"Glutamate receptors mediate signal integration and odorant discrimination in *C. elegans*"

A THESIS SUBMITTED

BY

ASWATHY ARAVIND

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF PHILOSOPHY

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM – 695 012

DECLARATION

I, **Aswathy Aravind**, hereby declare that I had personally carried out the work depicted in the thesis entitled "**Glutamate receptors mediate signal integration and odorant discrimination in *C. elegans***" under the direct supervision of **Dr. Anoopkumar Thekkuveettil, Scientist G, Division of Molecular Medicine,** Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

Aswathy Aravind

Name of Student

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY

THIRUVANANTHAPURAM – 695011, INDIA

(An Institute of National Importance under Govt. of India)

CERTIFICATE

This is to certify that the dissertation entitled "**Glutamate receptors mediate signal integration and odorant discrimination in *C. elegans***" submitted by **Aswathy Aravind** in partial fulfilment for the degree of Master of Philosophy Technology in Biomedical Research to be awarded by this Institute. The entire work was done by her under my supervision and guidance at **Division of Molecular Medicine**, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, 695012.

Thiruvananthapuram

Date: **Dr. Anoopkumar Thekkuveettil**

Name of Guide

The Thesis

Entitled

"Glutamate receptors mediate signal integration and Odorant discrimination in *C. elegans*"

Submitted

By

Aswathy Aravind

For

Master of Philosophy

of

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM

Evaluated and approved

by

Dr. Anoopkumar Thekkuveettil

Name of Supervisor

Examiner's name and Designation

ACKNOWLEDGEMENET

Foremost, I would extend my deepest gratitude to my mentor Dr. Anoopkumar Thekkuveetil, Scientist G, Division of Molecular Medicine, for his immense support, encouragement and guidance throughout the course of my thesis work.

I am indebted to the Director Dr. Asha Kishore, SCTIMST; Dr. Harikrishna Varma P. R, Head, BMT Wing, SCTIMST; Dean Dr. V. Kalliyana Krishnan, Deputy Registrar Dr. Santhosh Kumar for providing me with the necessary infrastructure support for the successful completion of my project work.

My sincere appreciation to all the MPhil co-ordinators Dr. Maya Nandkumar A, Dr. Manoj Komath and Dr. Francis Fernandez, for their valuable support and encouragement throughout the MPhil course. I am also grateful to all MPhil faculties, scientists and staff of BMT wing.

I would like to express my sincere gratitude to all my lab members Mr. Vishnu Raj, Ms. Aswathy A.R, Ms. Rasitha S.K, Ms. Swathy S Nair, Mr. Amal Wilson Varghese and Ms. Geethu Shaji for their guidance and motivation in all my worm works.

Lastly but not the least I am grateful to all my family and friends for their constant support.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

B	Butanone
Bz	Benzaldehyde
cAMP	Cyclic Adenosine Monophosphate
CGC	Caenorhabditis Genetics Centre
cGMP	Cyclic Guanosine Monophasphate
CI	Chemotaxis Index
GPCR	G protein coupled receptors
IAA	Isoamyl alcohol
NGM	Nematode Growth Medium
OR	Odorant Receptor

LIST OF FIGURES

SYNOPSIS

C.elegans, depend on olfactory mechanism, chemotax to different odour sources for food (bacteria), mating and defence. Both informative and uninformative cues are prevalent in its natural habitat. A well-developed neuroanatomy has aided the animal to detect changes in odorant concentration and a gradient of one odorant while ignoring ubiquitous odorants.

Saturation assay facilitated us to mock a model experimental habitat which depicted that of the worm's normal environment. In this assay the chemotaxis behaviour of the worm is analysed towards an odorant spot (isoamyl alcohol, butanone or benzaldehyde) while being presented with a saturated background odorant (in our case benzaldehyde and butanone saturated background) respectively. This assay helped us find answers to alteration in behaviour of worms under such conditional cues.

The results suggested significantly lower efficiency in detecting the spot odour in presence of a saturated background. It was observed that *C. elegans* showed an aversive behaviour when the spot odorant was the same as the saturated background. However, it is seen that detection of other solvent that were different to that of the saturated background was also significantly affected when compared to its chemo-attractiveness towards these solvents when presented individually.

Our interest progressed towards exploring different receptor genes that could be involved in the discrimination pathway. We subjected mutant strains with genes associated to olfactory receptors (*str-2*, *sra-11*); glutamate receptors (glc-3, glr-1, glr-2 and nmr-1) and motor neuron receptor (*tdc-1*) on two different background saturations of benzaldehyde and butanone respectively. The results obtained for these experiments indicated significant alteration in odour detection for isoamyl alcohol and butanone. Interestingly the glutamate receptors exhibited to be involved in odour discrimination. In addition, it is surprising to witness the interneuron olfactory receptor gene like the *sra-11* did not play a role in the odour discrimination pathway.

Overall, the results indicate that glutamate receptors are essential for butanone detection pathway while both isoamyl alcohol and benzaldehyde detection is independent of these receptors.

INTRODUCTION

1.1 Background:

Mammalians are gifted with complex sensing mechanisms to distinguish different stimuli from their environment. Humans rely on senses such as sight, hearing, touch, smell, and taste to recognize the external stimuli. Out of the senses mentioned above, the sense of smell is one of the oldest senses which allows the organisms constituting receptors for the odorant to identify food, prospective mating partners, dangers, and enemies. Smell has been an important means by which many living organisms and mankind interact with the environment.

Our nose is offered with millions of olfactory sensory neurons that detect volatile odorants [Shepherd 1988; Buck 1996]. These neurons are responsible to transmit signals to the olfactory bulb of the brain, which, in turn, sends signals to the primary olfactory cortex. From there, olfactory information is relayed both to higher cortical areas and to the limbic system, which allows for both the conscious perception of odours and their emotional and motivational effects. The olfactory perception begins in the nose via the olfactory sensory neurons. These neurons arise in the olfactory epithelium that occupies about $5cm^2$ of the back of nasal cavity. Several million neurons supported with glia-like cells are constituted in this area over-lined with basal layer of stem cells. Olfactory neurons possess a unique feature among other neurons in that they have a short life span of about 30-60 days and that they are replenished by the basal stem cell population [Buck, 2005].

The olfactory neurons consist about 50 million receptor cells with 8-20 cilia extending down in a layer of mucus secretion by the Bowmann glands in the olfactory epithelium [Elsaesser & Paysan, 2007]. Volatile substances that are soluble in mucus can only produce sensations as they efficiently reach the receptors and interact with them. The axonal ramifications of these neurons come together in groups of 10 to 100 fibres and traverse the cribriform plate and extend towards olfactory bulb where they

join to form glomeruli and from which they converge to mitral cells [Sarafoleanu D, 1999].

The complexity of mammalian olfactory system offers them an enormous discriminatory power. Although humans are known to have a poor sense of smell when compared to many other animals, they still can perceive a vast number of volatile solvents. Odorants are small organic molecules (< 400 Da) and are of diverse nature based on number of factors like shape, size, functional groups and charge [Amoore 1970]. They comprise of a range of aliphatic acids, alcohols, aldehydes, ketones, and esters; chemicals with aromatic, alicyclic, polycyclic, and heterocyclic ring structures; and large number of substituted chemicals of each of these types, as well as combinations of them. Diversifying nature of these chemicals can be detected and discriminated remarkably well by the olfactory system.

Olfactory perception studies on humans have revealed information that is both astonishing and baffling at the same time. It has been documented that slight change in the structure of an odorant can affect the manner in which that particular odour is perceived [Beets 1970; Polak 1973]. This can be well illustrated in case of octanol, where hydroxyl group replaced with carboxyl group forms octanoic acid and the same is sensed as orange and rose-like to rancid and sweaty [Arctander 1969]. Also it is been observed that the change in concentration of the odorant can in turn cause a change in the perceived quality of an odorant. Indole when concentrated gives a putrid odour while the same when diluted gives a floral odour and is an example of one such situations. Perception of odours also differ with some odour detectable at a lower concentration than others [Cain 1988)]. Such mechanisms by which the olfactory system achieves its perceptual task and the means of the puzzling features of olfactory system are yet to be understood. Studies on rodent olfactory system have deciphered information about the cellular functioning and structure of the mammalian olfactory system and also provided molecular tools that allow to solve mysteries of the molecular bases of olfactory system.

Rats (*Rattus rattus*) have a well-developed ability to detect and identify odorants in minimal concentration [Quignon *et al*. 2005]. Interestingly, food- deprived rats

possess increased detection of odorant at low concentrations indicating the fact that olfactory sensitivity is increased in food- deprived animals [Aime *et al.* 2007]. Improvement in olfactory performance is attributed to repeated exposure to a particular substance in mammals such as rats [Doty and Ferguson- Segall 1989, Wilson 2000, Wilson and Stevenson 2003] and mice [Wang *et al.* 1993, Yee and Wysocki 2001].

Duration of exposure to an odour has been shown to influence the degree of adaptation and the rate of recovery. Investigations to back up this concept have been conducted by employing olfactory adaptation in nematodes [Colbert and Bargmann, 1995] and *Drosophila* larvae [Wuttke, 1999; Wuttke and Tompkins, 2000]. The basic model of such experiments involve introducing the organism in the centre of a Petri plate on which odorant and water (control) is spotted equidistant from it on opposite sides. In their normal state they will be responsive to most, but not all odorants. However, upon adaptation they would be randomly distributed throughout the plate.

Although many vertebrate and invertebrate models are used to study olfactory aspects, the most prominent model for study are hermaphroditic, free-living nematodes, *Caenorhabditis elegans*. *C .elegans* was introduced as a model organism in the early 1960s by Sydney Brenner for research in developmental biology and neurobiology. These are used to widely investigate biological processes that have remained unchanged during evolution [Brenner. S, 1974]. They exist as both hermaphrodites and males. Under normal conditions they can self-fertilize and give rise to several hundred progeny. Reproductive maturity is attained within 3-5 days, depending on the cultivation temperature. In addition, they can mate with males, resulting in cross-progeny. But since the number of males emerge at 0.1- 0.2 % frequency, it requires a large brood size to set up genetic crosses.

Wild-type worm consists of 959 somatic cells, of which 302 cells are neurons. Its transparent body allows easy access to track cells, follow cell lineages and biological processes [Sulston. *et al.*, 1983]. The genome of the worm is well-mapped and is known to comprise a hundred million base pairs. The genome is completely sequenced and is remarkably identical to that of humans. Estimates suggest that 60-

80% of the genes have human counterparts [Harris *et al.*, 2004]. Working with such a model is flexible as it allows one to manipulate them genetically to efficiently generate viable mutant strains, strains that overexpress a gene or lack a gene function so as to identify the resulting phenotype [Hariharan *et al.*, 2003].

Complete information regarding gene structure, expression patterns, protein- protein interactions, mutant or RNA interference (RNAi) phenotypes and microarray data, is available in Wormbase, the online resource for nematode-related information (http://www. wormbase.org/) [Chen *et al.*, 2005]. Wild-type and mutant strains of the worm can be stored indefinitely in liquid nitrogen allowing large mutant collections and public mutant repositories to be set up. *C. elegans* has emerged as a powerful experimental system to study the molecular and cellular aspects of human disease *in vivo.* It has been estimated that about 42% of the human disease genes have an ortholog in the genome of *C. elegans*, including those genes associated with Alzheimer's disease (AD), juvenile Parkinson's disease (PD), spinal muscular atrophy (SMA), hereditary nonpolyposis colon cancer, and many others age-related disorders [Baumeister *et al.*,2002; Poulin *et al.*, 2004; Kenyon *et al.*, 2005]. Modelling a human disease in a simple invertebrate, such as *C. elegans*, allows the dissection of complex molecular pathways into their component parts, thus providing a meaningful insight into the pathogenesis of a complex disease phenotype.

Much of what is known about olfaction in *C. elegans* is based on classical genetic studies of mutant strains defective in chemosensory response [Hart and Chao, 2010]. *C. elegans* depend on olfactory mechanism to chemotax to different odour source for food (bacteria), mating, and defence. Both informative and uninformative cues are prevalent in its natural habitat like compost or mud. A well-developed neuroanatomy has aided the animal to detect changes in odorant concentrations and a gradient of one odorant while ignoring ubiquitous odorants.

Environmental signals have an impact on the behaviour in which the worm responds. For instance, in *C.elegans* prolonged exposure to a particular odorant decreases its response to that odorant [Colbert & Bargmann 1995]. This behavioural modification represents a primitive form of memory. Viable mutants with specific defects in

olfactory adaptation while retaining normal olfactory perception have been identified [Colbert & Bargmann 1995].

Interestingly, adaptation to different odorants require different receptors that are encoded by different genes. Olfactory adaptation is not fully understood and the inherent mechanism to detect a gradient of one odorant while disregarding ubiquitous odorants is not well- established. Hence our experiments focus to demystify such puzzles so as to find answers on how the neural connectome of the worm is able to be responsive towards a particular odorant when placed in an environment constituting a background odorant.

