-type (N2) exhibits a (91.66±2.887) immobilization by the 20th minute (Figure 4.4) compared to the

control($p<0.0001$). This can be attributed to receptors for tyramine intact and are highly susceptible to hyperpolarization of muscles due to tyramine action (Donnelly et al., 2013b). Meanwhile, SER-2 receptor mutant ser-2(ok2103) worms showed resistance to the immobilization (30.667±4.04) ($p<0.0001$). The SER-2 receptor mutant ser-2(ok2103) has other receptors activated by tyramine intact.

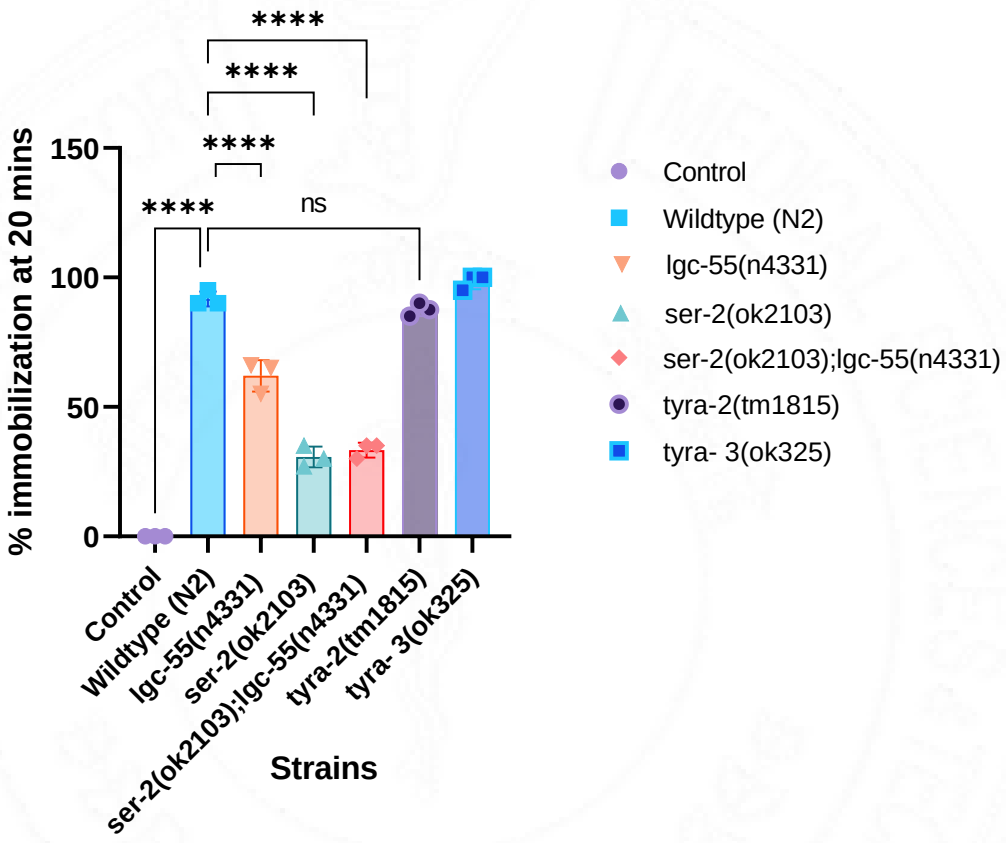


Figure 4.4: **Immobilization of tyramine mutants with exogenous tyramine 30mM TA**
The immobilization pattern of wildtype N2, receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X , AT255: lgc-55(n4331); ser-2(ok2103), QW42: tyra-2(tm1815) & VC125: tyra-3(ok325) respectively in 30mM TA. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented. Significance indicated as ****-$p<0.0001$; ns-non significant $n\geq 20$ worms 3 trials each

However, the resistance to immobilization shows the active role of the SER-2 receptor in locomotion and movement. The LGC-55 receptor mutant lgc-55(n4331) worms showed nearly (62.0±6.08) immobilization (Figure 4.4) but was statistically significant (P<0.0001) compared to wildtype(N2). However, there was no head twitching arrest, which is the characteristic feature attributed to the LGC-55 receptor through their presence in RMD and SMD neurons. The GPCR mutants tyra-2(tm1815) and tyra- 3(ok325) showed immobilization and head twitching arrest like N2 and exhibited omega turns at (87.5±2.5) and (98.33±2.887) respectively. This suggests the critical role of SER-2 and LGC-55 in locomotion regulated through tyramine.

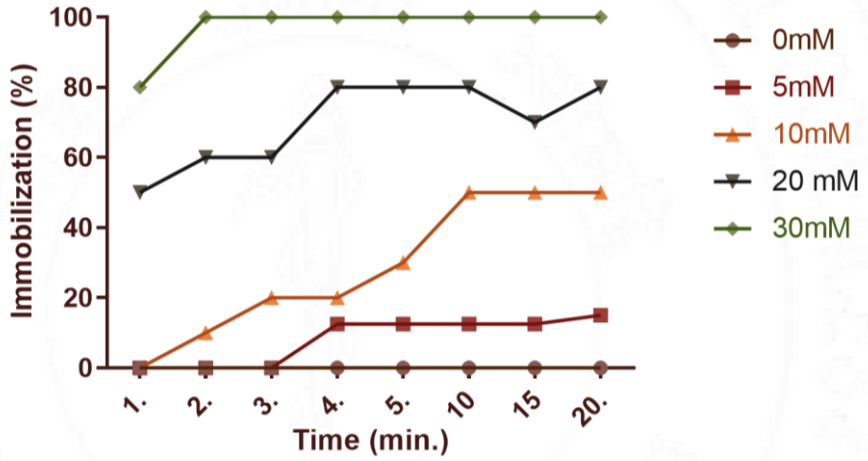


Figure 4.5: **Immobilization of Wildtype Bristol N2 worms**

The immobilization pattern of Wildtype Bristol N2 worms at 0mM,5mM,10mM, 20mM and 30mM respectively. n≥ 20 worms

The tyramine immobilization of worms was further explored by verifying concentrations ranging from 0 mM to 30mM. The immobilization was scored by prodding the non-motile worms to score their movement. The study looked into the immobilization pattern of the worm, and it was seen that by an initial 2-3 minutes 100% immobilization was observed at 30mM concentration and over 50%

immobilization was seen at an initial 1 minute (Figure 4.5- data points represented in diamond shapes). The 20mM concentration showed nearly 80% immobilization by the 20th minute, and 50% immobilization was observed by 1 minute of exposure (Figure 4.5-data points represented as inverted triangles). At a 10mM concentration of tyramine, the worms exhibited 50% immobilization by the 10th minute and this immobilization was sustained till the 20th minute (Figure 4.5- data points represented as upright triangles). The 5mM concentration of tyramine caused an immobilization of ~10% even at the 20th minute (Figure 4.5 -data point represented in -square box). Hence, the concentration of 5mM of tyramine was in further assays and it was also observed that at these concentrations the migration ability of the worm is minimally affected.

4.3 Tyramine influences the exploration/ migration of worms

The foraging pattern of the worm in its native environment, where they had to explore for food, show omega turns and reorientations to reach the food source using its olfactory and exploratory senses. The principle of the exploratory assay is described by Charnov's marginal value theorem (Charnov, 1976), which proposes that the optimal time for a worm to leave a foraging ground occurs when local resource levels fall below the average level in the entire habitat. For the exploratory assay, the food OP50 was placed at 0.2 O.D. in different zones and worms were placed worms in the centre of a 90mM plate (see methods for details). The zones till which the worms explored were observed post one hour and scored according to the track and food patches explored.

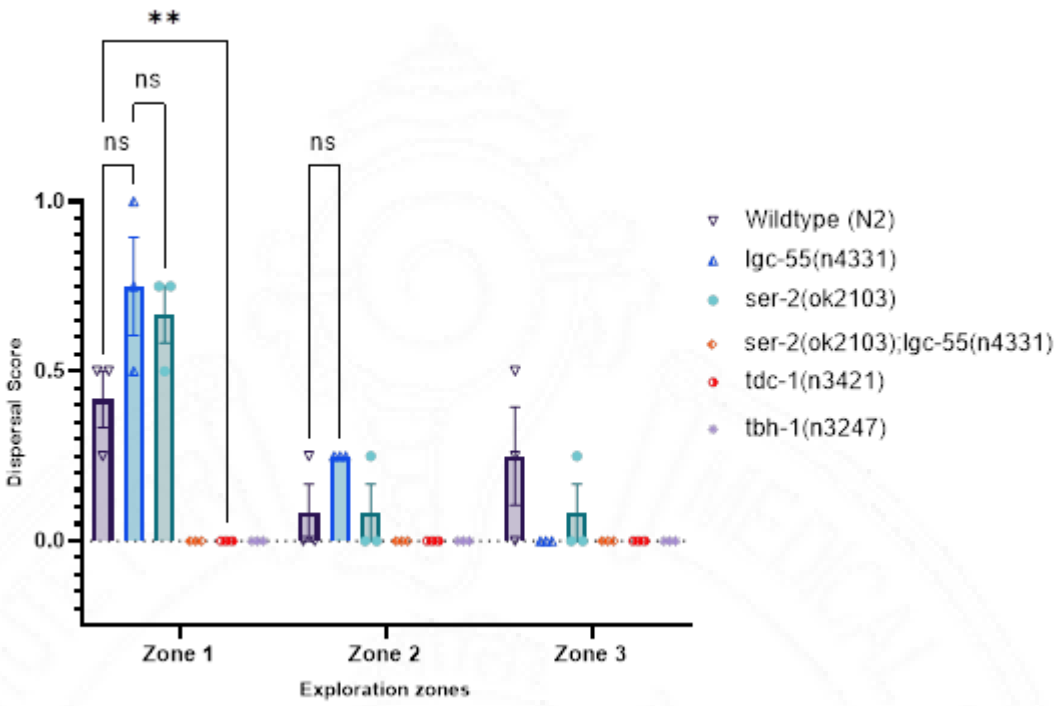


Figure 4.6: Exploration behaviour of tyramine mutants

Exploration behaviour of worms in different distances as zone 1, zone 2 and zone 3 respectively. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated **- $p<0.005$; ns- non significant $n\geq12$ worms 3 trials each.

The exploration of the worms would require olfactory cues and exploration manoeuvres to find food sources. The N2 worms exhibited exploration of food patches up to zone 3 for 1-hour time period (Figure 4.6). The SER-2 receptor mutant worms (RB1690 ser-2(ok2103) X) with the omega turn defects showed normal exploration similar to that of N2 worms (Figure 4.6), it was seen that ser-2 worms displayed exploration tendency reaching till zone 3. Surprisingly, the LGC-55 receptor mutant worms (MT14680 [lgc-55(n4331) V]) did not cross past zone 2 (Figure 4.6) - this raised the notion that LGC-55 might be defective in olfactory perception during chemotaxis. The double mutant AT255 worms (AT255: lgc-55(n4331); ser-2(ok2103)) with SER-2 and LGC-55 mutant receptors displayed exploration only limited to initial zone 1. (Figure 4.6).

Table 4.1: Exploration behaviour of tyramine mutants

Depicting the exploration pattern of different mutants according to different distances represented as zones (Zone 1 - 1.5cm diameter, Zone 2 -2.5cm diameter and Zone 3-3.5cm diameter respectively).

Strain	Zone 1 (1.5cm)	Zone 2 (2.5cm)	Zone 3 (3.5cm)
Wildtype (N2)	✓	✓	✓
lgc-55(n4331)	✓	✓	×
ser-2(ok2103)	✓	✓	✓
ser-2(ok2103);lgc-55(n4331)	×	×	×
tdc-1(n3421)	×	×	×
tbh-1(n3247)	×	×	×

4.4 Influence of exogenous tyramine on Short-Term Memory

To test the role of tyramine in olfactory adaptation, the study tested adaptive learning memory in the worms using a Pavlovian model classical conditioning paradigm (see Methods for details) using 1/10 butanone as a conditional stimuli. A significant increase in the 0^{th}-hour post-conditioning was observed in wild-type (N2) ($p<0.0001$) worms (Figure 4.7). When subjecting worms to short-term training, it was observed that in the presence of TA, an increased retention of memory post-normal 3^{rd} hour ($p<0.005$) was observed compared to the N2 control (Figure 4.7). The wild-type Bristol N2 worms exhibited normal short-term adaptation and showed a chemotaxis index (CI-0.737±.040) post-training (Figure 4.7). This was not significantly affected by the external addition of tyramine during the short-term conditioning at the 0^{th} hour.

Since 0th-hour memory was not affected by the presence of exogenous tyramine, this time point was used as the reference for various mutant strains.

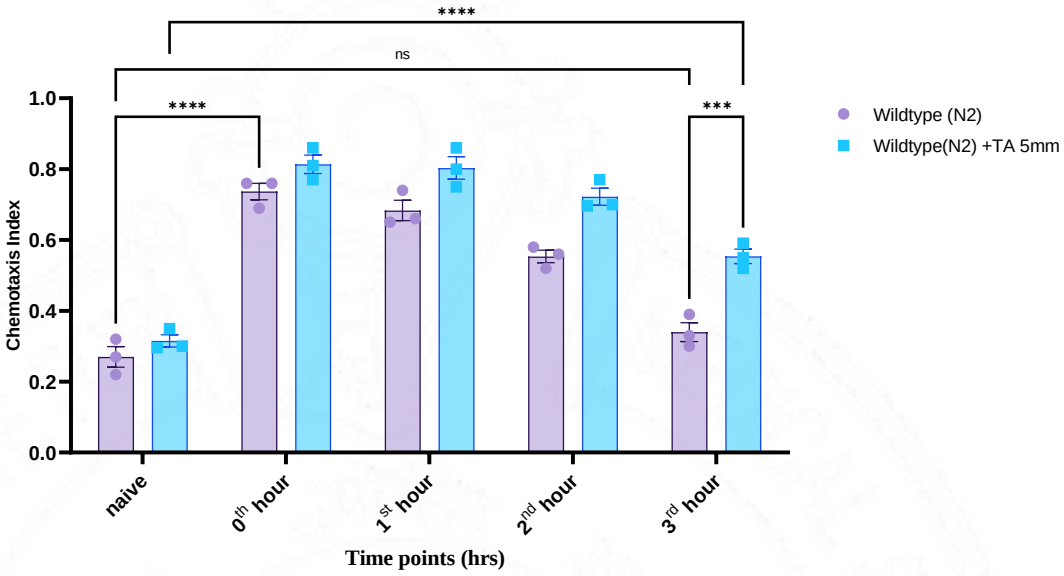


Figure 4.7 : Tyramine enhances Short-term associative memory in low doses

Short-term associative memory in wild-type N2 worms and wild-type N2 worms treated with 5mM TA. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons tests; Data are represented as the mean + S.E.M. Significance indicated as ****- p<0.0001; ***- p<0.0005; ns- non-significant (n≥52 worms N-3 trials)

The mutants of tyramine TDC-1, which lack tyrosine decarboxylase-1 tdc-1(n3421) displayed no chemotaxis to 1/10 butanone in the naïve as well as after conditioning (Figure 4.8). When tyramine was supplied externally, a significant increase in chemotaxis (p<0.0001) was observed.

The tbh-1(n3247) mutants showed no chemotaxis towards the solvent in naïve conditions (Figure 4.8). However, post-short-term training, minimal chemotaxis towards the solvent was seen but was non-significant in comparison with the naïve (Figure 4.8). Exogenous addition of tyramine seems to rescue the defect and to show a significant recovery (p<0.0005) in adaptive memory formation when compared to its naïve (Figure 4.8). This learning behaviour of enzymatic mutants of tyramine

biosynthesis suggested that the tyramine release could initiate a new set of neurons, through altered parallel pathways. This argument is backed by the fact that tyramine in the worms is not usually released during olfactory adaptation but only during threat situations.

