Universität Basel
Fakultät für Psychologie

Molecular mechanisms underlying age-dependent memory decline in *Caenorhabditis elegans*

Inauguraldissertation zur Erlangung der Würde EINER DOKTORIN/EINES DOKTORS der Philosophie vorgelegt der Fakultät für Psychologie der Universität Basel von

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aus Indien

Basel, 2023

Universität Basel

Fakultät für Psychologie

Genehmigt von der Fakultät für Psychologie auf Antrag von

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Datum des Doktoratsexamen: 23.01.2024

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"Role of GLR-1 in Age-Dependent Short-Term Memory Decline", accepted for publication in eNeuro

Authors: **Vaibhav Gharat**, Fabian Peter, Dominique J.-F. de Quervain, Andreas Papassotiropoulos, Attila Stetak

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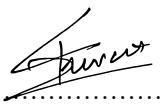
Signatur 

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Acknowledgement

First and foremost, I extend my heartfelt gratitude to my esteemed advisors, Prof. Dr. Andreas Papassotiropoulos and Prof. Dr. Dominique de Quervain, for granting me the invaluable opportunity to pursue my PhD in their lab. Their intellectual guidance and insightful feedback have been instrumental throughout this research journey.

A special acknowledgment goes to my co-supervisor, Dr. Attila Stetak, whose guidance has been an integral part of my success. I am grateful for your constant support and mentorship.

I am deeply grateful to the Freiwillige Akademische Gesellschaft (FAG) for their generous financial support through the Dissertations and habilitations grant for completion of this dissertation.

I extend my gratitude to the wonderful lab members: Pavlina, Csaba, Fabian, Kim, Andi, and Noelle. Their friendship, encouragement, and guidance have been truly invaluable throughout my studies.

Above all, I would like to express my profound appreciation to my parents, Nirmala and Ganpat. I cannot thank you enough for your unwavering love, encouragement, and the countless sacrifices you have made for me.

Finally, I want to express my profound gratitude to Lea for her never-ending support, and continuous encouragement throughout this journey. I am deeply thankful for the countless ways you have assisted me along the way. Thank you for everything!
Special thanks to her family for the sweet and kind support.

Abstract

Cognitive decline, particularly the decrease of memory, is a natural process during ageing. In a society with a growing elderly population, it is therefore critical to understand the impacts of aging on memory. This understanding may pave the way for finding new therapies to counteract physiological or pathological cognitive decline. From the many memory-related molecules, AMPA-type glutamate receptors are well known regulators of learning and memory, however their age-dependent alteration of dynamics, and their effect on age-dependent memory decline are largely unknown. Here, we investigated changes in the in vivo properties of the AMPA type glutamate receptor, GLR-1, during physiological ageing in the AVA interneuron of the C. elegans nervous system. We demonstrated that the abundance of total GLR-1 and membrane inserted GLR-1 receptors decreased with age in wild-type worms irrespective of the axonal position. Additionally, through the use of FRAP, we demonstrated that the reduction of GLR-1 abundance is associated with a decrease in the local synaptic receptor dynamics. Finally, the reduced GLR-1 levels strongly correlated with the observed age-dependent associative memory decline. In accordance, when we genetically manipulated GLR-1 stability through deletion of *msi-1* or expression of the ubiquitination-defective GLR-1 (4KR), we did not observe the reduction of receptor abundance with age. Moreover, these alterations were able to restore memory in aged animals to the levels seen in young animals. Altogether, our results demonstrate that AMPA-type glutamate receptor abundance and turnover dynamics play a key role in sustaining memory performance, and that decreased GLR-1 abundance and alteration of receptor dynamics are associated with age-dependent memory decline.

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1 Introduction

1.1 Ageing

Over the past century, there has been a remarkable and rapid global growth in both the number and proportion of elderly individuals within the population. According to the World Health Organization (WHO), global life expectancy at birth increased from 64.2 years in 1990 to 73.4 years in 2019 (WHO 2019). In developed countries, life expectancy at birth is even higher, reaching 81 years on average. By 2050, it is estimated that one in six people in the world will be over 65 years old, with the fastest growing segment being individuals aged 80 years and above (United Nations 2019). This shift in the distribution of the population towards older ages is called population ageing and is caused by several factors. Advancements in medicine, including improved disease prevention, better medical treatments, and enhanced public health measures, have particularly contributed to an increase in human longevity. However, the number of years spent in good health have broadly remained the same, implying that the additional years of life are spent in poor health (WHO 2019).

Ageing is an inevitable and irreversible process, characterized by the interplay between numerous molecular, cellular, and physiological changes. The most important changes include altered gene expression, epigenetic changes, changes in tissue structure and function, and the accumulation of cellular damage (Hou et al. 2019). This decline in physiological function affects all body systems, diminishes the body's ability to maintain internal stability in response to stressors (Carmona and Michan 2016) and leads to an increased risk of disease and death (Hou et al. 2019). Importantly, age is the primary risk factor for major debilitating and life-threatening condition such as cancer, cardiovascular disease, and neurodegenerative disease

(Hou et al. 2019; Partridge, Deelen, and Slagboom 2018; Smetana et al. 2016; Walker et al. 2019; Yan et al. 2021).

1.1.1 Ageing and neurodegenerative diseases

The impact of ageing is particularly pronounced in tissues that consist primarily of postmitotic cells, such as the brain. In a tissue with limited capacity for cell renewal, hallmarks of ageing, including genomic instability, telomere attrition, loss of proteostasis, mitochondrial disfunction and cell senescence become increasingly problematic, as the tissue undergoes the aging process (López-Otín et al. 2023). In fact, ageing is the main risk factor for most neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD). Age-related neurodegenerative diseases are progressive and irreversible in nature and one of the leading causes of death worldwide (WHO 2019). The very limited availability of effective treatments for these diseases, coupled with the significant associated personal and socioeconomic costs, represents a particularly urgent challenge for societies with an aging population (Hou et al. 2019). This underscores the pressing need of conducting further research to gain a comprehensive understanding of the effects of ageing on the brain, not only in individuals affected by neurodegenerative diseases but also in healthy individuals. This will allow us to understand the underlying mechanisms that differentiate healthy aging from disease, thereby paving the way for the development of new prevention and treatment approaches.

1.1.2 Physiological ageing and cognition

In healthy individuals, ageing is characterized by a gradual decline in multiple cognitive functions, with processing speed, executive cognitive functions, and in particular memory performance being impaired (Bishop, Lu, and Yankner 2010). An accumulating body of evidence suggests that age-related cognitive impairment is predominantly caused by region-specific changes in neuronal morphology and synaptic plasticity, rather than a significant loss of neuronal cells as suggested earlier (Henley and Wilkinson 2013). More specifically, ageing leads to decreased dendritic arborization and spine density, loss of synapses, alterations in the composition and distribution of neurotransmitters, changes in the expression, trafficking, and degradation of neurotransmitter receptors, alterations in the structural and functional connections between different brain regions, and an increase in oxidative stress and inflammation (Bishop et al. 2010; Burke and Barnes 2006; Mattson and Arumugam 2018).

Despite this growing body of knowledge, there is still a lack of understanding of the causal factors and temporal dynamics that lead to the neurobiological alterations correlated with age-dependent cognitive decline (López-Otín et al. 2023). Importantly, both cognitive and neurobiological age-related changes are highly complex and variable between individuals, which makes understanding mechanisms that lead to physiological as well as pathological cognitive decline extremely challenging. For this reason, using model organisms with simpler nervous systems and controlled laboratory environments can be incredibly valuable to dissect the underlying mechanisms that govern cognitive processes and to find new drug targets (White, 2016).

1.2 *C. elegans* as a model organism

Caenorhabditis elegans is a free-living soil-dwelling nematode, which was first established as a genetic model organism by Sydney Brenner in the second half of the twentieth century (Brenner 1974). Today this simple and transparent nematode has become the world's best understood animal. *C. elegans* has a number of features which have made it a popular model organism for biomedical research. Notably, it's small size (1mm), short life cycle (3 days from eggs to adults), and ease of cultivation on a feeder layer of E.*coli* (Brenner 1974). The worm's transparent body further allows the use of *in vivo* fluorescent markers and the easy observation of internal structures and cellular processes under a microscope (Chalfie et al. 1994). *C. elegans* primarily exist as a hermaphrodite, allowing for the production of a genetically homogeneous population. This, combined with a rapid life cycle facilitates genetic studies and allows researchers to observe the effects of genetic modifications over multiple generations in a relatively short time frame.

Furthermore, adult hermaphrodites display an invariant cell lineage and relatively simple morphology with only 959 cells and a very simple nervous system composed of 302 neurons (Sulston et al. 1983; Sulston and Horvitz 1977). The complete connectome of *C. elegans* has been meticulously mapped using serial electron micrographs, revealing approximately 500 chemical synapses, 600 gap junctions, and 2000 neuromuscular junctions which are remarkably consistent in their location across individuals of this species. (White JG, Southgate E, Thomson JN 1986). These features contribute to *C. elegans* being a highly robust and reliable model system.

Despite its simplicity, *C. elegans* shares numerous molecular pathways and genes with more complex organisms, including humans. 83% of its protein coding genes have a human

homologue, enabling researchers to study conserved biological processes and gene functions (Lai et al. 2000).

1.3 *C. elegans* as a model organism to study ageing

The nematode *Caenorhabditis elegans* is an ideal model organism for investigating the process of ageing. Under laboratory conditions, the average lifespan of worms is 14 to 21 days, which makes ageing studies in this model organism highly feasible and practical. Despite having a short life span, *C. elegans* shows normal progressive age-related deterioration in anatomical and functional features which recapitulate some of the ageing processes observed in humans (Chew et al. 2013).

In presence of abundant food and optimal temperature of 20° C the *C. elegans* reproductive cycle completes in approximately 4 days. After hatching from the eggs, *C. elegans* larva undergo 4 larval stages, L1-L4, before becoming adults. During the period of adulthood, the hermaphrodites are self-fertile for approximately 3-4 days, producing about 300 progenies. After the end of the reproduction phase, animals enter a post-reproductive phase of approximately 2-3 weeks before death (Corsi, Wightman, and Chalfie 2015). During this period, worms experience declines in various bodily functions such as feeding, locomotion, tissue integrity, immunity and also in learning and memory abilities (Golden and Melov 2007). At the tissue level, the primary age dependent changes affect the reproductive, muscular, and nervous system. The rate of reproduction reduces significantly with age along with the structural deterioration of the reproduction system (Son et al. 2019). At the muscular level, the reduction in the muscle integrity has been widely reported to occur, which results in impaired motility and increased susceptibility to infection (Garigan et al. 2002; Glenn et al. 2004). The

nervous system also shows subtle changes which include dendritic restructuring and synaptic deterioration (Herndon et al. 2002). Given the similarities between some of the fundamental processes of ageing in *C.elegans* and vertebrates, elucidating the factors that cause age-related deterioration in *C. elegans* may also lead to new insights regarding human ageing.

1.4 *C. elegans* as a model organism to study learning and memory

Historically, it was believed that simple invertebrate organisms are not capable of exhibiting any form of complex behavior or cognitive processes similar to those observed in humans or other mammals. However, starting in the 1960s with the pioneering work of Kandel and colleagues, researchers gradually demonstrated that even the simplest organisms possess remarkable abilities for learning, memory, and responding to environmental cues. Kandel and colleagues began to study learning in the mollusk *Aplysia* (Kandel and Tauc 1965) and proved that this organism displays behavioral plasticity associated with distinct changes at synapses. They were able to identify the neurons and biochemical changes that underly synaptic plasticity and learning in its most basic form, discovering molecules such as cAMP, PKA, and CREB. Shortly after, Benzer and colleagues started working on the fruit fly *Drosophila melanogaster* (Dudai et al. 1976; Quinn, Harris, and Benzer 1974) and established the first associative learning assay and forward genetic screen, identifying the first genes crucial for learning.

Similarly, with its invariant cell lineage and connectome and only 302 neurons, it was initially thought that behavior would be hardwired in *C. elegans* and that there would be no mechanism for learning and memory. However, subsequent findings have revealed that this

organism possesses remarkable behavioral plasticity, displaying an exquisite sensitivity to its environment (McEwan and Rankin 2013). *C. elegans* displays an impressive ability to learn and retain information concerning environmental cues that indicate the presence or absence of food, pathogens, or aversive stimuli.

The neural system of *C. elegans* comprises 60 ciliated sensory neurons, which enable it to sense various environmental cues such as temperature, oxygen levels, odorants, mechanical and chemical stimuli (Metaxakis, Petratou, and Tavernarakis 2018). This sensory capability allows the worms to utilize chemotaxis, thermotaxis, or aerotaxis to navigate towards more favorable environments. The worm integrates these sensory inputs in a highly recurrent network of approximately 80 interneurons and generates a behavioral response through a set of 120 motor neurons that control locomotion. Similarly, as in vertebrates, information is encoded, stored, and retrieved within these networks via coordinated neuronal activity and changes in synaptic strength between cells (Varshney et al. 2011). In the case of *C. elegans*, instead of having thousands of cells and synapses, and different brain regions involved, circuits are often composed of a handful of individual neurons (Varshney et al. 2011). The simplicity of these networks is a great advantage for scientists that want to study fundamental properties of synapses and neuronal communication involved in learning and memory processes.

In *C.elegans*, by using specific assays, simple behaviors can be easily quantified, manipulated, and used as a readout for learning and memory phenotypes. Scientists have utilized behavioral assays in numerous studies to demonstrate that worms are capable of various forms of learning and memory, including non-associative and associative learning, as well as short- and long-term memory (Ardiel and Rankin 2010; Ishihara et al. 2002; Mohri et al. 2005; Torayama, Ishihara, and Katsura 2007).

Importantly, the research conducted during the last four decades has proven that many of the uncovered genes and nervous circuits discovered in these simple model organisms are highly conserved and therefore relevant to study learning and memory in organism with higher order cognition (Barco, Bailey, and Kandel 2006; Byrne and Hawkins 2015; Grammenoudi, Kosmidis, and Skoulakis 2006; Fenyves et al. 2021; Hadziselimovic et al. 2014; Rose et al. 2003; Stetak et al. 2009; Vukojevic et al. 2012). This allows researchers to translate findings back and forth between humans and animal model organisms.

1.4.1 Nonassociative learning assays in *C. elegans*

Nonassociative learning refers to a type of learning where an organism's behavior or response to a stimulus change as a result of repeated exposure to the stimulus, without the need for any association with other stimuli or events (Kandel et al. 2012). One of the forms of nonassociative learning which is studied most extensively in *C. elegans* is mechanosensory habituation.

The assay performed to study mechanosensory habituation is called tapping assay. In 1990, Rankin et al. showed that exposing *C. elegans* to a non-threatening mechanical stimulus like light tapping on the culture plate, triggers changes in locomotion and a retraction response (Rankin, Beck, and Chiba 1990). This is caused by activation of mechanoreceptor neurons that generate electrical signals and trigger the behavioral response. However, with repeated administration of the tap, the worm’s response gradually diminishes, both in amplitude and frequency. This decline in the behavioral response is the result of habituation. There are several factors contributing to the underlying mechanism of mechanosensory habituation in *C. elegans*. One possible mechanism is related to changes in synaptic strength or efficiency within

the neural circuits involved in processing the mechanosensory information. Repeated stimulation may lead to synaptic depression, where the strength of the connections between neurons is reduced, resulting in a decreased response to the stimulus (Bozorgmehr et al. 2013).

1.4.2 Associative learning assays in *C. elegans*

Associative learning on the other hand is a type of learning that involves forming associations or connections between different stimuli or events. It occurs when an organism learns about the relationship between two stimuli, or between a stimulus and a behavioral response, based on their temporal and spatial relationship (Kandel et al. 2012).

There are two main types of associative learning, classical and operant conditioning. Classical conditioning involves learning a relationship and connection between different stimuli. In contrast, operant conditioning involves learning a relationship between the organism's behavior and the consequences of that behavior (Kandel et al. 2012).

Classical conditioning can be observed in *C. elegans* through various behavioral paradigms. In this type of learning, the organism establishes an association between an unconditioned stimulus (US), which elicits an innate physiological response (unconditioned response), and a conditioned stimulus (CS), which typically does not elicit such a response. Through repeated pairing of the US and the CS (conditioning), the animals learn the connection between these stimuli, leading to the display of the innate response in reaction to the CS (conditioned response).

