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**Effects of a Functional Diet on Cognitive Function and Behaviour
in Alzheimer's Disease Mouse Model**

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ABBREVIATIONS

AD – Alzheimer's disease
ADAD – autosomal dominant Alzheimer's disease
ALS – amyotrophic lateral sclerosis
APOE – Apolipoprotein E
APP – amyloid precursor protein
A β – amyloid beta
BBB – blood-brain barrier
CNS – central nervous system
CSF – cerebrospinal fluid
FTD – frontotemporal dementia
FUS – fused-in-sarcoma protein
HD – Huntington's disease
MIND – Mediterranean-DASH intervention for neurodegenerative delay
MRI – magnetic resonance imaging
MS – multiple sclerosis
ND – neurodegenerative disease
NFTs – neurofibrillary disease
NMDA – N-methyl-D-aspartate
PD – Parkinson's disease
PET – positron emission tomography
SAD – sporadic Alzheimer's disease
WHO – World Health Organization

INTRODUCTION

Neurodegenerative diseases (ND) are becoming a growing public health challenge due to aging population and increasing life expectancy. There are multiple ND that share similar mechanisms and clinical symptoms. One of the most severe clinical manifestations of ND is dementia (Wei et al., 2023). It is one of the leading causes of disability among older adults, affecting around 60 million people, ranking as the seventh leading cause of death worldwide (Xu et al., 2024). According to the World Health Organization (WHO), dementia is considered as a global public health priority, estimating that nearly 10 million people develop dementia each year (WHO, 2017). It is projected that the number of dementia cases will increase to reach 152 million by the year of 2050 (Luo et al., 2021).

One of the most common ND is Alzheimer's disease (AD), which is responsible for 60 – 80 % of all dementia cases (Wei et al., 2023). AD is characterised by the extracellular accumulation of amyloid beta (A β) plaques, intracellular aggregation of neurofibrillary tangles, synaptic and neuronal loss (Long and Holtzman, 2019). AD not only affects the brain, but it is also associated with a wide range of clinical symptoms, including memory loss, cognitive decline, language difficulties, behavioural changes, apathy and hallucinations (B. Kim et al., 2021).

Studies show that the development of AD is multifactorial, suggesting an interaction between genetic, environmental and lifestyle factors. However, age and genetics remain the most significant risk factors. Increasing evidence suggests that poor dietary habits, such as malnutrition, high-sugar, high-fat diets and chronic alcohol consumption, increase systemic inflammation, oxidative stress and alterations in the gut-brain axis, all of which have been shown to accelerate neurodegeneration and contribute to AD progression (McGrattan et al., 2019).

Poor dietary habits are considered as a modifiable risk factor, making nutritional interventions a promising preventative approach for AD (McGrattan et al., 2019). Diets enriched with antioxidants, B vitamins, vitamin E, polyunsaturated fatty acids and healthy fats have been shown to have neuroprotective effects, reducing neuroinflammation and oxidative stress, potentially slowing neurodegenerative processes (Stefaniak et al., 2022). Despite significant research advances, the potential for an effective preventive approach remains unclear. Therefore, this longitudinal study aims to investigate whether the functional diet has a positive effect on behaviour and cognition in a mouse model of Alzheimer's disease.

The aim of the research: To investigate the effects of a functional diet on cognitive function and behaviour in a mouse model of Alzheimer's disease.

Objectives:

1. To analyse the impact of a functional diet on body weight;
2. To assess the effects of a functional diet on physical activity in mice using the running wheel test;
3. To assess the effects of a functional diet on spatial working (short-term) memory in mice using the Y-maze;
4. To assess the effects of a functional diet on long-term memory and object recognition using the novel object recognition test;
5. To assess the effects of a functional diet on spatial (long-term) memory and learning in mice using the Barnes maze.

1. Literature review

1.1. Neurodegenerative diseases

Neurodegenerative diseases (ND) are one of the main causes of disability and currently affect over 60 million people, and rank as the seventh leading cause of death worldwide (Xu et al., 2024). Due to increasing life expectancy, ND are rapidly becoming a societal and economic challenge (Johri, 2021).

There are many types of ND, and despite their differences, they share many similar processes, like protein misfolding, oxidative stress and neuroinflammation. These mechanisms contribute to the progressive loss of neurons, leading to cognitive and motor impairments (Gülyeşil and Erbas, 2024). ND are classified according to their clinical symptoms, anatomical distribution of degeneration or the underlying molecular abnormalities (Dugger & Dickson, 2017).

Table 1. Summary of the main ND diseases, their pathogenesis, associated risk factors and primary symptoms.

Disease	Pathogenesis	Risk factors	Symptoms
Alzheimer's disease	Accumulation of A β plaques and tau tangles, neuroinflammation	Age, genetics, systemic inflammation, environmental and lifestyle factors	Memory loss, cognitive decline, language difficulties, behavioural changes
Parkinson's disease	Loss of dopaminergic neurons in the substantia nigra	Age, genetics and environmental toxins	Tremor, bradykinesia, rigidity, cognitive decline
Amyotrophic lateral sclerosis	Motor neuron degeneration in the brain and spinal cord	Age, male gender and environmental factors	Muscle weakness, paralysis, spasticity, cognitive and behavioural changes
Multiple sclerosis	Autoimmune attack on the myelin sheath of CNS neurons, causing inflammation, demyelination and neurodegeneration	Age, genetic and environmental factors	Fatigue, vision problems, numbness, tingling, muscle weakness
Frontotemporal dementia	Progressive degeneration of the frontal and temporal lobes	Head injury, genetic mutations, type 2 diabetes and smoking	Personality and behavioural changes, language difficulties

Alzheimer's disease is one of the most common types of ND, which is responsible for 60 – 80 % of all dementia cases (Wei et al., 2023). AD is characterised by the extracellular accumulation of amyloid- β (A β) plaques, intracellular aggregation of neurofibrillary tangles (NFTs), synaptic and neuronal loss (Long & Holtzman, 2019). The development of AD is mostly associated with interaction between different risk factors, including systemic inflammation, chronic health conditions, lifestyle and environmental factors (Zheng and Wang, 2025). Older age and genetics are among the strongest risk factors. Interestingly, women are at higher risk of developing AD due to a combination

of longer life expectancy and potentially higher levels of tau, despite similar levels of A β (Scheltens et al., 2021). AD presents with a wide range of symptoms, including memory loss, cognitive decline, language disorders, behavioural changes, apathy and hallucinations (B. Kim et al., 2021).

Parkinson's disease (PD) is another common, progressive and complex ND. It is considered as a multisystem disease that affects central nervous system (CNS), enteric nervous system, autonomic nervous system and gastrointestinal tract. PD is primarily characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta, a region in the midbrain, and the accumulation of intracellular protein aggregates known as Lewy bodies. These aggregates are composed of the alpha-synuclein protein, which accumulates within dopaminergic neurons, leading to their neurodegeneration. The development of PD is strongly associated with older age, genetics and pesticide exposure. Males have a higher risk of developing PD, and in women, symptoms tend to appear later and progress more slowly. Additionally, PD has a wide range of motor symptoms, such as tremor, bradykinesia and rigidity, as well as non-motor symptoms, like cognitive impairment, depression and constipation (Horsager & Borghammer, 2024).

Amyotrophic lateral sclerosis (ALS) is rare, progressive and fatal neurodegenerative disease. The diagnosis of ALS is frequently delayed due to difficulty in recognising early symptoms, especially in the early stages. ALS was initially considered as a neuromuscular disorder. However, it has been recognised that ALS also presents with cognitive and behavioural impairments, such as executive dysfunction, personality changes and frontotemporal dementia (Feldman et al., 2022). Therefore, it is considered as a complex genetic disorder that occurs either hereditarily or sporadically (Hardiman et al., 2017). The underlying pathophysiology of ALS involves protein misfolding, oxidative stress and excitotoxicity, causing neuronal death (Wei et al., 2023). ALS symptoms typically start in one localised area, spreading progressively to adjacent muscle groups or regions of the CNS (Ilieva et al., 2023). Various environmental factors have been linked to ALS, like smoking, alcohol, metabolic factors and pesticide, heavy metal or electromagnetic field exposures. However, there are only a few well-studied risk factors that contribute to the development of ALS, with age being the strongest risk factor. Additionally, males are at a higher risk of developing ALS (Riva et al., 2024).

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the CNS, causing neurodegeneration by attacking neurons myelin sheath, leading to inflammation, demyelination, gliosis and neuronal death (Haki et al., 2024). The progression of MS is accelerated due to remyelination and neuroplasticity impairment (Kuhlmann et al., 2023). MS presents with typical acute symptoms, including visual, numbness, tingling, fatigue, muscle weakness, balance, coordination problems, spasticity and cognitive impairment (Makhani & Tremlett, 2021).

Approximately 25 % of MS is inherited, while the remaining cases are influenced by environmental, epigenetic and gene-gene or gene-environment interaction (Kuhlmann et al., 2023).

Frontotemporal dementia (FTD) is the second most common cause of early-onset dementia after AD. The average estimated life expectancy is 6 – 9 years after the first symptoms appear. FTD is characterised by progressive loss of neurons in the frontal and temporal lobes. This disease presents with personality and behavioural changes, as well as cognitive, executive and language impairments. The clinical features of FTD and ALS overlap, with 5 – 10 % of FTD cases appearing with motor neuron symptoms, while 40 – 50 % of ALS cases have FTD symptoms. The pathogenesis of FTD is believed to be caused by the accumulation of tau, TDP-43, FUS proteins in the cortex and subcortex, with impaired clearance of neurotoxic proteins. FTD has several risk factors, including head injury, genetic mutations, type 2 diabetes and smoking (Rasmussen et al., 2018).

Overall, ND develop similarly, mostly by protein misfolding, oxidative stress and neuroinflammation, leading to neuronal death. Although the mechanisms of these diseases are similar, each has distinct molecular and clinical features that differentiate it from the others.

1.2. Alzheimer's disease

Alzheimer's disease (AD) is characterised by the accumulation of amyloid plaques and neurofibrillary tangles, which cause neurodegeneration, leading to memory loss, disorientation, language difficulties, mood swings, confusion, difficulty with daily tasks and behavioural changes. Ultimately, leading to loss of independence (Passeri et al., 2022). There are two forms of AD: Sporadic Alzheimer's disease and Autosomal Dominant Alzheimer's disease, each characterised by different patterns of onset and progression (Passeri et al., 2022).