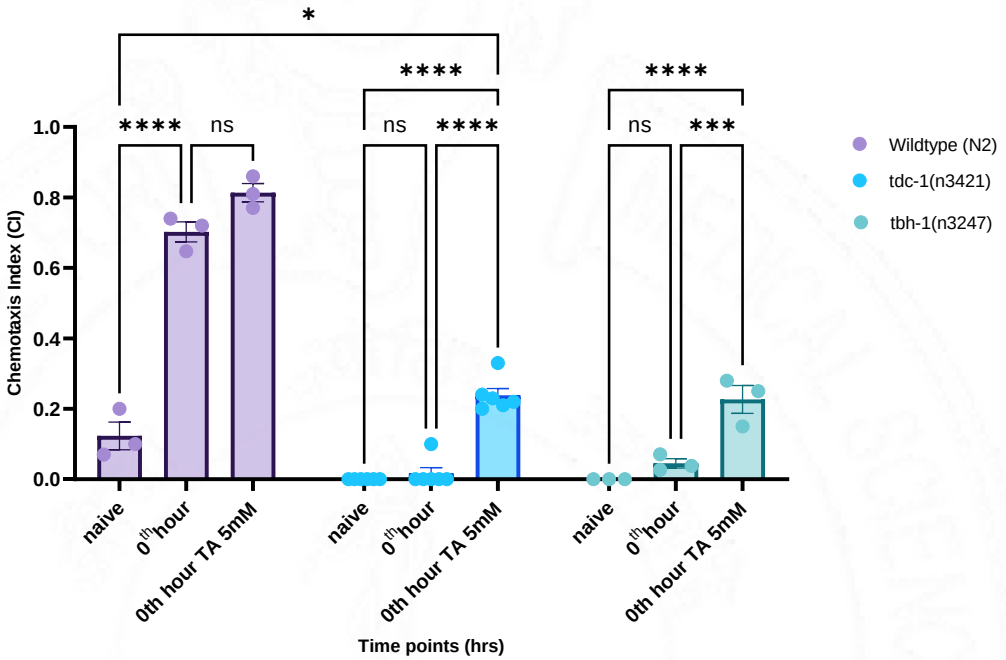


Figure 4.8: Short-term associative memory in tyramine biosynthesis enzyme mutants

Short-term associative memory in Wildtype (N2), MT10549 [tdc-1(n3421) II] and MT9455 [tbh-1(n3247) X] enzyme mutants respectively. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; ***- $p<0.0005$; *- $p<0.05$; ns- non significant ($n\geq40$ worms N-3 trials)

To further elucidate the role of tyramine in olfactory adaptation, the study tested tyramine receptor mutant worms. The wild-type(N2) worms were used as the control exhibited normal short-term adaptation and a significant increase in chemotaxis index($p<0.0001$). The wildtype (N2) worms post short term training in the presence of exogenous tyramine (Figure 4.9.) did not display any significant increase over the untreated wildtype (N2) (Figure 4.9).

The LGC-55 receptor mutant lgc-55(n4331) shows higher naïve chemotaxis migration (CI-0.52±0.045) before STAM conditioning (Figure 4.9). In the lgc-55(n4331) worms showed a slight increase post STAM conditioning. However, this increase was statistically non-significant in comparison with the naïve (Figure 4.9). Similarly, no significant change was seen with extrasynaptic TA addition in STAM-conditioned worms (Figure 4.9) (ns-non-significant). In LGC-55 receptor mutants lgc-55(n4331), all other receptors for tyramine are intact other than LGC-55 receptor but still exhibit impaired chemotaxis. It was suspected that this might be due to the fast-acting nature of this receptor and also due to its positioning in the head region. This puts forward the potential role of LGC-55 receptor in chemotaxis, migration and learning.

The SER-2 receptor mutant ser-2(ok2103) showed lower naïve chemotaxis towards 1/10 butanone, which was comparable with N2 (Figure 4.9). However, after short-term conditioning, the worms displayed a significant chemotaxis index (CI~0.6), suggesting adaptive memory generation post-STAM conditioning (Figure 4.9). The exogenous addition of tyramine during conditioning did not significantly change memory formation (Figure 4.9). In SER-2 receptor mutants ser-2(ok2103), all receptors for tyramine including LGC-55 are intact except for SER-2 receptor. The data suggest that the G-protein coupled receptor SER-2 might be helping in omega turns but is not critical in memory formation. This might be due to the positioning of SER-2 in VD and VB neurons in the mid-body region (Donnelly et al., 2013b).

To further investigate the role of LGC-55 and SER-2 receptors together, AT255 AT255: lgc-55(n4331); ser-2(ok2103), double mutant for for SER-2 and LGC-55 receptors was tested. AT255: lgc-55(n4331); ser-2(ok2103), mutant worms displayed

higher chemotaxis towards 1/10 butanone in naïve condition, and post STAM training no significant increase in memory was seen in the 0th hour (Figure 4.9) the data was similar to LGC-55 receptor mutant strain lgc-55(n4331). The chemotaxis index remained unaltered when exogenous tyramine was added during conditioning (Figure 4.9). This data further confirms the role of LGC-55 receptor in learning and memory-associated migrations.

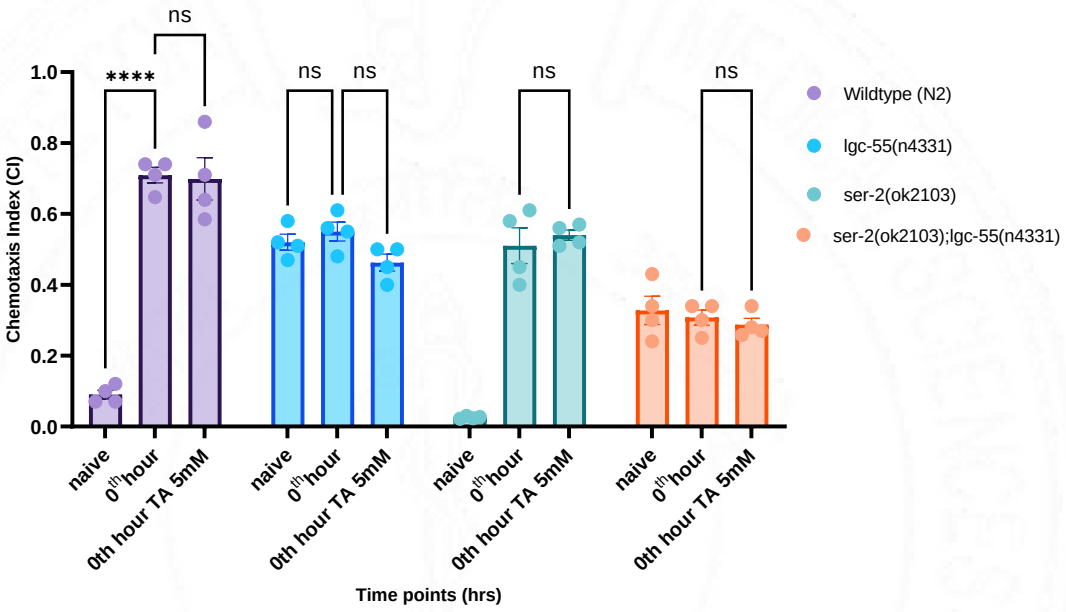


Figure 4.9 : Short-term associative memory in tyramine receptor mutants
Short-term associative memory in wild-type(N2), ionotropic receptor mutant (MT14680 [lgc-55(n4331) V]), RB1690 ser-2(ok2103),AT255: lgc-55(n4331); ser-2(ok2103)). STAM is done in the presence and absence of 5mM Tyramine. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****-p<0.0001; ns- non significant (n≥45 worms N-4 trials)

4.5 Tyramine-Cyclodextrin complex for slow release of tyramine

To induce the slow release of tyramine to the worms, a tyramine-cyclodextrin complex was generated (see Methods for details)(Vyas et al., 2008). It was observed that at 15 mM TA , the worms exhibited 50% immobilization by the 8th minute and by the 20th minute a 60±5% immobilization was observed (Figure 4.10). The CD-TA

complex with 15mM (1:1) complex when introduced to the worms, showed lower immobilization (11.67±2.8%) by 20 mins (Figure 4.10). This low-paced immobilization is the result of the slow release of tyramine from the CD inclusion complex. The complex was evaluated to ensure the inclusion complex formation and the resulting complex ratio (15mM:15mM) of tyramine and cyclodextrin could be used to generate a slow-release model for tyramine in the plate.

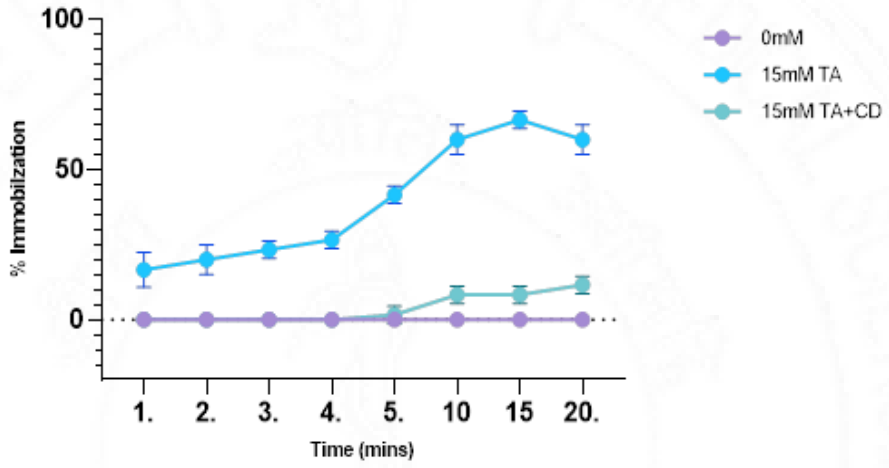


Figure 4.10 : Immobilization of Wild-type N2 with exogenous tyramine
The wild-type (N2) worms were treated with 0 mM, 15mM TA and CD-TA inclusion complex for slow drug release. (n≥20 worms N-3 trials)

We then analysed the influence of slow-released tyramine in short-term associative memory, as the direct extra-synaptic application of tyramine at 5mM concentration resulted in increased short-term memory in wild-type N2 (Figure 4.11). The short-term associative memory with CD-TA inclusion complex generated short-term associative memory in wild-type N2 worms (Figure 4.11). The 0^{th}-hour chemotaxis index of untreated N2 was more significant than the CD-TA-treated group ($p<0.0001$). Meanwhile, the 3^{rd}-hour chemotaxis index of CD-TA treated worms was

considerably higher than the untreated group ($p \leq 0.05$), suggesting increased memory retention with TA addition at the 3rd hour. The slow release of tyramine can facilitate controlled delivery of tyramine, and our observations substantiate the generation of an extended memory pathway mediated by tyramine in *C.elegans* as observed in Figure 4.7.

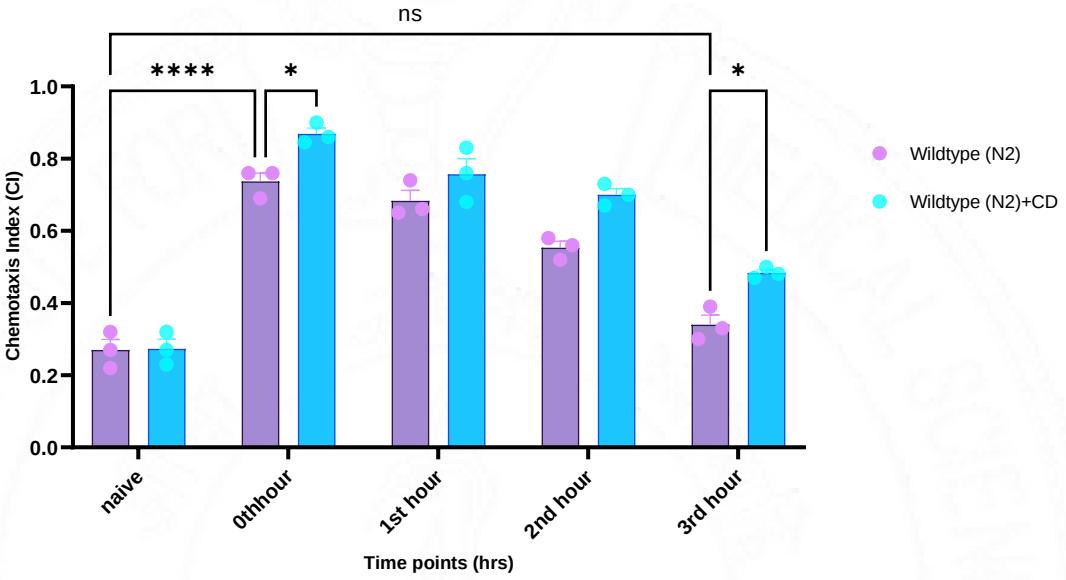


Figure 4.11 : Slow release of tyramine facilitates the short-term associative memory
Short-term associative memory in wild-type N2 worms with worms treated with (CD-TA) inclusion complex at 1:1 ratio. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; *- $p<0.05$; ns-non significant ($n \geq 53$ worms N-3 trials)

4.6 Exogenous tyramine does not affect Long-Term Memory

The wildtype (N2) worms were tested for long-term associative memory. The memory generated (refer to materials and methods for details) is observed to be retained until the 24th hour after training (Figure 4.12). When tyramine was added during conditioning, a significant ($p<0.0001$) increase in 0th-hour memory was observed (Figure 4.12). However, no significant increase in memory was seen in the 16th hour and 24th hour compared N2 suggesting that exogenous tyramine doesn't alter the long-term memory formation in the wild-type worms.

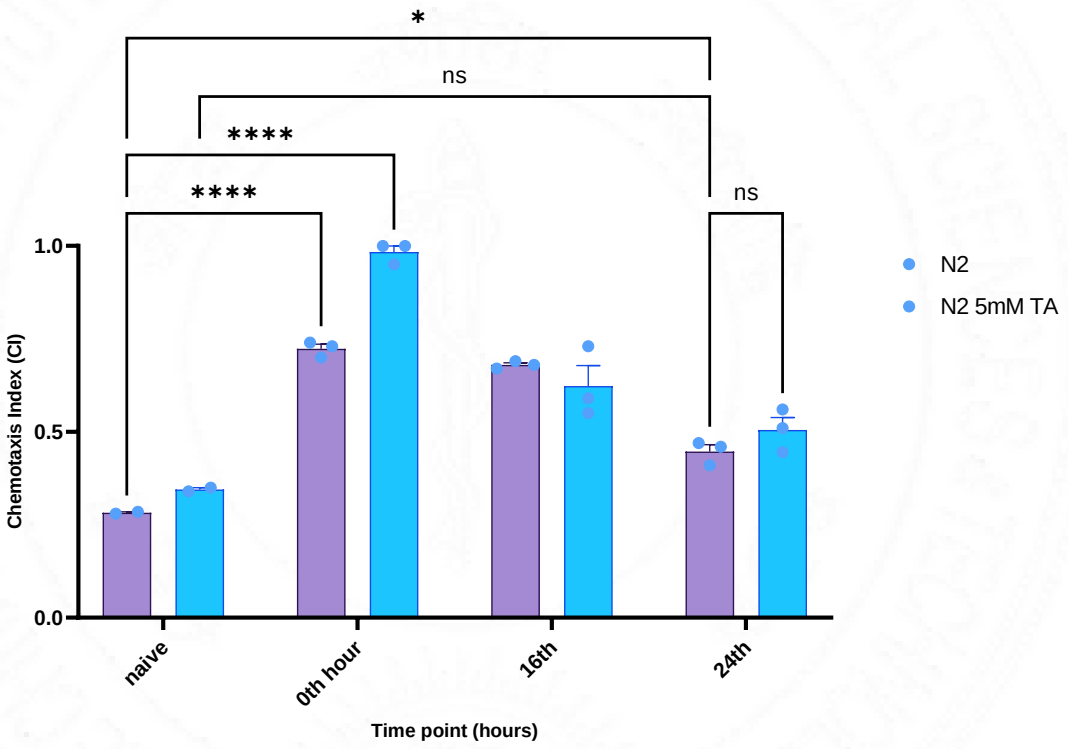


Figure 4.12 : Long-term associative memory is not affected by exogenous tyramine treatment
Long-term associative memory in wild-type N2 worms and wild-type N2 worms treated with 5mM TA (blue). Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; *- $p<0.05$: ns-non significant ($n\geq50$ worms N-3 trials)

4.7 Role of LGC-55 and SER-2 receptors in olfactory signalling in worms

Since LGC-55 mutant strains were showing significant defects in 1/10 butanone attraction and in adaptive behaviour, we further elucidated the function of the LGC-55 receptor mutant and the SER-2 receptor mutant in solvent recognition. We tested out different solvents ranging from neutral/mild repellent to highly attractant solvents on naïve worms. N2 worms showed attraction towards 1/200 benzaldehyde, 1/1000 butanone,1/300 Isoamyl alcohol and 1/10 isoamyl alcohol, respectively (Figure 4.13) and a neutral attraction towards 1/10 butanone.