1.2 Literature Review:

Evolution has facilitated all organisms with unique mechanisms to decode the information from their external milieu for adaption and survival. Most of the organisms are endowed with spectacular ways to recognize information from their environment. Sensory cues are perceived from the environment and relevant information is transmitted to the brain where it is processed to depict an internal image of the external environment. Animals explore the outer world via various senses such as taste, vision, touch and smell. An organism's perception about the environment is only based on the surrounding that it is subjected to and this varies among different organisms. The brain is then accountable to create its own selective image from that perceived by the external world. This image is critical for the survival and reproduction of the species.

For humans, smell is regarded to have an aesthetic sense, as it can be associated to elicit enduring emotions, thoughts and memories. Being of primal origin, smell is the sense that enables most of the organism with the ability to detect food, mate and predators. Humans are capable of recognizing varied different odours. Representing the central modality for many animals, smell has fascinated neuroscientist studying the brain to understand how external odorant molecules convey important information to be imaged in the brain for its molecular recognition and perceptual discrimination.

1.2.1 The Olfactory System:

For past 20 years, comprehensive knowledge of the mechanisms behind the detection of odours and reconstruction of the signals extracted from our external milieu to generate a 'smell map' in the brain has greatly advanced. Most of the work in this area was revealed from the remarkable work done by two Nobel laureates, Richard Axel and Linda Buck, who won the 2004 Nobel Prize in Physiology or Medicine "for their discoveries of odorant receptors and the organization of the olfactory system" [Rinaldi, 2007]. Their breakthrough work enabled us a clearer understanding of the process by which the odorants are detected by sensory neurons in the olfactory epithelium of the nose. The signals from here are in turn transmitted to the olfactory

bulb that passes the signal on to the olfactory cortex as represented in the **Figure: 1** below.

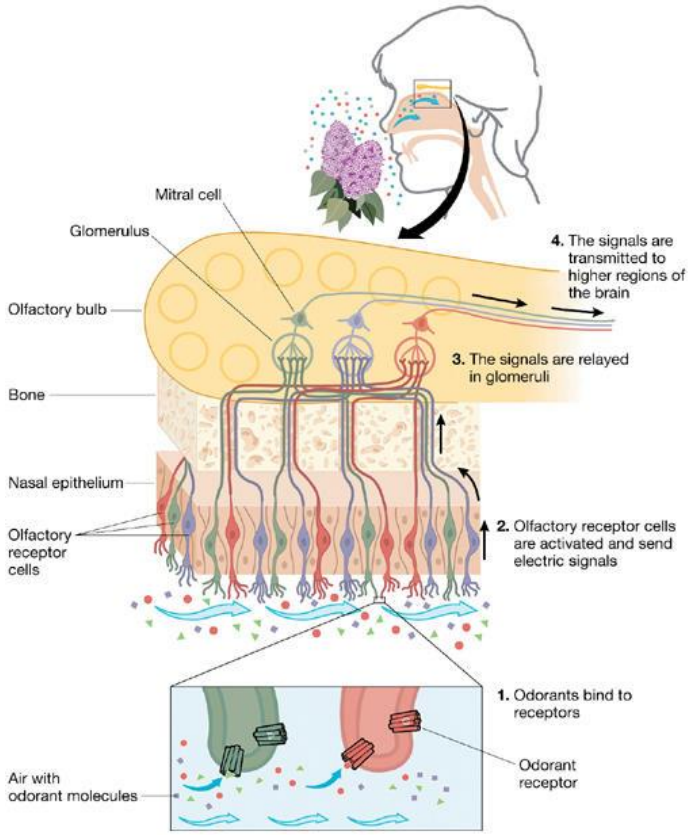

Fig 1. The human olfactory system. The odorant receptors are localized on olfactory sensory neurons, which occupy a small area in the upper part of the nasal epithelium. Every olfactory receptor cell expresses only one odorant receptor. On activation, signals from olfactory receptor cells are relayed in the glomeruli—well defined micro-regions in the olfactory bulb. Receptor cells of the same type are randomly distributed in the nasal mucosa but converge on the same glomerulus. In the glomerulus, the receptor nerve endings excite mitral cells that forward the signal to higher regions of the brain [Rinaldi, 2007].

The odorant receptors (ORs) present on the surface of the neurons show species dependent variability and specificity. For example the mouse has approximately 1,200 ORs, whereas humans comprise of much less than 400 ORs (Malnic, *et al.*, 2010)). Each OR binds to several odorants, and each odorant is specifically detected by a combination of ORs. This results in a primary input which is used in a combinatorial manner to encrypt odour identities. Neurons expressing the same OR, segregate with other neurons in an area in the olfactory bulb where the ‘first map’ of the odour input is formed. Axons which arise from the olfactory bulb link with several partly

overlapping clusters of neurons in the olfactory cortex. The resultant is a sensory map that differs from that obtained in the olfactory bulb as the olfactory cortex receives signals from other different ORs. This could build a possibility that single cortical neurons could assimilate signals obtained from different ORs that detect the same odorant to perform an initial step in the generation of an odour image from its deconstructed features [Zou and Buck, 2006]. Subsequently, neurons in the olfactory cortex might act coincidently to detect the required input from more than one OR to be active. The idea about the synthetic capacity of the olfactory cortex was illustrated by an experiment which inferred that binary odorant mixtures stimulated cortical neurons that were not active in presence of initial individual odorants, thereby creating a new odour image in the brain [Zou & Buck, 2006]. Although, further work needs to be carried out to understand how humans and animals in general are able to sense odorants in the first place.

1.2.2 Olfactory Discrimination

A combination of 275 unique component smells is known to combine to give the actual smell of a rose [Ohloff *et al.,* 1994], however each of these smells when present independently smell identical to that of the the flower as a whole.

For animals that are widely dependable on olfaction as one of their sensory modes of survival, accurately finding their ways to identify concoction of odours is a crucial problem to be solved. The enormous diversity of odours that differ from each other on the basis of quality and intensity, and the fact that the same has to be perceived, brands olfaction as an vital sensory mechanisms from which the information acquired can be utilised even in humans whose olfactory perceptual abilities are known to be weak when compared with other animal species [Castellucci *et al.,* 1970; Rawson *et al.,* 1997]. Estimates suggest that olfactory stimuli are 100,000 folds more discriminable in comparison to that of sight which is the next most sensitive modality [Bushdid *et al.,* 2014]. This however is claimed to be controversial [Gerkin & Castro, 2015]. This distinct information assimilated from the primary sensory organs must be successfully transmitted to the brain for higher processing and persists a challenge for animal signal transduction.

Chapter 1

The history of scientific research into olfaction is short. Early researches on olfaction was based on the idea that the varied range of smell must be simplified to combinations of some very small number of essential components [Daniel Merrit, 2016]. A classifier himself, Linneaus was the first to have attempted this, classifying enormous diverse smells to various combination of intensities of seven constituents namely, camphoraceous, musky, floral, pepper minty, ethereal, pungent and putrid [Weinstock, 1985]. Following this various other forms of representations came into picture, of which included Hendrik Zwaardemaker's classification in the late 19 th century. Such mode of influential classification extended the key components of smell to nine in number and were categorised as ethereal, aromatic fragnant, ambrosiaac, alliaceous, empyreumatic, hircine, foul and, nauseous [Zwaardemaker, 1889]. Even though the various classification schemes came into existence and was approved and disapproved, allowing all the efforts to reach to fruition in a publication in 1927 which was then termed as the "Crocker- Henderson System". This system organised smells into four primary types- fragrant, acid, burnt and, caprylic. Each of this smells when ranked in a scale of 1 and 8, were adequate enough to appropriately describe all possible smells [Crocker and Henderson, 1927].

Ultimately in the 1960's, a much clearer perspective was attained about the physiology of olfaction. A broader picture about the mammalian olfactory system came into light. The mammalian olfactory system consisted of the mammalian epithelium which lines the nasal cavity, and was found to constitute networks of olfactory sensory neurons, known to be responsible for olfactory signal transduction. This region also consisted of sub-sets of basal cells that played an important role to replenish the olfactory sensory neurons; brush cell, which functioned as non-olfactory modalities; and supporting cells [Haryran, 2013; Moulton and Beidler, 1967]. Olfactory sensory neurons protrude to single, long, unbranched axons through the cribriform plate, where it forms dense bundle in a region of glomeruli. Here these neurons are known to synapse onto dendrites of mitral and tufted cells [Pinching and Powell, 1971; Read, 1908; Ressler, Sullivan and Buck, 1994].

The mid 1970's the biochemical understanding of odorants and its perception by our system in turn lead to the discovery, that the odorant molecules bind to olfactory

sensory neuron via the cilia of the olfactory sensory neurons [Bronstein and Minor, 1997]. In addition during this time it was also understood that olfactory signal transduction progressed through a cyclic adenosine monophosphate (cAMP) dependent mechanism [Pace *et al.,* 1985]. Further studies suggested finding of an olfactory sensory neuron specific G protein alpha subunit [Jones and Reed, 1989], together with the exogenous Guanosine triphosphate (GTP) stimulated olfactory cells [Sklar *et al.,* 1986]. These findings gave some notion to the researchers working in the field, that olfactory receptors might constitute a specialised subgroup of heterotrimeric G protein coupled receptors (GPCR). Cloning experiments and analysis of the genes representative of the diverse family of GPCRs solely expressed in the olfactory epithelium confirmed this concept. Both mouse and human genome were known to contain genes that encoded approximately 1000 olfactory receptors [Ressler *et al.,* 1994; Rouquier *et al.,* 1998]. All these genes in the mouse genome are known to be functional, whereas 70% of olfactory receptor like open reading frame in the human genome are non-functional pseudogenes [Rouquier *et al.,* 1998].

Olfactory sensory neurons in mammals were found to express one or only few of the olfactory receptors in their genome [Ngal *et al.,* 1993; R. Vassar *et al.,* 1993]. Moreover, in mammals the olfactory epithelium are split into four zones. Each of these zones expresses a particular receptor specific to that zone and the location is conserved across animals [R. Vassar *et al.,* 1993]. The choice of a receptor or receptors a given olfactory sensory neuron expresses appears to be determined by the process of allelic inactivation which ensures that only one receptor from a large cluster of genes is expressed per cell [Chess *et al.,* 1994].

The axon of the olfactory sensory neuron expressing a particular kind of receptor tend to congregate and meet up to the same glomerulus, in such a manner that a given glomerulus in the olfactory bulb contains projections from neurons that express one, or at the most few olfactory receptors [Robert Vassar *et al.,* 1994]. An odorant molecule activates the olfactory receptor which results in signal transduction directed to a particular corresponding glomerulus. Many odorants can activate individual olfactory receptor or several olfactory receptors can be activated by a single odorant. The resultant is a multitude encrypt for olfaction wherein the uniqueness of a

particular odorant is conveyed to the brain by the respective combinatorial codes from distinctive combination of olfactory receptors activated by it [Malnic *et al.,* 1999]. Since each axon of the neurons expressing one or a few olfactory receptors converge on single glomeruli, the problem of cross-talk between mammalian olfactory systems is largely avoided. Even if this is the case still, the ability of an animal to discriminate between odours activating a partially overlapping combinatorial set of glomeruli remains limited, with the odour activating highly similar sets of glomeruli being less distinguishable that those activating more divergent sets [Rokni *et al.,* 2014].

Initially researches focused on agonists of olfactory receptors to determine the molecular mechanisms underlying olfaction. However recent studies have been deviated to resolve the antagonism of olfactory discrimination [Yuki Oka *et al.,* 2004]. As anticipated olfactory antagonists share some structural association with agonist like having similar functional groups even with different length of carbon chains when compared to that of its agonist's kind [Sanz *et al.,* 2005] or in other being oxidatively dimerized versions of the agonists [Y Oka *et al.,* 2004]. It can be predicted that antagonisms by odorants at olfactory receptor with respect to agonism by related odorants notably improves the complexity of the combinatorial olfactory code thus allowing for greater olfactory discrimination to be achieved by a mechanism making use of receptor alone [Sanz *et al.,* 2005].

Although diseases associated with olfaction is of limited importance, it is known to affect around one- fifth of the population and is said to have a significant detrimental impact on the quality of life. Olfactory disorder could arise as an abnormal condition as post nasal infections or head injuries [Croy *et al.,* 2014]. But it could be genetic in origin as in case of the developmental disorder Kallmann Syndrome [MacColl *et al.,* 2002]. Loss of olfaction (anosmia), or reduced olfaction (hyposmia) is attributed as a causative factor of depression. About approximately a quarter to a third of people suffering anosmia are being afflicted [Croy *et al.,* 2014].

In Alzheimer's patients, it is common to observe failure to detect and discriminate between odours [Talamo *et al.,* 1989] and the same has been proposed as an early indicator of the disease [Devanand *et al.,* 2000]. Relatedly idiopathic hyposmia can be

an early prophet of Parkinson's disease [Haehner *et al.,* 2007]. Early involvement of the olfactory system in these neurodegenerative diseases, fascinated researchers to come up with the concept of the "olfactory vector hypothesis", which stated that either or both of these diseases are caused due to the entry of a chemical or virus in the nasal passage, wherein it affects the olfactory bulb and the tract before invading and expanding the remainder of the brain [Hawkes *et al.,* 1999], this concept however poses conflicts among researchers [Doty, 2008].