Sporadic Alzheimer's disease is the most common form of AD, also known as late-onset disease, which occurs after the age of 65 (Andrade-Guerrero et al., 2023). People with this form exhibit all classical AD symptoms. (D'Alessandro et al., 2025). Late-onset AD is considered as a multifactorial condition, influenced by a combination of modifiable and non-modifiable risk factors. Moreover, the rarest form of AD is autosomal dominant Alzheimer's disease, also known as early-onset AD, which accounts for 1 – 5 % of all cases (Andrade-Guerrero et al., 2023). Early-onset symptoms appear before the age of 65 and progress more rapidly than the sporadic form. In some cases, classic symptoms are accompanied by neurological manifestations that are rarely seen in late-onset AD (Long & Holtzman, 2019).

Studies show that AD develops through the interaction of modifiable and non-modifiable risk factors. Although aging is considered the strongest known risk factor, the exact cause of AD remains unclear due to its distinctive mechanisms and pathogenesis.

1.2.1. Mechanisms and pathogenesis

AD pathogenesis involves both structural and functional damage within the CNS. It develops due to a variety of complex mechanisms that lead to neurodegeneration. Pathologically, AD is characterised by the loss of neurons in the hippocampus and cerebral cortex, particularly affecting cholinergic projections from basal forebrain to the cortex. The main features of AD are extracellular A β plaques, intracellular NFTs, gliosis accompanied by cerebrovascular amyloidosis, inflammation and synaptic dysfunction. Together, these pathological mechanisms lead to neuronal damage and brain atrophy, resulting in cognitive and functional decline (Pozzilli et al., 2023).

The most widely accepted hypothesis of the AD development is the amyloid cascade hypothesis, which suggests that an imbalance between the accumulation and clearance of A β plaques initiates a neuronal degeneration (Zha et al., 2025). The process begins when the amyloid precursor protein (APP) is abnormally cleaved by β -secretase and γ -secretase, producing insoluble A β peptides. These peptides initially form as oligomers, which then aggregate into fibrils and ultimately form into extracellular amyloid plaques. Among these forms, A β oligomers are considered as the most neurotoxic (Lublin & Gandy, 2010). They bind to several neuron receptors, such as EphA4, EphB2 and NMDA. This interaction disrupts synaptic communication, reduces long-term potentiation and increases long-term depression, which weakens synapses (Zheng & Wang, 2025).

The accumulation of A β plaques begins in the temporal basal and frontomedial regions and gradually spreads to the associative neocortex, primary sensory motor regions and medial temporal lobe, eventually reaching the striatum (Zheng & Wang, 2025). However, based on clinical observations, the causation between A β and dementia has been questionable. The severity of cognitive decline correlates more with NFTs than A β plaques, suggesting that although A β may initiate AD progression, neuroinflammation and tau pathology may be primary factors of neurodegeneration (Zheng & Wang, 2025). During the development of AD, tau protein undergoes post-translational modifications: hyperphosphorylation and truncation, which cause it to detach from microtubules. Tau protein aggregates similarly to A β . Firstly, they form oligomers, then aggregate into paired helical filaments and eventually into NFTs. Tau aggregates potentially spread to healthy neurons, contributing to this progressive pathology and interfering with intracellular transport. It disrupts energy production by damaging mitochondria, blocking vesicle movement, leading to impaired neurotransmitter release and presynaptic dysfunction (Guo et al., 2020).

Additionally, glial cells are activated by A β and tau pathology. Microglia cells normally help by clearing neurotoxins and waste. They use TREM2, LRP1, TLR4 and CD36 receptors to detect and respond to harmful A β , tau or other neurotoxins. When these receptors are exposed to excessive A β , microglia becomes overstimulated and starts releasing pro-inflammatory cytokines – IL-1 β , TNF- α ,

IL-6, and IL-8. This chronic inflammatory response contributes to permanent neuronal damage (Guo et al., 2020). Moreover, astrocytes become activated in response to A β , tau or inflammatory signals from microglia. Once it is activated, they release excess glutamate, inflammatory mediators, further exacerbating excitotoxicity, neuronal damage and synaptic dysfunction (Zheng & Wang, 2025). This neuroinflammation can disrupt the blood-brain barrier (BBB), contributing to further impaired clearance of harmful A β , tau or other neurotoxins, worsening neuroinflammation (Guo et al., 2020).

Increasing evidence suggests that A β and mitochondrial dysfunction are bidirectionally correlated (Monzio Compagnoni et al., 2020). Mitochondrial dysfunction alters APP cleavage, leading to the production of A β peptides (Rusek et al., 2019). Mitochondria is essential for cellular energy production and calcium homeostasis, which is critical for neuronal function. In AD, mitochondrial function is impaired, resulting in decreased ATP levels and increased oxidative stress (D'Alessandro et al., 2025). This dysfunction also increases reactive oxygen species production, leading to calcium imbalance, oxidative stress and neuronal death. All these mechanisms lead to the release of proapoptotic factors, leading to neuroinflammation and increased accumulation of A β plaques and tau tangles (Guo et al., 2020).

Although amyloid and tau pathology are considered as the primary factors in AD pathogenesis, increasing evidence suggests that glial overactivation and mitochondrial dysfunction may be the first contributors to the development of the disease. These complex and interconnected mechanisms suggest that additional factors may influence the progression of neurodegeneration.

1.2.2. Disease progression and risk factors

AD develops through the interaction of genetics, environmental and lifestyle factors. There are modifiable and non-modifiable risk factors that influence disease progression. As a result, the rate of progression depends not only on risk factors but also on the disease form. Although the cause and form vary from each case, they share similar symptoms and the same clinical stages (Guo et al., 2020):

- Pre-clinical (pre-symptomatic) stage – early pathological changes in the cortex and hippocampus, with mild memory loss, without functional impairment in daily activities or clinical symptoms. This stage can last for several years;
- The mild (early) stage – difficulties in daily life, loss of concentration and memory, disorientation of place and time, mood changes, depression;
- Moderate stage – pathological changes spread to the cerebral cortex, with increased memory loss, trouble recognising family and friends, loss of impulse control, reading, speaking and writing difficulties.
- Severe (late) stage – the disease takes over the whole cerebral cortex with severe accumulation of plaques and tangles, leading to progressive functional and cognitive impairment. During

this stage, patients can not recognise their family members, they become bedridden, have difficulties in swallowing and urination, eventually dying due to complications, such as respiratory distress syndrome, infection and sepsis (Breijyeh & Karaman, 2020; Guo et al., 2020).

Older age remains the strongest non-modifiable risk factor. Normal aging is associated with structural changes in the brain, with changes in cortical thickness and decreased white matter integrity. However, these changes are usually less severe than the neuronal loss and synaptic dysfunction found in late-onset AD (Passeri et al., 2022). Additionally, metabolic disorders such as glucose hypometabolism, cholesterol imbalance and mitochondrial damage are associated with increased neuronal loss and synaptic dysfunction. These pathologies usually develop with aging, making it difficult to distinguish age-related changes from late-onset AD (Breijyeh & Karaman, 2020). Therefore, advanced age remains one of the most significant risk factors of late-onset AD (Long & Holtzman, 2019).

Genetic predispositions influence the underlying pathophysiological processes of late-onset AD, which accounts for 58 -79 % of all cases (Zheng & Wang, 2025). The apolipoprotein E (APOE) gene is the most influential genetic factor, accounting for more than half of late-onset AD cases (Raulin et al., 2022). This gene has three main isoforms - APOE ϵ 2, APOE ϵ 3 and APOE ϵ 4 (Riedel et al., 2016). APOE ϵ 4 significantly increases the risk of developing AD (Riedel et al., 2016), due to being associated with hippocampal atrophy, abnormal A β accumulation, increased amyloid deposits, cerebral hypometabolism, tau pathology, synaptic plasticity, lipid transport, vascular function and neuroinflammation (C.-C. Liu et al., 2013). In contrast, the APOE ϵ 2 is a relatively rare gene that is considered as the strongest protective factor against late-onset AD, potentially delaying the accumulation of A β (Raulin et al., 2022; Serrano-Pozo et al., 2021). However, APOE ϵ 3 has no direct impact on late-onset AD (Lambert et al., 2013). Individuals carrying both APOE ϵ 2 and APOE ϵ 4 alleles may still be at risk of developing late-onset AD, suggesting that the protective effect of APOE ϵ 2 may be reduced in the presence of APOE ϵ 4 (Raulin et al., 2022). In contrast, early-onset AD is caused by mutations in one of the three genes: the presenilin 1 gene (PSEN1), the presenilin 2 gene (PSEN2) and the APP. These mutations increase A β accumulation and disrupt the clearance of neurotoxic waste much faster than the APOE gene. The most prominent and severe mutation occurs in the PSEN1 gene, which is present in up to 70 % of all early-onset cases. These mutations can cause symptoms to appear as early as 25 years of age (Andrade-Guerrero et al., 2023).

Gender is another non-modifiable risk factor. Women account for approximately two thirds of late-onset AD patients. Biological mechanisms, such as chromosomal, epigenic and hormonal factors, like menopause, are believed to be associated with an increased risk of developing late-onset AD

(Lopez-Lee et al., 2024). Women often present with verbal memory and word-finding difficulties in the early stages of the disease (Aggarwal & Mielke, 2023). Studies show that women with mild dementia may progress more quickly than similar-age men (Rosende-Roca et al., 2025).

Additionally, people with Down syndrome are at significantly higher risk of developing early-onset AD (Zheng & Wang, 2025). This increased risk is primarily due to the presence of three copies of the chromosome that contains the APP gene. Overexpression of the APP gene leads to increased A β production. It is estimated that over 90 % of individuals with Down syndrome will develop early-onset AD, making it the leading cause of death. Clinical symptoms present by the age of 40 show similar features to both sporadic and autosomal dominant AD forms (Forte et al., 2020).

While attention has focused on non-modifiable risk factors, there is growing interest in understanding modifiable risk factors and their impact on the progression of AD. Studies show that poor diet, lack of physical activity and smoking can significantly increase the risk of developing AD (Litke et al., 2021). Ongoing studies continue to research both modifiable and non-modifiable risk factors that could potentially help with AD diagnosis, treatment strategies and slow down the progression.

1.2.3. Diagnosis and clinical management

Early detection of AD can significantly improve both survival rate and the quality of life (Luo et al., 2021). Recent research primarily focuses on improving diagnostic and treatment strategies by integrating invasive and non-invasive methods. The main tools used in AD diagnosis are neuroimaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), along with biomarker assessments from cerebrospinal fluid (CSF) (Karlawish & Grill, 2024).