The lgc-55(n4331) mutant seems to show significantly higher attraction towards 1/200 benzaldehyde and 1/10 butanone. (Figure 4.13). The lgc-55(n4331) is found to exhibit attraction to all the solvents tested and cannot distinguish between attractive 1/200 benzaldehyde, 1/1000 butanone,1/300 Isoamyl alcohol and 1/10 isoamyl alcohol and 1/10 butanone neutral odorant concentrations ($p<0.0001$). SER-2 mutant seems to show attraction similar to N2 in the case of 1/200 benzaldehyde, 1/1000 butanone,1/300 Isoamyl alcohol and 1/10 isoamyl alcohol, but seems to exhibit a lower attraction to 1/10 butanone compared to N2 (Figure 4.13). LGC-55 receptor mutant lgc-55(n4331) lacked to show an inherent chemotaxis pattern towards the solvents, while SER-2 receptor mutants ser-2(ok2103) behaved like N2 wild-type worms except for the lower attraction for 1/10 butanone($p<0.05$). These data suggest that the absence of the LGC-55 receptor has a critical role in distinguishing the solvents and the receptor is playing crucial in generating associated behaviour in worms.

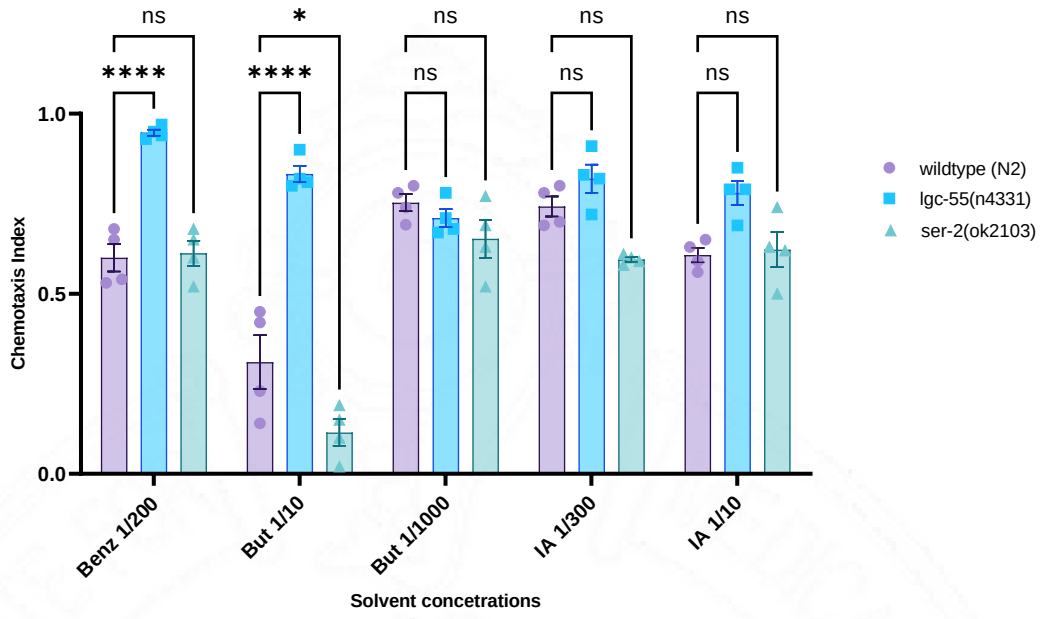


Figure 4.13: Solvent adaptation of SER-2 and LGC-55 receptor mutants

The LGC-55 receptor mutants cannot distinguish between dilutions of solvents

Solvent adaptation of 1/200 benzaldehyde, 1/10 butanone 1/1000 butanone, 1/300 Isoamyl alcohol and 1/10 isoamyl alcohol respectively in wild-type N2 worms ,LGC-55 receptor mutants lgc-55(n4331) and SER-2 receptor mutant ser-2(ok2103). Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; *- $p<0.05$; ns-non significant ($n\geq45$ worms N-4 trials)

The double mutants of tyramine ser-2(ok2103);lgc-55(n4331), which lack both SER-2 and LGC-55 receptors, were tested for chemotaxis behaviour towards attractive and neutral solvents. The worms showed reduced chemotaxis towards the solvents 1/200 benzaldehyde ($p<0.0001$), 1/1000 butanone($p<0.0005$),1/10 butanone ($p<0.005$),1/300 Isoamyl alcohol($p<0.0005$) and 1/10 isoamyl alcohol($p<0.005$) in comparison with wildtype (N2) respectively (Figure 4.14). The double mutant's display of significantly reduced attraction towards all the solvents suggests that the tyramine receptor LGC-55 in relation to SER-2, have an essential role in altering the solvent attraction and chemotaxis of the worm.

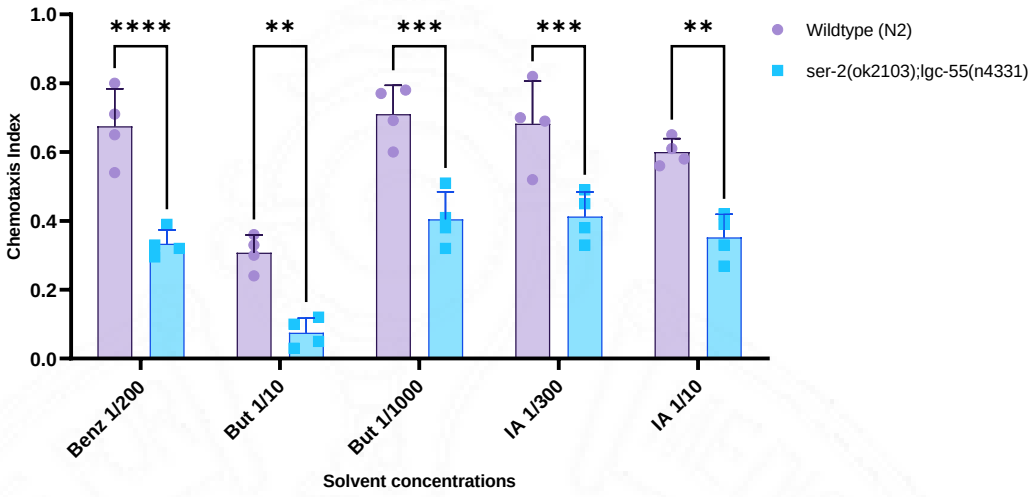


Figure 4.14 : Solvent adaptation of SER-2 and LGC-55 receptor double mutant (AT255)
Solvent adaptation of double mutant of TA receptor mutant ser-2(ok2103);lgc-55(n4331) which lacks both LGC-55 and SER-2 receptor against 1/200 benzaldehyde, 1/10 butanone 1/1000 butanone, 1/300 Isoamyl alcohol and 1/10 isoamyl alcohol respectively. Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; ***- $p<0.0005$; **- $p<0.005$; ($n\geq25$ worms N-4 trials)

4.8 Change in ion selectivity of LGC-55 receptor rescues the variation in learning and adaptation.

LGC-55 is a chloride channel, initiating an inhibitory pathway in the neuronal circuit (Pirri et al., 2015b). To test whether channel property or the downstream molecular pathways are involved in its functional role, we used a strain with a modified LGC-55 having cationic properties. The ion selectivity of the LGC-55 is changed to cationic sodium channels by genetic manipulation of the loop that joins the M1 and M2 domains(Pirri et al., 2015b). Thus, the inhibitory ion channel functions as an excitatory channel in the expressing neurons. lgc-55 cation-II(zfEx41) worm unlike the LGC-55 receptor mutant lgc-55(n4331) showed a similar chemotaxis index comparable to wild-type N2 (Figure 4.15). The chemotaxis index post Short-term memory training showed a similar pattern to that of the wild-type N2 worms (Figure

4.15). This result suggests that the downstream pathways of the LGC-55 receptor binding cascade could be playing a critical role, rather than the receptor alone, in the olfactory associated behaviour generation.

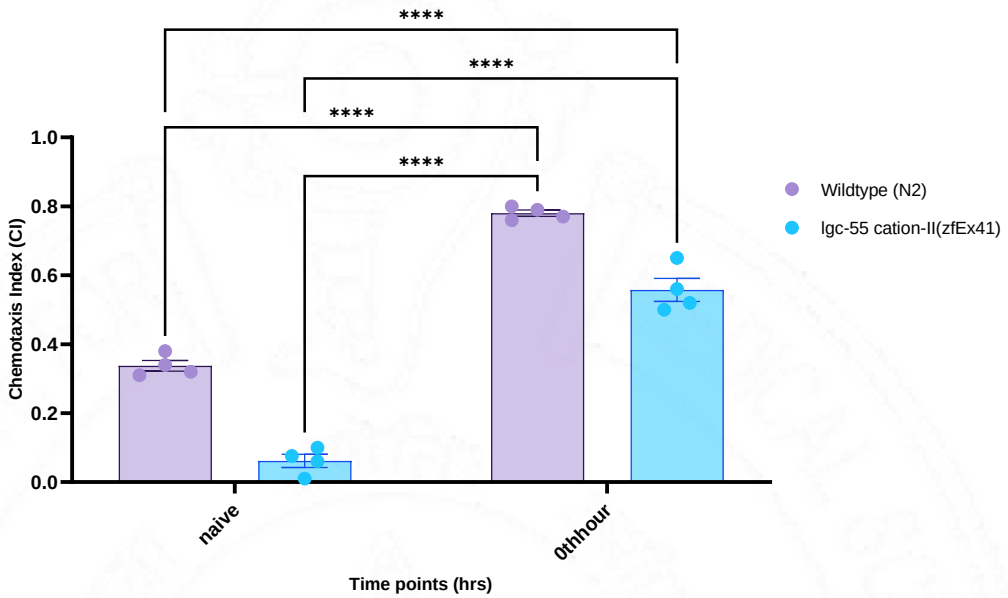


Figure 4.15 : Cationic LGC-55 receptor rescues the short-term associative memory
Short-term associative memory in wild-type (N2) worms and LGC-55 cationic receptor mutant QW224 Pmyo-3::lgc-55 cation-II(zfEx41). Statistical analysis was done by mean of two-way ANOVA with Tukey's multiple comparisons test; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; ($n\geq36$ worms N-4 trials)

4.9 Role of tyramine in threat response and decision-making

To further elucidate the role of LGC-55 and SER-2 receptors in the threat response and decision making a fructose barrier assay was conducted. Contact with fructose could result in the desiccation of the worms, hence well-fed worms won't cross the barrier (Ghosh et al., 2016). The worms would risk desiccation and move towards an attractive solvent when they are starved. Thus, the assay defines the decision-making depending on the internal state of the worm. When tested, the wild-type well-fed N2 worms stayed inside the fructose barrier in the presence of an attractive solvent (Figure 4.16). However, lgc-55(n4331) and AT255 ser-2(ok2103);lgc-55(n4331) explored and crossed the fructose ring suggesting the alteration in the internal state of

the worm. ser-2(ok2103) and tdc-1(n3421), and tbh-1(n3247) mutant strains did not cross the barrier (Figure 4.16). This data further confirms that the LGC-55 receptor is critical in responding to the olfactory signals and assessing the surrounding environment. The LGC-55 receptor mutation results in excessive attraction to the olfactory signal, and that in turn alters the behaviour of the worms.

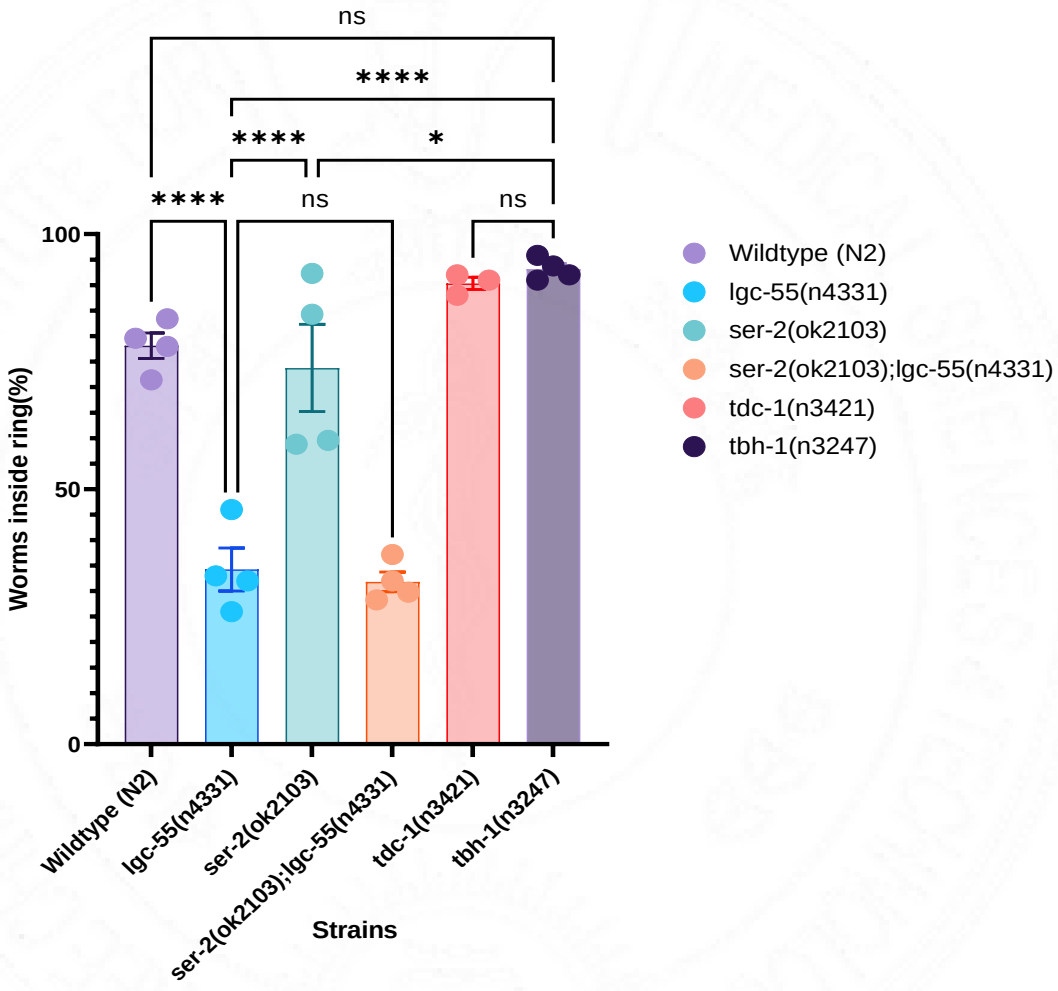


Figure 4.16 : Tyramine receptor lgc-55 plays an important role in decision-making during threat

Fructose ring barrier assay with wildtype N2, receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X , AT255: lgc-55(n4331); ser-2(ok2103)) and tyramine biosynthesis mutants (MT10549 [tdc-1(n3421) II] , MT9455 [tbh-1(n3247) X]) respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented as ****- $p<0.0001$; *- $p<0.05$: ns-non significant ($n\geq43$ worms N-4 trials)