1.2.3 Invertebrates as model system for olfactory research:

1.2.3.1 *Drosophila*

The remarkable work of evolutionary biologists Thomas Hunt Morgan in establishing the utility of fruit flies for the study of biology paved way to give importance to small model organisms for understanding the principles governing mammalian biology [Morgan, 1910]. Utilising small, invertebrate model organisms for research has proven to be advantageous due to low cost of maintenance, well- understood short life span and the fact that it does not pose any ethical issue as compared to the experiments conducted in large vertebrates.

Early studies on olfaction in *Drosophila* began with the assays understanding learning and memory behaviours associated with odours presented as a conditioned stimulus. These assays included punishing (electric shock) the flies in presence of one or a combination of odours, which would, in turn affect the fly's behaviours towards that odour [Quinn *et al.,* 1974]. Odorant based behavioural studies paved way to the discovery of specific olfactory genes [Ayyub *et al.,* 1990] as well as the mapping brain regions linked to olfaction [de Belle *et al.,* 1985].

Advancement in studies of *Drosophila* genetics, directed researchers to identify genetic mutants that was relative to odour detection at an early stage [Benzer & Konopka, 1971; Kikuchi, 1973]. Both ethological and behavioural researches cleared the cellular and physiological basis of odour perception. Remarkable works by researchers have highlighted that individual glomeruli in the anterior lobe are

functional units that receive antennal inputs in distinctive combinations [Stocker *et al.*, 1983].

With the discovery of mammalian odorant receptors [Buck & Axel, 1991] paired with algorithms to search for these genes in the fully sequenced *Drosophila* genome [Adams *et al.,* 2000; Clyne *et al.,* 1999] led to the neuroscientific tools and methods used today that thrust *Drosophila* into the forefront of olfactory research.

1.2.3.2 *Caenorhabditis elegans*

In the twentieth century Sydney Brenner introduced *Caenorhabditis elegans* as a model organism to decipher the molecular and cellular biology affecting animal behaviour [Tissenbaum, 2015]. A large body of work has been carried such as to delineate the principles in cell biology, developmental biology, cell lineages, apoptosis, and siRNA to name a few. These innovative works on the nematode worm earned five Nobel Prize winning discoveries.

The adult hermaphrodites constitute totally 959 cells, an invariant cell lineage and, a very superficial genetics thus making it a powerful model by which developmental and behavioural processes might be understood in their totality. Progress of research in *C. elegans* has made is feasible of our profound understanding of the concepts governing aging [Dorman *et al.,* 1995], embryogenesis [Sulston *et al.,* 1983] apoptosis [Ellis & Horvitz, 1986] and RNA regulation [Fire *et al.,* 1998], etc. Apart from these, studies on *C. elegans* have also provided important knowledge about complex phenomena that seem difficult to be modelled in a 1mm long worm, along with olfactory processing of information from the external milieu [Sengupta *et al.,* 1996], and the mechanisms influencing learning and memory functions [L'Etoile *et al.,* 2002]. In spite of its simple nature, still the in depth understanding about such process yet remains a puzzle that poses some challenges to be solved.

Olfaction research on nematode *C. elegans* embarked upon a realization that *C. elegans* demonstrated naïve chemoattraction or chemorepulsion from volatile odorants [Bargmann *et al.,* 1993], however chemotaxis by the worm towards the water soluble odorants was long known [Ward, 1973]. Captivatingly it emerged that

over half of the odorant when tested, provoked some chemotactic behaviour by the worms, with most being chemoattractive to the odorant [Bargmann *et al.,* 1993]. Furthermore it became clear from laser ablation studies of selected individual neurons revealing that some odorants are sensed widely by the AWA, AWB or AWC chemosensory neurons. Extensively varied variety of chemical structures were detected by the worms, which included alcohols, ketones, aromatic compounds, pyrazines, etc. Generation and screening of mutants further enlightened us about the information about the genes involvement on the olfactory behaviour of the worm. Some of these mutant strains were unable to detect some or all odorants, an example of which included mutations in the genes *odr-1*, *odr-2*, *odr-3*, *odr-4*, and *odr-5* respectively. Consequently research has also disclosed that unlike mammals, worms appear to express many chemosensory olfactory receptor genes, an inevitable evidence that the worm possesses many olfactory receptors and only 32 chemosensory neurons [Bargmann, 2006].

C. elegans ability to perceive diversity of chemical structures encouraged scientists to question whether different odorants were distinguishable from each other by the worms, or whether the signals generated exhibited crosstalk either by sharing a common receptor or by corresponding across a shared pathway [Merrit D, 2016]. To answer this question an experiment was formulated, where in worms were placed in a petri dish on agar which was previously saturated uniformly with one odorant at a high concentration, and on to which another spot of second odorant was applied. Worms showed directed chemotaxis towards the point source of odorant despite being surrounded by a background of saturated odorant. This was observed for many combination of odorants. In contrast, when the point odorant was same as the background of saturated odorant, no chemotaxis to the point source of the odorant was seen, even if the point odorant was spotted at a higher concentration than the saturated odorant. This skill of differentiating between odorants was found to be the case for pairs of odorants sensed by the same cells, as seen in the case of isoamyl alcohol and benzaldehyde, each of which is sensed by both AWC neurons [Merrit D, 2016].

Conversely even though the worms express many olfactory receptors per sensory neurons and mammals are known to express only one or very few, much is conserved

between olfaction in *C. elegans* and mammals. The olfaction in worms is perceived when the odorant molecules bind to the olfactory receptors that line the cilia of amphid sensory neurons. Like in mammals, these olfactory receptors are 7-transmembrane GPCRs, however they are not closely related to mammalian GPCRs [Robertson, 1998]. Upon activation these receptors results in release of G-protein alpha subunit. This in turn is responsible for downstream signal transduction [Roayaie et al., 1998] through an ion channel. For both cases, the ion channel is cyclic nucleotide gated, but the particular cyclic nucleotide differ in that for *C. elegans* it is cGMP and cAMP for mammals [Komatsu *et al.,* 1999]. Conversely, much of the machinery of olfactory signal transduction is conserved between worms and humans, thus allowing functional expression of worm's olfactory receptors in human cells [Zhang *et al.,* 1997]. However along with such similarities there are significant differences between mammalian and nematode olfaction. The particular sensory neuron olfactory receptors are expressed to play important role in determining responses to the corresponding ligands. *Odr-1*0 is the first olfactory receptor to have identified and studied in *C. elegans*. *Odr-10* was learnt to be required for chemmoattraction to low-concentrations of diacetyl, but with the mutations in the gene or loss of the gene has no significant effect on chemotaxis toward other odorants. Including those known to be sensed by the diacetyl- responsive AWA neuron pair [Sengupta *et al.,* 1996]. Attractive olfactory responses are mediated by receptors and expressed in the chemosensory neurons AWA and AWC, however those associated with repulsive responses are expressed in the chemosensory neurons AWB and ASH [Bargmann & Mori, 1997]. Transgenic expression of *odr-10* in AWB neurons resulted diacetyl to switch from being chemo attractive to being chemo repulsive respectively. This suggested that the entire valence of odours is determined by the identity of the neuron the corresponding receptor is expressed in [Troemel, Kimmel, & Bargmann, 1997]. By demonstrating that the valence of the response to chemosensory stimuli depends exclusively on the cell on which the receptor is expressed in, and is independent to the identity of that receptor, these experiments further come up with a curiosity about how the identity of various ligands binding to a receptor on a single cell can be encoded after initial binding at olfactory receptors.

Lastly but not the least problem of perception can be solved with an understanding of how the sensory input is conveyed into meaningful neural output in the forms of thoughts and behaviours.

HYPOTHESIS

Olfactory signalling pathway is complex. It is however not clear whether behaviour of an organism can get altered in the presence of two different background signals compare to its behaviour to a single signal.

It is also not clear how olfactory receptors help in differentiating multiple odours when presented simultaneously.

Since there are indications that there is involvement in Ca^{2+} in odour recognition, it would be interesting to see whether Ca^{2+} gated glutamate receptors in the neurons play any critical role in the odour recognition pathway.

OBJECTIVES

1. To understand the worms behaviour towards odorants such as isoamyl alcohol, butanone, and benzaldehyde when presented with a saturated benzaldehyde or butanone background.
2. To identify the role of following receptors using receptor mutants in odour recognition :
    - olfactory receptors like *sra-11, str-2.*
    - glutamate receptors like *glc-3, glr-1, glr-2* and *nmr-1.*
    - motor neuron receptors like *tdc-1.*

MATERIALS AND METHODS

2.1 WORM MAINTENNANCE AND STRAINS:

C. elegans strains were maintained under standard conditions (Brenner. S, 1974). Growth and maintenance of all the strains were on Nematode Growth Medium (NGM) at 20^0C (LABLINE incubators: BOD Incubator). Plates were seeded with *E.coli* OP50 strain, which is a uracil auxotroph that serves as a food source for the worms at 37^0C (TORREY PINES SCIENTIFIC- ECHO therm chilling incubator).

2.2 STRAINS:

The following strains were used: N2 Bristol (Wild Type), JC2209 (*str-2* mutant), RB594 (*glc-3* mutant), RB816 (*sra-11* mutant), MT10549 (*tdc-1* mutant), VM487 (*nmr-1* mutant), KP4 (*glr-1* mutant), and RB1808 (*glr-2* mutant).

All the strains were procured from Caenorhabditis Genetics Centre (CGC), University of Minnesota. All experiments were performed on the day-1 adult stage worms.

2.3 SYNCHRONISATION OF *C. ELEGANS*

Healthy gravid adult worms were washed with M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 litre. Sterilize by autoclaving) and collected in Eppendorf tube (1.5mL). The worms were washed thrice with M9 buffer. 1mL of Bleach solution (Sodium hypochlorite + NaOH [20%]) was added and the tube was vortexed (SPINIX) every 2 minutes for 10-12 minutes until the worm cuticle lysed off completely to obtain a clear solution. This solution was centrifuged at 7000 RPM (Biofuge pico- Heraus Instruments) for a minute. Supernatant was discarded and the pellet obtained was suspended in M9 buffer and centrifuged. This step was repeated

twice to wash off any excess bleach content in the pellet. The pellet consisting of the eggs were re-suspended in few drops of M9 buffer and dropped on to OP50 seeded NGM plates and were kept at 20^0C. Eggs are hatched after 9-12 hours to give synchronised worms.

2.4 CHEMOTAXIS ASSAY

Test and control spots were marked behind on a 8.6 cm chemotaxis plate at about 0.5 cm from the edge of the plate. A drop of M9 buffer was placed on the centre of the plate. 15 worms were pick transferred onto the drop. Sodium azide (1µL, 100mM), an anaesthetic agent was spotted on both spots. Test and control solvents (1µL) were spotted on the respective marks 3.8cm equidistant from both the sides. The M9 drop was dried of to remove any excess moisture with a Kim wipe and lid was closed immediately. The assay time for all the assays was set to 60 minutes [Bargmann *et al.*, 1993]. The number of worms at each spot was counted after 60 minutes under microscope (LEICA S8APO) and the chemotaxis index was calculated using the following formula:

$$\text{Chemotaxis index(CI)} = \frac{\text{Number of worms in the solvent spot} - \text{number of worms in the control spot}}{\text{Total number of worms}}$$

2.5 SATURATION ASSAY:

Saturation assay is analogous to the above mentioned chemotaxis assay. The assay utilises a saturated chemotaxis plate and a normal assay plate (which is the control plate). The chemotaxis assay is performed as mentioned above and chemotaxis index (CI) calculated [Bargmann *et al.*, 1993**]**.

2.6 STATISTICAL ANALYSIS

All statistical analysis and graphs were generated using Graph pad prism 6th edition (Graphpadprism.inc).

Each experiments were conducted in three sets for every individual dilutions. The significance for both the test and control values for each strains were analysed using two way ANOVA.

Further the values for both control and test for N2 and each mutant strain were compared and analysed using Fisher's test respectively.

RESULTS AND DISCUSSIONS

3.1 Chemotaxis Behaviour in *C. elegans* alters:

Chemoattractive behaviour of *C.elegans* to different volatile odorants in well characterised. *C. elegans* demonstrate a peculiar behaviour towards solvent benzaldehyde, in that it's seen to be attractive towards benzaldehyde at lower concentration but shows an aversive behaviour when presented with a higher concentration. Interestingly, isoamyl alcohol and butanone attracted worm at higher concentrations [Bargmann *et al.,* 1993]. This typical behaviour by the worm was observed when it was presented with individual solvents at a time. Conversely, this further interested us to understand the alteration in behaviour of the worm when exposed to a solvent in the presence of a background solvent.