Clinical management of AD begins with a comprehensive evaluation that includes patients' medical and family history. Neurologists determine whether patients meet diagnostic criteria by identifying cognitive and behavioural impairments, ruling out other medical conditions that could contribute to cognitive decline (Atri, 2019). To evaluate cognitive decline, clinicians typically use the Montreal Cognitive Assessment (MoCA) and Blessed Dementia Scale-Information-Memory-Concentration (BS-IMC) tests. MoCa is widely used for its sensitivity in detecting mild cognitive impairment, while the BDS-IMC is useful in monitoring progression and measuring various dementia stages, from mild to severe (Atri, 2019).

Additionally, if the cognitive test indicates dementia, then biomarker analysis is used for a more accurate diagnosis of AD (Cheng et al., 2024). One of the main diagnostic tools is the cerebrospinal fluid (CSF) analysis. During this procedure, CSF is collected by inserting a needle between two

lumbar vertebrae into the subarachnoid space, below the end of the spinal cord. CSF analysis reveals decreased A β 42/40 ratio, phosphorylated tau (pT217/T217 and pT205/T205) and elevated levels of tau associated with NFT (MTBR-tau243) or other tau disorders (Salvadó et al., 2024). Moreover, blood biomarkers are new, alternative, minimally invasive diagnostic methods that detect early pathological changes, such as the A β 42/40 ratio in the blood, indicating abnormal amyloid deposition in the brain. It detects phosphorylated tau proteins and glial proteins, which indicate an inflammatory reaction and astrocyte activation (Altuna-Azkargorta & Mendioroz-Iriarte, 2021). The blood biomarker tool is not a standard method, but it is starting to become increasingly recognised as an alternative to CSF analysis (Hampel et al., 2023).

Once biomarkers are identified, neurologists use PET scans to visualise amyloid or tau accumulation and MRI to assess brain atrophy. These neuroimaging tools help to determine the extent of neurodegeneration and track the disease progression. MRI, FDG-PET, amyloid PET and tau PET have been validated as reliable biomarkers for AD diagnosis. Tau PET imaging shows patterns of tau accumulation that typically occur after amyloid accumulation, while amyloid PET can detect early brain changes (Veitch et al., 2022).

Although there is no actual cure for AD, there are several approved treatments that temporarily relieve symptoms. Currently approved treatments for AD are cholinesterase inhibitors, N-methyl-D-aspartate (NMDA) receptor antagonists and combination therapies. Cholinesterase inhibitors increase acetylcholine levels, which are usually decreased during AD pathology. Meanwhile, memantine is an NMDA receptor antagonist that reduces calcium influx, excitotoxicity and neuronal death. Combination therapy is a combination of memantine and donepezil, which improves cognition, language and behavioural functions in patients with moderate AD, but it is less effective in the early stages (Athar et al., 2021). Additionally, non-pharmacological interventions have become an essential intervention in maintaining independence and quality of life in AD patients. The most widely used are cognitive behavioural and occupational therapies. These therapies help patients improve cognitive, physical, sensory functions and social integration, while also reducing psychological stress (Smallfield et al., 2024). A newer therapeutic approach is recollection therapy, which aims to help patients discover new meaning in life, improve emotional well-being and reduce depressive symptoms. During therapy sessions, patients are exposed to familiar stimuli, such as music or pictures, to evoke long-term memories that may remain intact even in later stages of AD. Recollection therapy is particularly effective when it is combined with occupational therapy, as it allows patients to perform tasks they used to do before their diagnosis. This approach strengthens emotional resilience and cognitive function (B. Kim et al., 2021).

Lifestyle interventions, such as regular physical activity, balanced diet, ongoing social engagement and cognitive function exercises have shown potential to slow the progression of symptoms and improve the overall well-being of AD patients (D. Kim, 2020). While currently approved medications only provide temporary relief from symptoms, ongoing research is aimed to develop more effective treatments. Meanwhile, simple lifestyle changes may help to reduce the risk of rapid AD progression.

1.3. The role of nutrition in brain health and neurodegeneration

Diet is believed to be closely related to brain health, primarily through the gut-brain axis and is considered a modifiable risk factor for AD or other neurodegenerative diseases (McGrattan et al., 2019). Specific healthy dietary patterns and nutrients are shown to have neuroprotective effects, while others increase the risk of oxidative stress, neuroinflammation, insulin resistance and vascular damage, all of which contribute to neurodegeneration (Stefaniak et al., 2022). Understanding how specific dietary components interact with brain function is essential for identifying effective strategies for AD prevention.

1.3.1. Association between the gut-brain axis and nutrition in neurodegeneration

The gut-brain axis is a bidirectional communication pathway between the gastrointestinal tract and the CNS, mediated by the ENS, vagus nerve, endocrine signaling and the immune system (Savulescu-Fiedler et al., 2025). Research suggests that cognitive decline is not only related to brain changes, but is also closely related to systemic disorders, such as insulin resistance, obesity and systemic inflammation. These disorders are associated with dysbiosis and impaired epithelial integrity, resulting in increased levels of cytokines, reactive oxygen species and nitric oxide, activating microglia and astrocytes, further increasing neuroinflammation (Park et al., 2026).

The gut-brain axis primarily occurs through the vagus nerve, as it serves as the direct pathway for afferent sensory signals from the gut to the brain, influencing brain activity and inflammatory processes (Savulescu-Fiedler et al., 2025). Additionally, about 90% of all serotonin is produced in the gut, where it regulates epithelial function and intestinal motility. Dysbiosis directly affects serotonin production and decreases its levels, increasing the risk of AD (Tahiri et al., 2024). Neuroinflammation is associated with increased gut permeability due to dysbiosis, which allows immune mediators to leak through the intestinal walls into the bloodstream. Inflammatory mediators disrupt the blood-brain barrier, allowing them to enter the brain, where they activate glial cells, further increasing neuroinflammation (Kincaid et al., 2021). Therefore, dietary patterns that influence gut composition may promote good brain health or increase the risk of AD development.

1.3.2. Diets and nutrients associated with increased risk of neurodegeneration

Due to rapid industrial growth and a fast pace of modern life, dietary habits have shifted significantly, with many people relying on fast and highly processed, low-nutrient foods. Health decline has been associated with low nutritional value diets, such as malnutrition, the Western diet, high sugar diets and chronic alcohol consumption. These unhealthy habits contribute to various diseases, including certain types of cancer, cardiovascular disease, stroke, obesity and diabetes. Research suggests that unhealthy dietary habits increase systemic inflammation, oxidative stress and dysbiosis, causing neuroinflammation leading to neurodegeneration (McGrattan et al., 2019).

Table 3. Main unhealthy diets, their components and neurodegenerative effects

Diet	Nutrients and components	Neurodegenerative effect
Malnutrition – undernutrition and overnutrition	Vitamins B6, B12, C, E, D, folate, zinc, selenium deficiency	High homocysteine levels, neurotoxicity, impaired neuronal repair and plasticity
Western diet	High saturated fats, refined sugars, processed meat	Neuroinflammation and oxidative stress, insulin resistance and metabolic dysfunction
High sugar diets	Refined carbohydrates, high fructose	Insulin resistance, oxidative stress, cognitive impairment, impaired metabolism
Alcohol abuse, chronic drinking	Ethanol	Neuronal damage, oxidative stress, neuroinflammation

Malnutrition refers to a deficiency, excess or imbalance of nutrients and energy in a persons diet. According to the WHO, there are three types of malnutrition (Fact Sheets - Malnutrition, n.d.):

- Undernutrition, wasting – low weight for height; stunting – low height for age and underweight – low weight for age;
- Micronutrient malnutrition – deficiency of essential vitamins and minerals or excesses of micronutrients;
- Overweight, obesity and diet-related non-infectious diseases.

Additionally, malnutrition is a common complication of AD, which is associated with accelerated disease progression. Malnutrition is not only caused by age-related changes, but also by direct influence of alterations in the arcuate nucleus of the hypothalamus, which is responsible for appetite control and regulation of food intake. These alterations can lead to decreased hunger or thirst, leading to skipping or forgetting to eat. In addition, individuals with AD may consume prepared, processed foods that often lack essential nutrients. Micronutrient deficiencies are one of the biggest problems in AD. Deficiency of antioxidants, such as vitamins E or C is considered as a risk factor for the development of AD (Stefaniak et al., 2022). Moreover, low levels of folate, vitamin B6 and vitamin B12 are associated with elevated homocysteine levels, which can lead to oxidative stress and

neuronal damage. Vitamin B12 deficiency may lead to oxidation of lipids, proteins and nucleic acids, which increases oxidative stress and contributes to the development of AD (Lauer et al., 2022). Furthermore, vitamin D deficiency has been associated with increased glial cell activation, increased levels of pro-inflammatory cytokines and A β accumulation (Grant & Blake, 2023).

Although malnutrition is often associated with nutrient deficiency, it can also be considered as overnutrition. People with AD are at risk of consuming too many calories and becoming obese by impulse eating, episodic overeating and repetitive eating due to memory impairment. Research suggests that obesity increases chronic inflammation, insulin resistance and mitochondrial dysfunction (Stefaniak et al., 2022). Chronic inflammation in obese adipose tissue leads to excess reactive oxygen species, oxidative stress, all of which exacerbate the inflammatory process, leading to neurodegeneration (Grant & Blake, 2023). In addition, metabolic dysfunction is another complication of AD, due to impaired regulation of homeostasis. This causes impaired metabolism, sleep and neuroendocrine function. Up to 80 % of all AD patients suffer from impaired glucose metabolism and insulin resistance (Abi-Ghanem et al., 2024).

Additionally, one of the fastest spreading diets is the Western diet, which has been proven to cause various diseases. It is characterised by a high consumption of processed foods, red and processed meats, saturated fats, fried foods, refined sugars, while being low in fruits, vegetables, whole grains, fish, legumes, micronutrients and antioxidants (McGrattan et al., 2019). Numerous studies have shown that Western diet increases the risk of hypercholesterolemia, obesity, non-alcoholic fatty liver disease, hyperglycemia and insulin resistance. These metabolic disorders are associated with increased activity of peripheral pro-inflammatory cytokines (Więckowska-Gacek et al., 2021). In addition, the Western diet can impair digestion and promote an imbalance in the gut microbiota, activating glial cells and causing neuroinflammation (Mackey-Alfonso et al., 2024). High intake of fats and saturated fatty acids (SFAs) are related to hyperinsulinemia and systemic inflammation. There have been indications that a high-cholesterol diet is associated with AD, as oxysterols accumulate in the AD brain (Stefaniak et al., 2022). Animal studies have shown that mice fed a fat-sugar-salt diet had impaired cognitive function, with increased amyloid plaques, neurofibrillary tangles, gliosis and vascular damage (Sweetat et al., 2024). In contrast, another study found that amyloid plaques and tau accumulation did not significantly increase. However, they observed glial overactivation, elevated pro-inflammatory cytokine release and synaptic pruning by microglia, indicating early neuroinflammatory and neurodegenerative changes (Mackey-Alfonso et al., 2024).