4.10 Worm extract acts as a cue for healthy worms in the colony and aids in risk avoidance

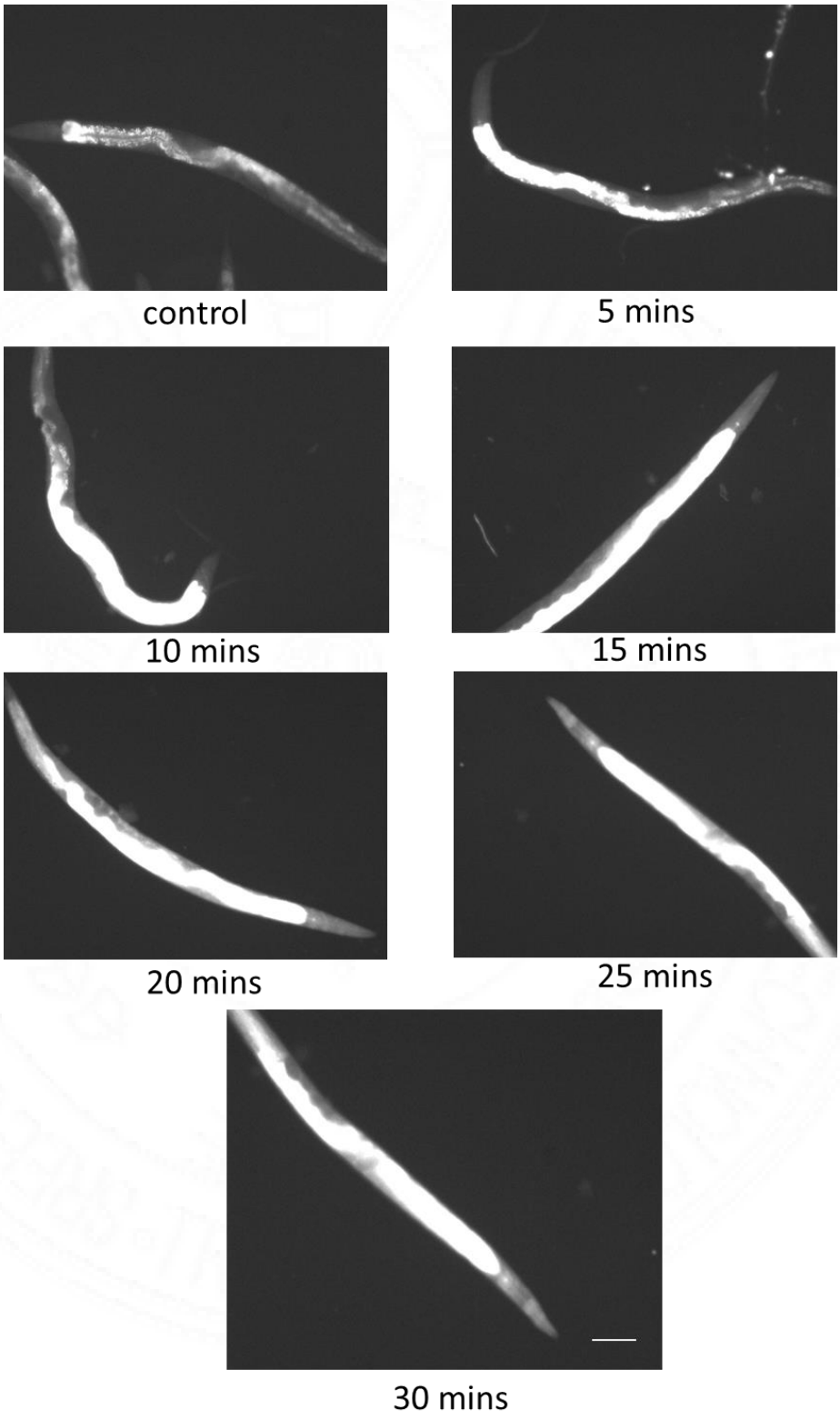


Figure 4.17: Death fluorescence observed in wildtype (N2)

Death fluorescence was observed in wild-type N2 worms at 5 mins, 10 mins, 15 mins, 20 mins, 25 mins and 30 mins respectively. Image obtained at 10X magnification **(**Ex-280nm Em-480nm)

We created a fear response paradigm which would mimic the real-world scenario where leachates from worms that underwent sudden death is used for the assay. It is previously known that a calcium-based necrotic wave is propagated in worms that undergo death by anthranilate fluorescence(Coburn et al., 2013). To establish that worms undergo death with a burst of blue fluorescence called death fluorescence. We imaged the worms when subjecting them to heat at 42ºC at time points 5 mins,10 mins,15 mins,20 mins, 25 mins and 30 mins respectively. The death fluorescence had initiated by the 5^{th} minute, and an increased fluorescence was observed originating from the anterior region to the posterior end of the worm (Figure 4.17). We subjected the healthy worms to heat stress and took the leachates from the dead worms. The extract was presented to healthy worms and observed for the repulsion response towards the extract. The wildtype (N2) worms show fear-evoked reversal response when presented with “dead worm” extracts of 10 mins, 20 mins and 30 mins each (Figure 4.18). At 30 minute extract the highest repulsion (75±4.08%) was observed in comparison with wildtype N2($p<0.0001$). Thus we decided to make use of 30 min heat-treated worm extract for further assays on tyramine mutants.

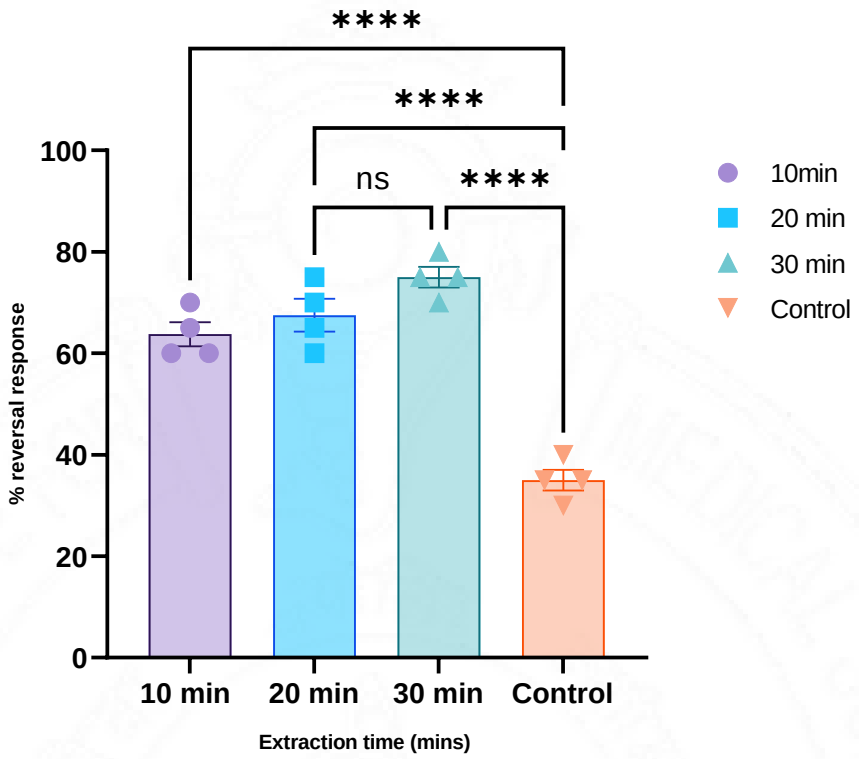


Figure 4.18: Risk avoidance behaviour towards heated worm extract

Reversal response with worm extract collected at 10mins,20mins and 30mins respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented as ****- $p<0.0001$; ns-nonsignificant ($n \geq 20$ worms 4 trials)

On presenting the worm extract from 30 min heat exposure to the worms, the wildtype (N2) worms showed a sudden reversal response when 30-min worm extract was presented($p<0.0001$). However, this sudden reversal response was absent in the LGC-55 receptor mutants lgc-55(n4331) (Figure 4.19). SER-2 receptor mutants ser-2(ok2103) showed normal reversal behaviour comparable to wildtype (N2) (Figure 4.19). The double mutant AT255: lgc-55(n4331); ser-2(ok2103) also showed reduced reversals similar to the LGC-55 receptor mutant lgc-55(n4331). This result gives the first evidence that the LGC-55 receptor plays a critical role in threat avoidance as well as threat eviction.

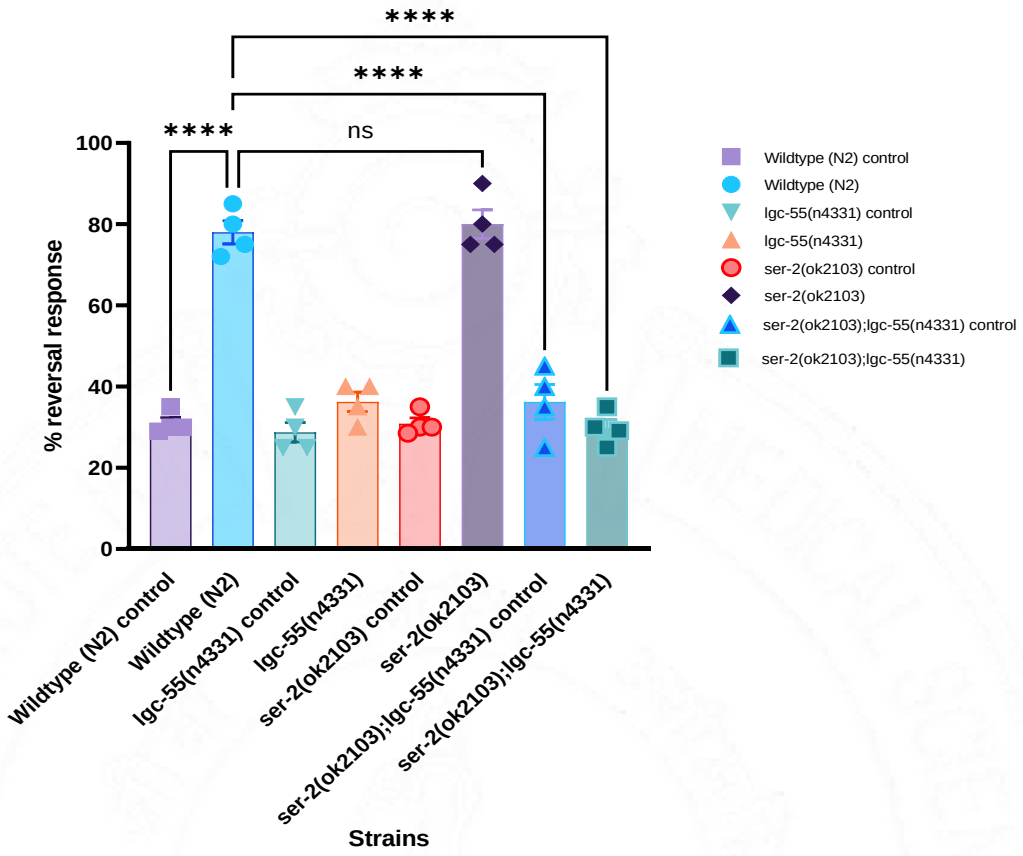


Figure 4.19: Olfactory aversive response towards worm extract

Reversal response towards worm extract collected after 30 min heat treatment was collected and presented on wildtype (N2), receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X , AT255: lgc-55(n4331); ser-2(ok2103)) respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented as ****-$p<0.0001$;ns-non significant ($n \geq 20$ worms 4 trials)

4.11 Anthranilic acid produced during the death of the worm acts as a threat signal

The major component in death fluorescence is anthranilic acid (Coburn et al., 2013). We hypothesized that anthranilic acid released during the death of the worm might be acting as a signal for other worms. Anthranilic acid was tested at various concentrations ranging from 1%, 1%:10, 1%:100 and 1%:1000 to see the immediate reversal behaviour of worms and observed a maximum repulsion at 1% concentration (Figure 4.20.).Thus, for the further assays, we decided to use 1% anthranilic acid to introduce the repulsive cue to the worm.

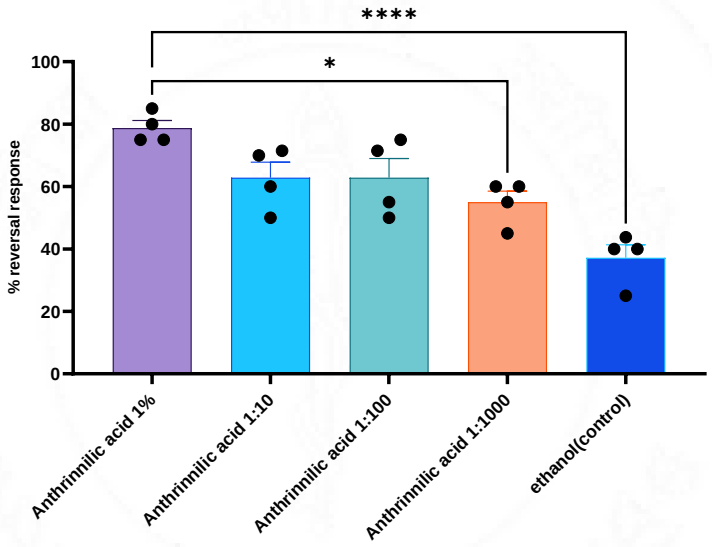


Figure 4.20: Reversal response towards anthranilic acid

a.) Anthranilic acid was presented to worms in dilutions ranging from 1%,1%:10, 1%:100 and 1%:1000 respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented as ****- $p<0.0001$; *- $p<0.05$ $n\geq 20$ worms

On scoring the reversal response, the wildtype (N2) worms showed a sudden reversal response when 1% anthranilic acid was presented($p<0.0001$).To verify that if the anthranilic acid is recognized with the help of LGC-55 receptors in combination with the sensory neurons, we presented the 1% anthranilic acid to the worms and it was seen that LGC-55 receptor mutant lgc-55(n4331) worms did not show reversal

behaviour to the anthranilic acid (Figure 4.21). A normal repulsion was seen in the case of SER-2 receptor mutant ser-2(ok2103), which was similar to that of the N2 (Figure 4.21). The double mutant AT255: lgc-55(n4331); ser-2(ok2103) showed a reduced reversal response to the anthranilic acid (Figure 4.21). These data suggest that anthranilic acid could be one of the active components in the worm extract, which could act as a signal to other worms and create a behavioural response in worms. The sensing and respective avoidance response might be regulated through the LGC-55 receptor in the interneurons. This inhibitory channel, LGC-55 is essential in slowing down the forward locomotion and triggering the reversal response through fast-acting ligand-gated ion channels.

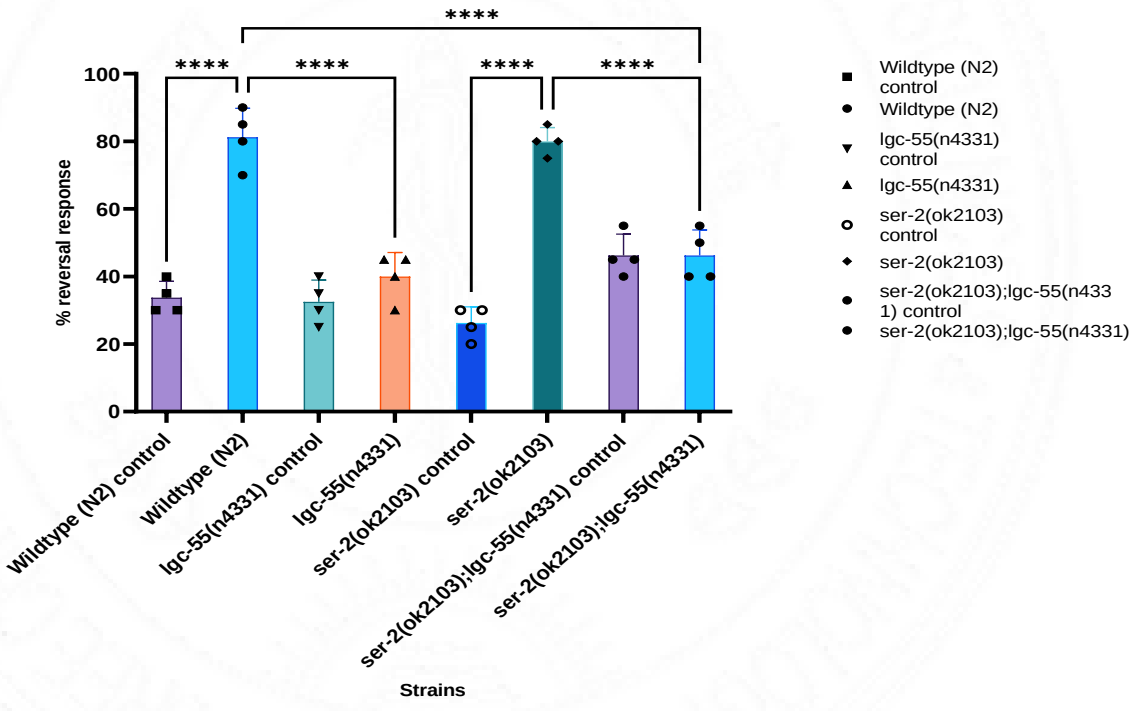


Figure 4.21: Olfactory aversive response towards 1% anthranilic acid

Reversal response towards 1% anthranilic acid wildtype (N2), receptor mutants (MT14680 [lgc-55(n4331) V], RB1690 ser-2(ok2103) X , AT255: lgc-55(n4331); ser-2(ok2103)) respectively. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented as ****- $p<0.0001$; ($n \geq 20$ worms N-4 trials)

A choice-based exploration assay was conducted where the worms were presented with a “death extract” of worms treated 30 minutes in heat on one half of the plate and the other half was spotted with the control solvent at spots equidistant to each other (see Methods). This assay would help in assessing the inherent repulsion or attraction behaviour of the worms. The results showed that the wildtype (N2) worms choose the half without the worm extract (Figure 4.22). Suggesting the avoidance response of the worms towards the leachates of heated worm extract($p<0.0001$). Similarly 1% anthranilic acid was tested for choice for exploration in worms. The worms migrated towards the quadrant containing the control solvent rather than the quadrant with 1% Anthranilic acid ($p<0.0001$) after 1 hour of exposure (Figure 4.22). These data confirm that the “death extract” and anthranilic acid induces behavioural alterations in the worm. The difference of worms preference for the exploration quadrent between the worm extract and 1% Anthranilic acid was significant ($p<0.0005$),The results suggest that there might be additional compounds in the worm extract that might be causing the strong repulsion in case of heated worm extract in comparison with the chemical cue 1% Anthranilic acid.