Table 1.1 *C. elegans* behavioral paradigms that model associative learning (adapted from Rahmani and Chew 2021)

	US	CS	UR	CR
Diacetyl aversive learning	Starvation (food deprivation)	Diacetyl	Strong attraction to diacetyl	Avoidance of diacetyl
Butanone appetitive learning	Satiety (food abundance)	Butanone	Weak attraction to butanone	Strong attraction to butanone
Salt aversive learning	Starvation (food deprivation)	Sodium chloride	Strong attraction to sodium chloride	Avoidance of sodium chloride
Temperature aversive learning	Starvation (food deprivation)	The temperature at which worms were cultivated (within 15–25°C)	Strong attraction to cultivation temperature	Avoidance of cultivation temperature

Most of the associative learning paradigms used in *C. elegans* research are based on pairing attractive or neutral/repellent chemical cues or favorable and unfavorable cultivation temperatures and pH ranges as the CS with starvation (aversive conditioning) or the presence of food (appetitive conditioning) as the US (see Table 1.1). When given the choice, unconditioned worms will spontaneously move towards attractants or favorable temperatures and pH ranges or away from neutral or repellent substances and unfavorable culture conditions. Conditioning is carried out by incubating the animals on a plate without food and the attractant substance or culture condition (aversive conditioning) or on a plate with abundant food and the neutral or repellent substance or unfavorable culture condition (positive conditioning). After repeated pairing of the two stimuli, the animals will display an opposite behavior compared to their initial response. Specifically, they will exhibit a migration towards substances that were originally neutral or repellent or unfavorable culture conditions and move away from substances or culture conditions that were initially attractive.

In our laboratory and in this project, we mainly used an aversive associative olfactory learning paradigm that is based on pairing the chemical attractant diacetyl with starvation (as described in Fenyves et al. 2021; Hadziselimovic et al. 2014; Vukojevic et al. 2012).

1.4.3 Short-and long-term memory in *C. elegans*

The aforementioned assays are not only useful for examining various types of learning but also for evaluating different forms of memory by assessing the duration of information retention after a short, intermediate, or prolonged delay (Rahmani and Chew 2021). For example, in associative learning task, it can be tested whether worms still show the unconditioned response to the conditioned stimulus after a period of delay without training.

Retention time varies depending on the type of stimulus and the training regime. For example, worms maintain a decreased tap withdrawal response after 24 hours after the training session when they are subjected to four blocks of 20 taps, each separated by 1 hour, with a 60-second interstimulus interval (Beck and Rankin 1995; Rose, Kaun, and Rankin 2002). In contrast, there was no evidence of long-term retention of habituation in worms that were mass-trained with 80 taps or given distributed training with a 10-second interstimulus interval (Beck and Rankin 1995; Rose et al. 2002). Consistent with the characteristics of long-term memory, blocking protein synthesis during training hindered the long-term consolidation of the memory (Beck and Rankin, 1995) but did not affect learning and short-term habituation, indicating that different mechanisms underly short- and long- term memory in *C. elegans*.

Similarly, in the context of aversive associative learning with diacetyl, worms exhibit the retention of the learned association 24 hours after the training when they are exposed to two 1-hour long blocks of diacetyl paired with starvation. On the other hand, when conditioned

with one 1-hour long block, the worms retained the association for less than 4 hours (Hadziselimovic et al. 2014; Vukojevic et al. 2012).

This shows how these different learning paradigms can also be used to study the distinct genes, molecular pathways and neuronal circuits associated with short- and long-term memory retention, extinction and forgetting.

1.5 *C. elegans* as a model organism to study age related cognitive decline

Despite its well-established role as a model for studying aging (Friedman and Johnson 1988; Kenyon et al. 1993; Klass 1977), synaptic function (Lewis et al., 1980; White et al., 1986), and behavior (Bono and Villu Maricq 2005), *C.elegans* has only recently emerged as a model organism in the field of age-related cognitive decline (Ardiel and Rankin 2010; Fenyves et al. 2021; Kauffman et al. 2010; Stein and Murphy 2012).

Age-related changes in learning and memory have been observed in *C. elegans*, with both non-associative and associative forms being affected. Non-associative learning, such as habituation to tapping, decreases at day 9 of adulthood, when animals start to show reduced response to taps and slower recovery from habituation compared to worms at day 1 or 4 (Beck and Rankin 1993). Associative learning and memory are even more sensitive to the effects of ageing (Ardiel and Rankin 2010; Kauffman et al. 2010). Kauffman et al. (2010) for example

assessed various phenotypes including motility, chemotaxis, olfactory learning, 2-hour short-term, and 16-hour long-term positive olfactory memory with butanone during the first week of adulthood. While movement and chemotaxis to butanone remained the same during this period, 16-hour long-term memory decreased by day 2 of adulthood and became undetectable by day 5, indicating that long-term memory is particularly susceptible to ageing processes. This decline in memory was associated with the decline of CREB at day 4, an essential protein for long-term memory and with the insulin receptor *daf-2* that maintains neuronal integrity in aged animals.

Similarly, in a recent study (Fenyves et al. 2021) from our group we used an associative-learning assay with chemotaxis to salt and showed that while chemotaxis stays intact in 6-day old animals, learning significantly decreases between 4-and 5-day old animals and 16-h long-term memory is already impaired in 3-day old animals. We further showed that specifically the decrease in long-term memory was associated with the repression of MPS-2, a protein regulating potassium channels during associative-learning tasks.

Taken together, these findings support the notion that ageing processes particularly impact learning and long-term memory in *C. elegans*, mirroring two of the main forms of age-dependent cognitive decline observed in humans. Further, they also emphasize that age-dependent changes in learning and memory are likely regulated by several different genes, molecular pathways, and neuronal circuits. This underscores the need for further research to unravel the distinct factors regulating these processes.

1.6 Genes and molecular pathways involved in learning and memory

As previously stated, learning and memory are characterized by numerous molecular and cellular changes that are tightly regulated and precisely orchestrated within neuronal circuits. During a learning event, specific neuronal circuits that encode and integrate information from the environment and compute a behavioral output are repeatedly activated. This increase in activity triggers molecular and cellular changes which allow neurons to either form new connections (synapses) or strengthen/weaken the pre-existing connections within a circuit. This leads to either temporary or sustained changes in the neuronal network activity, which represents the subcellular correlates of short- and long-term memory formation and maintenance. This capability of the nervous system to modulate and change the connections within neuronal networks is termed synaptic plasticity (Lynch 2004; Malenka and Nicoll 1999; Wigström and Gustaffson 1985).

More specifically, during learning, an increase of neuronal activity triggers the release of the neurotransmitter glutamate from the pre-synaptic neuron, which binds to AMPA and NMDA-type glutamate receptors on the post-synaptic neuron. This causes a Ca2+ influx in the post-synaptic neuron, which triggers the activation of complex downstream molecular pathways that can ultimately induce strengthening or weakening of synapses. A brief but large influx of Ca2+ results in the activation of Ca2+ sensitive kinases such as cAMP which activates the transcription factor CREB and triggers the expression of several new genes and the synthesis of proteins that stabilize, enlarge, and strengthen synapses. Importantly, this leads to the insertion of new AMPA-type glutamate receptors in the cell membrane. As a result, this will lead to a greater response of the post-synaptic cell to the activity of the pre-synaptic cell.

This synaptic strengthening is termed long-term potentiation (LTP) (Bliss and Collingridge 1993; Larkman and Jack 1995; Lisman 1994). On the other hand, a small and sustained rise in Ca2+ activates Ca2+ sensitive phosphatases that decrease synaptic transmission and strength through removal of AMPA-type receptors from the post-synaptic membrane. This weakening of synapses is termed long-term depression (LTD) (Mayford et al. 1995; Otmakhov et al. 2004). An overview of the different signaling cascades and molecules discovered so far is provided in Figure 1.1.

Using forward and reverse genetic approaches and gene expression studies, several genes, and molecular pathways that contribute to learning and memory have been identified in *C. elegans* (Kandel 2012). Most of these pathways either directly or indirectly modulate neuronal signaling and synaptic plasticity. For example, the genes encoding subunits of AMPA-type glutamate receptor GLR-1 (*glr-1*) (Rose et al. 2003; Vukojevic et al. 2012), and NMDA-type glutamate receptor NMR (*nmr-1* and *nmr-2*) (Kano et al. 2008) are both crucial for an intact learning and long-term memory phenotype in behavioral assays. Similarly, this was also observed for other genes encoding proteins that modulate ion channels such as activity-dependent potassium channels (potassium channel regulator *mps-2*) (Fenyves et al. 2021). Further, genes and proteins that regulate the cAMP-CREB pathway or structural proteins that modulate size and stability of the synaptic terminus (MSI-1, ADD-1)(Hadziselimovic et al. 2014; Vukojevic et al. 2012) have also been reported as crucial players of learning and memory in *C. elegans* (Rahmani and Chew 2021).

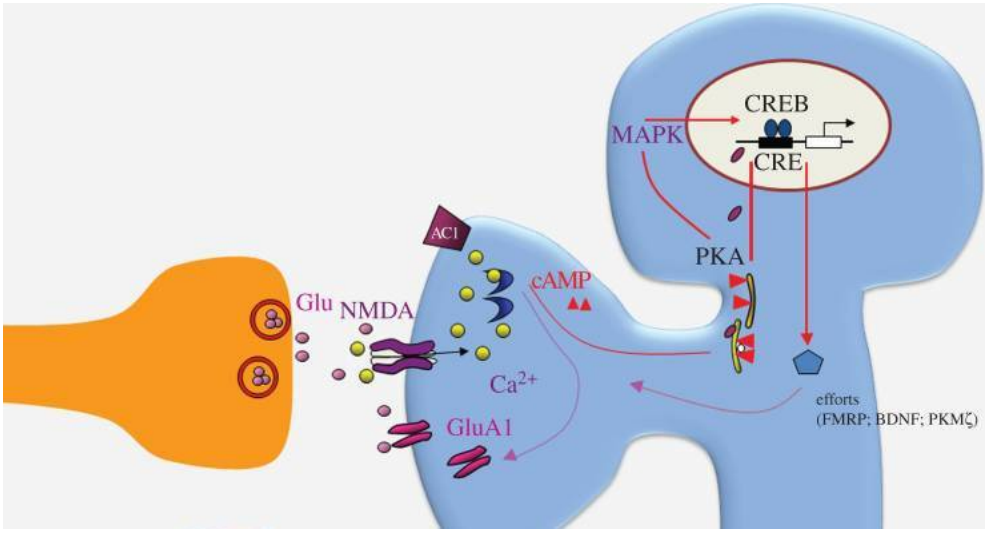

Figure 1.1 Schematic representation of NMDA-dependent. NMDA activation leads to Ca2+ influx in the postsynaptic neuron. Increased Ca2+ levels trigger a series of molecular events and activate downstream pathways. The signal is transmitted via kinase cascades and ultimately results in increased AMPA-type glutamate receptor (GluA1) trafficking to the synaptic membrane as well as CREB activation, which is necessary for the establishment of the gene expression-dependent late-LTP phase. Adapted from (Zhuo 2014)

Previous studies in vertebrates demonstrated that decay of LTP increased in aged animals and correlated with forgetting, suggesting that impaired acquisition and retention of information with ageing are related, among other functions, to impairment in the induction and maintenance of LTP (Dong et al. 2015). Several mechanisms have been described so far that could contribute to this shift from LTP to LTD during ageing, including changes in intra- and extracellular Ca2+ signaling and changes in abundance, distribution, and functional properties of NMDR and AMPA type glutamate receptors (Kumar and Foster, 2019).

1.7 Glutamate receptors

Glutamate receptors are a family of neurotransmitter receptors that play a fundamental role in mediating excitatory synaptic transmission in the central nervous system (CNS). They are the primary receptors for the neurotransmitter glutamate, which is the most abundant excitatory neurotransmitter in the brain (Bliss and Collingridge 1993).

In vertebrates, the glutamate receptor family includes three ionotropic (NMDA, AMPA and Kainate receptors) and the metabotropic receptor subfamilies (mGluRs I-III) (Traynelis et al. 2010). In the mammalian brain the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are the main receptors responsible for fast excitatory synaptic transmission (Bliss and Collingridge 1993). AMPARs are composed of four subunits GluA1, GluA2, GluA3 and GluA4 and can be organized into homo- or heterotetrameric complexes (Greger, Watson, and Cull-Candy 2017).

The *C. elegans* genome encodes at least 10 putative ionotropic glutamate receptor (iGluR) subunits which include members of the non-NMDA class (*glr-1 – glr-8*) and 2 NMDA class subunits (*nmr-1 and nmr-2*). One of the iGluR, *glr-1* encodes a receptor subunit of a non-NMDA excitatory inotropic glutamate receptor subtypes and has 40% homology with mammalian AMPA receptors GRIA2 and GRIA3 (Brockie 2006; P. J. Brockie et al. 2001; Brockie and Maricq 2003; Hart, Sims, and Kaplan 1995). From the many glutamate-receptor types in worms, GLR-1 is the most well-studied and has been implicated in various behaviors such as locomotion, egg-laying, learning, and memory (Maricq et al. 1995; Morrison and Van Der Kooy 2001; Rose et al. 2003).

Studies in vertebrates suggest that alterations in abundance, distribution, and functioning of glutamate receptors are correlated with age-dependent impairments in learning and memory.

However, especially in the case of AMPA-type glutamate receptors, little is known about the temporal dynamics and the molecular mechanism responsible for the observed changes in receptor properties during the natural process of ageing (Jurado 2018).

2 Research question

Previously, we have shown that GLR-1 is necessary for *C. elegans* associative learning and memory performance and demonstrated that cell-specific downregulation of GLR-1 activity in the command interneuron AVA selectively affect olfactory associative memory without impact on learning (Vukojevic et al. 2012). AVA plays a critical role in the nervous circuit regulating reversal and backward movement in *C. elegans* and is a key regulator of short- and long-term memory in aversive olfactory associative learning tasks (Brockie et al. 2001; Fenyves et al. 2021; Hadziselimovic et al. 2014; Stetak et al. 2009; Vukojevic et al. 2012). Knock-down of GLR-1 impairs the increase of synaptic volume and synaptic strengthening during conditioning at AVA and leads to failure of long-term retention of associative memory. However, how GLR-1 is regulated during ageing and how this relates to memory performance has not been investigated so far. Together with the evidence from studies in vertebrates and humans, GLR-1 seems to be an interesting candidate to study how properties of AMPA-type glutamate receptors change during the natural process of ageing and how this could be related to age-dependent memory decline. This is why we decided to focus on studying different properties of GLR-1 during ageing in *C.elegans* in my thesis.

In the current study, we were specifically interested in investigating changes in abundance, distribution, transport, and turnover at synapses of GLR-1 in the AVA interneuron and how this relates to age-dependent memory decline in the nematode *C. elegans*.

To track GLR-1 abundance, distribution, transport, and turnover at synapses, we used a transgenic strain expressing a construct capable of specifically labelling membrane bound GLR-1 receptors as well as total GLR-1 receptors in the AVA interneuron pair (Hoerndli et al., 2013). This allowed us to simultaneously visualize global, as well as plasma-membrane inserted

receptor levels at synapses. This is of particular importance as AMPARs have been described as having a highly dynamic turnover at synapses, which is crucial for synaptic plasticity during learning and memory (Choquet and Triller, 2013).

In our experiments, we compared 1-day and 3-day old animals. We specifically selected these timepoints based on our previous research (Fenyves et al., 2021), which demonstrated that between one and two days of adulthood, worms begin to exhibit a decline in memory performance in the aversive associative olfactory learning assays (Mastrandreas et al., 2022). Consequently, we decided to monitor GLR-1 before memory decline begins and compare it to a timepoint where memory decline has already happened at the behavioral level.

Our results show that GLR-1 abundance declines significantly with age. Moreover, ageing influences GLR-1 dynamics at the synapses by affecting the local short distance synaptic delivery and insertion of GLR-1 to the cell membrane. The observed effect on GLR-1 abundance correlates with decline in memory performance during ageing. Finally, we demonstrate that reverting the decline of GLR-1 abundance in aged animals restores GLR-1 dynamics and ultimately improves memory performance.

3 Methods

3.1 General methods and strains used

Standard methods were used for maintaining and manipulating *C. elegans* (Brenner, 1974). *C. elegans* Bristol strain, variety N2, was used as the wild-type reference strain. The experiments were performed with synchronized worm populations of young adult hermaphrodites. Gravid adult worms were washed with M9 and bleached using alkaline hypochlorite solution (0.5M NaOH, 5% HOCl). Eggs were allowed to hatch on non-seeded CTX agar plates overnight at 20°C. The synchronized L1 worms were always grown on NGM-OP50 plates to adulthood at 20°C and experiments were conducted at RT in acclimatized rooms. For all the ageing experiments, worms were transferred away from progeny to fresh plates every day.