A diet in which sugar makes up a large portion of daily calories, especially from added sugars, is associated with various diseases, including diabetes, metabolic syndrome and obesity. Sugars are

one of the main carbohydrate compounds. Carbohydrates are essential for nutrition, as they are the main source of energy for cellular metabolism and biosynthesis (Beilharz et al., 2015). Research suggests that when excessive amounts of carbohydrates are consumed, it is stored in adipose tissue, leading to weight gain and increased metabolic disorders. This can lead to fat accumulation in the liver, contributing to non-alcoholic fatty liver disease and obesity. Moreover, disrupted glucose metabolism is associated with neurological diseases, resulting in decreased glucose levels and neurodegeneration (Witek et al., 2022). In AD, impaired brain glucose metabolism leads to synaptic dysfunction and neurodegeneration. Additionally, insulin receptors are responsible for synaptic plasticity, memory, cognition and overall energy metabolism. Therefore, dysregulated insulin significantly increases the risk of developing AD (Beilharz et al., 2015).

Chronic and excessive alcohol consumption increases the risk of developing AD (Peng et al., 2024). Additionally, chronic ethanol consumption enhances GABA receptors and inhibits excitatory glutamate receptors (V.k et al., 2019) resulting in glutamate-induced excitotoxicity, oxidative stress, irreversible neuronal damage, decreased cognitive and executive function (Peng et al., 2024). Research suggests that chronic alcohol consumption increases A β accumulation and tau phosphorylation (Huang et al., 2018). Which also affects nerve growth and brain-derived neurotrophic factors, leading to impaired intracellular signaling (Hammoud & Jimenez-Shahed, 2019). Furthermore, alcohol consumption causes gut dysbiosis, which contributes to systemic inflammation, neuroimmune imbalance and increased neuroinflammation (X. Liu et al., 2022)

These unhealthy eating patterns share similar pathological features that contribute to neurodegeneration and the development of AD. They are associated with impaired metabolism, inflammation, oxidative stress and gut dysbiosis, further exacerbating inflammation and neurodegenerative progression.

1.3.3. Neuroprotective nutrients and diets

Diet and nutrition are known to modulate the immune system, which influences neuroinflammatory processes involved in AD pathogenesis (McGrattan et al., 2019). A neuroprotective diet is rich in antioxidants, B vitamins, D, E vitamins, polyunsaturated fatty acids and healthy fats, all of which contribute to reduced progression of AD (Stefaniak et al., 2022). These diets are based on vegetables, fruits, healthy fats, lean proteins, with minimal consumption of processed foods, saturated fats and refined sugars (McGrattan et al., 2019). Anti-inflammatory dietary patterns reduce neuroinflammation, oxidative stress, increase healthy gut microbiota and protect against cognitive decline (McGrattan et al., 2019).

Table 4. Main anti-inflammatory diets, their components and neuroprotective effects

Diet	Nutrients and components	Neuroprotective effect
Mediterranean diet	Polyphenols, omega-3 fatty acids, antioxidants, extra virgin oil, plant-based foods, lean proteins	Reduces neuroinflammation and oxidative stress, improves insulin sensitivity and increases gut microbiota health
MIND diet	Berrie, leafy greens, olive oil, nuts, fish, whole grains	A combination of MediDiet and DASH slows cognitive decline, anti-inflammatory
Keto diet	Low-carbohydrate, high-fat meats	Produces alternative energy sources in the glucose-deficient AD brain
High-antioxidant foods	Vitamins C and E, selenium, zinc, polyphenols	Protects neuronal membranes from oxidative damage
Vitamin B rich diet	B6, B12, folic acid	Reduces homocysteine concentration, prevents inflammation and oxidative stress

The Mediterranean diet is one of the most well-studied anti-inflammatory diets, originating from the Mediterranean region. It is characterised by a high intake of vegetables, fruits, nuts, whole grains, legumes and olive oils as the main source of fat, with moderate intake of fish or poultry, low intake of red and processed meats (Guasch-Ferré & Willett, 2021). This diet provides body with essential nutrients, such as vitamins C and E, selenium and potassium, antioxidants or phytosterols. These components are associated with cardiovascular disease prevention, anti-cancer effects, anti-aging effects and improved cognition (Davis et al., 2015). Studies have shown that this diet is associated with low rates of chronic disease and a longer life expectancy (Guasch-Ferré & Willett, 2021), as well as better cognitive abilities and slower rates of cognitive decline. The antioxidants in this diet inhibit oxidative stress and neuroinflammation (McGrattan et al., 2019) and reduce A β toxicity and lipid peroxidation (Stefaniak et al., 2022). Compared to the Western diet, the Mediterranean diet also contains more dietary fiber, which is the main source of energy for the gut microbiota. Research suggests that the Mediterranean diet has beneficial effects on brain health by reducing neuroinflammation (Solch et al., 2022). Meta-analysis suggests that the Mediterranean diet is associated with an 11 % reduced risk of all types of dementia and a 27 % reduced risk of AD (Nucci et al., 2024). While other studies suggest that this diet is associated with reduced neurodegeneration, greater cortical thickness, slower hippocampal atrophy, improved structural connectivity and reduced A β accumulation (McGrattan et al., 2019). These results support that the Mediterranean diet may benefit brain health due to its synergistic antioxidant, anti-inflammatory, metabolic and gut microbiota modulating effects (Guasch-Ferré & Willett, 2021; Stefaniak et al., 2022).

Mediterranean-DASH intervention for neurodegenerative delay (MIND) diet is a mix of both Mediterranean and DASH diets, emphasizing the neuroprotective properties of food groups, such as berries, olive oil, leafy vegetables, nuts, fish and olives (Devranis et al., 2023). This diet consists of

high levels of polyphenols and antioxidants from fruits and vegetables. Vitamin E from nuts and olive oil, omega-3 fatty acids from fish, B vitamins from leafy vegetables and whole grains (van Soest et al., 2024). These nutrients work synergistically to support brain health and reduce the risk of neurodegeneration (Barnes et al., 2023). Although the MIND diet has been associated with improved cognitive function and a reduced risk of AD. However, the findings are inconclusive and inconsistent with the limited AD findings (van Soest et al., 2024).

The ketogenic diet was originally used to treat epilepsy, but recently attention has shifted to its neuroprotective effects against neurodegeneration (Hersant & Grossberg, 2022). This diet consists of low carbohydrates and high fat, with moderate protein intake (Masood et al., 2025), which induces a metabolic state called ketosis. During this diet, the body switches from glucose to fatty acid metabolism, resulting in the production of ketone bodies, like acetoacetate and β -hydroxybutyrate (Rusek et al., 2019). Ketone bodies can cross the blood-brain barrier and be used as an alternative energy source for cells (McDonald & Cervenka, 2018). Research suggests that ketone bodies improve mitochondrial function, reduce the expression of inflammatory, apoptotic mediators and the production of reactive oxygen species (Rusek et al., 2019). This is particularly important for AD, due to increased levels of oxidative stress and glucose hypometabolism (Hersant & Grossberg, 2022). Additionally, some studies suggest that a ketogenic diet may potentially slow down cognitive decline in dementia patients (Devranis et al., 2023). Based on animal and clinical studies, this diet may have beneficial effects on mitochondrial function, cellular metabolism and cognitive abilities in older individuals with AD. However, the evidence is still inconclusive (Rusek et al., 2019).

Another dietary approach with neuroprotective effects is an antioxidant-rich diet. Antioxidants are compounds that neutralise reactive oxygen species, reducing oxidative stress. Exogenous antioxidants are found in fruits, vegetables and other plant-based diets. Research suggests that increased antioxidants may help to reduce oxidative stress and neuroinflammation (Rahaman et al., 2023). Antioxidants, like vitamin C and E, polyphenols and flavonoids can be either obtained naturally from food or synthetically from supplements (Rahaman et al., 2023). Studies have shown that individual vitamins and polyphenols are associated with a lower risk of dementia (X. Liu et al., 2021). Vitamin C is an essential antioxidant that modulates inflammation, epigenetic signaling and cellular senescence (Omidifar et al., 2021). It may also have neuroprotective effects by reducing A β toxicity and ion chelation (Mielech et al., 2020). Additionally, vitamin E, particularly the tocopherol and tocotrienol isoforms, have been shown to cross the blood-brain barrier, reducing lipid peroxidation and A β aggregation (Mielech et al., 2020). This vitamin may have anti-inflammatory properties, affect immune function, cell signaling and lower cholesterol levels. Although the evidence is inconclusive, some studies suggest that vitamin E may have a beneficial effect when taken in combination with other nutrients (Browne et al., 2019). Moreover, polyphenols are considered an

important element of healthy aging, found in berries, tea, onions and garlic. Animal studies by Hegazy et al. have shown that garlic and onion extracts rich in polyphenols can reduce oxidative DNA damage, alter the expression of apoptosis genes and reduce intracellular levels of reactive oxygen species (Hegazy et al., 2022). However, the benefits are unclear due to conflicting research results. Therefore, it has been proven that dietary patterns rich in antioxidants, healthy fats and anti-inflammatory compounds have greater neuroprotective benefits through synergistic effects rather than consuming isolated nutrient supplements (Grabska-Kobylecka et al., 2023).

A rich vitamin B diet can be achieved from a variety of fresh, unprocessed foods, including meat, poultry, fish, eggs, milk, cheese, some legumes and nuts. The B vitamins are essential water-soluble micronutrients that must be consumed in adequate amounts through the diet (Lauer et al., 2022). These vitamins consist of a variety of different structures and functions, such as folic acid (vitamin B9), vitamin B6 and vitamin B12. They are involved in the methionine cycle. In particular, B12 is essential for the conversion of homocysteine to methionine and vitamin B6 and folic acid act as essential cofactors in this reaction (Mielech et al., 2020). Vitamin B12 is important for maintaining neuronal health and supporting hematopoiesis. Additionally, it has been shown to protect against inflammation induced oxidative stress by modulating the production of cytokines and growth factors, which are implicated in AD pathology (Lauer et al., 2022). Research suggests that vitamin B12 is important for the development of the cholinergic system, which is essential for memory, attention and other cognitive functions. It is also believed that this compound may modulate the cholinergic system and contribute to restoring blood-brain barrier integrity in AD (Lee et al., 2024). Furthermore, studies suggest that a combination of vitamin B6 and other B complex vitamins may improve cognition, structural integrity and functional connectivity, although other studies show conflicting results in AD pathology (An et al., 2024).