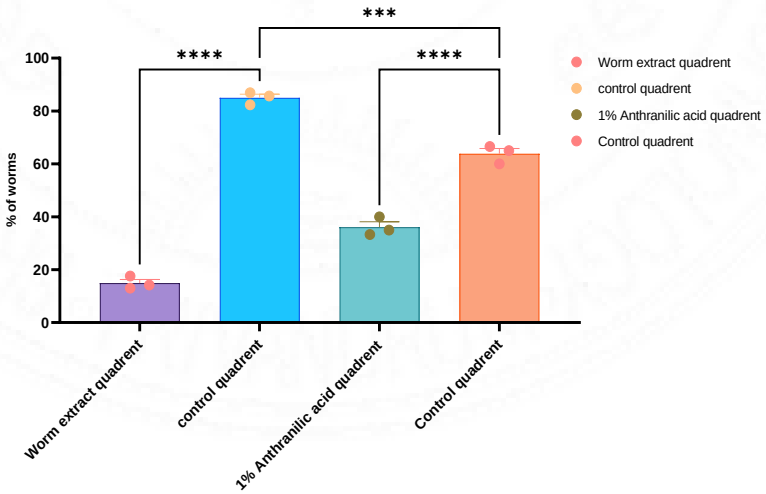


Figure 4.22: Percent accumulation of worms in plate dispersed with worm extract and 1% anthranilic acid respectively.

The test solvent (worm extract/1% Anthranilic acid) was placed at equidistant spots in one half of the plate and the worms were allowed to explore the plate. The number of worms in each quadrant was counted post 60 mins. Statistical analysis was carried out by mean of one-way ANOVA with Dunnett's multiple comparisons test; and significance represented; Data are represented as the mean + S.E.M. Significance indicated as ****- $p<0.0001$; ***- $p<0.0005$; ($n\geq20$ worms N-3 trials)

5. DISCUSSION

Animal behaviour alters when performing various actions such as finding food, searching for mates, escaping danger, and navigating. External stimuli from the environment or other surroundings can cause important behavioural changes in the animal. In *C. elegans* chemosensory network of various sensory neurons could detect signals of repellents or attractants and initiates corresponding behavioural responses (Albeg et al., 2011; Melkman and Sengupta, 2004). *C. elegans* frequently has to deal with changing environmental conditions over a short timescale, and a sudden change in its surroundings, such as a threat or unfavourable conditions, necessitates a subtle change in its behaviour and locomotion. Comparably, an important foraging decision is frequently a choice between the exploitation of existing resources and the exploration of alternative options when resources are limited. The capability of an organism to integrate the information and to function as a coordinated individual in a colony is crucial for its survival (Pessoa et al., 2021). Chemosensory neurons in the local search circuit control acute reorientations by activating excitatory and inhibitory ionotropic receptors on downstream interneurons(López-Cruz et al., 2019). This present study tries to understand how sensory inputs received by an organism in its natural habitat influence the learning and behaviour of the organism through the neurotransmitter tyramine and octopamine which is synonymous with epinephrine and norepinephrine in vertebrate systems (Roeder, 2005).

Complex behaviours of organisms are thought to be the result of dynamic sensory input. We hypothesized that monoamine neurotransmitters such as tyramine and octopamine may influence the organism's adaptive memory and exploration

behaviour. Tyramine receptors located near sensory neurons could play an active role in recognizing and responding to a threat signal, thereby influencing behaviour. In *C.elegans* tyramine have very few receptors (Gainetdinov et al., 2018), and that could be easily traced in a small set of neurons. The single neuron RIM facilitates the extra synaptic release of tyramine(Donnelly et al., 2013b), hence making this model apt for inducing and studying the escape-related complex behavioural outcomes associated with the tyramine pathway.

This study evaluated the behaviour of the worm in growth plates, and it was observed that the worms take normally closed omega turns with head-to-tail touch in a predictable frequency in their natural environment. In the touch response assay, wildtype N2 took a proper omega turn as a threat response behaviour. SER-2, a GPCR receptor for tyramine, (Donnelly et al., 2013b) is essential to initiate this behaviour GPCR receptor for tyramine(Donnelly et al., 2013b) in *C. elegans*. SER-2 receptors are activated through the extrasynaptic release of tyramine from the RIM neurons(Donnelly et al., 2013b). On binding to SER-2 receptors in the VD motor neuron, a GABAnergic neuron, results in the hypercontraction of body wall muscles and thereby facilitating the omega turn. This event occurs in its habitat for the exploration movement of the organism. The SER-2 receptor mutant strain (RB1690 ser-2(ok2103)), was defective in taking proper omega bends during touch response supporting Donnelly et al observation (Donnelly et al., 2013b). In addition, the data showed that the ser-2(ok2103) mutant had a significantly different escape response where the head-to-tail touch during an omega turn does not occur. This confirms that

mutant worms generate defective omega turns and result in an altered trajectory during an escape response (without a head-to-tail touch).

LGC-55 is yet another important ligand-gated chloride channel found primarily in the anterior region of the worm in close proximity to the nerve ring (Donnelly et al., 2013b; Pirri et al., 2015b, 2009). LGC-55 is a tyramine-gated chloride channel inhibitory in nature and is found to aid in the head twitching behaviour during an escape response. The omega turn escape response and escape angle of the LGC-55 mutant (MT14680 [lgc-55(n4331) V]) were similar to that of the wild type. The mutant strain, TDC-1(MT10549 [tdc-1(n3421) II]), lacking tyramine decarboxylase-1 enzyme (TDC-1), an essential part of the tyramine synthesis pathway responsible for the conversion of tyrosine to tyramine in neurons showed incomplete omega bends and escape response without the characteristic head-to-tail touch confirming that tyramine has a critical role in this behavioural response. Since the TDC-1 mutant strain also lacks octopamine in addition to tyramine, the study used a control strain lacking the tyrosine beta hydroxylase-1 (TBH-1), which will have the octopamine synthesis blocked but will have normal tyramine synthesis. The TBH-1 enzyme mutant (MT9455 [tbh-1(n3247) X]) showed proper omega turns with head-to-tail touch and angle of escape when compared to the wild-type worms. The tyramine receptor mutants TYRA-2 (QW42: tyra-2(tm1815)) and TYRA-3 (VC125: tyra-3(ok325)) did not show the omega turn angle defect in the worms. The double mutant AT255 AT255: lgc-55(n4331); ser-2(ok2103), lacking LGC-55 and SER-2 receptors had an incomplete omega turn and an altered angle of escape in comparison with the wild type. This increased angle of escape is explained in terms of optimal foraging theory

(Charnov, 1976). The defective mutant worms take turns with an increased angle and spend more energy and time to reach the food spots, leading them to spend more energy. The study shows that mutations in the LGC-55 receptor result in reduced fitness of the organism.

The parallel processing of sensory and nutritional cues is a characteristic of mammalian feeding and satiety circuits (López-Cruz et al., 2019). Chemosensory neurons play a major role in exploratory behaviour (Gray et al., 2005). The principle of the exploratory assay is described by Charnov's marginal value theorem (Charnov, 1976), which proposes that the optimal time for an animal to leave a foraging ground occurs when local resource levels fall below the average level in the entire habitat. To elucidate whether tyramine receptor mutation affects the exploratory behaviour for a quantitative exploratory behaviour assay was designed. In an agar plate, a minimum amount of food was placed at equidistant spots so that worms had to migrate and explore, taking turns and also had to rely on olfactory cues to discover the food source (Pradhan et al., 2018). The results showed that the wildtype N2 worms and SER-2 mutant ser-2(ok2103) worms explored to the farthest zone 3. Surprisingly the LGC-55 mutant lgc-55(n4331) worms did not cross past zone 2. This raised the notion that lgc-55(n4331) receptor mutants have a defect in olfactory perception during chemotaxis. In *C.elegans* control of body movements is designated to specialized body wall muscles, meanwhile, the head movements are precisely controlled by the action of head and neck muscles (Branicky and Schafer, 2009). The head region of the worm has numerous sensory endings that allows the worm to sense taste and smell the compounds around it(Chalasani et al., 2008; Gray et al., 2005). The head movements

are thought to facilitate the worm to explore the immediate surroundings, thereby helping in food search (Pirri and Alkema, 2012b). The loss of LGC-55 receptor, responsible for the arrest of head twitching, is suspected to activate parallel pathways that could hamper the exploratory sensation in worms.

The olfactory preferences in *C. elegans* alters depending on the odour and its concentration (Chalasani et al., 2008; Yoshida et al., 2012). The results suggested that in the absence of the LGC-55 receptor, there is an impairment in distinguishing solvents and associated behaviour compared to the SER-2 mutants. Altering the firing pattern in connectomes would play a role in the olfactory response (Evans et al., 2019; Pirri and Alkema, 2012b). It has been observed that biochemical abnormalities on trace amine-associated receptors (TAARs) can contribute to behaviours such as restlessness, anxiety. Similarly, oral administration of tyramine was found to cause Cluster headaches (CH) and can have profound alterations in the sense of smell and depression-like behaviours in humans (D'Andrea et al., 2007). In honeybees, the tyramine and its receptor AmTyr1 expressed in the antennal lobe and mushroom bodies play an active role in foraging, reproductive behaviours as well as olfactory learning (Sinakevitch et al., 2017). In *C. elegans*, hallmarks of such behaviours were observed in the absence of LGC-55 receptors resulting in loss of sensory perception and altered decision-making capabilities. Interestingly by altering the LGC-55 to a cationic receptor rescued the olfactory defect. The plausible reason for the differential behaviour of SER-2 and LGC-55 receptors could be due to their positioning in the worms in the nerve ring (Lemoine et al., 2012; Rex et al., 2004). SER-2 receptors are present close to the body wall musculature and aid in the turning behaviour (Donnelly

et al., 2013b). LGC-55 receptor, on the other hand, is present in the head neurons (RMD, SMD, AVB) of the worm (Donnelly et al., 2013b; Pirri et al., 2015b). The olfactory neurons, such as AIY and interneuron AVB, are interconnected to LGC-55 receptor-expressing neurons.

Exogenous tyramine is known to cause immobilization in worms, inducing immobilization through the activation of SER-2 receptor and Gαo signalling pathway in the GABAergic neurons (Donnelly et al., 2013b). Exogenous tyramine (TA) above 30mM concentration caused complete immobilization in wildtype N2 strains, while the ser-2(ok2103) and lgc-55(n4331) mutant strains displayed resistance to immobilization. We used the tyramine cyclodextrin inclusion complex for the slow release of TA (Gidwani and Vyas, 2015; Vyas et al., 2008). As expected, the tyrosine cyclodextrin lowered the immobilization risk compared to the direct exposure to TA. Similarly, based on the mobilization pattern of the N2 worms 5 mM TA was found to be ideal to test the memory assays because at these concentrations TA has a limited effect on the migration ability of the worm.

The fear response and avoidance behaviour using both death fluorescence extract and anthranilic acid showed that LGC-55 mutants are defective in sensing these environmental signals. One of the major components in “death fluorescence” is anthranilic acid(Gems and Coburn, 2013). The fluorescence is due to a necrotic wave of anthranilate during the death of an organism(Coburn et al., 2013). Anthriinillc acid derivates are also known to have activity by affecting the ryanodine receptor- RyR (Ren et al., 2022). RyR receptors are located in the sarcoplasmic reticulum of muscle cells and the endoplasmic reticulum of non-muscle cells. They play a crucial role in

regulating the outflow of calcium ions within cells, which is necessary for muscle contraction(Radermacher et al., 1994). We also found that anthranilic acid at 1% concentration can generate a strong repulsion behaviour in worms.

On employing the migration assay, the worms showed a preferred behaviour of exploring the areas without the anthranilic acid, suggesting the avoidance response of the worms towards this cue. LGC-55 receptor mutant worms lacked the ability to recognize the anthranilic acid-based responses and did not show reversal behaviour. Similarly, TA is thought to provide essential cues to shut down the forward locomotion and trigger the reversal response through fast-acting ligand-gated ion channels such as LGC-55 during the fear response. Tyramine released from RIM is found to suppress BAG activity through LGC-55 and inhibit the sensory perception of CO_2 (Riedl et al., 2022).These observations suggest the essential role of LGC-55 in sensory pathways.

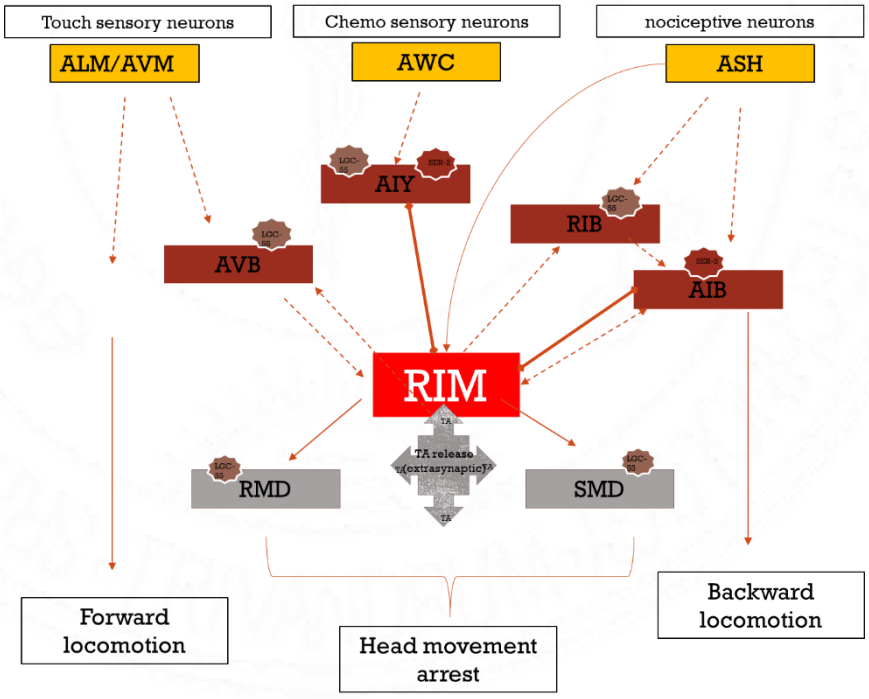


Figure 5.1 Locomotory circuit where LGC-55 receptor influence forward and backward movements and decision making through RMD,SMD and AVB neurons.

There is evidence in vertebrate systems that cyclic nucleotide-gated channels stimulate the activation of olfactory transduction and, in parallel, play a role in adaptation (Matthews and Reisert, 2003). However, there are very few reports on ligand-gated ion channels affecting olfaction and decision-making. The results from this study conclusively suggest that tyramine-associated receptor LGC-55 plays an active role in multisensory decision-making. Tyramine modulates the top-down extrasynaptic aminergic signals by their extrasynaptic release from RIM motor neurons, affecting RMD, SMD and AVB.