To understand this we performed saturation assays [Bargmann *et al.,* 1993], in which we questioned whether the worm would be able to discriminate between the spot of a solvent in the presence of a background solvent. When we analysed the chemotaxis of the wild type N2 worm in the saturated benzaldehyde background towards different solvents like isoamyl alcohol, butanone and benzaldehyde itself, the results show that there is significant lower efficiency in detecting the odour in the presence of a background **(Figure 1)**. It is seen that when spot odorant is the same as the saturated background the worm displayed an aversion to the odorant spot, by directing its movement towards the control solvent. However, when presented with a different solvent (isoamyl alcohol and butanone) other than the background, a significant reduction in the chemotaxis index is observed. These results were similar to that of Bargmann *et al.,* 1993.

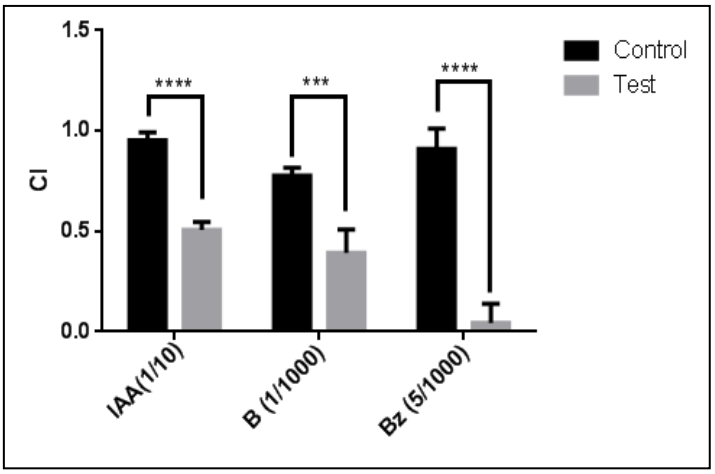

Fig.1. Chemotaxic index of worms control (without any background solvent) vs benzaldehyde (Bz) solvent as background

3.2 Involvement of olfactory receptors in the odorant recognition pathway:

With some insight about the altering behaviour of *C. elegans* in the presence of a background, we next wanted to understand if any olfactory receptor had a role to play in the odorant recognition pathway.

As documented, *C. elegans* constitute distinct genetic mechanisms to control the initial specification and maintenance of a pair of olfactory neuron AWC^{ON} and AWC^{OFF}. Both these neurons share similar morphologies. However, they have different functions and patterns of gene expressions [Troemel *et al.,* 1999]. AWC^{ON} senses the odorant butanone and is known to express the G protein-coupled receptor gene *str-2*, which promotes either attractive or repulsive behaviours based on inputs from guanylate cyclase [Troemel *et al.,* 1999; Wes and Bargmann, 2001; Tsunozaki *et al.,* 2008]. Upon performing a saturation assay on *str-2* mutant on a saturated benzaldehyde background, it showed a significantly low detection of butanone which is same for the control plate i.e. without any background saturation. This indicated butanone sensing defect due to the receptor mutation. In addition, *str-2* mutants also

show significantly low attraction towards isoamyl alcohol in the saturated background when compared to the control assay plate **(Figure: 2)**. Though the Fisher's test showed the change in attraction is not significantly different from that of N2 for isoamyl alcohol and butanone. This suggests that *str-2* in AWC neuron becomes a processing 'bottle neck' when *str-2* is mutated for butanone and affects the recognition of other odorants also.

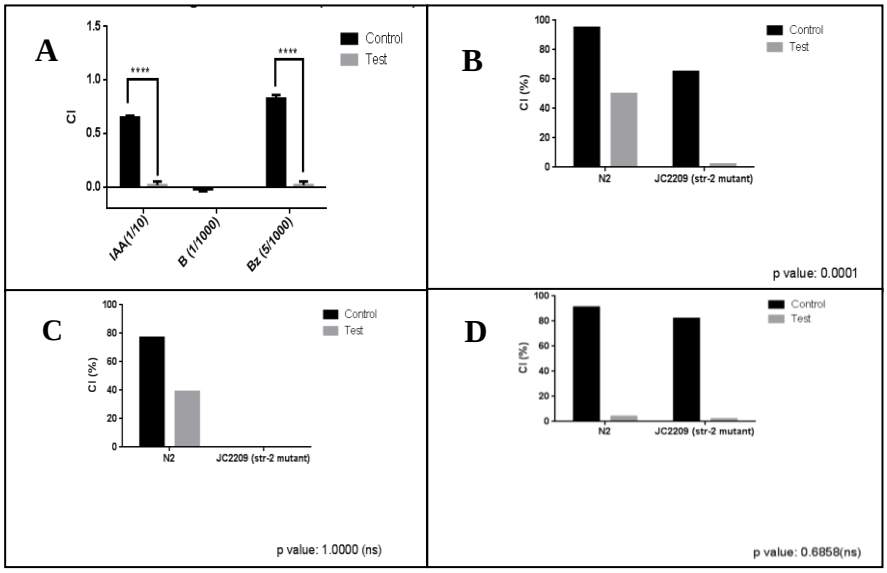

Fig 2. Chemotaxic behaviour and percentage of chemotaxis of *str-2* mutant worms. . **A:** Chemotaxis index for Saturation Assay for JC2209 (*str-2* mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and JC2209 (*str-2* mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and JC2209 (*str-2* mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and JC2209 (*str-2* mutants). The p value showed that both for isoamyl alcohol (IAA) and Benzaldehyde (Bz) the behaviour patterns were similar to that of N2 (p value <1.0 and <0.65 respectively).

Tyramine synthesis in *C.elegans* occurs via tyrosine decarboxylase (*tdc-1*), and a tyramine beta-hydroxylase (*tbh-1*) which is required for conversion of tyramine to octopamine [Alkema *et al.*, 2005]. Both *tcd-1* and *tbh-1* are co-expressed within a single pair of interneurons, the RICs, and in gonadal sheath cells that indicates that these are octopaminergic. Moreover, *tdc-1* is also expressed solitarily in the RIM motor neurons and the uterine UVI cells and is uniquely tyraminergic in *C. elegans*. The absence of tyramine and octopamine (*tdc-1 mutants*) in worms have shown to

retain behavioural defects like defects in egg laying, reversal behaviour and failure to supress head movements upon response to gentle anterior touch. Cell ablation and neural wiring diagram studies [White *et al.*, 1986] in *C.elegans*, reveal that the tyraminergic RIM neurons are associated to the neural circuits that control locomotion and head movements [Pirri *et al.*, 2009]. When *tdc-1* mutant is subjected to saturation assay under benzaldehyde background, no significant attraction were shown in recognition to isoamyl alcohol, benzaldehyde and butanone odours in the background of benzaldehyde compare to that of N2 (Fisher's test p value <0.2, <0.08 and <0.12 respectively suggesting that RIM neurons do not involve in odour discrimination **(Figure 3)**.

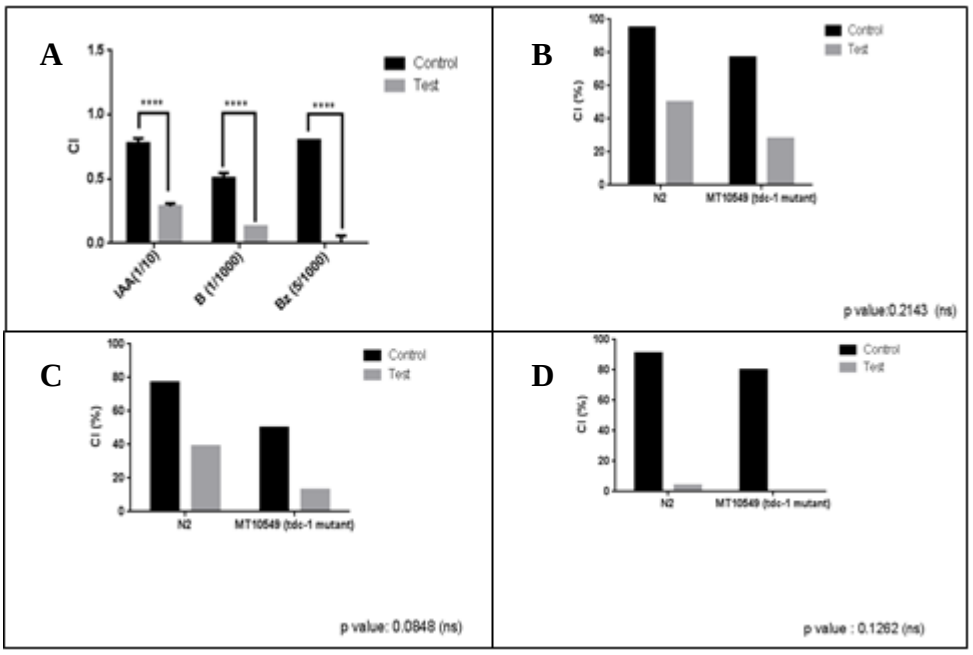

Fig. 3: Chemotaxic behaviour and percentage of chemotaxis of tdc-1 mutant worms. **A:** Chemotaxis index for Saturation Assay for MT10549 (tdc-1 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and MT10549 (tdc-1 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and MT10549 (tdc-1 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and MT10549 (tdc-1 mutants). The p value showed that isoamyl alcohol (IAA), butanone (B) and Benzaldehyde (Bz) the behaviour patterns were not significantly different from that of N2.

3.3 Role of receptors in the interneurons in the solvent recognition pathway

Further, we were interested to see if the receptors in the interneurons had a role to play in odour discrimination. Hence, we used *sra-11* receptor mutant for the assay. *Sra-11* is a serpentine receptor that is expressed in neurons and intestine during the late AIY development [Troemet *et al.,* 1995], and is said to have a role in determining the fine aspects of AWC-AIY connectivity that may be modulated upon olfactory imprinting (Remy and Hobert, 2005). There is a general defect in odorant recognition in this mutant as compared to N2 as observed from the results obtained **(see Figure 4 A)**. *Sra-11* when assayed on a saturated benzaldehyde background did not show any significant effect in odour discrimination when compared to its own control (Fisher test isoamyl alcohol, $p < 0.6191$, butanone $p < 0.5280$ and benzaldehyde 0.7140) **(Figure 4)**. This data suggest that sra-11 in AIY neuron might not be participating in odour discrimination pathway.

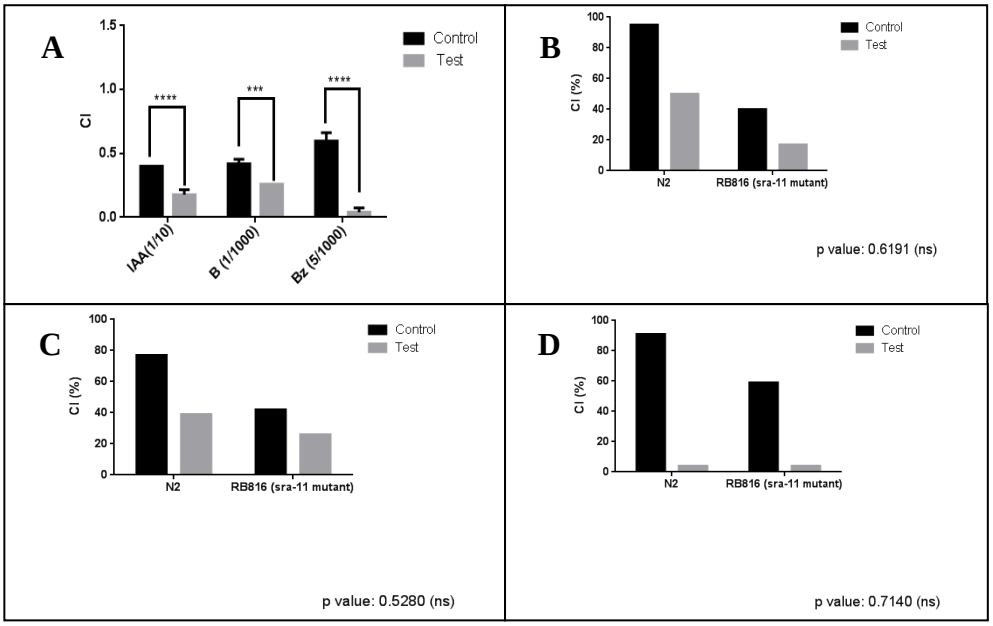

Fig. 4: Chemotaxic behaviour and percentage of chemotaxis of sra-11 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB816 (sra-11 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB816 (sra-11 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB816 (sra-11 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and RB816 (sra-11 mutants). The p value showed that both for isoamyl alcohol (IAA) and Benzaldehyde (Bz) the behaviour patterns were similar to that of N2.

We next checked *glc-3* mutants that lack the functional glc-3 receptor, a chloride channel glutamate receptor associated with the AIY neurons and whose function leads to polarization of AIY membrane potential. The results for *glc-3* mutants shows that there is a significantly lower detection of solvents when compared to N2 normally. However, after being assayed on a benzaldehyde saturated background this mutation significantly altered the isoamyl alcohol recognition, (Fisher's p value <0.01) while the butanone attraction was significantly better than that of the control (Fisher p value <0.04) **(Figure 5)**. It suggests that not switching off of AIY neurons through glc-3 channels would enhance butanone detection levels and isoamyl alcohol detection becomes significantly low.