A nutrient-rich, anti-inflammatory diet is increasingly being researched as a non-pharmacological strategy for reducing cognitive decline, AD pathogenesis and promoting healthy brain aging. Numerous studies have highlighted the importance of a healthy diet in reducing neuroinflammation, oxidative stress and metabolic dysfunction.

1.4. Dietary interventions in neurodegenerative and neuroprotective research

Dietary interventions are increasingly being researched to understand how specific nutrients and dietary patterns affect disease pathology, neuroinflammation and progression (Stefaniak et al., 2022). Researchers mostly focus on the interaction between specific nutrients and AD processes, such as antioxidants, vitamins B, E and C with neuroinflammation, oxidative stress and synaptic function. Additionally, they investigate the impact of different dietary patterns, especially Mediterranean and

Western diets on AD pathology and the correlation between metabolic syndrome with insulin resistance and neurodegeneration. (Hoscheidt et al., 2022; McGrattan et al., 2019; Stefaniak et al., 2022; Witek et al., 2022). By recreating human dietary patterns, researchers can determine the effects of diet on neuroinflammation, amyloid pathology and oxidative stress. These interventions not only provide mechanistic insights into the pathogenesis of AD but also provide public health recommendations. To investigate the effect of diet, experimental models have been developed, allowing direct observation of neurodegenerative processes and researching the underlying mechanisms of AD (Grant & Blake, 2023).

1.5. Animal models of Alzheimer's disease research

To better understand the correlation between diet and AD mechanisms, researchers use experimental models that have molecular, cellular and behavioural features of AD. They provide insights into dietary effects on cognitive function, neuroinflammation, amyloid and tau pathology.

The most widely used are animal models that have genetically expressed human-related genes, causing AD pathogenesis (Lorenzini et al., 2024; Puzzo et al., 2015). Genetically modified mice can be either transgenic or non-transgenic. Transgenic mice consist of one or multiple genetic modifications, overexpressing APP, PSEN1 and tau mutations. In contrast, non-transgenic mice are developed through toxin injections or direct administration of pathologies (Puzzo et al., 2015). (Puzzo et al., 2015).

One of the most common transgenic mouse strains is the Tg2576 mouse model, characterised by a mutated human APP gene, resulting in impaired cognition and increased A β production in the cortical and hippocampal regions from 5 months of age (Nyul-Toth et al., 2021). Additionally, the 3xTg-AD is a triple-transgenic mouse strain that has three genetically altered AD genes, overexpressing tau, PSEN1 and APP. A β accumulation, synaptic damage, neuroinflammation and cognitive deficits develop at the age of 3 – 4 months, while extracellular plaques appear at 6 – 9 months, with NFTs developing at 12 months (Pattanayak & Firdous, 2026). Another mouse model used in AD research is 5xFAD, characterised by amyloid deposition, gliosis and progressive neuronal loss, with cognitive decline and motor deficits. This model has 5 AD mutations, 3 APP, PSEN1 and PSEN2 mutations, causing rapid A β accumulation, starting as early as 1.5 – 2 months of age (Oblak et al., 2021). Furthermore, the APP/PSEN1 is a double transgenic mouse model that overexpresses human APP and PSEN1 genes, leading to increased amyloid accumulation from 6 months of age, which progressively increases over time, forming dense amyloid plaques in the hippocampus and dentate gyrus by the age of 18 months. Amyloid plaques consist of neuritic and non-neuritic plaques, which are similar in human AD. Oxidative stress and metabolic disorders are observed in this model,

starting from 1-2 months of age, while impaired hippocampal long-term potentiation, reactive gliosis and inflammatory cytokine production are observed in 14 months of age (Webster et al., 2013).

Mouse models are commonly used in behavioural and cognitive testing in AD research. Researchers use various behavioural tests and mazes to assess different memory types, including short-term, long-term and working memory. After behavioural assessment, brain tissues are usually collected for further cellular and molecular analysis to identify pathological changes affected by nutrients or dietary patterns.

Together, these experimental approaches provide insights into the relationship between diet, behaviour and neural AD pathology (Mackey-Alfonso et al., 2024). However, these models can have limitations that need to be considered before interpreting the results. Animal models do not fully replicate the complexity of AD pathology, mechanisms and cognitive decline. Dietary interventions in animal models may not fully reflect human nutrition, as they interfere with lifestyle and genetic factors (Puzzo et al., 2015). When choosing a model, it is important to consider the duration of the experiment and at what age AD pathology begins to develop (Puzzo et al., 2015).

2. Methodology

2.1. Laboratory animals and study protocol

This thesis analysed previously collected experimental data. The author performed a statistical analysis, interpretation of the results and conclusions. The experiment was performed in the animal laboratory of Vilnius University Life Sciences Center, in compliance with animal welfare and ethical requirements. It was conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council, with permit number 42-197 issued by the State Food and Veterinary Service. The sample size calculations were based on a statistical power analysis (G*Power), resulting in a total of 47 male mice. The number of animals was reduced accordingly to ensure reliable, significant results. The animals were bred and purchased from a specialised laboratory animal breeding facility, The Jackson Laboratory. Throughout the experiment, mice were housed in small groups of 1 – 5 per cage and kept under a 12-hour light/dark cycle, with unlimited access to food and water.

Experimental groups and timeline of the experiment

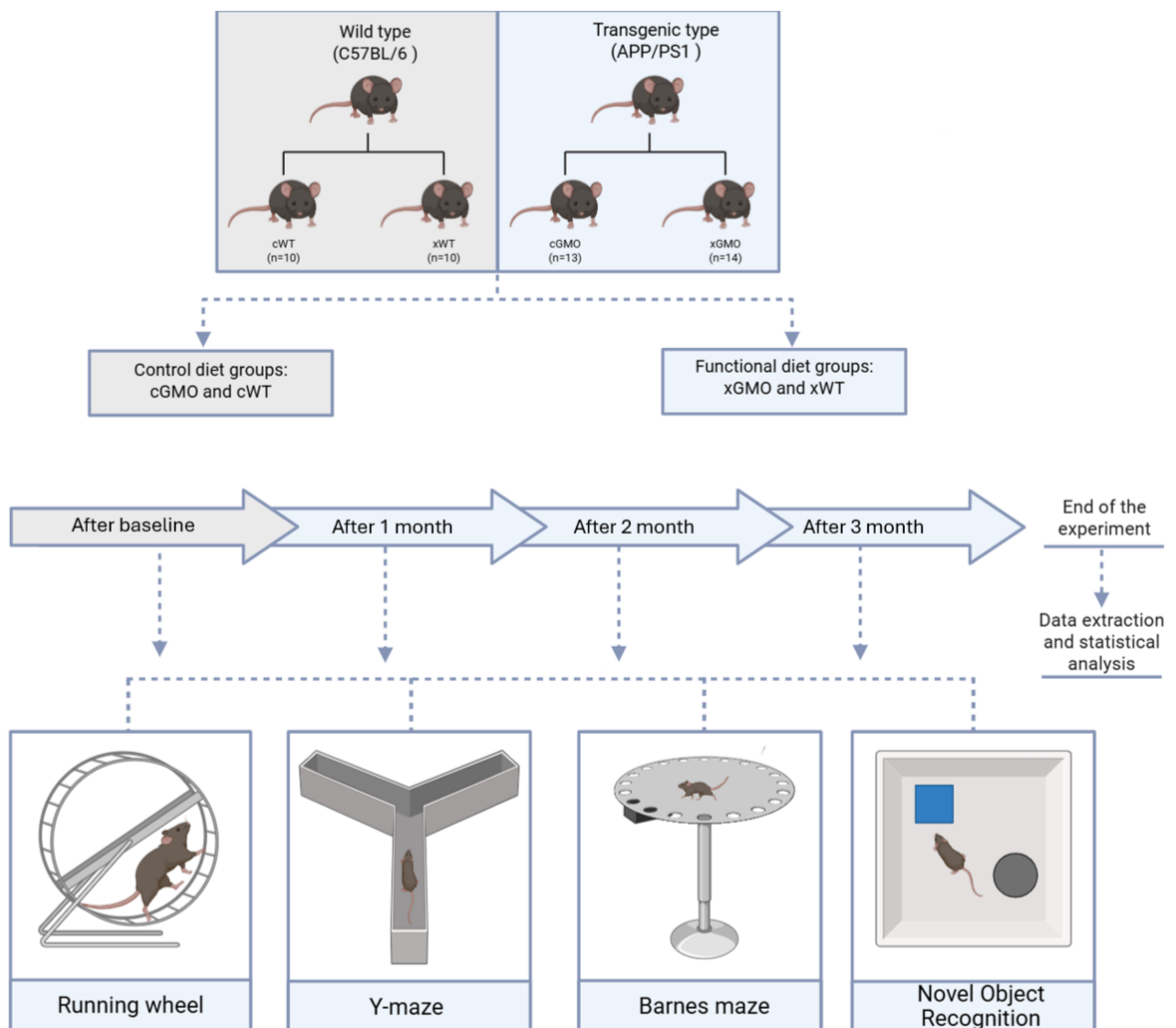


Figure 1. Schematic representation of experimental design with animal grouping, dietary interventions, and timeline of behavioural assessment.

Twenty wild type (WT) C57BL/6 mice and twenty-seven APP/PS1 transgenic (GMO) mice were used in this study. The APP/PS1 mice reflected AD pathogenesis by expressing human APP and PSEN1 mutated genes associated with early-onset AD. In this model, neurodegeneration occurs between 5 – 6 months of age. Meanwhile, C57BL/6 mice were used as healthy controls to compare results with the AD model and to assess the effect of diet. Before the experiment, all mice were randomly assigned to groups:

1. Control wild type group receiving standard food (cWT, n=10);
2. Experimental wild type group receiving functional food (xWT, n=10);
3. Control transgenic group receiving standard food (cGMO, n=14);
4. Experimental transgenic group receiving functional food (xGMO, n=13).

To avoid interference and ensure that baseline measurements were accurate, all mice were fed the same control diet for one month till 7 months of age. Then, baseline measurements were taken from all mice before the diet was changed for two groups, from control to functional diet. Behavioural measurements were taken at the end of each month to provide in-depth cognition and behaviour changes. Additionally, mice were weighed every week throughout the study. Mice remained on their respective diets for three months, and the experiment was concluded after the final measurements were taken (Figure 1).