6. SUMMARY AND CONCLUSION

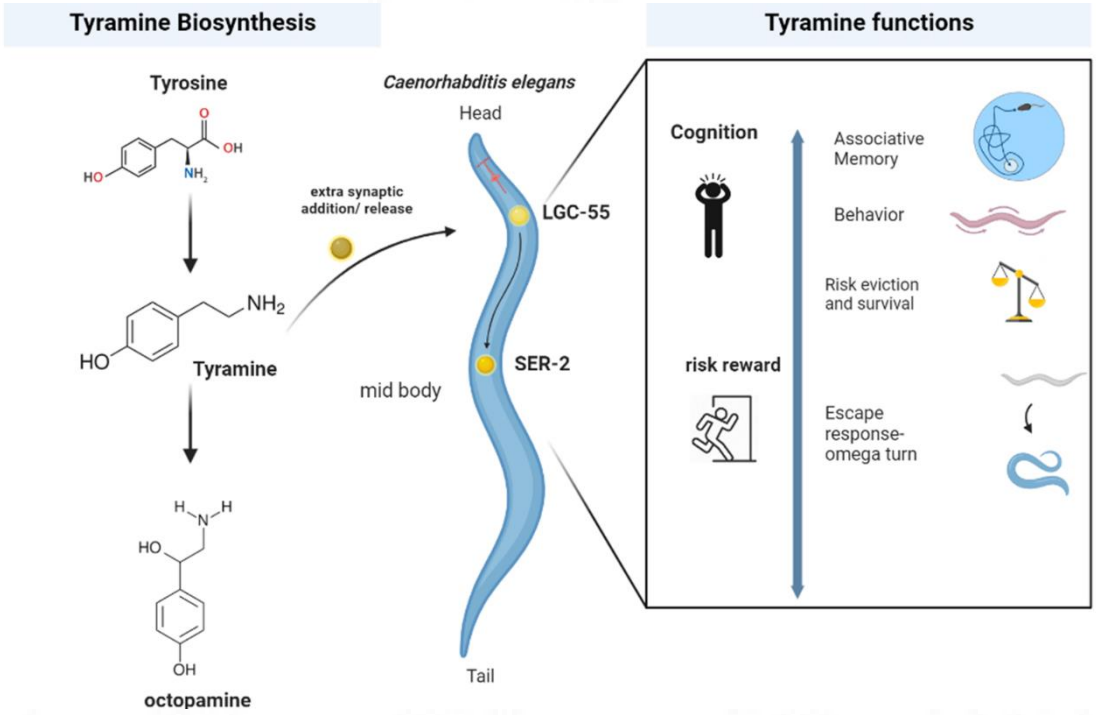


Figure 6.1 Pictorial representation depicting the role of extra synoptically released tyramine on LGC-55 and SER-2 receptors

Understanding how the nervous system facilitates the survival of an organism in the natural environment is fascinating. This study shows that the tyraminergic neurons are involved in risk-taking behaviour. With the aid of GPCR and fast-acting Ligand-gated adrenaline-like channels release the neurotransmitter tyramine from the master control neuron RIM. The work highlights the complexity of the tyramine inhibition pathway and the role of tyramine in influencing a widespread signalling cascade.

This study results indicate that TA which was initially thought as the precursor for dopamine in the system could act as a neurotransmitter having a critical function

in neurons. The TAergic neurons are critical in learning and memory, and the connectome associated with these neurons is critical in memory recall. Several theoretical studies (Schafer, 2015b)have suggested that the sinusoidal body movements underlying nematode locomotion might not require a central pattern generator circuit but instead, be generated exclusively by motor neuron sensory feedback. Based on our data on LGC-55 and SER-2, it is evident that TA influences the learning and migration in *C. elegans*. Further understanding of these connectomes and their active processing of information will enrich our understanding of the intrinsic relations between memory and decision-making at the cellular level.

7. REFERENCES

Albeg, A., Smith, C.J., Chatzigeorgiou, M., Feitelson, D.G., Hall, D.H., Schafer, W.R., Miller, D.M., Treinin, M., 2011. C. elegans multi-dendritic sensory neurons: Morphology and function. Molecular and Cellular Neuroscience 46, 308–317. https://doi.org/10.1016/j.mcn.2010.10.001

Albrecht, D.R., Bargmann, C.I., 2011. High-content behavioural analysis of Caenorhabditis elegans in precise spatiotemporal chemical environments. Nat Methods 8, 599–605. https://doi.org/10.1038/nmeth.1630

Alfonso, A., Grundahl, K., Duerr, J.S., Han, H.-P., Rand, J.B., 1993. The Caenorhabditis elegans unc- 17 Gene: A Putative Vesicular Acetylcholine Transporter. Science 261, 617–619. https://doi.org/10.1126/science.8342028

Ali, S.M., Asmat, F., Koketsu, M., 2007. 1H NMR spectroscopic investigation of β-cyclodextrin inclusion compounds with parecoxib. J Incl Phenom Macrocycl Chem 59, 191–196. https://doi.org/10.1007/s10847-007-9314-3

Alkema, M.J., Hunter-Ensor, M., Ringstad, N., Horvitz, H.R., 2005. Tyramine functions independently of octopamine in the Caenorhabditis elegans nervous system. Neuron 46, 247–260. https://doi.org/10.1016/j.neuron.2005.02.024

Anderson, M.C., Hasan, F., McCrodden, J.M., Tipton, K.F., 1993. Monoamine oxidase inhibitors and the cheese effect. Neurochem Res 18, 1145–1149. https://doi.org/10.1007/BF00978365

Anglada-Figueroa, D., 2005. Lesions of the Basal Amygdala Block Expression of Conditioned Fear But Not Extinction. Journal of Neuroscience 25, 9680–9685. https://doi.org/10.1523/JNEUROSCI.2600-05.2005

Ardiel, E.L., Rankin, C.H., 2010. An elegant mind: Learning and memory in Caenorhabditis elegans. Learn. Mem. 17, 191–201. https://doi.org/10.1101/lm.960510

Ashida, K., Hotta, K., Oka, K., 2019. The Input-Output Relationship of AIY Interneurons in Caenorhabditis elegans in Noisy Environment. iScience 19, 191–203. https://doi.org/10.1016/j.isci.2019.07.028

Ballestriero, F., Nappi, J., Zampi, G., Bazzicalupo, P., Di Schiavi, E., Egan, S., 2016. Caenorhabditis elegans employs innate and learned aversion in response to bacterial toxic metabolites tambjamine and violacein. Sci Rep 6, 29284. https://doi.org/10.1038/srep29284

Bargmann, C.I., Avery, L., 1995. Laser Killing of Cells in Caenorhabditis elegans. Methods Cell Biol 48, 225–250.

Bargmann, C.I., Hartwieg, E., Horvitz, H.R., 1993. Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell 74, 515–527. https://doi.org/10.1016/0092-8674(93)80053-H

Bendesky, A., Tsunozaki, M., Rockman, M.V., Kruglyak, L., Bargmann, C.I., 2011. Catecholamine receptor polymorphisms affect decision-making in C. elegans. Nature 472, 313–318. https://doi.org/10.1038/nature09821

Bieck, P.R., Antonin, K.H., 1989. Tyramine potentiation during treatment with MAO inhibitors: brofaromine and moclobemide vs irreversible inhibitors. J Neural Transm Suppl 28, 21–31.

Blanchard, D.C., Griebel, G., Pobbe, R., Blanchard, R.J., 2011. Risk assessment as an evolved threat detection and analysis process. Neuroscience & Biobehavioural Reviews 35, 991–998. https://doi.org/10.1016/j.neubiorev.2010.10.016

Bono, M.D., Maricq, A.V., 2005. Neuronal Substrates of Complex Behaviours. https://doi.org/10.1146/annurev.neuro.27.070203.144259
Branicky, R., Schafer, W.R., 2009. Tyramine: A New Receptor and a New Role at the Synapse. Neuron 62, 458–460. https://doi.org/10.1016/j.neuron.2009.05.005
Brede, M., Philipp, M., Knaus, A., Muthig, V., Hein, L., 2004. alpha2-adrenergic receptor subtypes - novel functions uncovered in gene-targeted mouse models. Biol Cell 96, 343–348. https://doi.org/10.1016/j.biolcel.2004.03.006
Brembs, B., Christiansen, F., Pflüger, H.J., Duch, C., 2007. Flight initiation and maintenance deficits in flies with genetically altered biogenic amine levels. Journal of Neuroscience 27, 11122–11131. https://doi.org/10.1523/JNEUROSCI.2704-07.2007
Brenner, S., 1974. The Genetics of CAENORHABDITIS ELEGANS. Genetics 77, 71–94.
Burns, C., Kidron, A., 2022. Biochemistry, Tyramine, in: StatPearls. StatPearls Publishing, Treasure Island (FL).
Caballero, B., Trugo, L.C., Finglas, P.M., 2003. Encyclopedia of food sciences and nutrition, 2nd ed. ed. Academic Press, Amsterdam New York.
Cai, C.-L., Martin, J.C., Sun, Y., Cui, L., Wang, L., Ouyang, K., Yang, L., Bu, L., Liang, X., Zhang, X., Stallcup, W.B., Denton, C.P., McCulloch, A., Chen, J., Evans, S.M., 2009. Cai et al. reply. Nature 458, E9–E10. https://doi.org/10.1038/nature07917
Card, G.M., 2012. Escape behaviours in insects. Current Opinion in Neurobiology 22, 180–186. https://doi.org/10.1016/j.conb.2011.12.009
Castellucci, V., Pinsker, H., Kupfermann, I., Kandel, E.R., 1970. Neuronal Mechanisms of Habituation and Dishabituation of the Gill-Withdrawal Reflex in *Aplysia*. Science 167, 1745–1748. https://doi.org/10.1126/science.167.3926.1745
Chalasani, S.H., Chronis, N., Tsunozaki, M., Gray, J.M., Ramot, D., Goodman, M.B., Bargmann, C.I., 2008. Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature 451, 102–102. https://doi.org/10.1038/nature06540
Chalfie, M., Sulston, J.E., White, J.G., Southgate, E., Thomson, J.N., Brenner, S., 1985. The neural circuit for touch sensitivity in Caenorhabditis elegans. J Neurosci 5, 956–964. https://doi.org/10.1523/JNEUROSCI.05-04-00956.1985
Chao, M.Y., Komatsu, H., Fukuto, H.S., Dionne, H.M., Hart, A.C., 2004. Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. Proc Natl Acad Sci U S A 101, 15512–15517. https://doi.org/10.1073/pnas.0403369101
Charnov, E.L., 1976. Optimal foraging, the marginal value theorem. Theoretical Population Biology 9, 129–136. https://doi.org/10.1016/0040-5809(76)90040-X
Cho, C.E., Brueggemann, C., L???Etoile, N.D., Bargmann, C.I., 2016. Parallel encoding of sensory history and behavioural preference during Caenorhabditis elegans olfactory learning. eLife 5, 1–20. https://doi.org/10.7554/eLife.14000
Clark, C.M., 2014. Neural Orchestration of the C. elegans Escape Response: A Dissertation. https://doi.org/10.13028/M24S4T
Coburn, C., Allman, E., Mahanti, P., Benedetto, A., Cabreiro, F., Pincus, Z., Matthijssens, F., Araiz, C., Mandel, A., Vlachos, M., Edwards, S.-A., Fischer, G., Davidson, A., Pryor, R.E., Stevens, A., Slack, F.J., Tavernarakis, N., Braeckman, B.P., Schroeder, F.C., Nehrke, K., Gems, D., 2013. Anthranilate Fluorescence Marks a Calcium-Propagated Necrotic Wave That Promotes Organismal Death in C. elegans. PLoS Biol 11, e1001613. https://doi.org/10.1371/journal.pbio.1001613
Cook, S.J., Crouse, C.M., Yemini, E., Hall, D.H., Emmons, S.W., Hobert, O., 2019. The connectome of the Caenorhabditis elegans pharynx.

D'Andrea, G., Nordera, G.P., Perini, F., Allais, G., Granella, F., 2007. Biochemistry of neuromodulation in primary headaches: focus on anomalies of tyrosine metabolism. Neurol Sci 28 Suppl 2, S94-96. https://doi.org/10.1007/s10072-007-0758-4

De Rosa, M.J., Veuthey, T., Florman, J., Grant, J., Blanco, M.G., Andersen, N., Donnelly, J., Rayes, D., Alkema, M.J., 2019. The flight response impairs cytoprotective mechanisms by activating the insulin pathway, Nature. https://doi.org/10.1038/s41586-019-1524-5

Dexter, J.P., Prabakaran, S., Gunawardena, J., 2019. A Complex Hierarchy of Avoidance Behaviours in a Single-Cell Eukaryote. Current Biology 29, 4323-4329.e2. https://doi.org/10.1016/j.cub.2019.10.059

Dieterich, C., 2006. Pristionchus pacificus genomics: from genetics to genome sequence. WormBook. https://doi.org/10.1895/wormbook.1.116.1

Domenici, P., Booth, D., Blagburn, J.M., Bacon, J.P., 2009. Escaping away from and towards a threat. Commun Integr Biol 2, 497–500.

Donnelly, J.L., Clark, C.M., Leifer, A.M., Pirri, J.K., Haburcak, M., Francis, M.M., Samuel, A.D.T., Alkema, M.J., 2013a. Monoaminergic Orchestration of Motor Programs in a Complex C. elegans Behaviour. PLoS Biology 11. https://doi.org/10.1371/journal.pbio.1001529

Donnelly, J.L., Clark, C.M., Leifer, A.M., Pirri, J.K., Haburcak, M., Francis, M.M., Samuel, A.D.T., Alkema, M.J., 2013b. Monoaminergic Orchestration of Motor Programs in a Complex C. elegans Behaviour. PLoS Biology 11. https://doi.org/10.1371/journal.pbio.1001529

Eaton, R.C., 1984. Neural mechanisms of startle behaviour. Springer Science & Business Media.

Eisenhofer, G., Aneman, A., Friberg, P., Hooper, D., Fåndriks, L., Lonroth, H., Hunyady, B., Mezey, E., 1997. Substantial production of dopamine in the human gastrointestinal tract. J Clin Endocrinol Metab 82, 3864–3871. https://doi.org/10.1210/jcem.82.11.4339

Elsworth, J.D., Roth, R.H., 1997. Dopamine Synthesis, Uptake, Metabolism, and Receptors: Relevance to Gene Therapy of Parkinson's Disease. Experimental Neurology 144, 4–9. https://doi.org/10.1006/exnr.1996.6379

Equence, C.E.S., Iology, T.O.B., The, C., Consortium, S., 1998. Genome sequence of the nematode C. elegans: A platform for investigating biology. Science 282, 2012–2018. https://doi.org/10.1126/science.282.5396.2012

Evans, D.A., Stempel, A.V., Vale, R., Branco, T., 2019. Cognitive Control of Escape Behaviour. Trends in Cognitive Sciences 23, 334–348. https://doi.org/10.1016/j.tics.2019.01.012

Fanselow, M.S., Lester, L.S., 1988. A functional behaviouristic approach to aversively motivated behaviour: Predatory imminence as a determinant of the topography of defensive behaviour, in: Evolution and Learning. Lawrence Erlbaum Associates, Inc, Hillsdale, NJ, US, pp. 185–212.

Fatt, H.V., Dougherty, E.C., 1963. Genetic Control of Differential Heat Tolerance in Two Strains of the Nematode *Caenorhabditis elegans*. Science 141, 266–267. https://doi.org/10.1126/science.141.3577.266

Fernald, R.D., 2017. Cognitive skills and the evolution of social systems. The Journal of Experimental Biology 220, 103. https://doi.org/10.1242/jeb.142430

Fernstrom, J.D., Fernstrom, M.H., 2007. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. J Nutr 137, 1539S-1547S; discussion 1548S. https://doi.org/10.1093/jn/137.6.1539S

Finberg, J.P.M., Gillman, K., 2011. Selective inhibitors of monoamine oxidase type B and the "cheese effect," in: Youdim, M.B.H., Douce, P. (Eds.), International Review of Neurobiology, Monoamine Oxidase and Their Inhibitors. Academic Press, pp. 169–190. https://doi.org/10.1016/B978-0-12-386467-3.00009-1

Frezal, L., Felix, M.A., 2015. C. elegans outside the Petri dish. eLife 2015, 1–14. https://doi.org/10.7554/eLife.05849.001

Frisch, K. von, 2014. Bees: Their Vision, Chemical Senses, and Language. Cornell University Press.

Fu, J., Zhang, H., Huang, W., Zhu, X., Sheng, Y., Song, E., Xu, T., 2018. AIM interneurons mediate feeding suppression through the TYRA-2 receptor in C. elegans. Biophys Rep 4, 17–24. https://doi.org/10.1007/s41048-018-0046-2

Fujiwara, M., Sengupta, P., McIntire, S.L., 2002a. Regulation of body size and behavioural state of C. elegans by sensory perception and the egl-4 cGMP-dependent protein kinase. Neuron 36, 1091–1102. https://doi.org/10.1016/S0896-6273(02)01093-0

Fujiwara, M., Sengupta, P., McIntire, S.L., 2002b. Regulation of body size and behavioural state of C. elegans by sensory perception and the egl-4 cGMP-dependent protein kinase. Neuron 36, 1091–1102. https://doi.org/10.1016/S0896-6273(02)01093-0

Füzesi, T., Daviu, N., Wamsteeker Cusulin, J.I., Bonin, R.P., Bains, J.S., 2016. Hypothalamic CRH neurons orchestrate complex behaviours after stress. Nat Commun 7, 1–14. https://doi.org/10.1038/ncomms11937

Gainetdinov, R.R., Hoener, M.C., Berry, M.D., 2018. Trace Amines and Their Receptors. Pharmacol Rev 70, 549–620. https://doi.org/10.1124/pr.117.015305

Gems, D., Coburn, C., 2013. The mysterious case of the C. elegans gut granule: death fluorescence, anthranilic acid and the kynurenine pathway. Frontiers in Genetics 4.