The *C. elegans* alleles and strains used in this study were:

akIs201; Prig-3::SEP GFP::mCherry::glr-1, utrIs51[Prig- 3::SEP GFP::mCherry::glr-1(4KR)], msi-1(os1); akIs201[Prig-3::SEP GFP::mCherry::glr-1], akIs141; Prig-3::HA::glr-1::gfp

Description of SEP::mCherry::glr-1 strain

The strain (SEP:mCherry::glr-1) was generated by expressing a functional GLR-1 protein which is fused to two different fluorophores at the extracellular N-terminal domain and specifically expressed in the AVA interneuron (Hoerndli et al. 2013). The two fluorophores are superecliptic pHluorin (SEP) and mCherry. SEP is a pH sensitive variant of GFP which is activated and becomes fluorescent only when the pH value exceeds 6, while it remains inactive when the pH is below 6. Consequently, SEP tagged GLR-1 detection occurs exclusively when GLR-1 is

inserted into the plasma membrane, where it is exposed to pH levels greater than 6. In contrast, if GLR-1 is present within a transport vesicle, characterized by an internal pH below 6, it will not display fluorescence (Fox-Loe, Henderson, and Richards 2017; Kennedy and Ehlers 2011).. On the other hand, the mCherry tag is not pH sensitive, meaning that it will always be fluorescent. mCherry represents the overall GLR-1 abundance in the AVA interneuron.

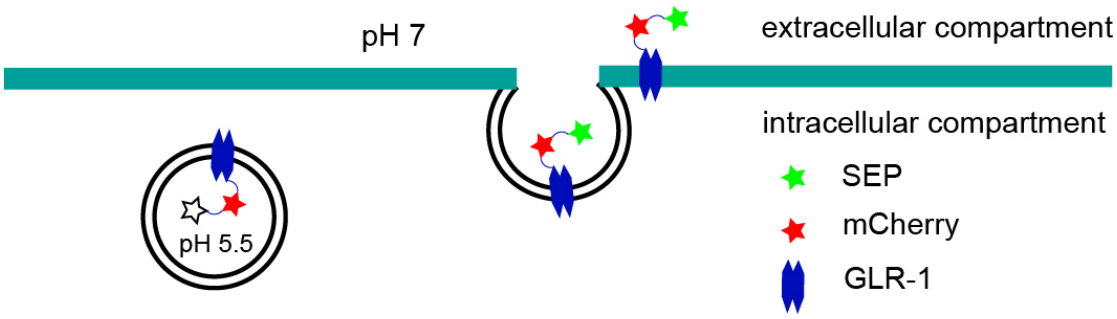

Figure 3.1 schematic of the dual-tagged SEP::mCherry::GLR-1 in transport vesicles and on the cell surface

3.2 Olfactory memory assays

C. elegans short-term aversive associative learning and memory were assessed as previously described (Stetak et al. 2009) with some modifications. Please refer to the schematic (Figure 3.2) for the timeline. Briefly, well-fed young adult worms were exposed for 1 h to starvation in the presence of 2 µl undiluted chemoattractant diacetyl (DA) spotted on the lid of 10 cm CTX plates (5 mM KH_2PO_4/K_2HPO_4 [pH 6.0], 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2% agar) and their attraction to DA was tested prior (naive), directly after (conditioned) and following a 1-h rest in absence of DA and food (1h delay). Naive and conditioned worms were given a choice between a spot of 0.1% diacetyl in ethanol with 20 mM sodium-azide and a counter spot with ethanol and sodium-azide. After a delay time, animals were counted and the chemotaxis index

was calculated as described previously (Bargmann, Hartwieg, and Horvitz 1993). A total of 50–200 animals were used in each technical and biological replicate.

For detailed protocol refer to **annex 7.1**

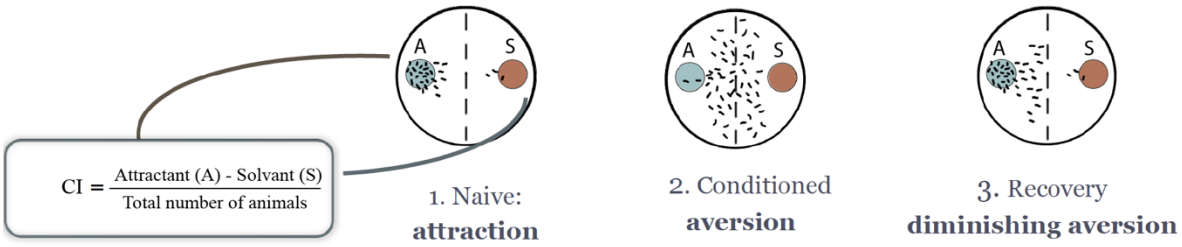

Figure 3.2 Schematic of chemotaxis assay plate

3.3 Fluorescence Microscopy

Worms were mounted on 7% agarose pads with 3µl of polystyrene beads (Polybead, catalog #00876-15, Polysciences), unless otherwise indicated. Images were acquired using Leica point scanning confocal SP8 with 63x/1.4 oil Plan APO objective using 488nm and 552nm excitation lasers. All the microscopy data was recorded in a proximal (posterior to AVA chiasma) and in the most distal region of AVA projections, along the z-axis, unless otherwise indicated.

3.4 Abundance analysis

For the intensity analysis, all recorded images were processed and quantified using FIJI. Total fluorescence signal intensities in a ~40-µm long region (proximal and distal) of AVA projections were quantified by drawing region of interest (ROI) on the maximal projection images of confocal stacks. The background fluorescence (i.e., outside of the AVA) from each

maximum projection was then subtracted from the average fluorescence of the ROI. The measured raw intensities were normalized to the mean of 1-day old adult signal from the respective strain.

3.5 Fluorescence recovery after photobleaching (FRAP)

The akIs201[Prig-3::SEPGFP::mCherry::glr-1] and *utrIs51[Prig-3::SEP GFP::mCherry::glr-1(4KR)]* strains were used to conduct FRAP on adult animals at day-1 and day-3. Animals were immobilized with 3 µl of a mixture containing equal measures of polystyrene beads (Polybead, catalog #00876-15, Polysciences) and 30 mM muscimol (catalog #195336, MP Biomedicals) on 7% agarose pads. Pre bleached image stack was acquired using the 488nm and 552nm laser. Bleaching of the region of interest was done with 488nm and 552nm laser using 4 iterations (~1s total illumination) at 100% laser energy. The imaging region was photobleached; and immediately after, one image stack (with 488nm and 552nm excitation) was acquired for the 0-minute time point. This was repeated at 2, 4, 6, and 8 min following the photobleaching of the imaging region. Recorded images were processed using FIJI to generate maximal projection. The signal intensities were manually quantified by drawing ROIs around the bleached spot. To compensate for the photobleaching over the time period, the recovery fluorescence signal at the bleached spot was normalized with the signal from a non-bleached area within the image. The same regions were used to quantify the membrane bound (SEP GFP) and total GLR-1 (mCherry) FRAP signals.

3.6 Transport-kymographs

Mutants from *akIs141[Prig-3::HA::glr-1::gfp]* strains were mounted for imaging as described above. Once the neurons were located using the 64x objective and a 488nm excitation laser (Solid state laser, 20mW) at 2% power, a proximal section of the neurites was photobleached using a using 200 iterations (~6s total illumination) at 100% laser energy. Transport images were acquired by taking a streaming movie in projected Z-section of 0.44µm (number of steps=2). Immediately following photobleaching, a 600-frame image stream was collected with the 488nm excitation laser at 8% power at a frame rate of 10 frames/sec. Kymographs were generated using the Multi Kymograph tool in FIJI with a 29-pixel line width and the Kymographs were analyzed using Kymobutler to yield the number of transport events and velocity (Jakobs, Dimitracopoulos, and Franze 2019).

3.7 Real-time RT-qPCR

GLR-1 RNA expression levels were measured from synchronized 1-day and 3-day old worms. For every sample 10 animals were collected in 2µl worm lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 8.2, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin) supplemented with 60 µg/ml proteinase K and digested for 1 h at 50°C followed by 10 min at 95°C. Immediately after digestion, the samples were processed with Maxima first strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer`s instructions. Real-time qPCR was performed with gene specific primers (Table 1) using SyBr fast kit (Kapa Biosystems, Wilmington, MA) in a Rotor Gene-6000 instrument (Corbett Research, Mortlake, New South Wales, Australia). Real-time qPCR was performed with gene specific primers (Table S1) using SyBr Fast Kit (Kapa Biosystems) according to the

manufacturer's recommendations in a Rotor Gene-6000 instrument (Corbett Research). Total RNA levels were normalized to the expression levels of *tba-1* and *cdc-42*. Fold differences were calculated using the $\Delta\Delta C_t$ method.

Table 3.1 List of primers

gene	forward	reverse
tba-1	CTGCTGACAAGGCTTACCATG	CAAACAGCCATGTACTTTC
Cdc-42	GCCTCTATCGTATCCACAG	TGAGTACCAACTAACAAGAATG
sep::gfp	CTGTTCCATGGCCAACACTTGTC	GACTTCAGCACGTGTCTTGTAG

3.8 Statistical analyses

All data and statistical analyses were carried out using Prism 9. Data is represented as Tukey's boxplot unless otherwise indicated. Outlier analysis was carried out using ROUT method with false discovery rate set to 1%. Detected outliers were excluded from the data analysis. Normality of the data was tested using D'Agostino & Pearson normality test. Main effects and interaction terms were tested using ANOVA. Statistical tests for significance were performed with F-tests using sum-of-squares type I. The p-value threshold was set to nominal significance ($p<0.05$). Pairwise group comparison was tested using post hoc t-tests corrected for multiple comparisons using Tukey correction ($P < 0.05$). All figures were created using Adobe Illustrator.

4 Results

4.1 GLR-1 abundance decreases with ageing in the AVA neuron

In order to study whether age-dependent cognitive decline and glutamate signaling are related, we first investigated whether GLR-1 receptor levels in the AVA interneuron decrease during ageing. To quantify potential GLR-1 abundance changes with age, we quantified the total and membrane bound GLR-1 levels in the SEP::mCherry::GLR-1 strain with confocal microscopy.

AVA interneurons project their axon along the entire length of the ventral nerve chord (VNC) and receive considerable synaptic inputs from many different neurons in the anterior and posterior part (White JG, Southgate E, Thomson JN 1986). Unfortunately, the circuitry involved in associative memory formation in *C. elegans* is still not fully elucidated, and it remains unknown which of the synaptic inputs are the most relevant. Therefore, we decided to measure the signal intensity of SEP and mCherry in both the proximal and distal region of the AVA axon relative to the soma (Figure 4.1A). These two regions likely represent different synaptic inputs (such as PVC input in the distal region of the AVA axon) and allowed us to investigate possible differences in GLR-1 abundance along the axon in 1-day and 3-day old adult animals (Figure 4.1B). In this experimental setup, we found a significant reduction in the total GLR-1 amount in 3-day old animals in AVA interneurons as compared to 1-day old animals, as represented by the mCherry signal (Figure 4.1C). Additionally, we also observed a significant reduction in membrane-bound GLR-1 levels (Figure 4.1D), represented by the SEP signal. This was true for both the proximal and distal part of the AVA axon, suggesting a global rather that localized subcellular decrease in GLR-1 levels (Figure 4.1E, F).

To understand the cause of the observed decrease in GLR-1 abundance, we tested if ageing alters mRNA levels or the translation rate of *glr-1*. To quantify age-related AVA interneuron specific changes in *glr-1* mRNA expression, we took advantage of the SEP::GFP sequence in the SEP::mCherry::GLR-1 strain. We measured the mRNA levels coding the fusion protein with quantitative RT-PCR and compared the expression levels between 1-day old and 3-day old animals. This experiment showed no significant decline of *glr-1* mRNA levels with age (Figure 4.1G)

In addition, we also measured the amount of GLR-1 protein in the soma of the AVA neuron in 1-day old and 3-day old animals by measuring the intensity of the mCherry signal in the SEP::mCherry::GLR-1 strain, which corresponds to the total GLR-1 amount. Interestingly, we found that GLR-1 levels were not reduced, but in contrast, significantly increased in the AVA soma with ageing (Figure 4.1H). This suggests that the observed overall decrease in GLR-1 abundance in AVA is not caused by a reduction of GLR-1 protein synthesis in the cell body. Hence, we wondered whether the observed decline in GLR-1 abundance is due to a reduction in long-distance transport of GLR-1 from the soma to the synaptic sites. By using real-time streaming confocal microscopy, we investigated anterograde and retrograde GLR-1 transport frequency to and from the AVA cell body and whether it changes with ageing (Figure 4.1I, L). Interestingly, we did not observe any significant decline in the frequency of anterograde and retrograde transport in the proximal part of the axon. On the contrary, we observed a significant increase in the anterograde and retrograde transport frequency in the distal part with ageing (Figure 4.1J-K, M-N).

Taken together, our results suggest that GLR-1 synthesis and long-distance transport along the axon are not affected by age and are likely not the key driving mechanism of the observed age-dependent reduction of GLR-1 at the synapses. Thus, we hypothesized that the

observed reduction of receptor content in AVA axon could be caused by age-related differences in local receptor dynamics.

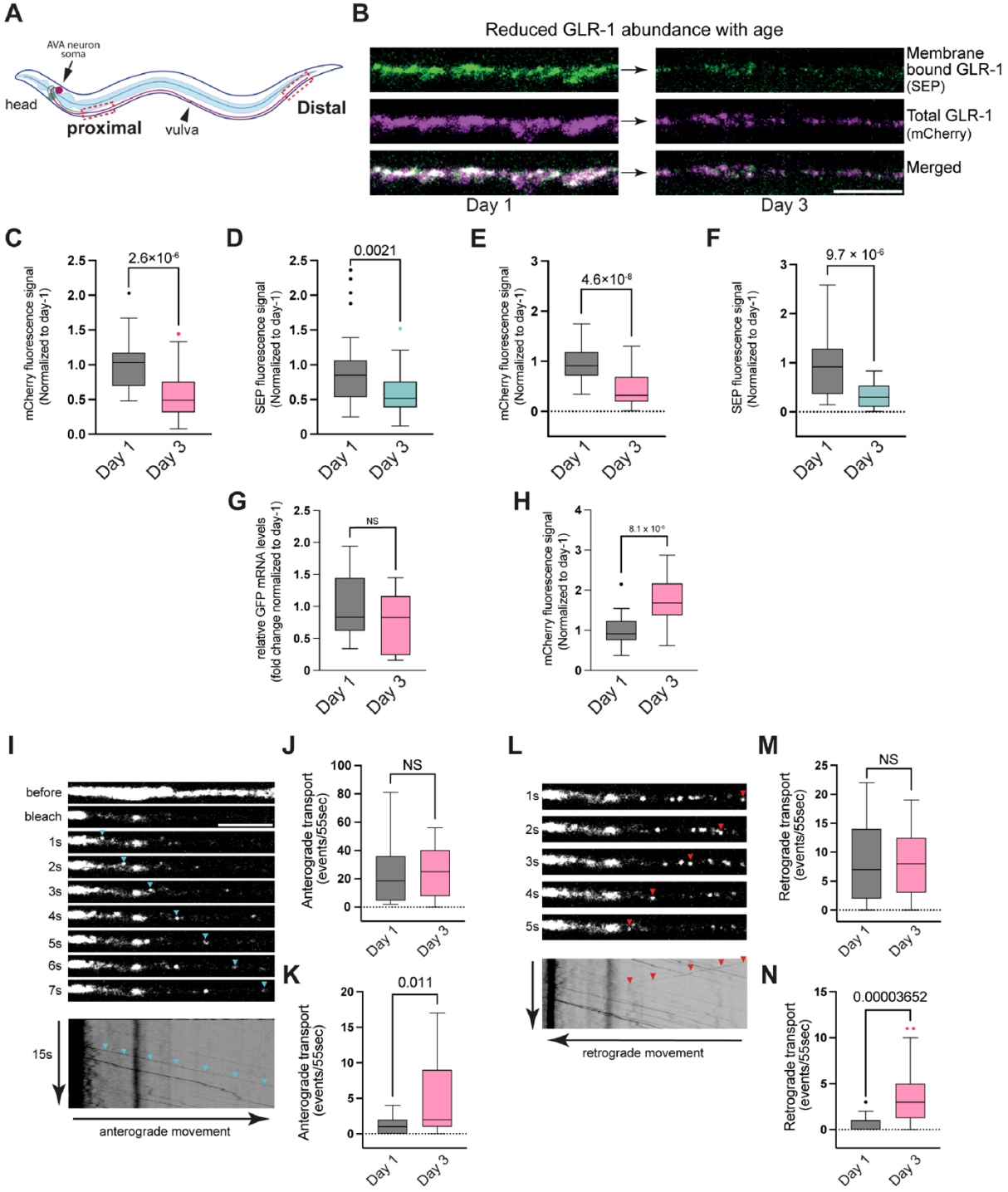

Figure 4.1 GLR-1 abundance declines in wild-type worms during ageing.