2.2. Dietary intervention

The control diet consisted of a standard laboratory chow (Altromin C1000, *Altromin International*), which contained essential vitamins, macronutrients and minerals needed to maintain normal mouse physiology. Meanwhile, the functional diet consisted of standard laboratory chow enriched with plant extracts (*Bacopa monnieri*, *Rosmarinus officinalis*, *Rhodiola rosea*, *Hericium erinaceus*, *Ginkgo biloba*) and bioactive compounds, such as bacosides, beta-glucans, flavonol glycosides, terpene lactones, phosphatidylserine, choline and B vitamins (B6, B9, B12), which are associated with neuroprotective effects. For the full nutritional list, see supplementary material (Table S1-S2).

2.3. Behavioural assessment

Behavioural assessment was conducted to evaluate cognitive function and physical activity in mice using Y-maze, running wheel test, novel object recognition test and Barnes maze. These tests are widely used in neurodegenerative disease research, as they provide comprehensive insights into disease progression. Before each test, mice were moved with their home cages next to the test laboratory and left for one hour to allow acclimatisation to the new environment.

All behavioural tests were performed during the day, from 9 a.m. to 3 p.m., and there was at least one day break between different tests. Finally, after each test, all the experimental surfaces and arenas were cleaned with distilled water to remove olfactory cues (odor, urine, feces) from previous animals from influencing other mice during the experiment. During the experiments, a specific lighting level in the room was set, adapted to each test.

Most of the tests were recorded using a high-resolution (FHD) video camera (DS-2CD1643G0-I(Z), manufactured by Hikvision) and HiLookVision software. The rest of the tests were recorded using a lower resolution (SD) video camera with the Badicam program. Camera was placed above the mazes. The recorded videos were used to extract behavioural data using a specialised video behaviour tracking program ANY-maze.

2.3.1. Running wheel test

The running wheel test was used to assess the physical activity of mice. Each mouse was placed in an individual cage (26.5 cm × 20 cm × 14 cm), equipped with a rodent running wheel (diameter of 12 cm), allowing them to use it freely and at their own pace, without any force or interference. Adjustable 100 lx lighting was used for this test. Mice were only allowed to run on the running wheel during these experiments. In addition, the physical activity was recorded for up to 24 mice at a time. All tests were performed in the span of 2 days.

All running wheel tests were conducted during the day and lasted 24 hours under a 12-hour light/dark cycle. During the first hour of the experiment, when mice were placed in the cage, physical activity was not recorded. This time was allocated for adaptation to the new cage and familiarisation with the running wheel. A sensor was attached next to the running wheel, which recorded rotations (one rotation = one electrical pulse). PowerLab (ADInstruments) signal acquisition and processing system and LabChart Pro software were used to record electrical signals from the sensor. This setup allowed measurement of wheel rotations with precise timing. Physical activity was assessed by calculating the physical activity index:

$$Activity\ index = \frac{(Night\ rotation - Day\ rotation)}{(Total\ rotation)}$$

If the AI value was positive, then the mouse was more active at night. On the contrary, if the AI value was negative, then mice were more active during the day. However, if the value was 0, then the physical activity during the day and night was equal. Lastly, more rotations per 24 hours indicate greater physical activity.

2.3.2. Y-maze test

The Y-maze was used to assess spatial working (short-term) memory in mice. This maze consisted of three identical arms, each 40 cm long, 15 cm high, and 10 cm wide, with no physical partitions in between the arms. Adjustable lighting was used 10 – 20 lx for this test.

During the test, mice were gently placed in the A arm and allowed to explore freely for 10 minutes. Definitions of arm entries and visits were based on the previously published study (J. Kim et al., 2023). Entrance was defined as at least 25 % of the entire arm's length, and visits were defined as at least 70% of the entire arm's length. This way, false entrances were prevented, and only true entrances and visits that met the right criteria were included for further analysis (Figure 2A, J. Kim et al., 2023). A correct alternation was defined as sequential entries into three different arms (A-B-C) (Figure 2B). On the contrary, repeated entries (A-B-B or C-B-C) were classified as incorrect alternation (Figure 2C). Also, if mice were visiting the same arm repeatedly, it was counted as a same-arm return. The percentage of spontaneous alternation was calculated as:

$$\text{Spontaneous alternation} = \frac{\text{Total alternations}}{(\text{Total arm entries} - 2)} \times 100$$

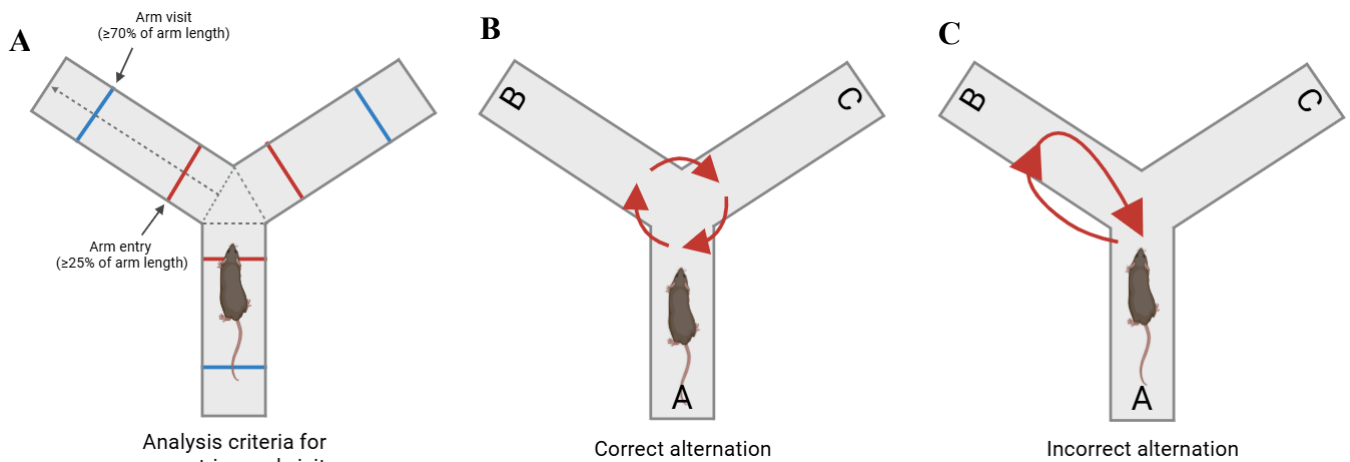


Figure 2. The structure of the Y-maze and possible alternations. (A) Blue lines indicate visits, and red lines indicate entries. (B) Correct alternation with the sequence of A-C-B. (C) Incorrect alternation with repeated arm A-B-A.

2.3.3. Novel object recognition test

The novel object recognition test was used to assess long-term memory and object recognition in mice. The test consisted of an open, square arena (40 cm × 40 cm × 30 cm) with two objects placed in the center. Adjustable lighting was used 10 – 15 lx for this test. During each phase, a mouse was gently placed in the center and allowed to explore freely. The test lasted for 10 minutes and was performed for three days in a row, with a 24-hour gap between measurements (Figure 4):

- Day 1 (habituation) – mice explored freely in an empty arena for 10 minutes to habituate to a new environment;
- Day 2 (learning phase) – during this phase, mice could freely explore, where two identical objects were placed in the center of the arena;
- Day 3 (test phase) – used to assess memory retention. One familiar object was replaced with a novel one, and the mouse was allowed to explore the arena for 10 minutes.

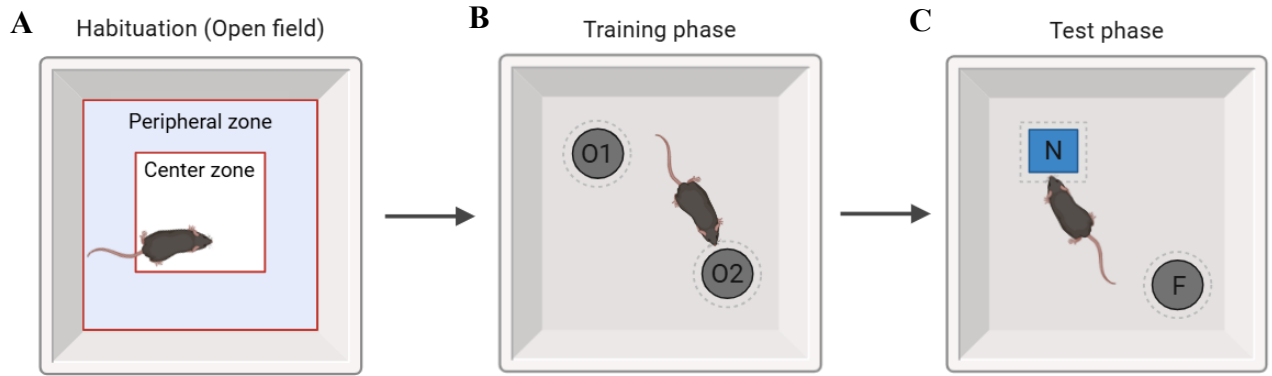


Figure 4. Novel object recognition test phases: (A) habituation with peripheral and center zones; (B) training phase with object 1 (O1) and object 2 (O2); (C) test with Novel (N) object and Familiar (F) object. The dashed line indicates a 2 cm distance from the object, indicating exploration.

Based on the previously described analysis methods, the habituation phase was analysed as an open field test (Denninger et al., 2018). The arena was divided into two zones: peripheral and center, which was 25 % of the total arena 20×20 cm size (Figure 4A). Locomotor activity was assessed by calculating total traveled distance, and anxiety-like behaviour was evaluated by measuring time spent in the periphery and central zones. More time spent in the periphery was interpreted as higher anxiety-like behaviour, while increased time spent in the center zone showed lower anxiety-like behaviour (Evans et al., 2024). Both the training and test phases were analysed based on the previously described methods (Koivisto et al., 2025; Lueptow, 2017). Moreover, in both phases, exploration was defined when the mouse nose was within 2 cm of the object. Therefore, during the learning phase, time spent exploring each object was measured to evaluate object preference and to prevent side bias. Equal exploration time for both objects indicated no preference, allowing the test phase to proceed. In contrast, mice that showed a preference or side bias were excluded from further analysis (Figure 4B). Additionally, before the main tests, the preference of selected figures was assessed. However, no preference was found, chosen figures were suitable for further testing. During the test phase, time spent exploring each object was measured to assess preferences for novel and familiar objects (Figure 4C). In addition, the discrimination index was calculated in a test phase to indicate a preference for a specific object, either a familiar or a novel one (Lueptow, 2017; Koivisto et al., 2025).

$$\text{Discrimination index} = \frac{(\text{Novel object time} - \text{Familiar object time})}{\text{Total object time}}$$

2.3.4. Barnes maze test

The Barnes maze was used to assess the spatial learning and long-term memory of mice. It consisted of a circular platform (diameter – 90 cm) raised 60 cm above the floor, with symmetrically placed 5 cm diameter holes along the edges (20 in total). Only one hole was open, leading to an escape box where the mouse could hide. An escape hole remained in the same spot throughout the whole experiment. Moreover, spatial cues were placed on the walls around the platform, helping mice to orient themselves. The experiment was conducted under a bright light (~500 lx), which caused stress for mice and motivated them to seek a darker place. During the experiment, a mouse was gently placed on the platform and allowed to explore freely, searching for a hiding spot (Figure 3). The test was conducted in 5 days:

- Day 1 (training phase) – mice were trained to seek and find an open hole with an escape box. This part of the experiment was not analysed;
- Days 2-4 (learning phase) – mice were tested for their ability to learn and remember where the location of the escape box was. The mice had to find the escape box within 2 minutes and enter within 3 minutes. If they failed to locate or enter the target hole, then they were gently guided to it. Once inside the escape box, the animal had to remain there for 1 minute;
- Day 5 (probe phase) – within 90 seconds, mice had to find the target hole of the escape box.