Ghosh, D.D., Sanders, T., Hong, S., McCurdy, L.Y., Chase, D.L., Cohen, N., Koelle, M.R., Nitabach, M.N., 2016. Neural Architecture of Hunger-Dependent Multisensory Decision Making in C. elegans. Neuron 92, 1049–1062. https://doi.org/10.1016/j.neuron.2016.10.030

Gidwani, B., Vyas, A., 2015. A Comprehensive Review on Cyclodextrin-Based Carriers for Delivery of Chemotherapeutic Cytotoxic Anticancer Drugs. Biomed Res Int 2015, 198268. https://doi.org/10.1155/2015/198268

Gillman, P.K., 2018. A reassessment of the safety profile of monoamine oxidase inhibitors: elucidating tired old tyramine myths. J Neural Transm 125, 1707–1717. https://doi.org/10.1007/s00702-018-1932-y

Gonzalez-Moragas, L., Roig, A., Laromaine, A., 2015. C. elegans as a tool for in vivo nanoparticle assessment. Advances in Colloid and Interface Science 219, 10–26. https://doi.org/10.1016/j.cis.2015.02.001

Goodman, M., 2006. Mechanosensation. WormBook. https://doi.org/10.1895/wormbook.1.62.1

Goodman, M.B., Sengupta, P., 2019. How Caenorhabditis elegans Senses Mechanical Stress, Temperature, and Other Physical Stimuli. Genetics 212, 25–51. https://doi.org/10.1534/genetics.118.300241

Gray, J.M., Hill, J.J., Bargmann, C.I., 2005. A circuit for navigation in Caenorhabditis elegans. Proceedings of the National Academy of Sciences 102, 3184–3191. https://doi.org/10.1073/pnas.0409009101

Guillermin, M.L., Carrillo, M.A., Hallem, E.A., 2017. A Single Set of Interneurons Drives Opposite Behaviours in C. elegans. Current Biology 27, 2630-2639.e6. https://doi.org/10.1016/j.cub.2017.07.023

Guo, Z.V., Hart, A.C., Ramanathan, S., 2009. Optical interrogation of neural circuits in Caenorhabditis elegans. Nat Methods 6, 891–896. https://doi.org/10.1038/nmeth.1397

Guthrie, S., 1997. Axon guidance: Netrin receptors are revealed. Current Biology 7, R6–R9. https://doi.org/10.1016/S0960-9822(06)00007-8

Hapiak, V., Summers, P., Ortega, A., Law, W.J., Stein, A., Komuniecki, R., 2013. Neuropeptides Amplify and Focus the Monoaminergic Inhibition of Nociception in Caenorhabditis elegans. J Neurosci 33, 14107–14116. https://doi.org/10.1523/JNEUROSCI.1324-13.2013

Hare, M.L.C., 1928. Tyramine oxidase. Biochem J 22, 968–979.

Hart, A., 2006. Behaviour. WormBook. https://doi.org/10.1895/wormbook.1.87.1

Hedgecock, E.M., Russell, R.L., 1975. Normal and mutant thermotaxis in the nematode Caenorhabditis elegans. PNAS 72, 4061–4065. https://doi.org/10.1073/pnas.72.10.4061

Homberger, B., Korner-Nievergelt, F., Jenni-Eiermann, S., Duplain, J., Lanz, M., Jenni, L., 2021. Integrating behaviour, physiology and survival to explore the outcome of reintroductions: a case study of grey partridge. Animal Behaviour 176, 145–156. https://doi.org/10.1016/j.anbehav.2021.04.005

Honda, H., 1925. Experimental and cytological studies on bisexual and hermaphrodite free-living nematodes, with special reference to problems of sex. Journal of Morphology 40, 191–233. https://doi.org/10.1002/jmor.1050400202

Jozefowiez, J., Witnauer, J.E., Miller, R.R., 2012. The temporal pattern of responding in conditioned bar-press suppression: the role of the context switch and training mode. Behav Processes 89, 239–243. https://doi.org/10.1016/j.beproc.2011.12.002

Kamath, R.S., Ahringer, J., 2003. Genome-wide RNAi screening in Caenorhabditis elegans. Methods 30, 313–321. https://doi.org/10.1016/s1046-2023(03)00050-1

Kaplan, J.M., Horvitz, H.R., 1993. A dual mechanosensory and chemosensory neuron in Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America 90, 2227–2231. https://doi.org/10.1073/pnas.90.6.2227

Kauffman, A., Parsons, L., Stein, G., Wills, A., Kaletsky, R., Murphy, C., 2011. C . elegans Positive Butanone Learning , Short-term , and Long-term Associative Memory Assays 1–10. https://doi.org/10.3791/2490

Kindt, K.S., Quast, K.B., Giles, A.C., De, S., Hendrey, D., Nicastro, I., Rankin, C.H., Schafer, W.R., 2007. Dopamine Mediates Context-Dependent Modulation of Sensory Plasticity in C. elegans. Neuron 55, 662–676. https://doi.org/10.1016/j.neuron.2007.07.023

Korn, H., 1988. The Mauthner Cell, in: Comparative Neuroscience and Neurobiology. Birkhäuser, Boston, MA, pp. 69–71. https://doi.org/10.1007/978-1-4899-6776-3_29

Kosinski, R.A., Zaremba, M., 2007. Dynamics of the Model of the Caenorhabditis Elegans Neural Network. Acta Physica Polonica B 38, 2201.

Laurent, P., Soltesz, Z., Nelson, G.M., Chen, C., Arellano-Carbajal, F., Levy, E., de Bono, M., 2015. Decoding a neural circuit controlling global animal state in C. elegans. eLife 4, e04241. https://doi.org/10.7554/eLife.04241

Law, C., 2014. Conditioned Reflexes . An Investigation of the Physiological Activity of the Cerebral Cortex by I . P . Pavlov ; G . V . Anrep Review by : Franklin Fearing Journal

of the American Institute of Criminal Law and Criminology , Vol . 20 , No . 1 (May , 20, 153–155.
Lemoine, D., Jiang, R., Taly, A., Chataigneau, T., Specht, A., Grutter, T., 2012. Ligand-Gated Ion Channels: New Insights into Neurological Disorders and Ligand Recognition. Chem. Rev. 112, 6285–6318. https://doi.org/10.1021/cr3000829
Lindemann, L., Hoener, M.C., 2005. A renaissance in trace amines inspired by a novel GPCR family. Trends Pharmacol Sci.
López-Cruz, A., Sordillo, A., Pokala, N., Liu, Q., McGrath, P.T., Bargmann, C.I., 2019. Parallel Multimodal Circuits Control an Innate Foraging Behaviour. Neuron 102, 407-419.e8. https://doi.org/10.1016/j.neuron.2019.01.053
Luchkina, N.V., Bolshakov, V.Y., 2019. Mechanisms of Fear Learning and Extinction: Synaptic Plasticity — Fear Memory Connection. Psychopharmacology 236, 163. https://doi.org/10.1007/s00213-018-5104-4
M. Stain, G., T. Murphy, C., 2010a. C. elegans Positive Olfactory Associative Memory is a Molecularly Conserved Behavioural Paradigm. Neurobiology of Learning and Memory 9, 19–22. https://doi.org/10.1016/j.nlm.2014.07.011.C.
M. Stain, G., T. Murphy, C., 2010b. C. elegans Positive Olfactory Associative Memory is a Molecularly Conserved Behavioural Paradigm. Neurobiology of Learning and Memory 9, 19–22. https://doi.org/10.1016/j.nlm.2014.07.011.C.
Maguire, S.M., Clark, C.M., Nunnari, J., Pirri, J.K., Alkema, M.J., 2011. The C. elegans Touch Response Facilitates Escape from Predacious Fungi. Current Biology 21, 1326–1330. https://doi.org/10.1016/j.cub.2011.06.063
Maheshwari, A., Sharma, M., Sharma, D., 2013. Complexation of sodium picosulphate with beta cyclodextrin: NMR spectroscopic study in solution. J Incl Phenom Macrocycl Chem 77, 337–342. https://doi.org/10.1007/s10847-012-0251-4
Mallo, G.V., Kurz, C.L., Couillault, C., Pujol, N., Granjeaud, S., Kohara, Y., Ewbank, J.J., 2002. Inducible antibacterial defense system in C. elegans. Current Biology 12, 1209–1214. https://doi.org/10.1016/S0960-9822(02)00928-4
Manni, M.E., De Siena, G., Saba, A., Marchini, M., Landucci, E., Gerace, E., Zazzeri, M., Musilli, C., Pellegrini-Giampietro, D., Matucci, R., Zucchi, R., Raimondi, L., 2013. Pharmacological effects of 3-iodothyronamine (T1AM) in mice include facilitation of memory acquisition and retention and reduction of pain threshold. Br J Pharmacol 168, 354–362. https://doi.org/10.1111/j.1476-5381.2012.02137.x
Markers, C.T., 2019. Discovery and analysis of the C . elegans Neuronal Gene Expression Network – CeNGEN 11–13.
Matthews, H.R., Reisert, J., 2003. Calcium, the two-faced messenger of olfactory transduction and adaptation. Curr Opin Neurobiol 13, 469–475. https://doi.org/10.1016/s0959-4388(03)00097-7
Maupas, E., 1900. Modes et formes de reproduction des nematodes.
Melkman, T., Sengupta, P., 2004. The worm's sense of smell: Development of functional diversity in the chemosensory system of Caenorhabditis elegans. Developmental Biology 265, 302–319. https://doi.org/10.1016/j.ydbio.2003.07.005
Monastirioti, M., Linn, C.E., White, K., 1996. Characterization of Drosophila tyramine beta-hydroxylase gene and isolation of mutant flies lacking octopamine. J Neurosci 16, 3900–3911. https://doi.org/10.1523/JNEUROSCI.16-12-03900.1996
Mori, I., Ohshima, Y., 1995. Neural regulation of thermotaxis in Caenorhabditis elegans. Nature 376, 344–348. https://doi.org/10.1038/376344a0

Morrison, G.E., Wen, J.Y., Runciman, S., van der Kooy, D., 1999. Olfactory associative learning in Caenorhabditis elegans is impaired in lrn-1 and lrn-2 mutants. Behav Neurosci 113, 358–367. https://doi.org/10.1037//0735-7044.113.2.358

O'Hagan, R., Chalfie, M., Goodman, M.B., 2005. The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. Nat Neurosci 8, 43–50. https://doi.org/10.1038/nn1362

Ostrom, J.H., 1974. Archaeopteryx and the Origin of Flight. The Quarterly Review of Biology. https://doi.org/10.1086/407902

Ouellette, M.-H., Desrochers, M.J., Gheta, I., Ramos, R., Hendricks, M., 2018. A Gate-and-Switch Model for Head Orientation Behaviours in Caenorhabditis elegans. eNeuro 5, ENEURO.0121-18.2018. https://doi.org/10.1523/ENEURO.0121-18.2018

Pessoa, L., Medina, L., Desfilis, E., 2021. Refocusing neuroscience: moving away from mental categories and towards complex behaviours. Philosophical Transactions of the Royal Society B: Biological Sciences 377, 20200534. https://doi.org/10.1098/rstb.2020.0534

Philips, S.R., Rozdilsky, B., Boulton, A.A., 1978. Evidence for the presence of m-tyramine, p-tyramine, tryptamine, and phenylethylamine in the rat brain and several areas of the human brain. Biol Psychiatry 13, 51–57.

Pirri, J.K., Alkema, M.J., 2012a. The neuroethology of C . elegans escape. Current Opinion in Neurobiology 22, 187–193. https://doi.org/10.1016/j.conb.2011.12.007

Pirri, J.K., Alkema, M.J., 2012b. The neuroethology of C . elegans escape. Current Opinion in Neurobiology 22, 187–193. https://doi.org/10.1016/j.conb.2011.12.007

Pirri, J.K., Mcpherson, A.D., Donnelly, J.L., Francis, M.M., Alkema, M.J., 2009. A Tyramine-Gated Chloride Channel Coordinates Distinct Motor Programs of a Caenorhabditis elegans Escape Response. Neuron 62, 526–538. https://doi.org/10.1016/j.neuron.2009.04.013

Pirri, J.K., Rayes, D., Alkema, M.J., 2015a. A Change in the Ion Selectivity of Ligand-Gated Ion Channels Provides a Mechanism to Switch Behaviour. PLoS Biology 13, 1–20. https://doi.org/10.1371/journal.pbio.1002238

Pirri, J.K., Rayes, D., Alkema, M.J., 2015b. A Change in the Ion Selectivity of Ligand-Gated Ion Channels Provides a Mechanism to Switch Behaviour. PLoS Biology 13, 1–20. https://doi.org/10.1371/journal.pbio.1002238

Pradhan, S., Quilez, S., Homer, K., Hendricks, M., 2018. Environmental programming of adult foraging behaviour in C. elegans. bioRxiv 400754. https://doi.org/10.1101/400754

Prevett, J.P., Prevett, L. s, 1973. Egg Retrieval by Blue Geese on JSTOR.

Quinn, W.G., Harris, W.A., Benzer, S., 1974. Conditioned behaviour in Drosophila melanogaster. Proc Natl Acad Sci U S A 71, 708–712. https://doi.org/10.1073/pnas.71.3.708

R, R., SC, C., HR, H., 2000. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in C. elegans. Nature 408, 470–475.

Radermacher, M., Rao, V., Grassucci, R., Frank, J., Timerman, A.P., Fleischer, S., Wagenknecht, T., 1994. Cryo-electron microscopy and three-dimensional reconstruction of the calcium release channel/ryanodine receptor from skeletal muscle. J Cell Biol 127, 411–423. https://doi.org/10.1083/jcb.127.2.411

Rafehi, M., Faltraco, F., Matthaei, J., Prukop, T., Jensen, O., Grytzmann, A., Blome, F.G., Berger, R.G., Krings, U., Vormfelde, S.V., Tzvetkov, M.V., Brockmöller, J., 2019. Highly Variable Pharmacokinetics of Tyramine in Humans and Polymorphisms in

OCT1, CYP2D6, and MAO-A. Front Pharmacol 10, 1297. https://doi.org/10.3389/fphar.2019.01297

Reineccius, T. a., Reineccius, G. a., Peppard, T. l., 2002. Encapsulation of Flavors using Cyclodextrins: Comparison of Flavor Retention in Alpha, Beta, and Gamma Types. Journal of Food Science 67, 3271–3279. https://doi.org/10.1111/j.1365-2621.2002.tb09577.x

Ren, J., Yuan, H., Liu, X., Yu, Z., Meng, F., Xiong, L., Yang, N., Li, Y., Li, Z., Fan, Z., 2022. Novel Fluorinated Aniline Anthranilic Diamides Improved Insecticidal Activity Targeting the Ryanodine Receptor. J. Agric. Food Chem. 70, 10453–10465. https://doi.org/10.1021/acs.jafc.2c03134

Rex, E., Komuniecki, R.W., 2002. Characterization of a tyramine receptor from Caenorhabditis elegans. Journal of Neurochemistry 82, 1352–1359. https://doi.org/10.1046/j.1471-4159.2002.01065.x

Rex, E., Molitor, S.C., Hapiak, V., Xiao, H., Henderson, M., Komuniecki, R., 2004. Tyramine receptor (SER-2) isoforms are involved in the regulation of pharyngeal pumping and foraging behaviour in Caenorhabditis elegans. Journal of Neurochemistry 91, 1104–1115. https://doi.org/10.1111/j.1471-4159.2004.02787.x

Riedl, J., Fieseler, C., Zimmer, M., 2022. Tyraminergic corollary discharge filters reafferent perception in a chemosensory neuron. Curr Biol 32, 3048-3058.e6. https://doi.org/10.1016/j.cub.2022.05.051

Robertson, H.M., Thomas, J.H., 2006. The putative chemoreceptor families of C. elegans. WormBook 1–12. https://doi.org/10.1895/wormbook.1.66.1

Roeder, T., 2005. Tyramine and octopamine: ruling behaviour and metabolism. Annu Rev Entomol 50, 447–477. https://doi.org/10.1146/annurev.ento.50.071803.130404

Roy, K., Bomzan, P., Roy, M.C., Roy, M.N., 2017. Inclusion of tyrosine derivatives with α-cyclodextrin in aqueous medium of various pH conditions by surface tension, conductance, UV–Vis and NMR studies. Journal of Molecular Liquids 230, 104–112. https://doi.org/10.1016/j.molliq.2016.12.104

Roy, M.N., Saha, S., Kundu, M., Saha, B.C., Barman, S., 2016a. Exploration of inclusion complexes of neurotransmitters with β-cyclodextrin by physicochemical techniques. Chemical Physics Letters 655–656, 43–50. https://doi.org/10.1016/j.cplett.2016.05.031

Roy, M.N., Saha, S., Kundu, M., Saha, B.C., Barman, S., 2016b. Exploration of inclusion complexes of neurotransmitters with β-cyclodextrin by physicochemical techniques. Chemical Physics Letters 655–656, 43–50. https://doi.org/10.1016/j.cplett.2016.05.031

Rutigliano, G., Bräunig, J., Del Grande, C., Carnicelli, V., Masci, I., Merlino, S., Kleinau, G., Tessieri, L., Pardossi, S., Paisdzior, S., Dell'Osso, L., Biebermann, H., Zucchi, R., 2019. Non-Functional Trace Amine-Associated Receptor 1 Variants in Patients With Mental Disorders. Frontiers in Pharmacology 10.