(A) Scheme illustrating the location for *in vivo* imaging of GLR-1 protein intensities and dynamics in AVA neuron. (B) GLR-1 receptor abundance was measured using confocal

microscopy in transgenic worms in wild-type background. Representative image of the membrane-bound and total GLR-1 content of the AVA axon in 1-day (left) and 3-day old (right) wild type animals. **(C-F)** Box plots displaying quantification of the fluorescence intensity of **(C)** total (mCherry) and **(D)** membrane bound (SEP) GLR-1 receptors normalized to day-1 signal in the proximal part of the axon of AVA neuron; **(E)** total (mCherry) and **(F)** membrane-bound (SEP) GLR-1 level in the distal parts of AVA axon of SEP::mCherry::GLR-1(wt) worms. **(G)** *glr-1* RNA levels were measured with real-time qRT-PCR in 1-day and 3-day old SEP::mCherry::GLR-1(WT) strain. Expression levels were normalized to *tba-1* and *cdc-42*. **(H)** Fluorescence intensity levels of the mCherry (total GLR-1) signal in young and aged SEP::mCherry::GLR-1(WT) animals in cell body of the AVA neuron. **(I, L)** Time-lapse images of GLR-1 vesicle movements along the axon of the AVA neuron. Moving vesicle is highlighted with arrowheads. Lower panel shows a kymogram of all merged timeframes where transport events can be seen as oblique lines crossing. **(I)** Anterograde transport from the soma (vesicle; blue arrowhead). **(L)** Representative example of the retrograde transport (vesicle; red arrowhead). **(J)** Average number of anterograde and **(K)** retrograde transport events in the proximal part of AVA. **(M)** Average number of anterograde and **(N)** retrograde transport events in the distal part of AVA of SEP::mCherry::GLR-1(wt) animals. Scale bars represent 5 µm. Data on **(C-H)** and **(J-K, M-N)** are visualized with Tukey's boxplots. See Table 2 for detailed statistical information.

Table 4.1 Statistical table for results 4.1

Figure	model	condition tested	Levels	n	Test	Test statistics	df	p-value
4.1C	2-tailed t-test	age	Day-1, Day-3	33,33	t	5.157	64	2.63E-06
4.1D	2-tailed Mann-Whitney test	age	Day-1, Day-3	32,32	U	286		0.0021
4.1E	2-tailed Mann-Whitney test	age	Day-1, Day-3	32,33	U	139		4.62E-08
4.1F	2-tailed Mann-Whitney test	age	Day-1, Day-3	32,24	U	129.5		9.66E-06
4.1G	2-tailed t-test	age	Day-1, Day-3	27,27	t	4.9864	49	8.10E-06
4.1H	2-tailed t-test	age	Day-1, Day-3	10,10	t	1.1051	18	0.3141
4.1J	2-tailed t-test	age	Day-1, Day-3	28,30	t	0.2374	56	0.8132
4.1K	2-tailed t-test	age	Day-1, Day-3	27,30	t	0.1188	55	0.9059
4.1M	2-tailed Mann-Whitney test	age	Day-1, Day-3	24,29	U	210.5		0.011
4.1N	2-tailed Mann-Whitney test	age	Day-1, Day-3	23,28	U	117		3.65E-05

4.2 Dynamics of GLR-1 decrease with age

To gain insight into the local receptor dynamics in young and aged animals, we performed fluorescence recovery after photobleaching (FRAP) experiments along the AVA axons using the SEP::mCherry::GLR-1 strain. We monitored recovery of mCherry (total GLR-1) (Figure 4.2A) and SEP (membrane bound) (Figure 4.2B) after photobleaching to quantify the local short-distance delivery of new GLR-1 to synaptic sites and GLR-1 membrane integration respectively, both in the proximal and distal region of the AVA axon. After photobleaching, the mCherry signal is bleached while SEP fluorescence is protected from photobleaching as the receptor is in a quenched state inside the acidic endosomes (Hoerndli et al. 2015; Kennedy et al. 2010).

We quantified the GLR-1::mCherry and the GLR-1::SEP fluorescence recovery signal for each animal for both proximal and distal part of AVA and plotted it against time to obtain a recovery curve (Figure 4.2C, E, G, I). To plot the values from individual worm, we used the curve fitting analysis (one phase association) (Prism 9 software), which also gave us the plateau value, which represents the maximum signal which can be recovered after photobleaching within a specific time (10 minutes in this experiment). Using plateau values form individual recovery curves we calculated the mobile fraction for the GLR-1::mCherry and the GLR-1::SEP. Mobile fraction of total GLR-1 represents the freely available pool of GLR-1 receptors which can be moved rapidly between nearby synaptic sites. The mobile fraction of membrane bound GLR-1 represents the freely available pool of GLR-1 receptors available for membrane insertion at a given synaptic site.

Quantification of the GLR-1::mCherry and the GLR-1::SEP FRAP signal demonstrated that at the proximal part of AVA, both the amount of the total GLR-1 and the membrane-bound

GLR-1 mobile fraction reduced significantly with age (Figure 4.2C-F). A similar effect was observed when analyzing the GLR-1::mCherry and the GLR-1::SEP FRAP for the distal part of AVA (Figure 4.2G-J). These experiments suggest that during ageing the GLR-1 synaptic receptor mobility and local turnover are significantly decreased compared to young animals.

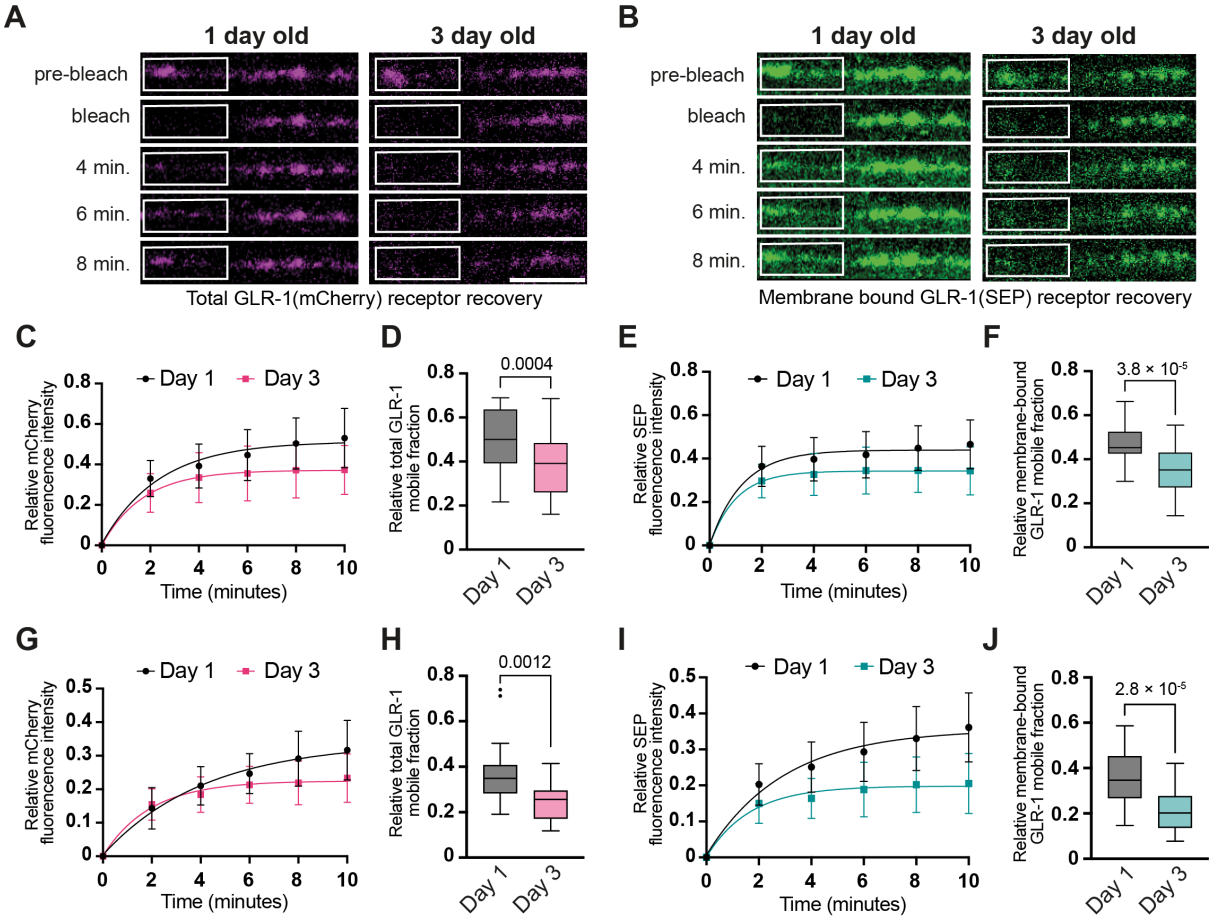

Figure 4.2 Age-dependent GLR-1 dynamics decline in wild type worms

(A, B) A representative image of the FRAP recovery of the membrane-bound **(A)** and total GLR-1 **(B)** content of the AVA axon in young and aged wild type animals over time. **(C, E)** Percentage FRAP recovery in the proximal part for **(C)** mCherry fluorescence over time at day-1 (plateau= 0.5136) and at day-3 (plateau= 0.3718), and **(E)** SEP fluorescence at day-1 (plateau= 0.4395) and at day-3 (plateau= 0.3428) (n >22 for each group). **(D, F)** Quantity of the mobile fraction of **(D)** total GLR-1 and **(F)** membrane-bound GLR-1 during ageing in the proximal part of the AVA neuron. **(G, I)** Percent recovery of **(G)** mCherry fluorescence after photobleaching over time at day-1 (plateau= 0.34) and at day-3 (plateau= 0.2250), and **(I)** SEP fluorescence at day-1 (plateau= 0.3555) and at day-3 (plateau= 0.1983) (n >20 for each group) in the distal part. **(H, J)** Relative quantity of the mobile fraction of **(H)** total GLR-1 and **(J)** membrane-bound GLR-1 during ageing in the distal part of the AVA neuron during ageing. Scale bars represent 5 µm.

Data on **D, F, H,** and **J** are visualized with Tukey's boxplots; and **C, E, G,** and **I** show mean ± SEM.

Table 4.2 Statistical table for results 4.2

Figure 2	model	condition tested	Levels	n	Test	Test statistics	df	p-value
4.2D	2-tailed t-test	age	Day-1, Day-3	28,37	t	3.72	63	0.0004
4.2F	2-tailed t-test	age	Day-1, Day-3	23,29	t	4.521	50	3.80E-05
4.2H	2-tailed t-test	age	Day-1, Day-3	19,20	t	3.515	37	0.0012
4.2J	2-tailed t-test	age	Day-1, Day-3	21,23	t	4.693	42	2.86933E-5

4.3 GLR-1 abundance is maintained with age in ubiquitination defective mutants

To test whether the reduced GLR-1 synaptic abundance is related to changes in local GLR-1 dynamics, we used a transgenic strain expressing SEP::mCherry::GLR-1(4KR), a ubiquitination-defective variant of GLR-1 that is predicted to increase the number of cell-surface synaptic receptors (Burbea et al. 2002; Grunwald et al. 2004). As the GLR-1 construct is under the control of *rig-3,* it is only expressed in the AVA neuron.

First, we measured GLR-1 abundance in AVA of 4KR mutants and the ubiquitination defective GLR-1 mutant showed no age-dependent decrease in the abundance of total GLR-1 in the proximal part (Figure 4.3A) of AVA neurons and a marginal, albeit significant reduction in the more distal part (Figure 4.3C). The same effect was observed for the abundance of membrane bound GLR-1 (Figure 4.3B, D).

Secondly, we measured GLR-1 dynamics using FRAP in 4KR mutants and quantified the results as described before. When quantifying the FRAP recovery signal for the proximal part of AVA, we did not see any significant age dependent change in the recovery curves or in the mobile fraction for the total and the membrane bound GLR-1 (Figure 4.3E-H). However, we found a subtle but significant decrease of recovery dynamics with age after photobleaching of the total GLR-1 in the distal part of the AVA axon (Figure 4.3I-J). This decrease could be due to the observed decrease in the abundance of total GLR-1 signal in the distal part of AVA axon with age (Figure 4.3C). Recovery dynamics of membrane bound GLR-1 however showed no such changes (Figure 4.3K-L). Altogether, our data suggests that when degradation of GLR-1 is inhibited and receptor abundance does not markedly decrease with age, receptor dynamics also remain intact.

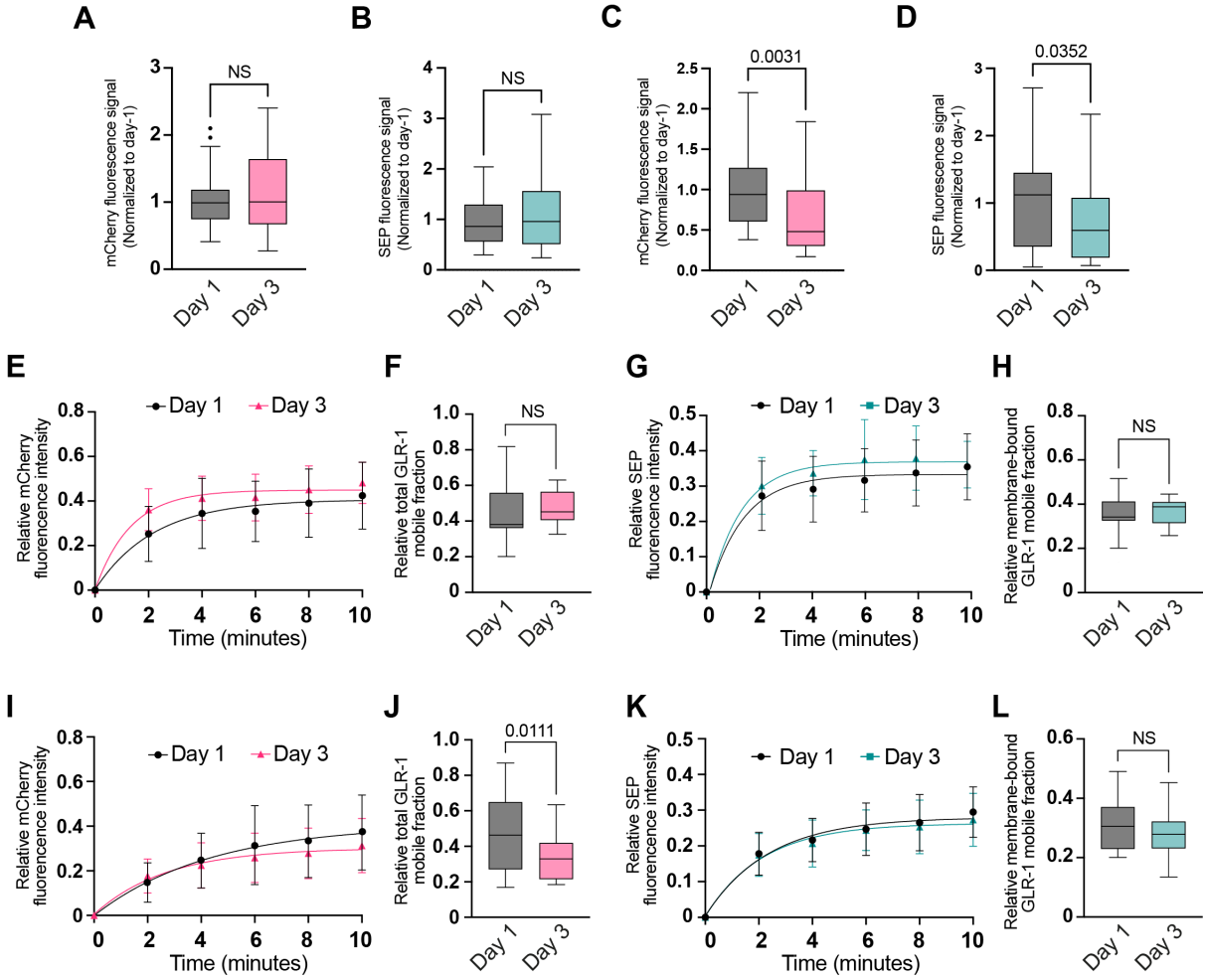

Figure 4.3 GLR-1 abundance is maintained with age in ubiquitination defective mutants

(A, B) Box plots displaying quantification of the fluorescence intensity of **(A)** total GLR-1 and **(B)** membrane bound receptors normalized to day-1 signal in the proximal part of the axon of AVA neuron of 4KR mutant worms. **(C, D)** receptor levels of **(C)** total GLR-1 and **(D)** membrane bound in the distal part of AVA axon of 4KR mutant worms. **(E, G)** Percent fluorescence recovery over time after photobleaching of **(E)** mCherry at day-1 (plateau= 0.4062) and at day-3 (plateau = 0.4493), and **(G)** SEP::GFP at day-1 (plateau= 0.3358) and at day-3 (plateau = 0.3728) (n >13 for each group) in the proximal part of AVA neuron. **(F, H)** mobile fraction of **(F)** total GLR-1 and **(H)** membrane-bound GLR-1 during ageing in the proximal part of the AVA neuron. **(I, K)** Percent fluorescence recovery of **(I)** mCherry after photobleaching over time at day-1 (plateau= 0.4079) and at day-3 (plateau= 0.3024), and **(K)** SEP at day-1 (plateau=0.2801) and at day-3 (plateau=0.2632) (n=>20 for each group) in the distal part. **(J, L)** Quantification of the mobile fraction of **(J)** total GLR-1 and **(L)** membrane-bound GLR-1 in the distal part of the

AVA neuron during ageing. Data on **A-D** and **F**, **H**, **J** and **I** are visualized with Tukey's boxplots. Panels **E**, **G**, **I** and **K** show mean ± SEM.