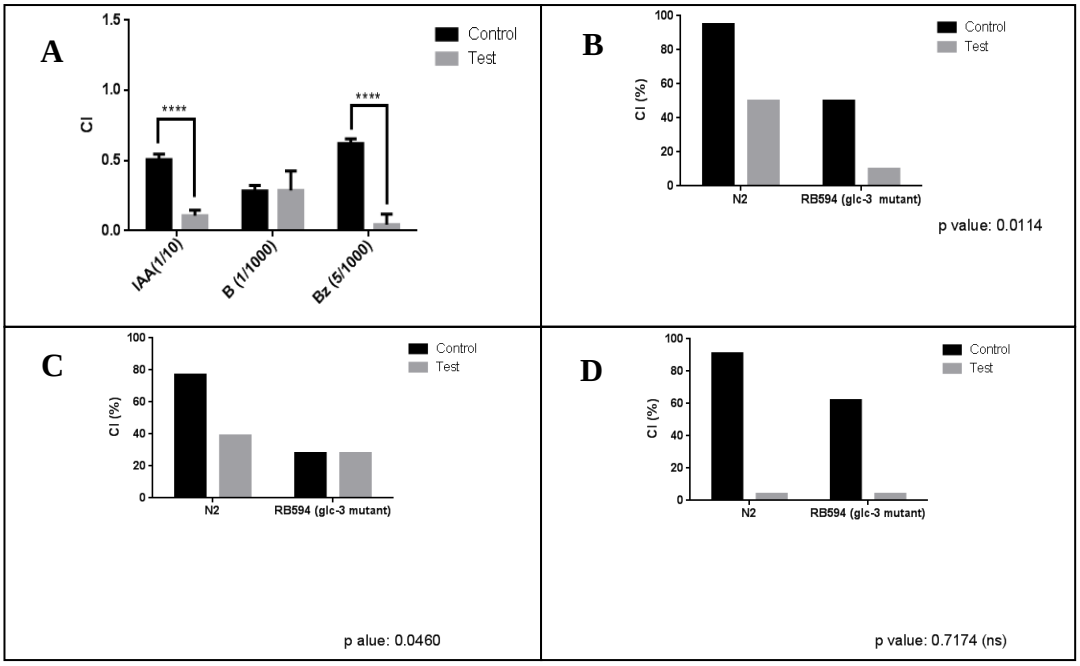

Fig 5: Chemotaxic behaviour and percentage of chemotaxis of glc-3 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB594 (glc-3 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB594 (glc-3 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB594 (glc-3 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and RB594 (glc-3 mutants). The p value showed that both for isoamyl alcohol (IAA) and Benzaldehyde (Bz) the behaviour patterns were significantly different from that of N2.

3.4 Role of Ca^{2+} channel NMDA or Non NMDA receptors in the solvent recognition pathway

nmr-1, a *C. elegans* NMDA-type ionotropic glutamate receptor subunit (N-Methyl-D-aspartic acid), plays a role in the control of movement and foraging behaviour. *nmr-1* mutants show a lower probability of switching from forward to backward movement and a reduced ability to navigate a complex environments [Brockie *et al.*, 2001]. *nmr-1* mutants when assayed results in no major attraction defect in odour detection as seen with respect to that of N2. However, in presence of a saturated benzaldehyde background, butanone recognition were significantly affected (Fisher's test p value <0.0001). In fact, it is seen that isoamyl alcohol attraction has not changed from that

of control suggesting nmr-1 is essential for butanone discrimination pathway **(Figure: 6)**.

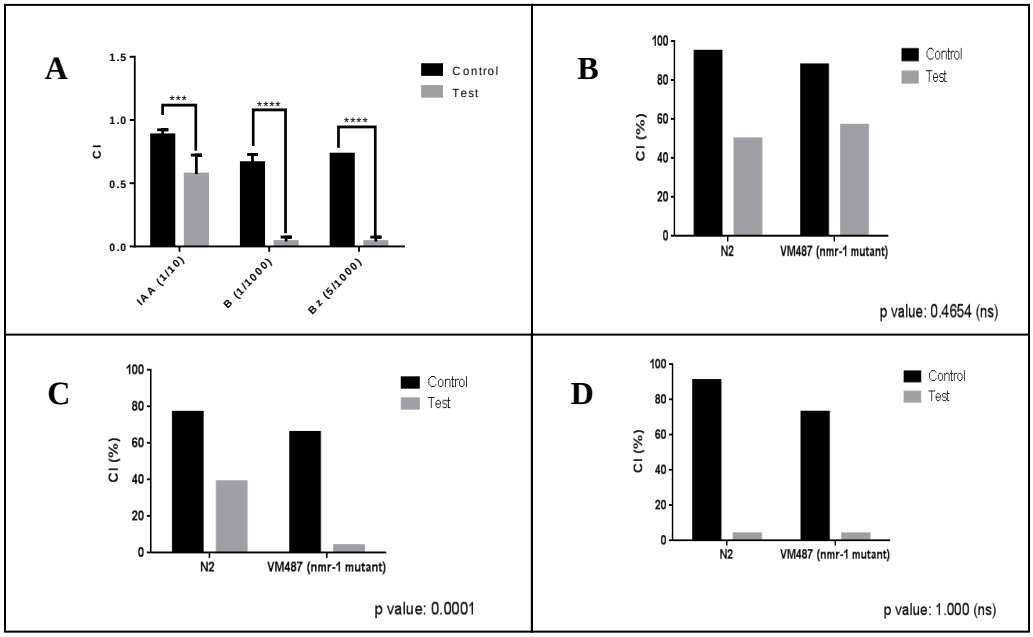

Fig. 6. Chemotaxic behaviour and percentage of chemotaxis of nmr-1 mutant worms. **A:** Chemotaxis index for Saturation Assay for VM487 (nmr-1 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and VM487 (nmr-1 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and VM487 (nmr-1 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and VM487 (nmr-1 mutants). The p value showed that for butanone, the behaviour patterns were significantly different from that of N2.

glr-1 is a gene encoding non-NMDA glutamate receptor sub -type which is expressed exclusively in a subset of neurons, including the interneurons AVA, AVD, and AVE (the backward command interneurons), and AVB and PVC (the forward command interneurons). It is also known to be critical for long term memory in *C. elegans* [Rose, *et al.*, 2003]. Mutations in this gene is associated to disrupted mechanosensation that is mediated by the ASH neurons [Hart *et al.*, 1995; Maricq *et al.*, 1995]. Saturation assay on a benzaldehyde background shows *glr-1* mutant to have no major changes in odour detection when compared with wild type N2 but when presented with a background, it significantly affects the solvent recognition pathway for both isoamyl alcohol and butanone (Fisher's test $p < 0.001$ for isoamyl

alcohol, p<0.001 for butanone) **(Figure:7)**. This data suggest glr-1 is essential for butanone and isoamyl recognition pathway.

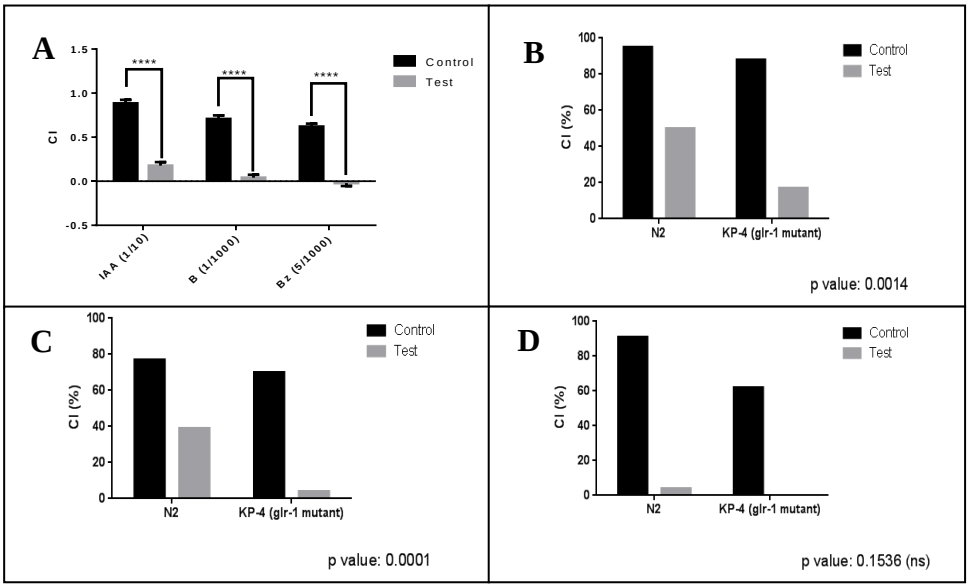

Fig. 7. Chemotaxic behaviour and percentage of chemotaxis of glr-1 mutant worms. **A:** Chemotaxis index for Saturation Assay for KP4 (glr-1 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and KP4 (glr-1 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and KP4 (glr-1 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and KP4 (glr-1 mutants). The p value showed that for butanone and isoamyl alcohol, the behaviour patterns were significantly different from that of N2.

The glr-2 subunit is expressed in many of the neurons that express glr-1, including the command interneurons. *Glr-1* and *glr-2* mutants share the same behaviour defects, although some are less severe in *glr-2* mutants [Mellem et al., 2002]. We next tested glr-2 mutants. Under normal conditions, *glr-2* mutants showed significantly lower butanone attraction compared to wild type N2. The presence of a background benzaldehyde affects the recognition pathway of the worm significantly for both isoamyl alcohol and butanone (Fisher's test p value <0.0001 and <0.0001

respectively) **(Figure: 8)**. This data suggest that similar to glr-1, glr2 is also essential in both isoamyl alcohol and butanone recognition.

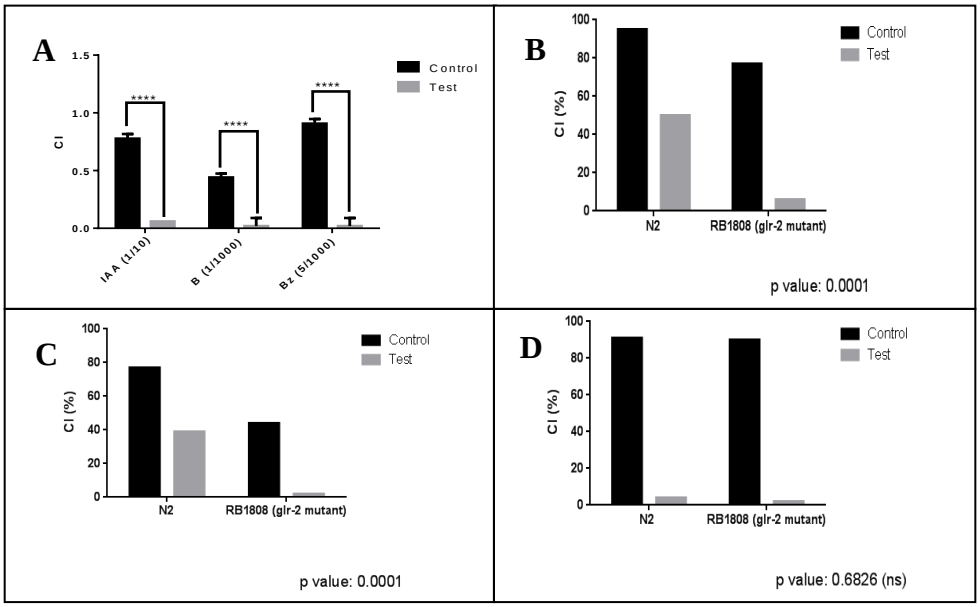

Fig. 8: Chemotaxic behaviour and percentage of chemotaxis of glr-2 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB1808 (glr-2 mutants) on a Benzaldehyde (Bz) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB1808 (glr-2 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB1808 (glr-2 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and RB1808 (glr-2 mutants). The p value showed that for butanone and isoamyl alcohol, the behaviour patterns were significantly different from that of N2.

3.5 Altered behaviour of *C. elegans* when presented with butanone as saturating background

After observing the above results on a saturated benzaldehyde background, we want to test whether similar patterns can be obtained on a butanone background. In the wild type N2, attraction to isoamyl alcohol and benzaldehyde is significantly affected when assayed on a butanone background **(Figure: 9) similar to that of benzaldehyde background (Figure 1)**.

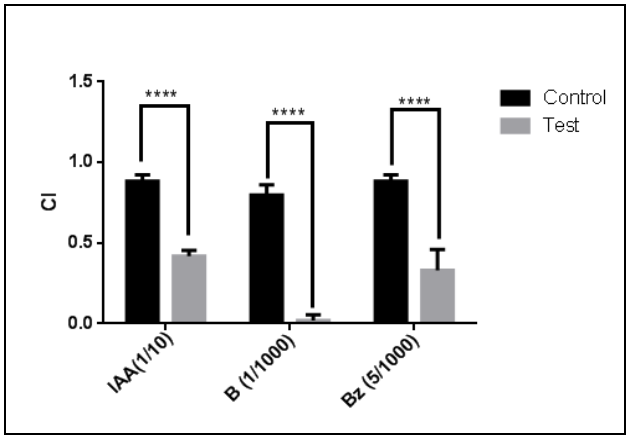

Fig.9. Chemotaxic index of worms control (without any background solvent) vs butanone (B) solvent as background.