Spatial learning and short-term memory were analysed based on previously described methods (Pitts, 2018). For each test phase, several parameters were recorded, including the latency to locate the target hole (primary latency) and the latency to enter the escape box (total latency). Additionally, the number of incorrect holes visited before locating the target hole (primary errors) and the total number of incorrect hole visits before entering the escape box (total errors) (Figure 3) (Pitts, 2018). Also, based on another study, the total time spent in each quadrant was recorded (Figure 3B) (Custodio et al., 2021).

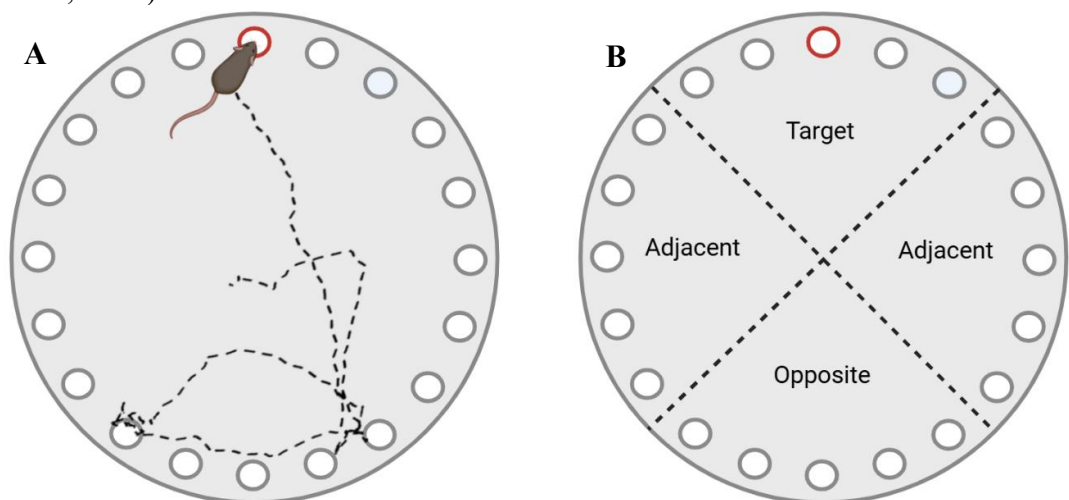


Figure 3. (A) Path trajectory to the target hole. (B) Barnes maze is divided into 4 quadrants (target, opposite, and adjacent). The red-marked hole is a target hole.

In addition to the analysis, another study was used as a reference to assess search strategies for the target hole as quickly as possible in the learning and probe phases (Rodríguez Peris et al., 2024). This strategy scoring system helped to identify and distinguish the different behaviours of mice seeking the target hole. The system goes from random search (score 1) to direct trajectory (score 11). It has four search strategy groups with different scores according to the type of strategy used (Table 5):

Table 5. Barnes maze test search strategy scoring system (Rodríguez Peris et al., 2024).

Strategy	Types		Description	Score
Direct			Straight direction to the target hole	11
Corrections	Target quadrant	Short correction 1	Directly to the target hole after correcting from the adjacent hole	10
		Short correction 2	Directly to the target hole after correcting through 2 adjacent holes	9
	Other quadrants	Long correction	From the opposite quadrant , checking holes and going through the center or through adjacent holes to the target hole	8
		Medium correction	Trajectory outside the target quadrant , checking holes, and going through the center to the target hole	7
Serial	One direction	Short serial	3-4 holes in sequence before the target hole	8
		Serial 1	5-6 holes in sequence before the target hole	7
		Serial 2	7-10 holes in sequence before the target hole	6
		Serial 3	11-15 holes in sequence before the target hole	5
		Serial 4	≥ 16 holes in sequence before the target hole	4
	Two direction	Combined 1	Serial strategy in both directions	3
	Multiple direction	Combined 2	Serial strategy with multiple direction changes	2
Random			Multiple changes in direction, checking holes not sequentially, ≥ 1 crossing the center	1

2.4. Data analysis

Data was analysed with RStudio. Data normality was assessed using the Shapiro-Wilk test. If the data met the normality assumptions, a one-way ANOVA with Holm correction was used to compare groups at baseline. However, when the normality assumptions were not met, Kruskal-Wallis with Holm correction was used. For repeated measures, a linear mixed model was used. Genotype, diet and time were used as fixed effects and animal number as a random effect. After significant effect was identified, within with Holm correction, between group with Tukey correction and change differences with Holm correction analyses were performed. Finally, for categorical data, Fisher's exact test with Monte Carlo simulation and Holm correction was used.

Illustrations were made using the Biorender program. Grammatical and stylistic errors were corrected using ChatGPT and Grammarly. References were added using Zotero.

3. Results

3.1. Body weight over time

No extreme body weight outliers were identified. All data were used in the analysis (cWT n=10; xWT n=10; cGMO n=14; xGMO n=13). Baseline body weight differed significantly between groups ($F(3,43) = 7.506$, $p < 0.001$). Post hoc analysis revealed that cWT group was heavier than cGMO ($p < 0.0001$) and xGMO ($p = 0.037$) groups, and xWT group was heavier than cGMO ($p = 0.007$) group. Furthermore, there was no difference between the genotype groups (Figure 5).

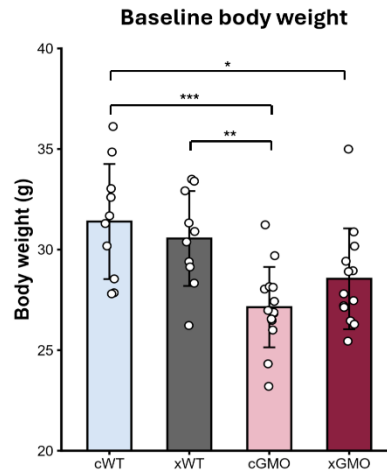


Figure 5. Baseline body weight before dietary intervention and differences between groups. Data is presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Within-group analysis revealed that body weight significantly increased in xWT by 14.29 % ($p < 0.001$), xGMO by 21.23 % ($p < 0.001$) and cGMO by 7.74 % ($p = 0.007$) groups, while no change was detected in cWT group ($p = 0.369$) from baseline compared to the end of the study (Figure 6).

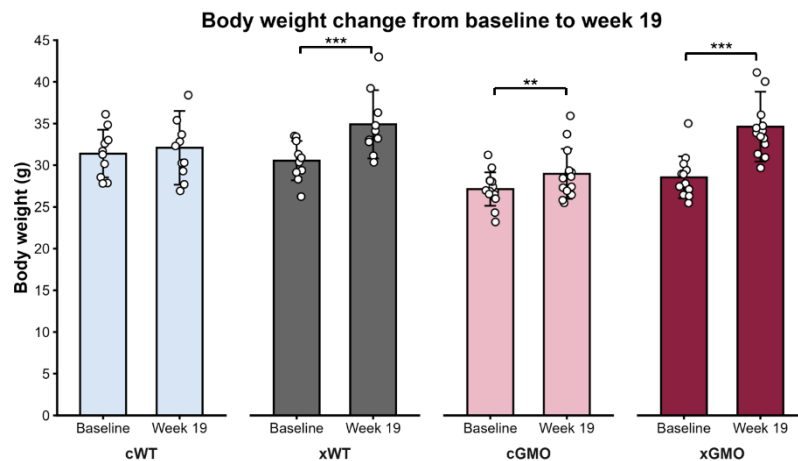


Figure 6. Body weight change of each group from the beginning to the end of the study. Data is presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Linear mixed model analysis revealed significant effects of time on body weight gain ($F(1,43) = 82.667$, $p < 0.001$), as well as genotype ($F(1,43) = 7.369$, $p = 0.009$) and diet ($F(1,43) = 6.447$, $p = 0.015$). The interaction between diet $\times$ time was also significant ($p < 0.001$). However, the genotype $\times$ diet $\times$ time interaction was not significant ($p = 0.684$). Post hoc analysis revealed that significant

weight changes began in the cGMO, xGMO, and xWT groups from the fifth week ($p < 0.001$) and in the cWT group from the sixth week ($p = 0.002$). Additionally, the functional diet significantly increased body weight in both the GMO ($p < 0.001$) and WT ($p = 0.001$) groups, compared to the standard diet (Figure 7).

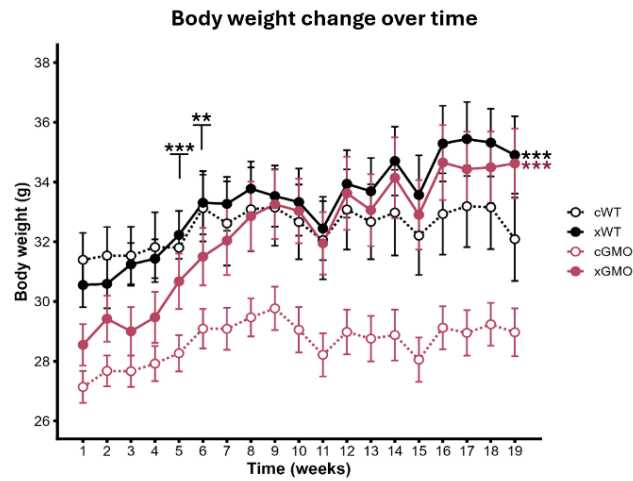


Figure 7. Body weight changes between groups throughout the study. Asterisks above the line plot indicate when significant weight gain was first observed for each group. While asterisks on the side indicate which groups significantly changed from the baseline. A significant increase in body weight was observed from the fifth week in the cGMO, xGMO, and xWT groups ($p < 0.001$) and in the cWT group ($p = 0.002$) from the sixth week. Both the xGMO ($p < 0.001$) and xWT ($p = 0.001$) groups showed a significant increase in body weight from baseline. Data is presented as mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$.