Table 4.3 Statistical table for results 4.3

Figure 3	model	condition tested	Levels	n	Test	Test statistics	df	p-value
4.3A	2-tailed Mann-Whitney test	age	Day-1, Day-3	40,39	U	743		0.72
4.3B	2-tailed Mann-Whitney test	age	Day-1, Day-3	41,42	U	778		0.453
4.3C	2-tailed Mann-Whitney test	age	Day-1, Day-3	41,32	U	467		0.0352
4.3D	2-tailed Mann-Whitney test	age	Day-1, Day-3	41,34	U	422		0.0031
4.3F	2-tailed t-test	age	Day-1, Day-3	18,12	t	0.5176	28	0.6088
4.3H	2-tailed t-test	age	Day-1, Day-3	21,14	t	0.9806	33	0.3339
4.3J	2-tailed t-test	age	Day-1, Day-3	20,25	t	2.654	43	0.0111
4.3L	2-tailed t-test	age	Day-1, Day-3	20,25	t	1.549	43	0.1288

4.4 GLR-1 abundance and dynamics remain unchanged during ageing in *msi-1(lf)* mutants

In a previous study, we identified the RNA-binding protein musashi (MSI-1) which promotes forgetting in *C. elegans* and has been shown to regulate the removal of AMPA receptors from synapses during long-term depression (LTD) (Hadziselimovic et al. 2014; Rocca et al. 2008). Interestingly, *msi-1(lf)* mutants have improved short- and long-term memory retention compared to wild-type and do not show age-dependent memory decline (Mastrandreas et al. 2022). We were curious to investigate whether the better memory performance of this mutant is associated with sustained levels of GLR-1 in aged animals and therefore compared GLR-1 abundance and dynamics between *msi-1* loss-of-function mutants and wild-type animals in day-1 and day-3 old adults.

As expected, we did not observe any age-dependent decrease in total (Figure 4.4A) or membrane-bound GLR-1 (Figure 4.4B) levels in the proximal part of AVA axons in *msi-1(lf)* mutants. Similarly, we observed sustained level of total (Figure 4.4C) as well as membrane-bound GLR-1 (Figure 4.4D) receptors between 1-day old and 3-day old mutants in the distal part of the AVA axon.

Furthermore, we performed FRAP analysis for mCherry and SEP signal and calculated the recovery curves and receptor mobile fraction values. We did not find any significant age dependent change in the recovery curves or in the mobile fraction for the total and the membrane bound GLR-1 in the proximal part (Figure 4.4E-H) nor in the distal part (Figure 4.4I-L) of the AVA axon. This shows that GLR-1 dynamics for total and membrane-bound GLR-1 do not change with age in *msi-1 (lf)* mutants. Importantly, *msi-1(lf)* showed normal life span (Figure 4.4M), suggesting that *msi-1* specifically affects memory decline rather than influencing

ageing in general. These results strengthen our hypothesis that GLR-1 synaptic abundance and turnover dynamics are tightly linked to memory performance and could be responsible for decreased memory performance in aged animals.

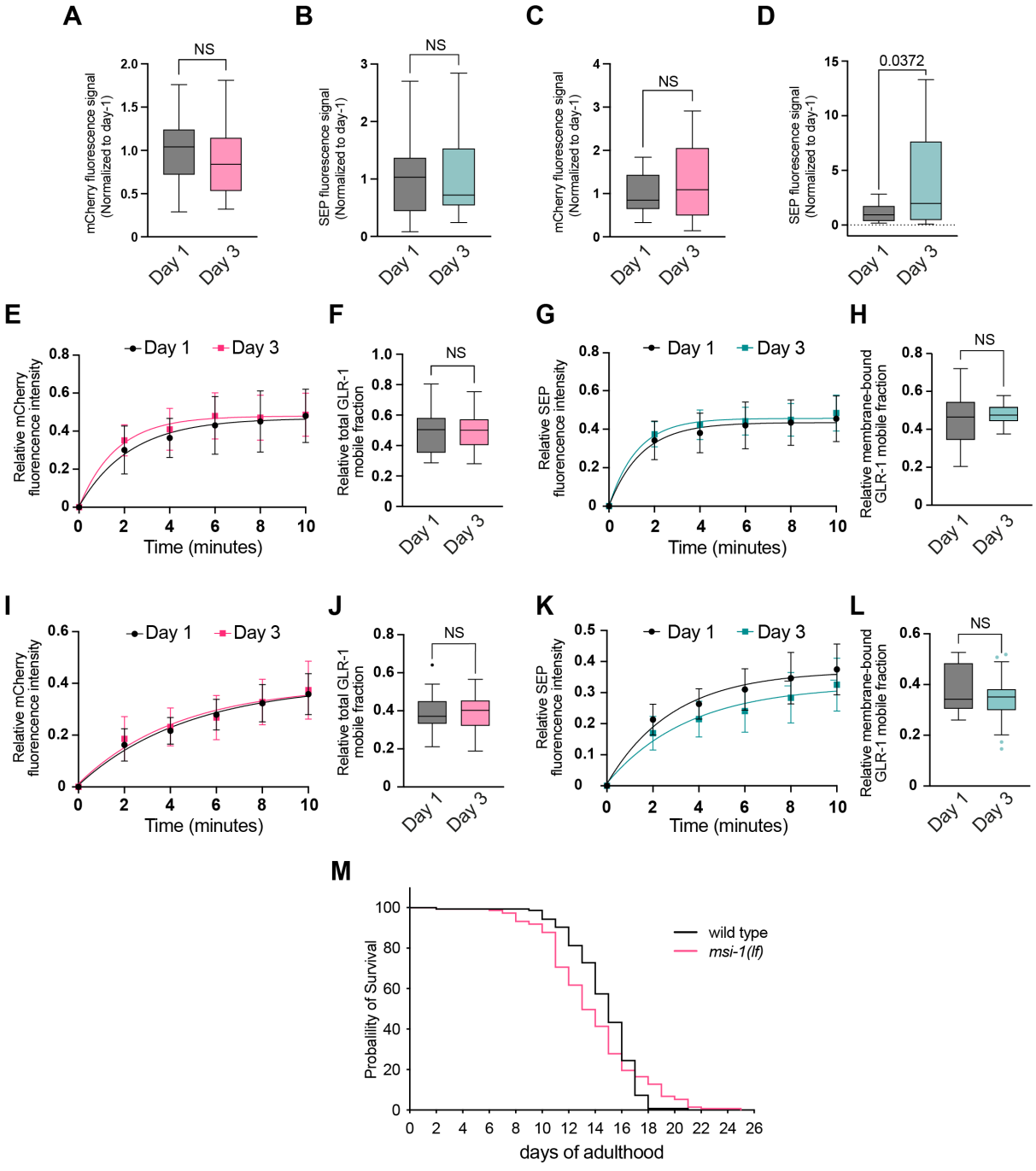

Figure 4.4 GLR-1 abundance and dynamics remain unchanged in *msi-1(lf)* mutants with ageing

(A, B) Quantification of the fluorescence intensity of (A) total and (B) membrane-bound GLR-1 receptors normalized to day-1 signal in the proximal part of the axon of AVA neuron of *msi-*

1(lf) mutant worms. **(C, D)** receptor levels of **(C)** total GLR-1 and **(D)** membrane bound in the distal part of AVA axon. **(E, G)** Fluorescence recovery following photobleaching of **(E)** mCherry at day-1 (plateau=0.4681) and at day-3 (plateau= 0.4793), and **(G)** SEP at day-1 (plateau=0.4341) and at day-3 (plateau= 0.4562) (n >16 for each group) in the proximal part of AVA neuron. **(F, H)** box plots displaying mobile fraction of **(F)** total GLR-1 and **(H)** membrane-bound GLR-1 during ageing in the proximal part of the AVA neuron. **(I, K)** Percent fluorescence recovery of **(I)** mCherry after photobleaching over time at day-1 (plateau=0.3988) and at day-3 (plateau= 0.3932), and **(K)** SEP::GFP at day-1 (plateau= 0.37) and at day-3 (plateau= 0.3240) (n >17 for each group) in the distal part. **(J, I)** Relative quantity of the mobile fraction of **(J)** total GLR-1 and **(I)** membrane-bound GLR-1 during ageing in the distal part of the AVA neuron of *msi-1(lf)* mutants at day-1 and day-3. **(M)** Life span of wild type and *msi-1(lf)* mutant animals. Data on **A-D** and **F**, **H**, **J** and **I** are visualized with Tukey's boxplots. Panels **E**, **G**, **I** and **K** show mean ± SEM.

Table 4.4 Statistical table for results 4.4

Figure 4	model	condition tested	Levels	n¶	Test	Test statistic	df	p-value
4.4A	2-tailed t-test	age	Day-1, Day-3	33,33	t	1.121	62	0.2665
4.4B	2-tailed Mann-Whitney test	age	Day-1, Day-3	30,31	U	419.5		0.5166
4.4C	2-tailed Mann-Whitney test	age	Day-1, Day-3	33,33	U	468.5		0.3336
4.4D	2-tailed Mann-Whitney test	age	Day-1, Day-3	32,33	U	369.5		0.0372
4.4F	2-tailed t-test	age	Day-1, Day-3	22,16	t	0.01765	36	0.986
4.4H	2-tailed t-test	age	Day-1, Day-3	25,18	t	0.4326	41	0.6676
4.4J	2-tailed t-test	age	Day-1, Day-3	16,15	t	0.1425	29	0.8877
4.4L	2-tailed t-test	age	Day-1, Day-3	17,19	t	1.199	34	0.2387
4.4M	Chi square	genotype	wt, *msi-1(lf)*	23, 18	Log-rank (Mantel-cox) test	0.8588	1	0.354

4.5 Memory is maintained in GLR-1(4KR) mutants with age

Our experiments strongly suggest that ageing alters local GLR-1 dynamics, a process that may be linked to physiological memory decline. Thus, we tested whether increasing GLR-1 abundance at synapses during ageing can rescue memory performance in aged animals. To assess memory, we used a previously described short-term aversive memory (STAM) assay (Hadziselimovic et al. 2014; Vukojevic et al. 2012) that measures attraction toward the chemoattractant diacetyl (DA) after aversive olfactory conditioning.

We performed the STAM assay in 1-day and 3-days old adult wild type animals, GLR-1(WT) overexpressing strain (SEP::mCherry::GLR-1) and GLR-1(4KR) mutant strain (SEP::mCherry::GLR-1(4KR)). 1-day old SEP::mCherry::GLR-1(WT) and SEP::mCherry::GLR-1(4KR) animals showed no significant difference in their memory performance as compared to age matched wild type control animals (Figure 4.5A). However, in line with our hypothesis, 3-day-old animals expressing the GLR-1(4KR) mutant form exhibited a significant increase in STAM retention compared to their wild-type counterparts (N2 and SEP::mCherry::GLR-1(WT)) (Figure 4.5B). This is in line with the *msi-1 loss-of-function* mutant results and shows that by maintaining GLR-1 receptor availability at synapses in AVA, memory performance decline can be prevented during ageing.

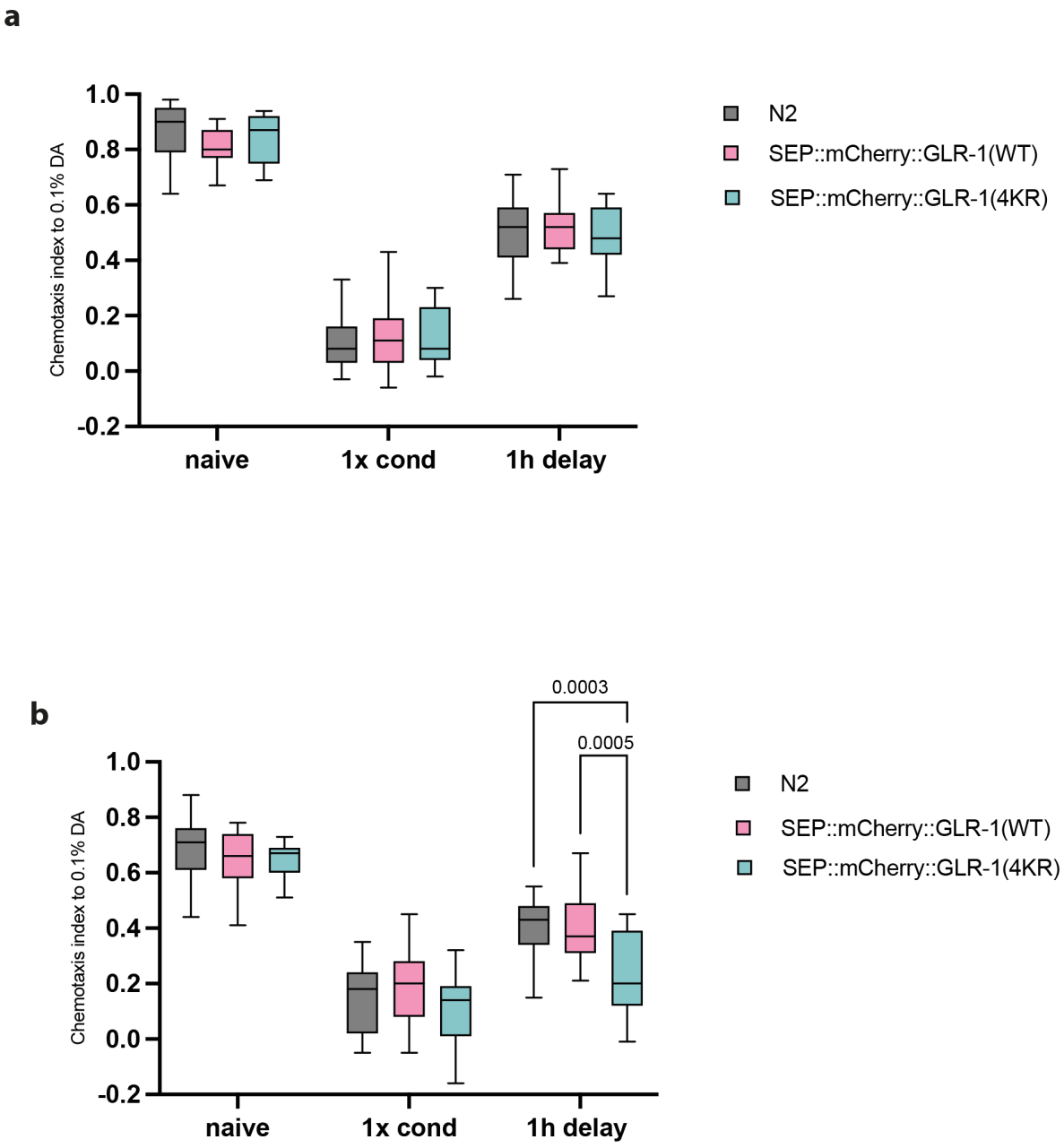

Figure 4.5 Memory is maintained in GLR-1(4KR) mutants with age

(A, B) Short-term aversive olfactory memory assay of wild-type, transgenic animals carrying either SEP::mCherry::GLR-1(wt) and SEP::mCherry::GLR-1(4KR) using DA was tested in **(A)** young adult and **(B)** 3-days old worms. Data are visualized with Tukey's boxplots See Data Table 3.5 for detailed statistical information.