The mutant strains that are used for the saturation assay with benzaldehyde background is also used for the assays that is performed with a butanone background. *str-2* mutant show the same behaviour towards isoamyl alcohol and butanone when presented under butanone background. When compared to N2, *str-2* mutants shows significantly better recognition for both isoamyl alcohol and benzaldehyde (Fisher's p value <0.0001 and <0.0001respectively) **(Figure 10)**, suggesting absence of str-2 enhanced the recognition of solvents under background noise. Under benzaldehyde background recognition of isoamyl alcohol was affected besides a significant loss of detection of butanone because of the mutation. This data suggest that both butanone and isoamyl recognition pathways are different. Besides when benzaldehyde is given as background isoamyl alcohol recognition also equally suffers. This suggest that both isoamyl alcohol and benzaldehyde recognition is through a single pathway.

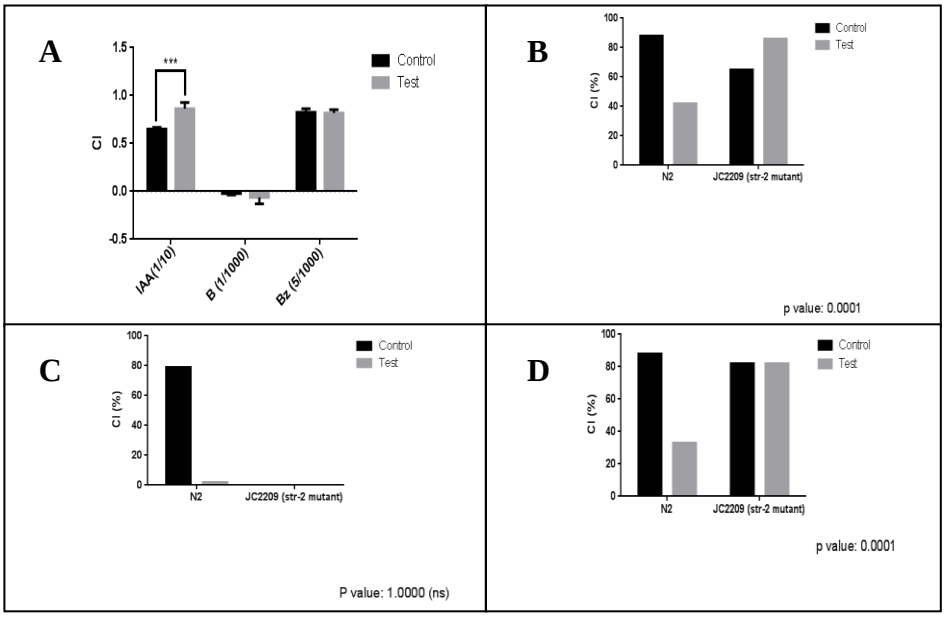

Fig. 10: Chemotaxic behaviour and percentage of chemotaxis of str-2 mutant worms. . **A:** Chemotaxis index for Saturation Assay for JC2209 (str-2 mutant) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and JC2209 (str-2 mutant). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and JC2209 (str-2 mutant). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and JC2209 (str-2 mutant).The p value showed that for benzaldehyde and isoamyl alcohol, the behaviour patterns were significantly different from that of N2.

Similar to str-2, *glc-3* mutants also showed a significantly higher recognition of isoamyl alcohol and benzaldehyde (Fisher's p value <0.0062 and <0.0021 respectively) under a saturated butanone background as compared to the normal N2 worms. These results indicate that absence of *glc-3* does not affect the recognition of these solvents. **(Figure 11)**.

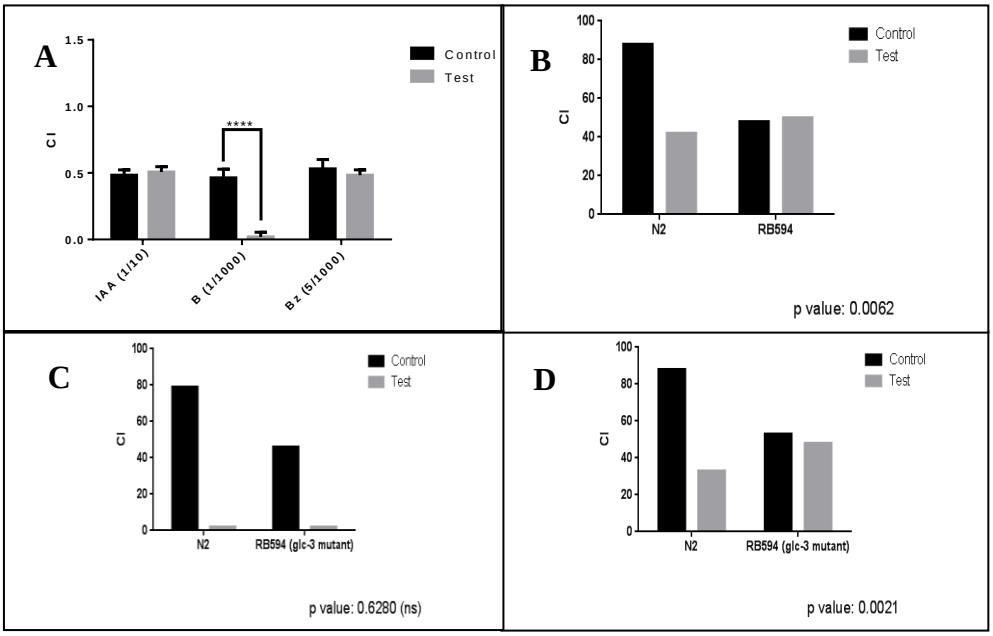

Fig. 11: Chemotaxic behaviour and percentage of chemotaxis of glc-3 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB594 (glc-3 mutants) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB594 (glc-3 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB816 (sra-11 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and RB594 (glc-3 mutants). The p value showed that for benzaldehyde and isoamyl alcohol, the behaviour patterns were significantly different from that of N2.

On the other hand, sra-*11* mutants show significantly better recognition of isoamyl alcohol. However, benzaldehyde recognition is similar to that of N2 worms (Fisher's test p value <0.002 and <0.4 for isoamyl alcohol and benzaldehyde respectively) **(Figure: 12)**. This suggest that even though there is a common circuit for isoamyl and benzaldehyde, the molecular pathway involved are different. For example sra-11 shutting down helps better recognition of isoamyl alcohol without affecting benzaldehyde pathway. Sra-11 is ODR receptor present in AIY interneurons.

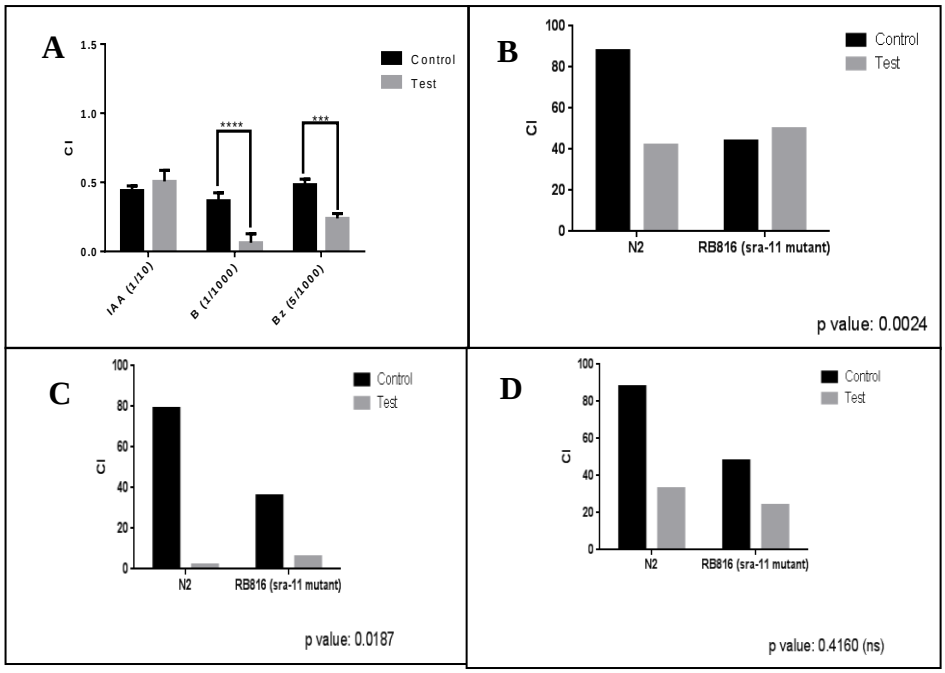

Fig. 12: Chemotaxic behaviour and percentage of chemotaxis of sra-11 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB816 (sra-11 mutants) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB816 (sra-11 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB816 (sra-11 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and RB816 (sra-11 mutants) The p value showed that for isoamyl alcohol, the behaviour patterns were significantly different from that of N2.

The glutamate receptor mutants like *nmr-1*, *glr-1*, and *glr-2* show significantly high recognition towards isoamyl alcohol and benzaldehyde when compared to that of the normal N2 **(Figure: 13, Figure: 14, Figure: 15)**. Fisher's test showed p values of <0.006 and <0.0001 for isoamyl and benzaldehyde for nmr-1. Similar pattern was observed for glr-2 mutant with p values <0.02 and <0.004 for isoamyl and benzaldehyde respectively. However for glr-1 only butanone detection was significantly altered (p, <0.01) while isoamyl alcohol showed a behaviour similar to that of N2 (p, <0.1). It is interesting to note that this behaviour is similar to that of sra-11 under butanone background with isoamyl alcohol becoming significantly recognized and that for benzaldehyde was similar to N2 **(see Figure 12)**. These data suggest that absence of these glutamate receptors even though essentIAAl for

butanone recognition they do not play a critical role in isoamyl and benzaldehyde recognition. Rather in the absence of these channels it enhances the recognition of the solvents.

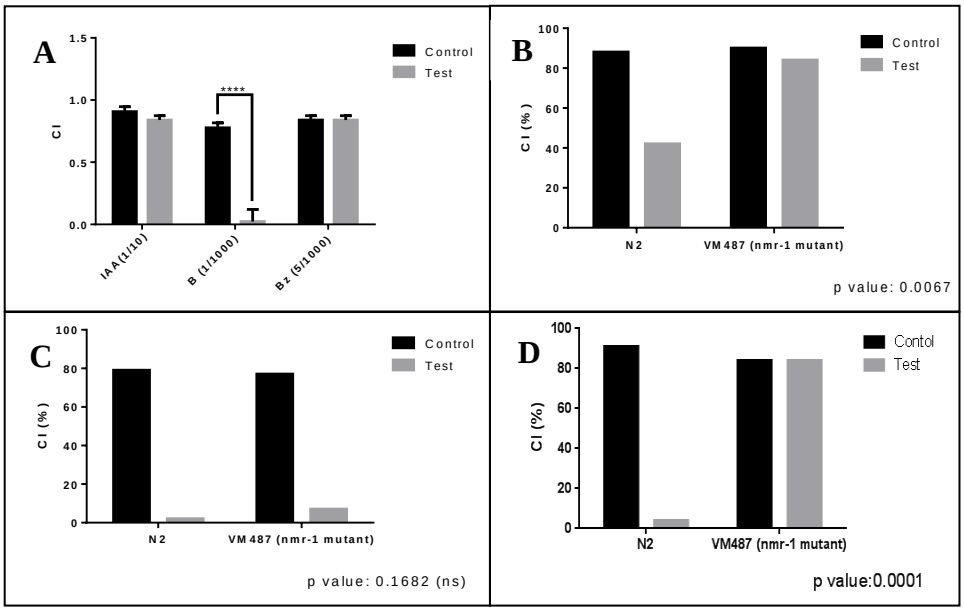

Fig. 13: Chemotaxic behaviour and percentage of chemotaxis of nmr-1 mutant worms. **A:** Chemotaxis index for Saturation Assay for VM487 (nmr-1 mutants) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and VM487 (nmr-1 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and VM487 (nmr-1 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and VM487 (nmr-1 mutants). The p value showed that for isoamyl alcohol and benzaldehyde, the behaviour patterns were significantly different from that of N2.