Sandoval, G.M., Duerr, J.S., Hodgkin, J., Rand, J.B., Ruvkun, G., 2006. A genetic interaction between the vesicular acetylcholine transporter VAChT/UNC-17 and synaptobrevin/SNB-1 in C. elegans. Nat Neurosci 9, 599–601. https://doi.org/10.1038/nn1685

Sasakura, H., Tsukada, Y., Takagi, S., Mori, I., 2013. Japanese studies on neural circuits and behaviour of Caenorhabditis elegans. Frontiers in Neural Circuits 7.

Schafer, W.R., 2015a. Mechanosensory molecules and circuits in C. elegans. Pflugers Archiv European Journal of Physiology 467, 39–48. https://doi.org/10.1007/s00424-014-1574-3

Schafer, W.R., 2015b. Mechanosensory molecules and circuits in C. elegans. Pflugers Archiv European Journal of Physiology 467, 39–48. https://doi.org/10.1007/s00424-014-1574-3

Schafer, W.R., 2005. Deciphering the Neural and Molecular Mechanisms of C. elegans Behaviour. Current Biology 15, R723–R729. https://doi.org/10.1016/j.cub.2005.08.020

Scheiner, R., Abramson, C.I., Brodschneider, R., Crailsheim, K., Farina, W.M., Fuchs, S., Grünewald, B., Hahshold, S., Karrer, M., Koeniger, G., Koeniger, N., Menzel, R., Mujagic, S., Radspieler, G., Schmickl, T., Schneider, C., Siegel, A.J., Szopek, M., Thenius, R., 2013. Standard methods for behavioural studies of *Apis mellifera*. Journal of Apicultural Research 52, 1–58. https://doi.org/10.3896/IBRA.1.52.4.04

Sengupta, P., Chou, J.H., Bargmann, C.I., 1996. odr-10 Encodes a Seven Transmembrane Domain Olfactory Receptor Required for Responses to the Odorant Diacetyl. Cell 84, 899–909. https://doi.org/10.1016/S0092-8674(00)81068-5

Sinakevitch, I.T., Daskalova, S.M., Smith, B.H., 2017. The Biogenic Amine Tyramine and its Receptor (AmTyr1) in Olfactory Neuropils in the Honey Bee (Apis mellifera) Brain. Frontiers in Systems Neuroscience 11.

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., Yamamoto, K., 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 34, 395–402. https://doi.org/10.1038/ng1206

Tabara, H., Grishok, A., Mello, C.C., 1998. RNAi in *C. elegans* : Soaking in the Genome Sequence. Science 282, 430–431. https://doi.org/10.1126/science.282.5388.430

Tabara, H., Sarkissian, M., Kelly, W.G., Fleenor, J., Grishok, A., Timmons, L., Fire, A., Mello, C.C., 1999. The rde-1 Gene, RNA Interference, and Transposon Silencing in C. elegans. Cell 99, 123–132. https://doi.org/10.1016/S0092-8674(00)81644-X

Tartar, V., 1961. The Biology of Stentor, in: Tartar, V. (Ed.), The Biology of Stentor, International Series of Monographs in Pure and Applied Biology. Division: Zoology. Pergamon, pp. 11–27. https://doi.org/10.1016/B978-0-08-009343-7.50008-9

Taylor, S.R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M.B., Xu, C., Varol, E., Oikonomou, P., Glenwinkel, L., McWhirter, R., Poff, A., Basavaraju, M., Rafi, I., Yemini, E., Cook, S.J., Abrams, A., Vidal, B., Cros, C., Tavazoie, S., Sestan, N., Hammarlund, M., Hobert, O., Miller, D.M., 2021. Molecular topography of an entire nervous system. Cell 184, 4329-4347.e23. https://doi.org/10.1016/j.cell.2021.06.023

ten Brink, B., Damink, C., Joosten, H.M., Huis in 't Veld, J.H., 1990. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 11, 73–84. https://doi.org/10.1016/0168-1605(90)90040-c

Timmons, L., Fire, A., 1998. Specific interference by ingested dsRNA. Nature 395, 854. https://doi.org/10.1038/27579

Troisi, A., 2002. Displacement activities as a behavioural measure of stress in nonhuman primates and human subjects. Stress 5, 47–54. https://doi.org/10.1080/102538902900012378

Vidal-Gadea, A., Topper, S., Young, L., Crisp, A., Kressin, L., Elbel, E., Maples, T., Brauner, M., Erbguth, K., Axelrod, A., Gottschalk, A., Siegel, D., Pierce-Shimomura, J.T., 2011. Caenorhabditis elegans selects distinct crawling and swimming gaits via dopamine and serotonin. Proceedings of the National Academy of Sciences 108, 17504–17509. https://doi.org/10.1073/pnas.1108673108

Vyas, A., Saraf, Shailendra, Saraf, Swarnlata, 2008. Cyclodextrin based novel drug delivery systems. J Incl Phenom Macrocycl Chem 62, 23–42. https://doi.org/10.1007/s10847-008-9456-y

Walsh, J.J., Christoffel, D.J., Wu, X., Pomrenze, M.B., Malenka, R.C., 2021. Dissecting neural mechanisms of prosocial behaviours. Current opinion in neurobiology 68, 9. https://doi.org/10.1016/j.conb.2020.11.006

Ware, R.W., Clark, D., Crossland, K., Russell, R.L., 1975. The nerve ring of the nematode Caenorhabditis elegans: Sensory input and motor output. Journal of Comparative Neurology 162, 71–110. https://doi.org/10.1002/cne.901620106

Wicks, S.R., Rankin, C.H., 1995. Integration of mechanosensory stimuli in Caenorhabditis elegans. J Neurosci 15, 2434–2444. https://doi.org/10.1523/JNEUROSCI.15-03-02434.1995

Wong, S.Q., Jones, A., Dodd, S., Grimes, D., Barclay, J.W., Marson, A.G., Cunliffe, V.T., Burgoyne, R.D., Sills, G.J., Morgan, A., 2018. A Caenorhabditis elegans assay of seizure-like activity optimised for identifying antiepileptic drugs and their mechanisms of action. J Neurosci Methods 309, 132–142. https://doi.org/10.1016/j.jneumeth.2018.09.004

Yao, Y., Yang, Y.Z., Gu, T.T., Cao, Z.F., Zhao, W.M., Qin, H.R., Xu, Q., Chen, G.H., 2019. Comparison of the broody behaviour characteristics of different breeds of geese. Poultry Science 98, 5226–5233. https://doi.org/10.3382/ps/pez366

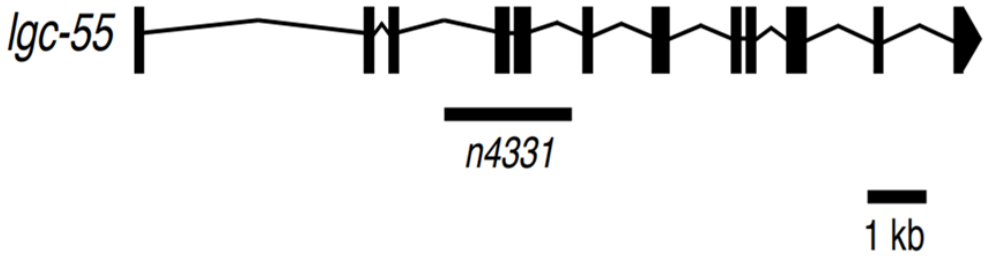
Yoshida, K., Hirotsu, T., Tagawa, T., Oda, S., Wakabayashi, T., Iino, Y., Ishihara, T., 2012. Odour concentration-dependent olfactory preference change in C. elegans. Nat Commun 3, 739. https://doi.org/10.1038/ncomms1750

Youdim, M.B.H., Edmondson, D., Tipton, K.F., 2006. The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci 7, 295–309. https://doi.org/10.1038/nrn1883

Zhou, H.-Y., Jiang, L.-J., Zhang, Y.-P., Li, J.-B., 2012. β-Cyclodextrin inclusion complex: preparation, characterization, and its aspirin release in vitro. Front. Mater. Sci. 6, 259–267. https://doi.org/10.1007/s11706-012-0176-2

Zhumakova, S., Ten, A., Zharkynbek, T., Yu, V., Seilkhanov, T., Basharimova, A., Bayazit, S., Aydemir, M., Zazybin, A., 2022. NMR study of the inclusion complexes of β-cyclodextrin with diphenhydramine, clonidine and tolperisone. SN Appl. Sci. 4, 75. https://doi.org/10.1007/s42452-022-04958-5

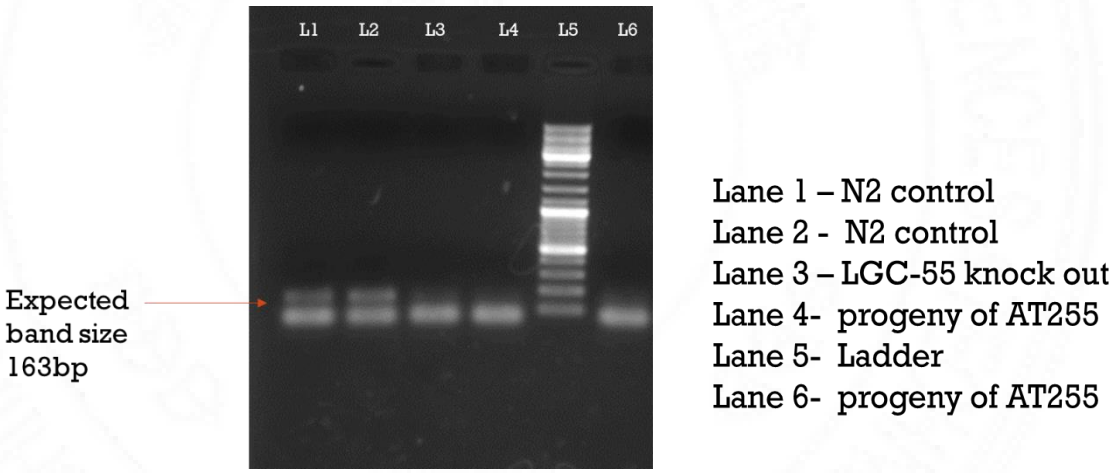
ANNEXURE I



The pictorial representation of LGC-55 gene with n4331 region spanning the 4th and 5th intron

Forward- LGC_55-F	CCCACTGCCTGACCAATTTA
Reverse-LGC_55-R	CGATAGTTCATCCAAACTGTTCC

PCR primers targeted towards LGC-55 receptor



Validation of LGC-55 knockout in progenies AT255 ser-2(ok2103);lgc-55(n4331) double mutant

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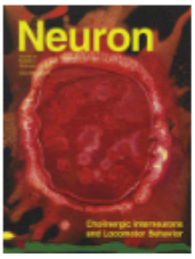

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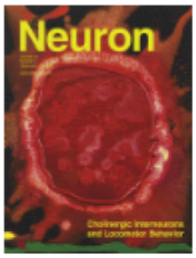

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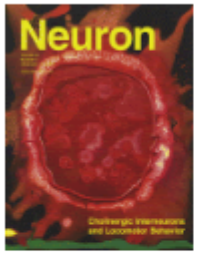

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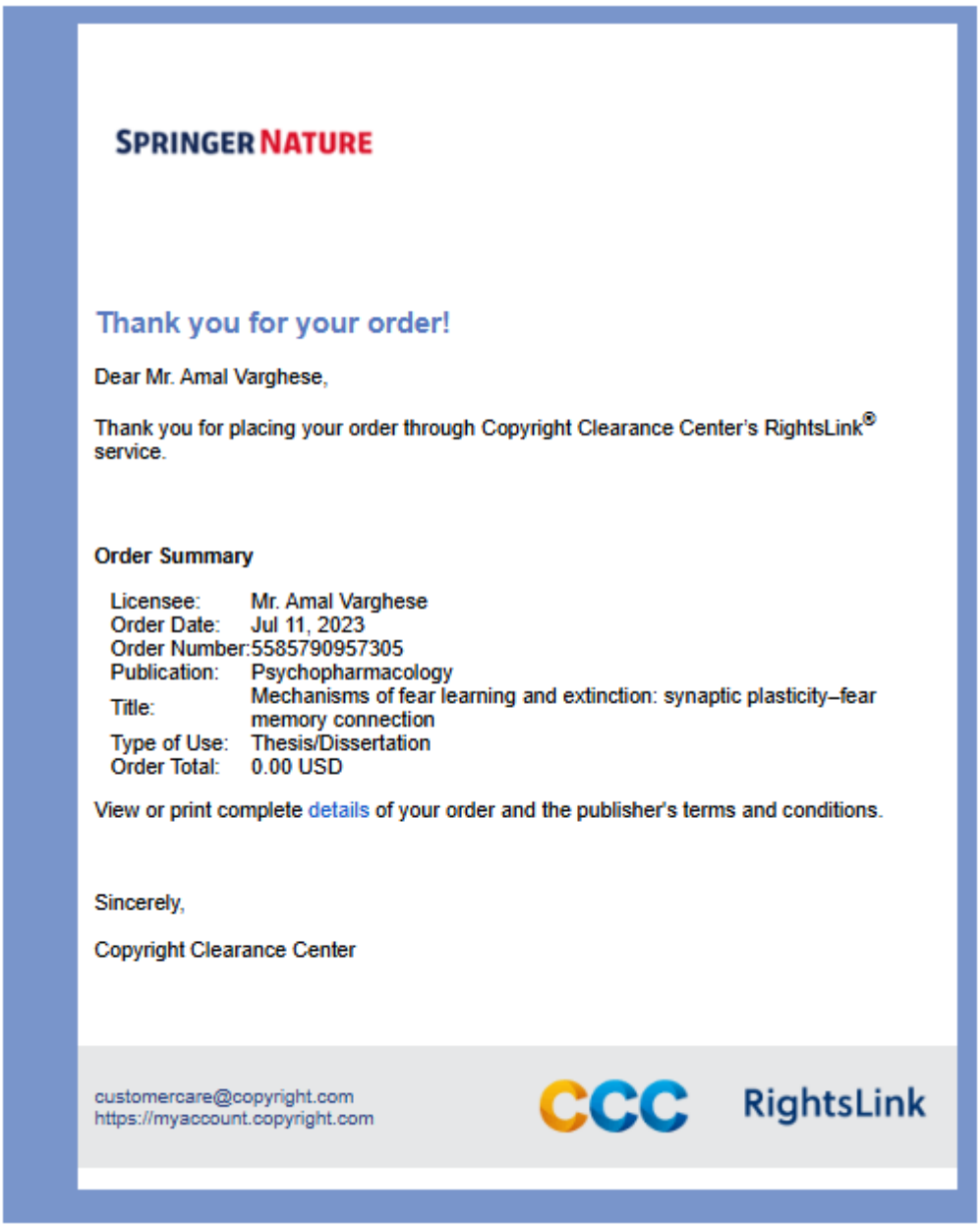

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