Table 4.5 Statistical table for results 4.5

Figure 5	model	condition tested	Levels	n	Test	Test statistics	df	p-value	corr p-value*
4.5A	Anova	genotype	wt, *SEP::mCherry::GLR-1(wt), SEP::mCherry::GLR-1(4KR)*		F	0.04913	2, 126	0.9521	
	Anova	treatment	naive, cond, 1h delay		F	573.4	2, 126	<1E-15	
	Anova	genotype:treatment			F	0.9238	4, 126	0.4524	
4.5B	Anova	genotype	wt, *SEP::mCherry::GLR-1(wt), SEP::mCherry::GLR-1(4KR)*		F	7.79	2, 126	0.0006	
	Anova	treatment	naive, cond, 1h delay		F	191.5	2, 126	<1E-15	
	Anova	genotype:treatment			F	2.74	4, 126	0.0316	
	Post-hoc	genotype (naive)	wt, *SEP::mCherry::GLR-1(wt)*	15,15	q	1.69	126	0.2343	0.4583
	Post-hoc	genotype (naive)	wt, *SEP::mCherry::GLR-1(4KR)*	15,15	q	1.628	126	0.2517	0.4845
	Post-hoc	genotype (naive)	*SEP::mCherry::GLR-1(wt), SEP::mCherry::GLR-1(4KR)*	15,15	q	0.06184	126	0.9652	0.9989
	Post-hoc	genotype (conditioned)	wt, *SEP::mCherry::GLR-1(wt)*	15,15	q	1.587	126	0.2639	0.5022
	Post-hoc	genotype (conditioned)	wt, *SEP::mCherry::GLR-1(4KR)*	15,15	q	1.216	126	0.3915	0.6665
	Post-hoc	genotype (conditioned)	*SEP::mCherry::GLR-1(wt), SEP::mCherry::GLR-1(4KR)*	15,15	q	2.803	126	0.0496	0.1208
	Post-hoc	genotype (1h delay)	wt, *SEP::mCherry::GLR-1(wt)*	15,15	q	0.2267	126	0.8729	0.9859
	Post-hoc	genotype (1h delay)	wt, *SEP::mCherry::GLR-1(4KR)*	15,15	q	5.689	126	9.86E-05	0.0003
	Post-hoc	genotype (1h delay)	*SEP::mCherry::GLR-1(wt), SEP::mCherry::GLR-1(4KR)*	15,15	q	5.462	126	0.0002	0.0005

5 Discussion

Age-dependent cognitive decline is commonly observed during healthy ageing. However, the molecular and cellular mechanisms underlying this phenomenon are still unclear. It has been proposed that a decline in neuronal plasticity is a main driver in age-dependent cognitive decline (Bishop et al. 2010; Burke and Barnes 2006) but the mechanisms leading to this impairment are still largely unknown. Likely wise, a combination of altered synaptic transmission, neurotransmitter imbalances and the synthesis, distribution, and degradation of glutamate receptors are at play (Temido-Ferreira et al., 2019).

In the current study we investigated age-dependent changes in the AMPA-type glutamate receptor GLR-1 abundance, transport, dynamics, and its relationship with age-dependent memory decline. We have previously shown that the AMPA-type glutamate receptor, GLR-1 plays a crucial role in both learning and memory performance in *C. elegans*. Specifically, we demonstrated that GLR-1 in the AVA interneuron pair is essential for memory retention (Vukojevic et al. 2012). Further, we showed that *C. elegans* exhibits age-dependent memory decline and that the AVA interneuron seems to play an essential role during this process (Fenyves et al. 2021). Taken together, this suggests that GLR-1 might impact neuronal plasticity in a cell-type specific manner and that these processes are affected by ageing. However, it had never been shown how GLR-1 properties change with ageing and why this affects memory performance. Therefore, in this study we sought to investigate how GLR-1 abundance, distribution, and dynamics are regulated during ageing in the AVA interneuron in *C. elegans*.

Using a transgenic line expressing SEP::mCherry::GLR-1, we showed that the abundance of the total AMPA type glutamate receptors GLR-1 as well as the availability of membrane bound receptors is significantly reduced with age along the AVA axon. As most receptors are produced in the cell soma (Henley and Wilkinson 2016), possible explanations could be that the observed reduction of GLR-1 in the AVA axon is caused by reduced GLR-1 protein synthesis or decrease in long-distance transport from the cell body along the axon. However, by measuring GLR-1 transport frequency, we observed no decline in GLR-1 transport with ageing. Furthermore, we also measured abundance of total GLR-1 in the AVA soma and, interestingly, we found that GLR-1 is not reduced but, on the contrary, increased in aged animals as compared to young ones. This indicates that the observed decline in GLR-1 abundance in the AVA axon is likely not caused by a decreased protein production in the cell body nor by reduced transport along the axon to the synapses.

We hypothesize that the observed reduction in receptor content in the AVA axon could be caused by a general increase in protein degradation, increased local removal or, alternatively, decreased local receptor synthesis. The observed increase in receptor synthesis in the soma with age could represent a compensatory mechanism to contrast these processes. In this scenario, unchanged receptor transport could be the limiting factor that explains why the quantity of delivered receptors to the synaptic sites, measured using FRAP by quantifying mCherry signal recovery after photobleaching, is reduced with ageing, and why GLR-1 could be stalled in the soma.

Although intriguing, this hypothesis needs further experimental validation. In future studies, it could be investigated whether GLR-1 degradation is increased in aged animals by measuring real-time protein degradation kinetics. For example, this can be achieved by directly measuring GLR-1 ubiquitination levels with specific assays like NanoBRET or interactions of

GLR-1 with elements of the protein degradation pathway with the HaloTag technology (Promega).

Further, using FRAP, we analyzed changes in local receptor dynamics at synapses to assess whether receptor delivery and turnover changes with ageing. Our results show that with age, the short-distance delivery of GLR-1 receptors to the synapse and also the membrane insertion of GLR-1 receptor reduced significantly. This could lead to reduced synaptic plasticity and explains why memory performance in aged animals is reduced. However, it was not clear if reduced GLR-1 dynamics are merely reflective of a lower abundance of total GLR-1 receptors in the AVA axon or if there are other mechanisms at play.

To test whether reduced GLR-1 abundance affects GLR-1 dynamics and also memory performance, we used a mutant (4KR) expressing a ubiquitination defective form of GLR-1. Ubiquitination of proteins is an essential step during protein degradation, which allows the proteasome to recognize proteins flagged for degradation (Cooper, 2000). In the 4KR mutant, GLR-1 degradation rates should be remarkably reduced, as the protein is not ubiquitinated. We found that in the 4KR mutant, GLR-1 synaptic abundance is maintained with ageing as expected, and that this restores GLR-1 dynamics in 3-day old animals. This suggests that by maintaining GLR-1 levels during ageing, the receptor dynamics remain intact. Therefore, we hypothesize that a decrease in the total GLR-1 abundance during ageing limits the level of receptors at the synapse, leading to reduced receptor dynamics, and ultimately affecting the plasticity of the system. In fact, using behavioural assays in the 4KR mutant, we could show that blocking GLR-1 degradation improves memory of aged animals as compared to wild-type, further strengthening our hypothesis.

We have previously identified several key genes and proteins that influence memory performance in *C. elegans*. For example, msi-1 gene encoding an RNA-binding protein from the

Musashi family, regulates forgetting via translational repression of the Arp2/3 acting-branching complex (Hadziselimovic et al. 2014). *msi-1(lf)* mutants have improved long-term memory retention and, interestingly, do not show age-dependent memory decline (Mastrandreas et al. 2022). It has been shown that inhibition of the Arp 2/3 complex leads to a decreased removal of AMPARs from synapses and suppressed LTD (Rocca et al. 2008). These previous findings are in line with our findings, as we observed that GLR-1 abundance and dynamics are unchanged in aged *msi-1(lf)* mutant animals, as opposed to the wild-type. This further strengthens our hypotheses that the observed decrease in receptor dynamics in wild-type animals and the reduced memory performance in aged animals are directly linked to the observed lower levels of GLR-1.

Altogether, our findings demonstrate that the decrease of GLR-1 levels at synapses in relevant cell types is responsible for a gradual decline in memory performance during physiological ageing and that the decrease in GLR-1 is likely caused by an increase in receptor degradation rather than impaired synthesis or transport. This decline in GLR-1 receptors most likely reduces the efficiency of excitatory neurotransmission, therefore leading to synaptic depression and impaired cognitive function (Henley and Wilkinson 2016; Huganir and Nicoll 2013).

Interestingly, similar synaptic depression-induced cognitive decline is observed in conditions like Alzheimer's disease, major depressive disorder (MDD), and chronic stress (Guntupalli et al. 2017; Lam et al. 2014; Yuen et al. 2012). Dysregulation of AMPAR, particularly a decrease in its function, has been implicated in the pathophysiology of these conditions. In Alzheimer's disease, AMPAR ubiquitination plays a critical role in Aβ-induced synaptic depression (Guntupalli et al. 2017; Rodrigues et al. 2016). In chronic stress, glucocorticoid receptor-dependent reduction in AMPAR-mediated synaptic transmission due to increased

ubiquitination of GluA1 subunits is involved (Popoli et al. 2012). Our study suggests that restoring local GLR-1 levels at synapses in memory relevant neuronal circuits could potentially prevent age-related memory decline, offering hope for the development of novel therapeutic approaches for memory-related disorders.

So far, therapeutic interventions targeting the ubiquitin system to restore AMPA levels have proven challenging, with only a few drugs approved by the FDA (Huang and Dixit 2016; Kadriu et al. 2021; Widagdo et al. 2017). One main reason for this is that the glutamatergic system is the major excitatory neurotransmitter system in the brain that regulates many different processes across the whole brain. In fact, although the underlying problem of AMPAR hypofunction is similar across conditions, the mechanisms and affected brain regions may vary (Kadriu et al. 2021; Widagdo et al. 2017). Therefore, unspecific targeting of AMPARs or ubiquitination across the brain will most likely not lead to improvements in specific conditions. Although promising, this highlights the need for more research exploring specific ways of targeting AMPARs and ubiquitination in the brain.

C. elegans is a very simple model organism that lacks a complex nervous system such as a blood-brain-barrier. However, it offers the possibility to perform fast screenings that allow the testing of drug targets, as demonstrated in our previous studies (Mastrandreas et al. 2022). In the context of studying glutamatergic synaptic transmission and glutamate receptor biology, it could offer us an efficient platform to perform *in vivo* screenings to discover new compounds that could interact with glutamate receptors or specific components of the ubiquitination machinery. This could be an important first step to increase the spectrum of potential therapeutic substances that could then be tested in vertebrates and humans in the context of specific conditions or disorders.

Finally, our study has a number of limitations which should be acknowledged and addressed in future experiments.

Firstly, it is important to note that the described changes in GLR-1 are likely not only specific to AVA but extend to all GLR-1 expressing neurons in *C. elegans*. However, we could show that restoring GLR-1 levels specifically in AVA in aged animals was sufficient to improve memory performance and to restore GLR-1 dynamics at synapses. In fact, the 4KR mutant strain used for these experiments expresses the ubiquitination defective form of GLR-1 under control of the rig-3 promoter which overlaps in its activity with GLR-1 expression only in the AVA neuron. Our study thus highlights the need to investigate the function of synaptic proteins in specific cells and circuits. Whether there are synergistic or alternative neuronal circuits involved in the described phenomena, however, remains to be investigated.

Secondly, we used a transgenic line with an integrated extrachromosomal array overexpressing *SEP::mCherry::glr-1* and *SEP::mCherry::glr-1(4kr)* transgenes. As a result, we did not directly measure the abundance, transport, and membrane integration of endogenous GLR-1. Fortunately, these processes are governed by protein complexes and enzymatic cascades that do not distinguish between proteins synthetized from endogenous genes and proteins synthetized from extrachromosomal arrays. However, on the other hand, gene expression regulation is, at least in part, dependent on the chromatin context and could differ between endogenous genes and extrachromosomal arrays (Du and Zheng 2021; Lund et al. 2004). Importantly, it has been shown that with ageing chromatin undergoes significant remodelling and that this can lead to significant changes in gene expression and protein abundance (Feser and Tyler 2011; Liu et al. 2022; Stevanovic et al. 2023; Sun, Yu, and Dang 2018). With our extrachromosomal array, we cannot detect whether there are changes in

endogenous GLR-1 expression caused by remodelling of the endogenous GLR-1 gene locus. This could be overcome by using CRISPR/Cas9 gene editing to tag endogenous GLR-1 with fluorophores. However, in this case, measuring the abundance of endogenous GLR-1 and performing FRAP analysis would be challenging due to the much lower signal intensity and current detection limits. This is the reason why for this study we choose to use the multicopy array overexpressing fluorescently labelled GLR-1. However, in future studies it would be important to address whether gene expression of endogenous GLR-1 changes with ageing, in order to understand whether this could also contribute to altered GLR-1 abundance in aged animals.

As mentioned in the introduction, the *C. elegans* genome encodes at least 10 putative ionotropic glutamate receptor (iGluR) subunits. However, in the current study, we only focused on GLR-1. It has been demonstrated in *C. elegans* that the AMPA receptor subunits GLR-1 and GLR-2 form heteromer, which are essential for receptor function, trafficking, and synaptic localization (Chang and Rongo 2005). The combination of different subunits contributes to the complexity and versatility of AMPA receptor function in the central nervous system (Diering and Huganir 2018) and therefore further investigation is necessary to fully explore the roles of other glutamate receptors subunits in memory and age-dependent cognitive decline. In future studies, we could assess the properties, abundance, and distribution of other receptor subunits and the interaction between them.

Further, it is widely acknowledged that glutamate receptors play a crucial role in various forms of learning and memory across different tasks and brain regions. As one of the fundamental principles of synaptic plasticity, we presume that our findings hold broader applicability. However, it's essential to acknowledge that in our study, in which we employed

one specific assay paradigm with one chemical compound, did not directly establish this generalizability. In future experiments, it would be valuable to analyze the effect of blocking GLR-1 ubiquitination and degradation on age-dependent memory decline using different assay paradigms, as mentioned in chapter 1.4. For example, not only short but also long-term memory could be assessed in aged animals and not only aversive but also appetitive conditioning with different compounds could be tested. One challenge in this regard is that the ability of *C. elegans* to sense various environmental cues gradually declines with age, which makes it difficult to evaluate learning and memory in assays involving those sensory cues. For example, chemosensation towards diacetyl significantly declines in 4-day old animals. Due to this limitation, we were unable to assess long-term aversive memory (LTAM) at day-3, as the test for long-term recovery occurs at day-4. To bolster the results, it would be worthwhile to test whether assays using different chemical compounds could still be applicable in aged animals.

It is important to acknowledge the considerable differences between *C. elegans* and mammals in terms of complexity of their nervous system, cognitive abilities, and genetics. Although roughly 83% of the *C. elegans* protein coding genes are homologous to humans, there can still be considerable differences in regulation, function, and contribution to complex processes of the same genes (Lai et al. 2000). In fact, as already mentioned in the introduction, GLR-1 itself has only 40% homology with mammalian AMPA-type glutamate receptor GRIA2 and GRIA3. To overcome this limitation, similar experiments as conducted in this study could be carried out in *in vitro* induced human neurons. For example, following a protocol of direct conversion of human fibroblast into glutamatergic neurons (Mertens et al. 2015) we could derive relevant cell types for cognitive functions from young and old donors and investigate

expression, trafficking, and turnover of human specific AMPA type glutamate receptor subunits.

Further, as discussed before, *C. elegans* has a very simple nervous system and lacks many higher cognitive functions found in humans. Human memory disorders are complex and involve multiple brain regions and other higher cognitive functions like attention and executive control, which cannot be recapitulated in *C. elegans*. The simplicity of learning and memory paradigms used in *C. elegans* studies further emphasizes these differences. While these assays offer valuable insights into the fundamental cellular and molecular mechanisms related to memory, they cannot full capture the diverse and complex memory processes observed in humans. Consequently, caution is required when trying to directly extrapolate findings from studies involving *C. elegans* to understand or address human memory disorders. To achieve a comprehensive understanding of memory disorders in humans, further research using more relevant model organisms such as mice, rats or primates is necessary.

Despite the inherent limitations of using *C. elegans* as a model organism for studying complex cognitive processes like learning and memory, this study yields valuable insights into the role of AMPA-type glutamate receptors in regulating age-dependent memory decline. The fact that GLR-1 manipulation could influence memory in *C. elegans* suggests its potential significance as a target for further investigation in mammalian models and humans. The study also highlights the practicality and usefulness of *C. elegans* as a model organism for initial investigations into learning and memory mechanisms.

We hope that our findings will contribute to a better understanding of the specific genes and pathways involved in memory regulation in ageing and that this research lays the groundwork for more targeted investigations in more complex organisms, potentially leading to insights into memory-related disorders in humans. Furthermore, we hope that we could

provide a foundation for future research that could lead to significant advancements in therapies to treat age-related cognitive decline and neurodegenerative disorders.

6 Bibliography

Ardiel, Evan L., and Catharine H. Rankin. 2010. “An Elegant Mind: Learning and Memory in Caenorhabditis Elegans.” *Learning and Memory* 17(4):191–201.

Barco, Angel, Craig H. Bailey, and Eric R. Kandel. 2006. “Common Molecular Mechanisms in Explicit and Implicit Memory.” *Journal of Neurochemistry* 97(6).

Bargmann, Cornelia I., Erika Hartwieg, and H. Robert Horvitz. 1993. “Odorant-Selective Genes and Neurons Mediate Olfaction in C. Elegans.” *Cell* 74(3). doi: 10.1016/0092-8674(93)80053-H.