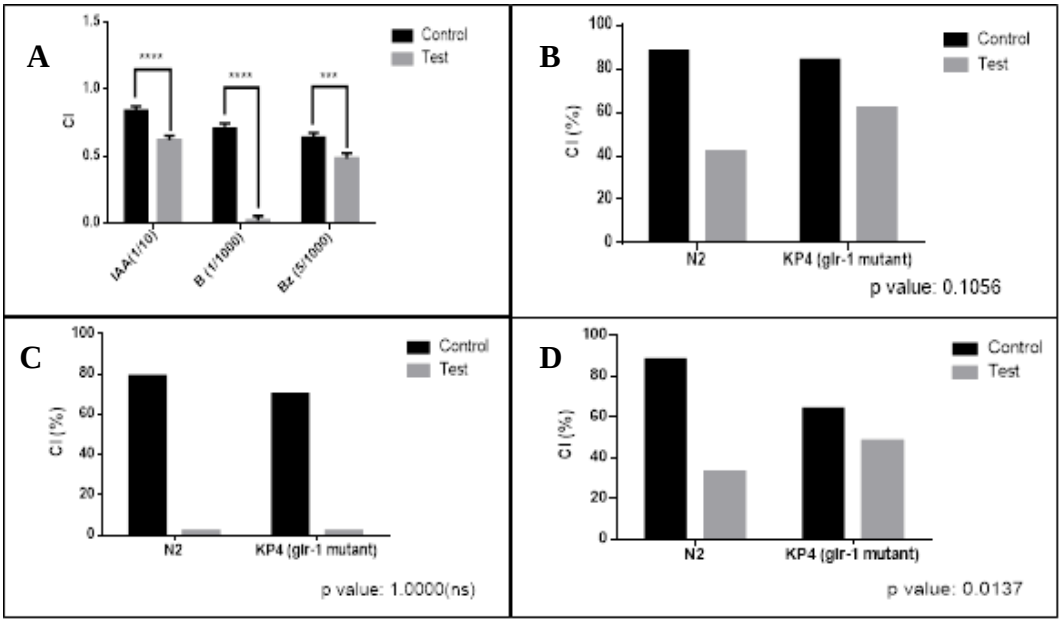

Fig. 14: Chemotaxic behaviour and percentage of chemotaxis of glr-1 mutant worms. **A:** Chemotaxis index for Saturation Assay for KP4 (glr-1 mutants) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and KP4 (glr-1 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and KP4 (glr-1 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and KP4 (glr-1 mutants). The p value showed that for benzaldehyde, the behaviour patterns were significantly different from that of N2.

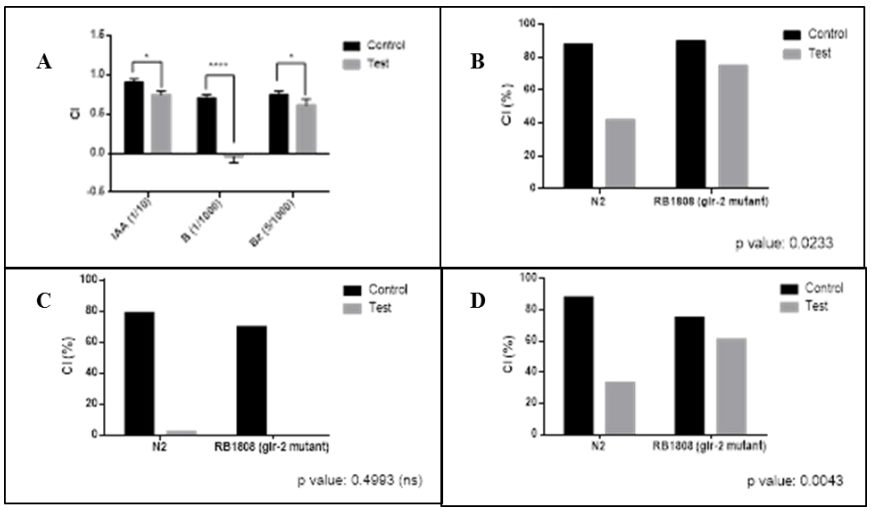

Fig. 15: Chemotaxic behaviour and percentage of chemotaxis of glr-2 mutant worms. **A:** Chemotaxis index for Saturation Assay for RB1808 (glr-2 mutants) on a Butanone (B) background. **B:** Fisher's Test comparing percent chemotaxis index (CI) for isoamyl alcohol (IAA) for N2 and RB1808 (glr-2 mutants). **C:** Fisher's Test comparing percent chemotaxis index (CI) for butanone (B) for N2 and RB1808 (glr-2 mutants). **D:** Fisher's Test comparing percent chemotaxis index (CI) for benzaldehyde (Bz) for N2 and KP4 (glr-1 mutants). The p value showed that for isoamyl alcohol and benzaldehyde, the behaviour patterns were significantly different from that of N2.

All the above results suggest that there is a significant alteration in odour detection for isoamyl alcohol and butanone. It is interesting to note that glutamate receptors do not involve in IAA and BZ detection pathway while butanone detection involve all the glutamate receptors tested **(Figure: 16)**.

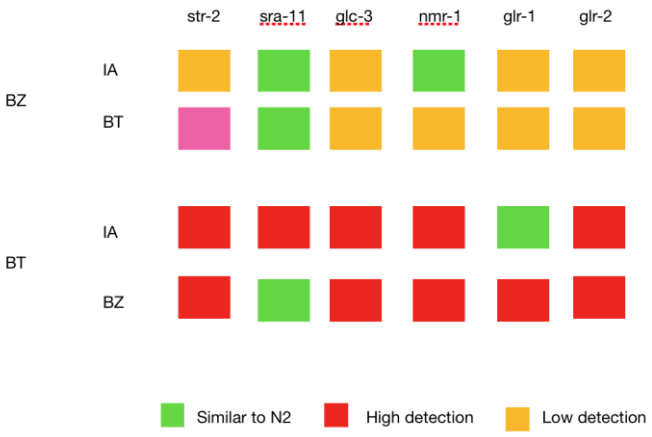

Fig 16. Summary of various behavioural differences between two background signals Benzaldehyde (BZ) and Butanone (B) towards Isoamyl alcohol (IAA), Butanone (B) and, Benzaldehyde (BZ). (Pink colour-indicative of mutation defect).

SUMMARY

All these results suggest that glutamate receptors play a critical role in the discriminatory pathway in presence of a background when compared to that of the results obtained for the wild type N2. Interestingly, involvement of NMDA receptors in the odour discrimination pathway is an interesting observation which needs to be further probed. Moreover, it was surprising to find that the interneuron receptor mutants like *sra-11* mutants, that is known to play a part among olfactory sensory receptors did not have a role in the odour discrimination pathway.

Most of the mutants assayed on butanone background show a consistent trend of better recognition of isoamyl alcohol and benzaldehyde under the presence of a butanone background. However, an exception in this case is *sra-11* mutants that show better recognition for isoamyl alcohol but recognition towards benzaldehyde is unchanged with respect to that of the normal wild type N2 worm.

These results suggest that glutamate receptors are essential for butanone detection pathway while both isoamyl alcohol and benzaldehyde detection is independent of these receptors. It would be interesting to see how neurons decode information based on these various calcium channel activation.

BIBLIOGRAPHY

1. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, et al. The genome sequence of *Drosophila melanogaster*. Science. 2000 Mar 24; 287(5461):2185–95.
2. Aimé P, Duchamp-Viret P, Chaput MA, Savigner A, Mahfouz M, Julliard AK. Fasting increases and satiation decreases olfactory detection for a neutral odour in rats. Behavioural Brain Research. 2007 May 16; 179 (2):258– 264.
3. Aimé P, Duchamp-Viret P, Chaput MA, Savigner A, Mahfouz M, Julliard AK. Fasting increases and satiation decreases olfactory detection for a neutral odour in rats. Behavioural brain research. 2007 May 16; 179(2):258-64.
4. Alkema MJ, Hunter-Ensor M, Ringstad N, Horvitz HR. Tyramine functions independently of octopamine in the Caenorhabditis elegans nervous system. Neuron. 2005 Apr 21; 46(2):247-60.
5. Amoore JE. Molecular basis of odour [Internet]. Springfield, Ill. : Thomas; 1970.
6. Arctander S. Perfume and flavour chemicals: (aroma chemicals). Allured Publishing Corporation; 1969.
7. Ayyub C, Paranjape J, Rodrigues V, Siddiqi O. Genetics of Olfactory Behaviour in *Drosophila melanogaster*. Journal of Neurogenetics. 1990 Jan 1; 6(4):243–62.
8. Bargmann CI, Hartwieg E, Horvitz HR. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell. 1993 Aug 13; 74(3):515–27.
9. Bargmann CI, Mori I. Chemotaxis and Thermotaxis. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors. C elegans II 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997.
10. Bargmann CI. Chemosensation in *C. elegans*. WormBook. 2006 Oct 25; 1–29.
11. Baumeister R, Ge L. The worm in us - *Caenorhabditis elegans* as a model of human disease. Trends Biotechnology. 2002 Apr; 20(4):147–8.
12. Beets MG. The molecular parameters of olfactory response. Pharmacological Reviews. 1970 Mar 1; 22(1):1-34.

13. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974 May 1; 77(1):71-94.
14. Brockie PJ, Mellem JE, Hills T, Madsen DM, Maricq AV. The C. elegans glutamate receptor subunit NMR-1 is required for slow NMDA-activated currents that regulate reversal frequency during locomotion. Neuron. 2001 Aug 30; 31(4):617-30.
15. Bronshteín AA & Minor AV. Regeneration of olfactory flagella and restoration of the electro-olfactogram following application of Triton X-100 to the olfactory mucosa of frogs. Tsitologia. 1977 19(1), 33–39.
16. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odour recognition. Cell. 1991 Apr 5; 65(1):175–87.
17. Buck LB. Information Coding in the Vertebrate Olfactory System. Annual Reviews Neuroscience. 1996 Mar 1; 19(1):517–44.
18. Buck LB. Unraveling the Sense of Smell (Nobel Lecture). Angewandte Chemie International Edition. 44(38):6128–40.
19. Bushdid C, Magnasco MO, Vosshall LB, Keller A. Humans can discriminate more than one Trillion Olfactory Stimuli. Science. 2014 Mar 21; 343 (6177):1370–2.
20. Castellucci V, Pinsker H, Kupfermann I, Kandel ER. Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in Aplysiaa. Science. 1970 Mar 27; 167(3926):1745–8.
21. Chen N, Harris TW, Antoshechkin I, Bastiaani C, Bieri T, Blasiaar D, Bradnam K, Canaran P, Chan J, Chen CK, Chen WJ. WormBase: a comprehensive data resource for Caenorhabditis biology and genomics. Nucleic acids research. 2005 Jan 1; 33(supplement-1):D383-9.
22. Chess A, Simon I, Cedar H, Axel R. Allelic inactivation regulates olfactory receptor gene expression. Cell. 1994 Sep 9; 78(5):823–34.
23. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron. 1999 Feb; 22(2):327–38.

24. Colbert HA, Bargmann CI. Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. Neuron. 1995 Apr 1; 14(4):803-12.
25. Crocker, E. C., & Henderson, L. F. Analysis and classification of odours: An Effort to Develop a Workable Method. American Perfumer and Essential Oil Review. 1927; 22(356): 325– 327.
26. Croy I, Nordin S, Hummel T. Olfactory disorders and quality of life- an updated review. Chemical Senses. 2014 Mar; 39(3):185–94.
27. De Belle JS, Heisenberg M. Associative odour learning in *Drosophila* abolished by chemical ablation of mushroom bodies. Science. 1994 Feb 4; 263(5147):692–5.
28. Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, Padilla M, Marder K, et al. Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. American Journal of Psychiatry. 2000 Sep; 157(9):1399–405.
29. Dorman JB, Albinder B, Shroyer T, Kenyon C. The Age-1 and Daf-2 Genes Function in a Common Pathway to Control the Lifespan of *Caenorhabditis elegans*. Genetics. 1995 Dec; 141(4):1399–406.
30. Doty RL, Ferguson-Segall M. Influence of adult castration on the olfactory sensitivity of the male rat: A signal detection analysis. Behavioural Neuroscience. 1989 Jun; 103(3):691–694.
31. Doty RL. The olfactory vector hypothesis of neurodegenerative disease: is it viable? Annals of Neurology. 2008 Jan; 63 (1):7–15.
32. Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode *C. elegans*. Cell. 1986 Mar 28; 44(6):817–29.
33. Elsaesser R, Paysan J. The sense of smell, its signalling pathways, and the dichotomy of cilia and microvilli in olfactory sensory cells. BMC Neuroscience. 2007 Sep 18; 8 Supplement 3:S1.
34. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 1998 Feb 19; 391(6669):806–11.