Beck, C. D. O., and C. H. Rankin. 1993. “Effects of Aging on Habituation in the Nematode Caenorhabditis Elegans.” *Behavioural Processes* 28(3). doi: 10.1016/0376-6357(93)90088-9.

Beck, C. D., and C. H. Rankin. 1995. “Heat Shock Disrupts Long-Term Memory Consolidation in Caenorhabditis Elegans.” *Learning & Memory (Cold Spring Harbor, N.Y.)* 2(3–4). doi: 10.1101/lm.2.3-4.161.

Bishop, Nicholas A., Tao Lu, and Bruce A. Yankner. 2010. “Neural Mechanisms of Ageing and Cognitive Decline.” *Nature* 464(7288).

Bliss, T. V. P., and G. L. Collingridge. 1993. “A Synaptic Model of Memory: Long-Term Potentiation in the Hippocampus.” *Nature* 361(6407).

Bono, Mario de, and Andres Villu Maricq. 2005. “ Neuronal Substrates of Complex Behaviors in C. Elegans .” *Annual Review of Neuroscience* 28(1):451–501. doi: 10.1146/annurev.neuro.27.070203.144259.

Bozorgmehr, Tahereh, Evan L. Ardiel, Andrea H. McEwan, and Catharine H. Rankin. 2013. "Mechanisms of Plasticity in a Caenorhabditis Elegans Mechanosensory Circuit." *Frontiers in Physiology* 4 AUG(August):1–11. doi: 10.3389/fphys.2013.00088.

Brenner, S. 1974. "The Genetics of Caenorhabditis Elegans." *Genetics* 77(1). doi: 10.1093/genetics/77.1.71.

Brockie, J. Mellem, T. Hills, D. Madsen, and A. Maricq. 2001. "The C. Elegans Glutamate Receptor Subunit NMR-1 Is Required for Slow NMDA-Activated Currents That …." *Neuron* 31:617–30.

Brockie, Penelope. 2006. "Ionotropic Glutamate Receptors: Genetics, Behavior and Electrophysiology." *WormBook* 1–16. doi: 10.1895/wormbook.1.61.1.

Brockie, Penelope J., David M. Madsen, Yi Zheng, Jerry Mellem, and Andres V. Maricq. 2001. "Differential Expression of Glutamate Receptor Subunits in the Nervous System of Caenorhabditis Elegans and Their Regulation by the Homeodomain Protein UNC-42." *Journal of Neuroscience* 21(5). doi: 10.1523/jneurosci.21-05-01510.2001.

Brockie, Penelope J., and Andres V. Maricq. 2003. "Ionotropic Glutamate Receptors in Caenorhabditis Elegans." *NeuroSignals* 12(3):108–25. doi: 10.1159/000072159.

Burbea, Michelle, Lars Dreier, Jeremy S. Dittman, Maria E. Grunwald, and Joshua M. Kaplan. 2002. "Ubiquitin and AP180 Regulate the Abundance of GLR-1 Glutamate Receptors at Postsynaptic Elements in C. Elegans." *Neuron* 35(1):107–20. doi: 10.1016/S0896-6273(02)00749-3.

Burke, Sara N., and Carol A. Barnes. 2006. "Neural Plasticity in the Ageing Brain." *Nature Reviews Neuroscience* 7(1).

Byrne, John H., and Robert D. Hawkins. 2015. "Nonassociative Learning in Invertebrates." *Cold Spring Harbor Perspectives in Biology* 7(5). doi: 10.1101/cshperspect.a021675.

Carmona, Juan José, and Shaday Michan. 2016. “Biology of Healthy Aging and Longevity.” *Revista de Investigacion Clinica; Organo Del Hospital de Enfermedades de La Nutricion* 68(1).

Chalfie, Martin, Yuan Tu, Ghia Euskirchen, William W. Ward, and Douglas C. Prasher. 1994. “Green Fluorescent Protein as a Marker for Gene Expression.” *Science* 263(5148). doi: 10.1126/science.8303295.

Chang, Howard Chia Hao, and Christopher Rongo. 2005. “Cytosolic Tail Sequences and Subunit Interactions Are Critical for Synaptic Localization of Glutamate Receptors.” *Journal of Cell Science* 118(9). doi: 10.1242/jcs.02320.

Chew, Yee Lian, Xiaochen Fan, Jürgen Götz, and Hannah R. Nicholas. 2013. “Aging in the Nervous System of Caenorhabditis Elegans.” *Communicative and Integrative Biology* 6(5).

Corsi, Ann K., Bruce Wightman, and Martin Chalfie. 2015. “A Transparent Window into Biology: A Primer on Caenorhabditis Elegans.” *Genetics* 200(2). doi: 10.1534/genetics.115.176099.

Diering, Graham H., and Richard L. Huganir. 2018. “The AMPA Receptor Code of Synaptic Plasticity.” *Neuron* 100(2).

Dong, Zhifang, Huili Han, Hongjie Li, Yanrui Bai, Wei Wang, Man Tu, Yan Peng, Limin Zhou, Wenting He, Xiaobin Wu, Tao Tan, Mingjing Liu, Xiaoyan Wu, Weihui Zhou, Wuyang Jin, Shu Zhang, Todd Charlton Sacktor, Tingyu Li, Weihong Song, and Yu Tian Wang. 2015. “Long-Term Potentiation Decay and Memory Loss Are Mediated by AMPAR Endocytosis.” *Journal of Clinical Investigation* 125(1). doi: 10.1172/JCI77888.

Du, Shuqi, and Hui Zheng. 2021. “Role of FoxO Transcription Factors in Aging and Age-Related Metabolic and Neurodegenerative Diseases.” *Cell and Bioscience* 11(1).

Dudai, Y., Y. N. Jan, D. Byers, W. G. Quinn, and S. Benzer. 1976. “Dunce, a Mutant of Drosophila Deficient in Learning.” *Proceedings of the National Academy of Sciences of the United States of America* 73(5). doi: 10.1073/pnas.73.5.1684.

Fenyves, Bank G., Andreas Arnold, Vaibhav G. Gharat, Carmen Haab, Kiril Tishinov, Fabian Peter, Dominique de Quervain, Andreas Papassotiropoulos, and Attila Stetak. 2021. “Dual Role of an Mps-2/KCNE-Dependent Pathway in Long-Term Memory and Age-Dependent Memory Decline.” *Current Biology* 31(3):527-539.e7. doi: 10.1016/j.cub.2020.10.069.

Feser, Jason, and Jessica Tyler. 2011. “Chromatin Structure as a Mediator of Aging.” *FEBS Letters* 585(13).

Fox-Loe, Ashley M., Brandon J. Henderson, and Christopher I. Richards. 2017. “Utilizing Phluorin-Tagged Receptors to Monitor Subcellular Localization and Trafficking.” *Journal of Visualized Experiments* 2017(121). doi: 10.3791/55466.

Friedman, D. B., and T. E. Johnson. 1988. “A Mutation in the Age-1 Gene in Caenorhabditis Elegans Lengthens Life and Reduces Hermaphrodite Fertility.” *Genetics* 118(1). doi: 10.1093/genetics/118.1.75.

Geoffrey M Cooper. 2000. “The Cell, 2nd Edition A Molecular Approach.” in *Sunderland (MA): Sinauer Associates;*. Vol. 63.

Golden, Tamara R., and Simon Melov. 2007. “Gene Expression Changes Associated with Aging in C. Elegans.” *WormBook : The Online Review of C. Elegans Biology*.

Grammenoudi, Sofia, Stylianos Kosmidis, and Efthimios M. C. Skoulakis. 2006. “Cell Type-Specific Processing of Human Tau Proteins in Drosophila.” *FEBS Letters* 580(19). doi: 10.1016/j.febslet.2006.07.045.

Greger, Ingo H., Jake F. Watson, and Stuart G. Cull-Candy. 2017. “Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins.” *Neuron* 94(4).

Grunwald, Maria E., Jerry E. Mellem, Nathalie Strutz, Andres V. Maricq, and Joshua M. Kaplan. 2004. “Clathrin-Mediated Endocytosis Is Required for Compensatory Regulation of GLR-1 Glutamate Receptors after Activity Blockade.” *Proceedings of the National Academy of Sciences of the United States of America* 101(9):3190–95. doi: 10.1073/pnas.0306156101.

Guntupalli, Sumasri, Se Eun Jang, Tianyi Zhu, Richard L. Huganir, Jocelyn Widagdo, and Victor Anggono. 2017. “GluA1 Subunit Ubiquitination Mediates Amyloid-β-Induced Loss of Surface α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) Receptors.” *Journal of Biological Chemistry* 292(20). doi: 10.1074/jbc.M116.774554.

Hadziselimovic, Nils, Vanja Vukojevic, Fabian Peter, Annette Milnik, Matthias Fastenrath, Bank Gabor Fenyves, Petra Hieber, Philippe Demougin, Christian Vogler, Dominique J. F. De Quervain, Andreas Papassotiropoulos, and Attila Stetak. 2014. “Forgetting Is Regulated via Musashi-Mediated Translational Control of the Arp2/3 Complex.” *Cell* 156(6):1153–66. doi: 10.1016/j.cell.2014.01.054.

Hart, Anne C., Shannon Sims, and Joshua M. Kaplan. 1995. “Synaptic Code for Sensory Modalities Revealed by C. Elegans GLR-1 Glutamate Receptor.” *Nature* 378(6552). doi: 10.1038/378082a0.

Henley, Jeremy M., and Kevin A. Wilkinson. 2013. “AMPA Receptor Trafficking and the Mechanisms Underlying Synaptic Plasticity and Cognitive Aging.” *Dialogues in Clinical Neuroscience* 15(1):11–27. doi: 10.31887/dcns.2013.15.1/jhenley.

Henley, Jeremy M., and Kevin A. Wilkinson. 2016. “Synaptic AMPA Receptor Composition in Development, Plasticity and Disease.” *Nature Reviews Neuroscience* 17(6):337–50. doi: 10.1038/nrn.2016.37.

Hoerndli, Frédéric J., Dane A. Maxfield, Penelope J. Brockie, Jerry E. Mellem, Erica Jensen, Rui Wang, David M. Madsen, and Andres V. Maricq. 2013. “Kinesin-1 Regulates Synaptic Strength by Mediating the Delivery, Removal, and Redistribution of AMPA Receptors.” *Neuron* 80(6):1421–37. doi: 10.1016/j.neuron.2013.10.050.

Hoerndli, Frédéric J., Colin Thacker, Angy Kallarackal, Penelope J. Brockie, Rui Wang, Jerry E. Mellem, David M. Madsen, and Andres V. Maricq. 2015. “Neuronal Activity and CaMKII Regulate Kinesin-Mediated Transport of Synaptic AMPARs.” *Neuron* 86(2):457–74. doi: 10.1016/j.neuron.2015.03.011.

Hou, Yujun, Xiuli Dan, Mansi Babbar, Yong Wei, Steen G. Hasselbalch, Deborah L. Croteau, and Vilhelm A. Bohr. 2019. “Ageing as a Risk Factor for Neurodegenerative Disease.” *Nature Reviews Neurology* 15(10).

Huang, Xiaodong, and Vishva M. Dixit. 2016. “Drugging the Undruggables: Exploring the Ubiquitin System for Drug Development.” *Cell Research* 26(4).

Huganir, Richard L., and Roger A. Nicoll. 2013. “AMPARs and Synaptic Plasticity: The Last 25 Years.” *Neuron* 80(3):704–17. doi: 10.1016/j.neuron.2013.10.025.

Jakobs, Maximilian Ah, Andrea Dimitracopoulos, and Kristian Franze. 2019. “Kymobutler, a Deep Learning Software for Automated Kymograph Analysis.” *ELife* 8. doi: 10.7554/eLife.42288.

Jurado, Sandra. 2018. “AMPA Receptor Trafficking in Natural and Pathological Aging.” *Frontiers in Molecular Neuroscience* 10(January):1–14. doi: 10.3389/fnmol.2017.00446.

Kadriu, Bashkim, Laura Musazzi, Jenessa N. Johnston, Lisa E. Kalynchuk, Hector J. Caruncho, Maurizio Popoli, and Carlos A. Zarate. 2021. "Positive AMPA Receptor Modulation in the Treatment of Neuropsychiatric Disorders: A Long and Winding Road." *Drug Discovery Today* 26(12).

Kandel, E. R., and L. Tauc. 1965. "Heterosynaptic Facilitation in Neurones of the Abdominal Ganglion of Aplysia Depilans." *The Journal of Physiology* 181(1). doi: 10.1113/jphysiol.1965.sp007742.

Kandel, Eric R. 2012. "The Molecular Biology of Memory: CAMP, PKA, CRE, CREB-1, CREB-2, and CPEB." *Molecular Brain* 5(1).

Kandel, Eric, James Schwartz, Thomas Jessell, Steven Siegelbaum, A. J. Hudspeth, Eric R. Kandel, James H. Schwartz, Thomas M. Jessell, and Steven A. Siegelbaum. 2012. "Principles of Neural Science, Fifth Edition (Principles of Neural Science (Kandel))." in *Principles of Neural Science*.

Kano, Takashi, Penelope J. Brockie, Toshihiro Sassa, Hiroyuki Fujimoto, Yasushi Kawahara, Yuichi Iino, Jerry E. Mellem, David M. Madsen, Ryuji Hosono, and Andres V. Maricq. 2008. "Memory in Caenorhabditis Elegans Is Mediated by NMDA-Type Ionotropic Glutamate Receptors." *Current Biology* 18(13):1010–15. doi: 10.1016/j.cub.2008.05.051.

Kauffman, Amanda L., Jasmine M. Ashraf, M. Ryan orces-Zimmerman, Jessica N. Landis, and Coleen T. Murphy. 2010. "Insulin Signaling and Dietary Restriction Differentially Influence the Decline of Learning and Memory with Age." *PLoS Biology* 8(5). doi: 10.1371/journal.pbio.1000372.

Kennedy, Matthew J., Ian G. Davison, Camenzind G. Robinson, and Michael D. Ehlers. 2010. "Syntaxin-4 Defines a Domain for Activity-Dependent Exocytosis in Dendritic Spines." *Cell* 141(3):524–35. doi: 10.1016/j.cell.2010.02.042.

Kennedy, Matthew J., and Michael D. Ehlers. 2011. “Mechanisms and Function of Dendritic Exocytosis.” *Neuron* 69(5).

Kenyon, Cynthia, Jean Chang, Erin Gensch, Adam Rudner, and Ramon Tabtiang. 1993. “A C. Elegans Mutant That Lives Twice as Long as Wild Type.” *Nature* 366(6454). doi: 10.1038/366461a0.

Klass, Michael R. 1977. “Aging in the Nematode Caenorhabditis Elegans: Major Biological and Environmental Factors Influencing Life Span.” *Mechanisms of Ageing and Development* 6(C). doi: 10.1016/0047-6374(77)90043-4.

Lai, Chun Hung, Chang Yuan Chou, Lan Yang Ch’ang, Chung Shyan Liu, and Wen Chang Lin. 2000. “Identification of Novel Human Genes Evolutionarily Conserved in Caenorhabditis Elegans by Comparative Proteomics.” *Genome Research* 10(5). doi: 10.1101/gr.10.5.703.

Lam, Raymond W., Sidney H. Kennedy, Roger S. McIntyre, and Atul Khullar. 2014. “Cognitive Dysfunction in Major Depressive Disorder: Effects on Psychosocial Functioning and Implications for Treatment.” *Canadian Journal of Psychiatry* 59(12).

Larkman, Alan U., and J. Julian B. Jack. 1995. “Synaptic Plasticity: Hippocampal LTP.” *Current Opinion in Neurobiology* 5(3). doi: 10.1016/0959-4388(95)80045-X.

Lisman, John. 1994. “The CaM Kinase II Hypothesis for the Storage of Synaptic Memory.” *Trends in Neurosciences* 17(10).

Liu, Zunpeng, Juan Carlos Izpisua Belmonte, Weiqi Zhang, Jing Qu, and Guang-Hui Liu. 2022. “Deciphering Aging at Three-Dimensional Genomic Resolution.” *Cell Insight* 1(3). doi: 10.1016/j.cellin.2022.100034.

López-Otín, Carlos, Maria A. Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. 2023. “Hallmarks of Aging: An Expanding Universe.” *Cell* 186(2).

Lund, P. Kay, Eileen C. Hoyt, Jennifer Bizon, Dani R. Smith, Rebecca Haberman, Kassie Helm, and Michela Gallagher. 2004. “Transcriptional Mechanisms of Hippocampal Aging.” in *Experimental Gerontology*. Vol. 39.

Lynch, M. A. 2004. “Long-Term Potentiation and Memory.” *Physiological Reviews* 84(1).

Malenka, Robert C., and Roger A. Nicoll. 1999. “Long-Term Potentiation - A Decade of Progress?” *Science* 285(5435).

Maricq, Andres V., Erin Peckol, Monica Driscoll, and Cornelia I. Bargmann. 1995. “Mechanosensory Signalling in C. Elegans Mediated by the GLR-1 Glutamate Receptor.” *Nature* 378(6552). doi: 10.1038/378078a0.

Mastrandreas, Pavlina, Andreas Arnold, Csaba Boglari, Dominique J. F. de Quervain, Attila Stetak, and Andreas Papassotiropoulos. 2022. “(-)- Gossypol Inhibition of Musashi-Mediated Forgetting Improves Memory and Age-Dependent Memory Decline in Caenorhabditis Elegans.” *Molecular Neurobiology* 7. doi: 10.1007/s12035-022-03116-7.

Mattson, Mark P., and Thiruma V. Arumugam. 2018. “Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States.” *Cell Metabolism* 27(6).

Mayford, Mark, Jian Wang, Eric R. Kandel, and Thomas J. O’Dell. 1995. “CaMKII Regulates the Frequency-Response Function of Hippocampal Synapses for the Production of Both LTD and LTP.” *Cell* 81(6). doi: 10.1016/0092-8674(95)90009-8.

McEwan, Andrea H., and Catharine H. Rankin. 2013. “Mechanosensory Learning and Memory in Caenorhabditis Elegans.” in *Handbook of Behavioral Neuroscience*. Vol. 22.

Mertens, Jerome, Apuã C. M. Paquola, Manching Ku, Emily Hatch, Lena Böhnke, Shauheen Ladjevardi, Sean McGrath, Benjamin Campbell, Hyungjun Lee, Joseph R. Herdy, J. Tiago Gonçalves, Tomohisa Toda, Yongsung Kim, Jürgen Winkler, Jun Yao, Martin W. Hetzer, and Fred H. Gage. 2015. “Directly Reprogrammed Human Neurons Retain Aging-

Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects." *Cell Stem Cell* 17(6). doi: 10.1016/j.stem.2015.09.001.

Metaxakis, Athanasios, Dionysia Petratou, and Nektarios Tavernarakis. 2018. "Multimodal Sensory Processing in Caenorhabditis Elegans." *Open Biology* 8(6).

Morrison, Glenn E., and Derek Van Der Kooy. 2001. "A Mutation in the AMPA-Type Glutamate Receptor, Glr-1, Blocks Olfactory Associative and Nonassociative Learning in Caenorhabditis Elegans." *Behavioral Neuroscience* 115(3). doi: 10.1037/0735-7044.115.3.640.

Otmakhov, Nikolai, Jung Hwa Tao-Cheng, Stephen Carpenter, Brent Asrican, Ayse Dosemeci, Thomas S. Reese, and John Lisman. 2004. "Persistent Accumulation of Calcium/Calmodulin-Dependent Protein Kinase II in Dendritic Spines after Induction of NMDA Receptor-Dependent Chemical Long-Term Potentiation." in *Journal of Neuroscience*. Vol. 24.

Partridge, Linda, Joris Deelen, and P. Eline Slagboom. 2018. "Facing up to the Global Challenges of Ageing." *Nature* 561(7721).

Popoli, Maurizio, Zhen Yan, Bruce S. McEwen, and Gerard Sanacora. 2012. "The Stressed Synapse: The Impact of Stress and Glucocorticoids on Glutamate Transmission." *Nature Reviews Neuroscience* 13(1).

Quinn, W. G., W. A. Harris, and S. Benzer. 1974. "Conditioned Behavior in Drosophila Melanogaster." *Proceedings of the National Academy of Sciences of the United States of America* 71(3). doi: 10.1073/pnas.71.3.708.

Rahmani, Aelon, and Yee Lian Chew. 2021. "Investigating the Molecular Mechanisms of Learning and Memory Using Caenorhabditis Elegans." *Journal of Neurochemistry* 159(3).

Rankin, Catherine H., Christine D. O. Beck, and Catherine M. Chiba. 1990. “Caenorhabditis Elegans: A New Model System for the Study of Learning and Memory.” *Behavioural Brain Research* 37(1). doi: 10.1016/0166-4328(90)90074-O.

Rocca, Daniel L., Stéphane Martin, Emma L. Jenkins, and Jonathan G. Hanley. 2008. “Inhibition of Arp2/3-Mediated Actin Polymerization by PICK1 Regulates Neuronal Morphology and AMPA Receptor Endocytosis.” *Nature Cell Biology* 10(3). doi: 10.1038/ncb1688.

Rodrigues, Elizabeth M., Samantha L. Scudder, Marisa S. Goo, and Gentry N. Patrick. 2016. “Aβ-Induced Synaptic Alterations Require the E3 Ubiquitin Ligase Nedd4-1.” *Journal of Neuroscience* 36(5). doi: 10.1523/JNEUROSCI.2964-15.2016.

Rose, Jacqueline K., Karla R. Kaun, Sylvia H. Chen, and Catharine H. Rankin. 2003. “GLR-1, a Non-NMDA Glutamate Receptor Homolog, Is Critical for Long-Term Memory in Caenorhabditis Elegans.” *Journal of Neuroscience* 23(29):9595–99. doi: 10.1523/JNEUROSCI.23-29-09595.2003.

Rose, Jacqueline K., Karla R. Kaun, and Catharine H. Rankin. 2002. “A New Group-Training Procedure for Habituation Demonstrates That Presynaptic Glutamate Release Contributes to Long-Term Memory in Caenorhabditis Elegans.” *Learning and Memory* 9(3). doi: 10.1101/lm.46802.

Smetana, Karel, Lukáš Lacina, Pavol Szabo, Barbora Dvoánková, Prokop Brož, and Aleksi Šedo. 2016. “Ageing as an Important Risk Factor for Cancer.” *Anticancer Research* 36(10).

Stein, Geneva M., and Coleen T. Murphy. 2012. “The Intersection of Aging, Longevity Pathways, and Learning and Memory in C. Elegans.” *Frontiers in Genetics* 3(NOV). doi: 10.3389/fgene.2012.00259.

Stetak, Attila, Frederic Hörndli, Andres V. Maricq, Sander van den Heuvel, and Alex Hajnal. 2009. “Neuron-Specific Regulation of Associative Learning and Memory by MAGI-1 in C. Elegans.” *PLoS ONE* 4(6). doi: 10.1371/journal.pone.0006019.

Stevanovic, Milena, Andrijana Lazic, Marija Schwirtlich, and Danijela Stanisavljevic Ninkovic. 2023. “The Role of SOX Transcription Factors in Ageing and Age-Related Diseases.” *International Journal of Molecular Sciences* 24(1).

Sulston, J. E., and H. R. Horvitz. 1977. “Post-Embryonic Cell Lineages of the Nematode, Caenorhabditis Elegans.” *Developmental Biology* 56(1). doi: 10.1016/0012-1606(77)90158-0.

Sulston, J. E., E. Schierenberg, J. G. White, and J. N. Thomson. 1983. “The Embryonic Cell Lineage of the Nematode Caenorhabditis Elegans.” *Developmental Biology* 100(1).

Sun, Luyang, Ruofan Yu, and Weiwei Dang. 2018. “Chromatin Architectural Changes during Cellular Senescence and Aging.” *Genes* 9(4).

Traynelis, Stephen F., Lonnie P. Wollmuth, Chris J. McBain, Frank S. Menniti, Katie M. Vance, Kevin K. Ogden, Kasper B. Hansen, Hongjie Yuan, Scott J. Myers, and Ray Dingledine. 2010. “Glutamate Receptor Ion Channels: Structure, Regulation, and Function.” *Pharmacological Reviews* 62(3).

United Nations. 2019. “World Population Prospects 2019 - Highlights.” *Department of Economic and Social Affairs. World Population Prospects 2019.* (141).

Varshney, Lav R., Beth L. Chen, Eric Paniagua, David H. Hall, and Dmitri B. Chklovskii. 2011. “Structural Properties of the Caenorhabditis Elegans Neuronal Network.” *PLoS Computational Biology* 7(2). doi: 10.1371/journal.pcbi.1001066.

Vukojevic, Vanja, Leo Gschwind, Christian Vogler, Philippe Demougin, Dominique J. F. De Quervain, Andreas Papassotiropoulos, and Attila Stetak. 2012. “A Role for α-Adducin

(ADD-1) in Nematode and Human Memory." *EMBO Journal* 31(6):1453–66. doi: 10.1038/emboj.2012.14.

Walker, Lauren, Kirsty E. McAleese, Daniel Erskine, and Johannes Attems. 2019. "Neurodegenerative Diseases and Ageing." in *Subcellular Biochemistry*. Vol. 91.

White, Benjamin H. 2016. "What Genetic Model Organisms Offer the Study of Behavior and Neural Circuits." *Journal of Neurogenetics* 30(2). doi: 10.1080/01677063.2016.1177049.

White JG, Southgate E, Thomson JN, Brenner S. 1986. "The Structure of the Nervous System of the Nematode Caenorhabditis Elegans." *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 314(1165):1–340. doi: 10.1098/rstb.1986.0056.

WHO. 2019. "Global Health Estimates: Life Expectancy and Leading Causes of Death and Disability." *The Global Health Observatory* 1–6.

Widagdo, Jocelyn, Sumasri Guntupalli, Se E. Jang, and Victor Anggono. 2017. "Regulation of AMPA Receptor Trafficking by Protein Ubiquitination." *Frontiers in Molecular Neuroscience* 10.

WIGSTRÖM, H., and B. GUSTAFSSON. 1985. "On Long-lasting Potentiation in the Hippocampus. a Proposed Mechanism for Its Dependence on Coincident Pre- and Postsynaptic Activity." *Acta Physiologica Scandinavica* 123(4). doi: 10.1111/j.1748-1716.1985.tb07621.x.

Yan, Mingjing, Shenghui Sun, Kun Xu, Xiuqing Huang, Lin Dou, Jing Pang, Weiqing Tang, Tao Shen, and Jian Li. 2021. "Cardiac Aging: From Basic Research to Therapeutics." *Oxidative Medicine and Cellular Longevity* 2021.

Yuen, Eunice Y., Jing Wei, Wenhua Liu, Ping Zhong, Xiangning Li, and Zhen Yan. 2012. "Repeated Stress Causes Cognitive Impairment by Suppressing Glutamate Receptor

Expression and Function in Prefrontal Cortex." *Neuron* 73(5). doi: 10.1016/j.neuron.2011.12.033.

Zhuo, Min. 2014. "Long-Term Potentiation in the Anterior Cingulate Cortex and Chronic Pain." *Philosophical Transactions of the Royal Society B: Biological Sciences* 369(1633).

7 Annex

7.1 Aversive olfactory short-term associative memory assay protocol.

Reagents and materials for the assay

- CTX buffer – (5 mM KH_2PO_4/K_2HPO_4 [pH 6.0], 1 mM $CaCl_2$, 1 mM $MgSO_4$)
- NGM plates -
- Non-vent CTX plates - (5 mM KH_2PO_4/K_2HPO_4 [pH 6.0], 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2% agar)
- 95% EtOH
- Diacetyl stock and 1% Diacetyl (in 95% EtOH)
- 10% Sodium azide

Preparation of animals for assay

1. Cultivate worms on 10cm Nematode Growth Medium (NGM) plates seeded with 600 µl OP50 *E.coli.*
2. To get a clean and synchronized worm population, treat gravid adult worms with hypochlorite solution.
3. Evenly divide the synchronized population of eggs and transfer onto seeded NGM plates
4. Incubate worms at 20°C for 72 hours, to let the worms grow and reach the young adult stage.

Preparation for Naïve testing

1. After 72 hours of incubation, check the NGM plates to make sure that the young adults have plenty of food, and that there are enough worms for the assay.
2. Prepare chemotaxis assay plates by marking odorant and control spot on the bottom of the unseeded CTX plates as displayed in figure 3.2. Prepare at least 3 replicates per genotype, per condition.
3. Prepare one conditioning(training) plates per genotype by putting a white sticker on the lid of the non-seeded CTX plate.
4. Harvest the worms from NGM plates into 15 mL falcon tubes by gently washing the NGM plates with CTX buffer. Let worms settle by gravity. Repeat 2X for a total of 3 washes to get rid of any bacteria.
5. After the 2nd wash while the worms are settling in the conical tube, spot 1 µl of 10% sodium azide at the odorant and control spot on the marked CTX plate.
6. After the 3rd wash, spot 1 µL each of 1% diacetyl and 95% of EtOH at the odorant and control spot respectively on the marked CTX plate.
7. Gently pipette around 100-150 worms in the middle of the plates using Pasteur pipette. Be careful not to pipette too much CTX buffer. Transfer the leftover worms onto the conditioning plates.
8. Under the hood pipette 2 µL diacetyl on the lid of the conditioning plate. Seal the plates with parafilm and leave it under the hood.
9. Incubate for 1 hour at room temperature (RT). Transfer the Naïve testing plates to a fridge to stop the test.

Preparation for conditioning testing

1. Prepare the CTX plates for testing conditioning as mentioned in the previous step.

2. At the end of 1 hour incubation period collect the worms from the conditioning plates with 5-10 mL of CTX buffer into 15 mL falcon tubes.
3. Once the worms settle down, remove the supernatant out with the glass Pasteur pipette.
4. Gently pipette around 100-150 worms in the middle of the plates using Pasteur pipette as described before. Transfer the leftover worms on a CTX plate and incubate them at RT for 1 hour recovery.
5. Transfer the conditioning testing plates to a fridge to stop the test after 1 hour.

Recovery for short-term memory assay

1. Prepare the CTX plates for testing recovery as mentioned before.
2. Collect the leftover worms kept aside on the CTX plates after 1 hour recovery period with 5-10 mL of CTX buffer into 15 mL falcon tubes.
3. Once the worms settle down, remove the supernatant out with the glass Pasteur pipette.
4. Gently pipette around 100-150 worms in the middle of the plates using Pasteur pipette as described before.
5. Incubate the plates for 1 hour at RT, then transfer to the fridge to stop the test.

Plate counting

1. Count the worms paralyzed at the attractant spot, at the solvent (EtOH) spot separately and the remailing worms on the plate separately.
2. Calculate the chemotaxis index (CI) using the formula -

$$CI = \frac{Number\ of\ animals\ at\ attractant\ spot - Number\ of\ animals\ at\ solvent\ spot}{Total\ number\ of\ animals\ in\ the\ assay}$$

8 Curriculum vitae

EDUCATION

UNIVERSITY OF BASEL — Basel, Switzerland
PhD candidate at the faculty of psychology — 2019 - 2023

UNIVERSITY OF TRENTO — Trento, Italy
MSc. in Cellular and Molecular Biotechnology — 2014- 2017

UNIVERSITY OF MUMBAI (M.G.M College of Engineering & Technology) — Mumbai, India
Bachelor of Engineering (B.E.), Biotechnology — 2008- 2012

RESEARCH EXPERIENCE

PhD student — Basel, Switzerland
Department of Molecular and Cognitive Neurosciences, University of Basel — Jan 2019 – present
Supervisor: Prof. Dr. Andreas Papassotiropoulos

- Deciphering the molecular mechanisms underlying age-dependent memory decline using *C. elegans* as a model organism.

MSc Internship — Trento, Italy
Center of Integrative Biology (CIBIO) — Feb 2016-Mar 2017
Supervisor: Stefano Biressi, Ph.D.

- Investigating the cellular and molecular events responsible for the defective muscle regeneration, such as the interplay between Wnt signaling and TGFβ2 pathway, during ageing and variety neuromuscular diseases.
- Independent sample processing of mouse muscles, muscle fiber preparation, satellite cell culture, imaging, FACS and data analysis.

Junior research fellow (JRF) — Mumbai, India
ACTREC- Advanced Centre for Treatment, Research and Education in Cancer — July 2017- May 2018
Supervisor: Shilpee Dutt, Ph.D.

- Investigation of proteins involved in Cell-Cell Adhesion-Mediated Radioresistance in glioblastoma.
- Chromatin immunoprecipitation to identify novel binding partners of phosphorylated gamma H2AX in radioresistant glioblastoma cell line.

Undergraduate researcher — Mumbai, India
MGM College of Engineering & Technology — Jan 2012-May 2012
Supervisor: Selvaa Kumar C, Ph.D.

- Investigated interaction and binding affinity of mutated forms of dihydrofolate reductase enzyme with anti-malarial drugs using Hex docking program.

PUBLICATIONS

Fenyves BG*, Arnold A*, **Gharat VG***, Haab C, Tishinov K, Peter F, de Quervain D, Papassotiropoulos A, Stetak A. Dual Role of an mps-2/KCNE-Dependent Pathway in Long-Term Memory and Age-Dependent Memory Decline. Curr Biol. 2021 Feb 8;31(3):527-539.e7.
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