35. Gerkin RC, Castro JB. The number of olfactory stimuli that humans can discriminate is still unknown. E Life Sciences. 2015 Jul 7; 4:e08127.
36. Haehner A, Hummel T, Hummel C, Sommer U, Junghanns S, Reichmann H. Olfactory loss may be a first sign of idiopathic Parkinson's disease. Movement Disorders. 2007 Apr 30; 22(6):839–42.
37. Harris TW, Chen N, Cunningham F, Tello-Ruiz M, Antoshechkin I, Bastiani C, Bieri T, Blasiar D, Bradnam K, Chan J, Chen CK. WormBase: a multi-species resource for nematode biology and genomics. Nucleic acids research. 2004 Jan 1; 32(supplement-1):D411-7.
38. Hart AC, Chao MY. From odours to behaviours in Caenorhabditis elegans. The neurobiology of olfaction. 2010.
39. Hart AC, Sims S, Kaplan JM. Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. Nature. 1995 Nov; 378(6552):82.
40. Hayran, M. Nasal Physiology and Pathophysiology of Nasal Disorders. (T. M. Önerci, Ed.). Springer. 2013.
41. Jones DT, Reed RR. Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. Science. 1989 May 19; 244(4906):790–5.
42. Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell. 2005 Feb 25; 120(4):449-60.
43. KIKUCHI T. Genetic alteration of olfactory functions in *Drosophila melanogaster*. The Japanese Journal of Genetics. 1973 Jan 1; 48:105–18.
44. Komatsu H, Jin YH, L'Etoile N, Mori I, Bargmann CI, Akaike N, et al. Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of *Caenorhabditis elegans* in cultured cells. Brain Research. 1999 Mar 6; 821(1):160–8.
45. Konopka RJ, Benzer S. Clock mutants of *Drosophila melanogaster*. PNAS, USA. 1971 Sep; 68(9):2112–6.
46. L'Etoile ND, Coburn CM, Eastham J, Kistler A, Gallegos G, Bargmann CI. The Cyclic GMP-Dependent Protein Kinase EGL-4 Regulates Olfactory Adaptation in C. elegans. Neuron. 2002 Dec 19; 36(6):1079–89.

47. MacColl G, Bouloux P, Quinton R. Kallmann syndrome: adhesion, afferents, and anosmia. Neuron. 2002 May 30; 3 4(5):675–8.
48. Malnic B, Hirono J, Sato T, Buck LB. Combinatorial Receptor Codes for Odours. Cell. 1999 Mar 5; 96(5):713–23.
49. Maricq AV, Peckol E, Driscoll M, Bargmann CI. Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor. Nature. 1995 Nov; 378(6552):78.
50. Moulton DG, Beidler LM. Structure and function in the peripheral olfactory system. Physiological Reviews. 1967 Jan 1; 47(1):1–52.
51. Ngai J, Dowling MM, Buck L, Axel R, Chess A. The family of genes encoding odorant receptors in the channel catfish. Cell. 1993 Mar 12; 72(5):657–66.
52. Ohloff G. Scent and Fragrances: The Fascination of Odours and their Chemical Perspectives. Softcover reprint of the original 1st ed. 1994 edition. Place of publication not identified: Springer; 2012. 238 p.
53. Oka Y, Nakamura A, Watanabe H, Touhara K. An Odorant Derivative as an Antagonist for an Olfactory Receptor. Chemical Senses. 2004 Nov 1; 29(9):815–22.
54. Oka Y, Omura M, Kataoka H, Touhara K. Olfactory receptor antagonism between odorants. EMBO J. 2004 Jan 14; 23(1):120–6.
55. Pace U, Hanski E, Salomon Y, Lancet D. Odorant-sensitive adenylate cyclase may mediate olfactory reception. Nature. 1985 Jul 18; 316 (6025):255–8.
56. Pinching AJ, Powell TPS. The Neuropil of the Glomeruli of the Olfactory Bulb. Journal of Cell Science. 1971 Sep 1; 9(2):347–77.
57. Pirri JK, McPherson AD, Donnelly JL, Francis MM, Alkema MJ. A tyramine-gated chloride channel coordinates distinct motor programs of a Caenorhabditis elegans escape response. Neuron. 2009 May 28; 62(4):526-38.
58. Polak EH. Multiple profile-multiple receptor site model for vertebrate olfaction. Journal of Theoretical Biology. 1973 Aug 22; 40(3):469-84.

59. Poulin G, Nandakumar R, Ahringer J. Genome-wide RNAi screens in Caenorhabditis elegans: impact on cancer research. Oncogene. 2004 Nov; 23(51):8340.
60. Quignon P, Giraud M, Rimbault M, Lavigne P, Tacher S, Morin E, Retout E, Valin AS, Lindblad-Toh K, Nicolas J, Galibert F. The dog and rat olfactory receptor repertoires. Genome biology. 2005 Oct; 6(10):R83.
61. Quinn WG, Harris WA, Benzer S. Conditioned behaviour in *Drosophila melanogaster*. Proceedings of the National Academy of Science, U S A. 1974 Mar; 71(3):708–12.
62. Rawson NE, Gomez G, Cowart B, Brand JG, Lowry LD, Pribitkin EA, et al. Selectivity and Response Characteristics of Human Olfactory Neurons. Journal of Neurophysiology. 1997 Mar; 77(3):1606–13.
63. Read EA. A contribution to the knowledge of the olfactory apparatus in dog, cat and man. American Journal of Anatomy. 1908; 8(1):17–47.
64. Remy JJ, Hobert O. An interneuronal chemoreceptor required for olfactory imprinting in C. elegans. Science. 2005 Jul 29; 309(5735):787-90.
65. Ressler KJ, Sullivan SL, Buck LB. A molecular dissection of spatial patterning in the olfactory system. Current Opinion in Neurobiology. 1994 Aug 1; 4(4):588–96.
66. Rinaldi A. The scent of life. The exquisite complexity of the sense of smell in animals and humans. EMBO Reports. 2007 Jul; 8(7):629–33.
67. Roayaie K, Crump JG, Sagasti A, Bargmann CI. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron. 1998 Jan; 20(1):55–67.
68. Robertson HM. Two large families of chemoreceptor genes in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* reveal extensive gene duplication, diversification, movement, and intron loss. Genome Research. 1998 May; 8(5):449–63.

69. Rokni D, Hemmelder V, Kapoor V, Murthy VN. An olfactory cocktail party: figure-ground segregation of odorants in rodents. Nature Neuroscience. 2014 Sep; 17(9):1225–32.
70. Rose JK, Kaun KR, Chen SH, Rankin CH. GLR-1, a non-NMDA glutamate receptor homolog, is critical for long-term memory in Caenorhabditis elegans. Journal of Neuroscience. 2003 Oct 22; 23(29):9595-9.
71. Rouquier S, Taviaux S, Trask BJ, Brand-Arpon V, van den Engh G, Demaille J, et al. Distribution of olfactory receptor genes in the human genome. Nature Genetics. 1998 Mar; 18(3):243–50.
72. S Arctander, Perfume and Flavor Chemicals (Aroma Chemicals). Las Vegas, Steffen's Arctander's Publications, NV (1969).
73. Sanz G, Schlegel C, Pernollet J-C, BrIAAnd L. Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. Chemical Senses. 2005 Jan; 30(1):69–80.
74. Sarafoleanu D. Explorarea paraclinica si functionala in otorinolaringologie. Bucuresti: Ed Didactica si Pedagogica; 1999. pp. 190–243.
75. Sengupta P, Chou JH, Bargmann CI. *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell. 1996 Mar 22; 84(6):899–909.
76. Shepherd GM. Neurobiology. Third Edition. Oxford, New York: Oxford University Press; 1994: 776.
77. Sklar PB, Anholt RR, Snyder SH. The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. Journal of Biological Chemistry. 1986 Nov 25; 261(33):15538–43.
78. Stocker RF, Singh RN, Schorderet M, Siddiqi O. Projection patterns of different types of antennal sensilla in the antennal glomeruli of *Drosophila melanogaster*. Cell and Tissue Research. 1983 Jul 1; 232(2):237–48.

79. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Developmental Biology. 1983 Nov 1; 100(1):64–119.
80. Sulston JE. Neuronal cell lineages in the nematode *Caenorhabditis elegans*. Cold Spring Harbor SymposIAA Quantitative Biology. 1983; 48:443–52.
81. Tacher S, Quignon P, Rimbault M, Dreano S, Andre C, Galibert F. Olfactory Receptor Sequence Polymorphism Within and Between Breeds of Dogs. Journal of Heredity. 2005 Nov 1; 96 (7):812– 816.
82. Talamo BR, Rudel R, Kosik KS, Lee VM, Neff S, Adelman L, et al. Pathological changes in olfactory neurons in patients with Alzheimer's disease. Nature. 1989 Feb 23; 337(6209):736–9.
83. Tissenbaum HA. Using C. elegans for aging research. Invertebrate Reproduction and Development. 2015 Jan 30; 59(sup1):59–63.
84. Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI. Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans. Cell. 1995 Oct 20; 83(2):207-18.
85. Troemel ER, Kimmel BE, Bargmann CI. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell. 1997 Oct 17; 91(2):161–9.
86. Troemel ER, Sagasti A, Bargmann CI. Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in C. elegans. Cell. 1999 Nov 12; 99(4):387-98.
87. Tsunozaki M, Chalasani SH, Bargmann CI. A behavioural switch: cGMP and PKC signaling in olfactory neurons reverses odour preference in C. elegans. Neuron. 2008 Sep 25; 59(6):959-71.
88. Vassar R, Chao SK, Sitcheran R, Nuñez JM, Vosshall LB, Axel R. Topographic organization of sensory projections to the olfactory bulb. Cell. 1994 Dec 16; 79(6):981–91.
89. Vassar R, Ngai J, Axel R. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. Cell. 1993 Jul 30; 74(2):309–18.

90. W.S Cain. Olfaction. R.C Atkinson, R.J Herrnstein, G Lindzey, R.D Luce (Eds.), Steven's Handbook of Experimental Psychology: Perception and Motivation, Wiley, New York. 1998: 409–459.

91. Wang HW, Wysocki CJ, Gold GH. Induction of olfactory receptor sensitivity in mice. Science. 1993 May 14; 260 (5110): 998–1000.

92. Ward S. Chemotaxis by the Nematode Caenorhabditis elegans: Identification of Attractants and Analysis of the Response by Use of Mutants. PNAS, U S A. 1973 Mar; 70(3):817–21.

93. Weinstock, J. M. Contemporary perspectives on Linnaeus. University Press of America. 1985.

94. Wes PD, Bargmann CI. C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature. 2001 Apr; 410(6829):698.

95. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode Caenorhabditis elegans. Philosophical Transactions of the Royal Society B: Biological Sciences. 1986 Nov 12; 314(1165):1-340.

96. Wilson DA, Stevenson RJ. The fundamental role of memory in olfactory perception. Trends in Neurosciences. 2003 May 1; 26(5):243–247.

97. Wilson DA, Stevenson RJ. The fundamental role of memory in olfactory perception. Trends in neurosciences. 2003 May 1; 26(5):243-7.

98. Wilson DA. Comparison of odour receptive field plasticity in the rat olfactory bulb and anterior piriform cortex. Journal of Neurophysiology. 2000 Dec 1; 84(6): 3036–3042.

99. Wilson DA. Comparison of odour receptive field plasticity in the rat olfactory bulb and anterior piriform cortex. Journal of Neurophysiology. 2000 Dec 1; 84(6):3036-42.

100. Wuttke MS, Tompkins L. Olfactory adaptation in *Drosophila* larvae. Journal of Neurogenetics. 2000 Apr; 14(1):43–62.

101. Wuttke, M. S. Olfactory adaptation in *Drosophila melanogaster* larvae.Unpublished doctoral dissertation, Temple University. 1999.

102. Yee KK, Wysocki CJ. Odorant exposure increases olfactory sensitivity: olfactory epithelium is implicated. Physiology & Behaviour. 2001 Apr 1; 72(5):705-11.

103. Zhang Y, Chou JH, Bradley J, Bargmann CI, Zinn K. The *Caenorhabditis elegans* seven-transmembrane protein ODR-10 functions as an odorant receptor in mammalian cells. PNAS, U S A. 1997 Oct 28; 94(22):12162–7.

104. Zou Z, Buck LB. Combinatorial effects of odorant mixes in olfactory cortex. Science. 2006 Mar 10; 311(5766):1477–81.

105. Zwaardemaker H. ON MEASUREMENT OF THE SENSE OF SMELL IN CLINICAL EXAMINATION. The Lancet. 1889 Jun 29; 133(3435):1300–2.

APPENDIX

PREAPARATION OF CHEMICALS AND REAGENTS:

- **Preparation of Nematode Growth Medium (NGM) (**Brenner, 1974):

100 mL of the media constitute the following components:

Agar	1.7g
NaCl	0.3g
Peptone	0.25g
Distilled Water	100mL

All the above components are measured and added onto a conical flask, which is autoclaved at 121^0 C for 15 minutes. The media is then cooled to 55^0 C before adding the following minerals and chemicals

1M KPO_4 buffer (pH: 6)	2.5mL
1M $MgSO_4$	0.1mL
1M $CaCl_2$	0.1mL
Cholesterol (5mg/mL)	0.1mL

The media is poured into sterile petri plates under aseptic conditions and allowed to set. The media is stored at 4^0C until use.

- **Preparation of Chemotaxis Media:**

100 mL of chemotaxis media includes the following composition:

Distilled water	100mL
Agar	1.5g

The agar media was autoclaved at 121^0C for 15 minutes. It was then cooled up to 55^0C to add the remaining minerals and chemicals.

1M KPO_4 buffer (pH: 6)	0.5mL
1M $MgSO_4$	0.1mL
1M $CaCl_2$	0.1mL

The media is poured onto sterile petri plates and allowed to set. The plates were kept upside down over night for drying and to prevent excess moisture until the assay is to be performed the next day.