In summary, at baseline GMO were significantly lighter than WT groups. During the third month, xGMO, cGMO and xGMO had a significant increase in body weight compared to baseline. However, only xWT and xGMO groups had a significant body weight gain compared to the other groups.

3.2. Running wheel test

Extreme running wheel outliers were identified. However, their removal did not affect the significance of the results. Therefore, all data were included in the final analysis (cWT $n=10$; xWT $n=10$; cGMO $n=14$; xGMO $n=13$). Baseline total rotations differed significantly between groups ($\chi^2(3) = 12.276$, $p = 0.006$). Post hoc analysis revealed that the cGMO group had a significantly higher number of total rotations compared to cWT ($p = 0.009$) and xWT ($p = 0.041$) groups (Figure 8A).

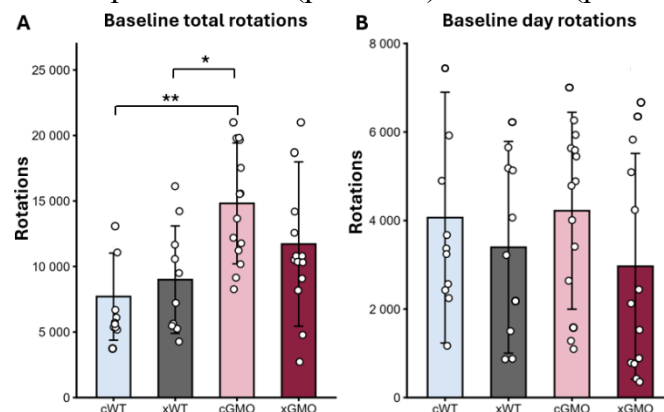


Figure 8. Baseline total (A) and day (B) rotations before dietary intervention and differences between groups. Data is presented as mean $\pm$ SD * $p < 0.05$, ** $p < 0.01$

The number of night rotations differed significantly between groups ($\chi^2(3) = 20.819$, $p < 0.00$) (Figure 9A), while no differences were found in day rotations ($p = 0.515$) (Figure 8B). The cGMO group had a higher number of night rotations than both cWT and xWT groups ($p < 0.001$), while the xGMO group had more night rotations than the cWT group ($p < 0.00$) (Figure 9A). Additionally, the activity index differed significantly between groups ($\chi^2(3) = 11.885$, $p = 0.008$). Both cGMO ($p = 0.028$) and xGMO ($p = 0.007$) groups had significantly higher activity index than the cWT group (Figure 9B).

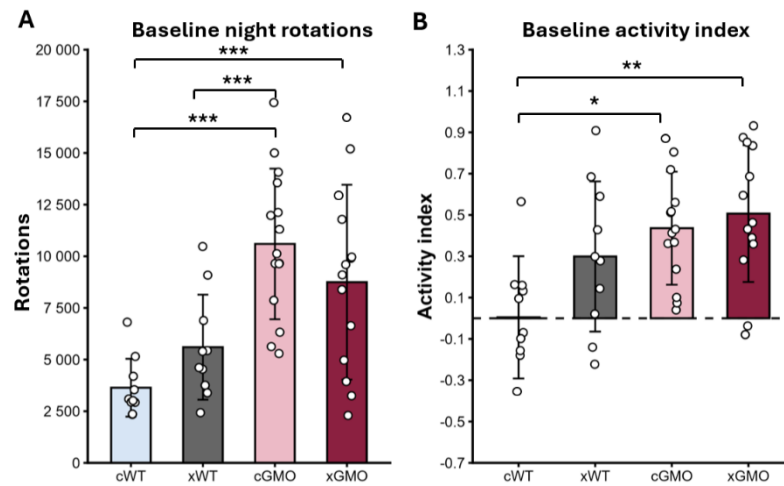


Figure 9. Baseline night rotations (A) and activity index (B) before dietary intervention and differences between groups. Data is presented as mean $\pm$ SD * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Linear mixed model analysis showed significant effects of time on total rotations ($F(3,129) = 12.287$, $p < 0.001$), genotype ($F(1,43) = 15.900$, $p < 0.001$) and diet ($F(1,43) = 5.486$, $p = 0.024$). A significant interaction between diet $\times$ time was also observed ($p = 0.009$). Post hoc analysis revealed that total rotations began to increase from the second month in both cGMO ($p = 0.011$) and cWT ($p = 0.017$) groups. During the last month of the study, the cGMO group had significantly higher total rotations than xGMO ($p < 0.001$) and cWT ($p = 0.016$) groups (Figure 10B). Within-group analysis showed that total rotations significantly increased in the cWT by 65.8 % ($p < 0.001$) and in the cGMO by 28.4 % ($p < 0.001$) groups (Figure 10A).

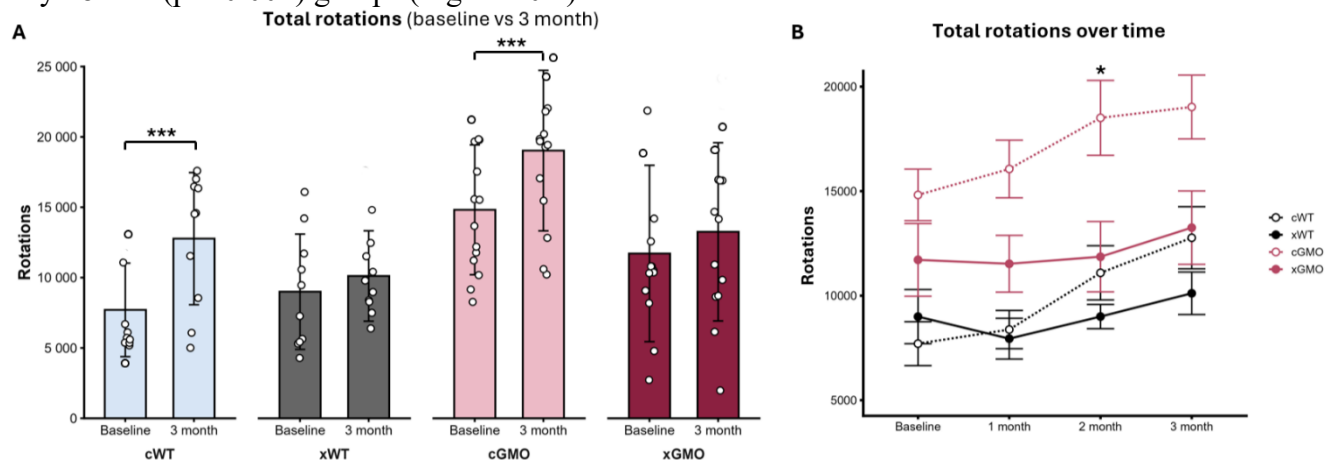


Figure 10. Total rotation (A) difference between baseline and third month. (B) Total rotation changes between groups throughout the study. Asterisks above the line plot indicate when a significant increase was first observed. A significant increase in total rotations was observed from the second month in both cGMO ($p = 0.011$) and cWT ($p = 0.017$) groups. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, *** $p < 0.001$.

Significant effect of time was observed in day rotations ($F(3,129) = 18.827$, $p < 0.001$). Day rotations increased from the second month in the cWT group ($p = 0.023$), and for cGMO and xGMO groups ($p < 0.001$) from the last month. However, no significant differences between groups were observed after the third month or changes throughout the experiment (Figure 11B). Day rotations increased in cWT by 65.4 % ($p < 0.001$), cGMO by 44.4 % ($p = 0.001$) and xGMO (by 65.2 %, $p = 0.002$) groups from baseline compared to the end of the study (Figure 11A).

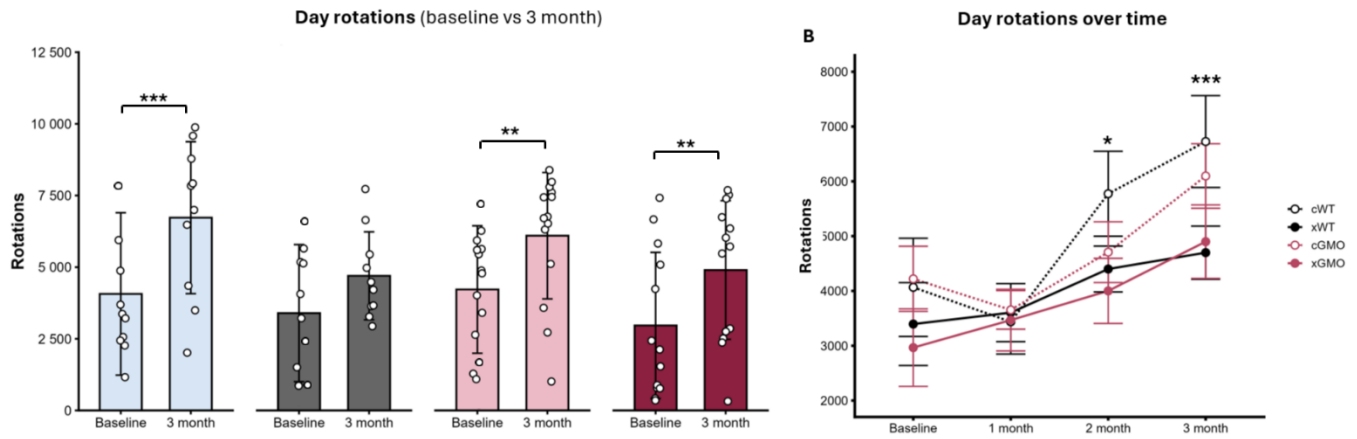


Figure 11. Day rotation (A) difference between baseline and third month. (B) Day rotation changes between groups throughout the study. Asterisks above the line plot indicate when a significant increase was first observed. A significant increase in day rotations was first observed from the second month in the cWT group ($p = 0.023$), and in xGMO and cGMO from the third month ($p < 0.001$). Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

A significant effect of genotype ($F(1,43) = 34.613$, $p < 0.001$) and diet ($F(1,43) = 5.314$, $p = 0.026$) were observed in night rotations. The interaction between diet $\times$ time ($p = 0.002$) and genotype $\times$ diet ($p = 0.026$) was also significant. A significant increase in night rotations was first observed from the second month in the cGMO group ($p = 0.016$), and from the third month in the cWT group ($p = 0.029$) (Figure 12B). During the third month, the cGMO group had higher night rotations than the cWT ($p < 0.001$) and xGMO ($p = 0.009$) groups. Night rotations significantly increased in cWT by 66.1 % ($p = 0.018$) and cGMO by 22 % ($p = 0.007$) groups (Figure 12A).

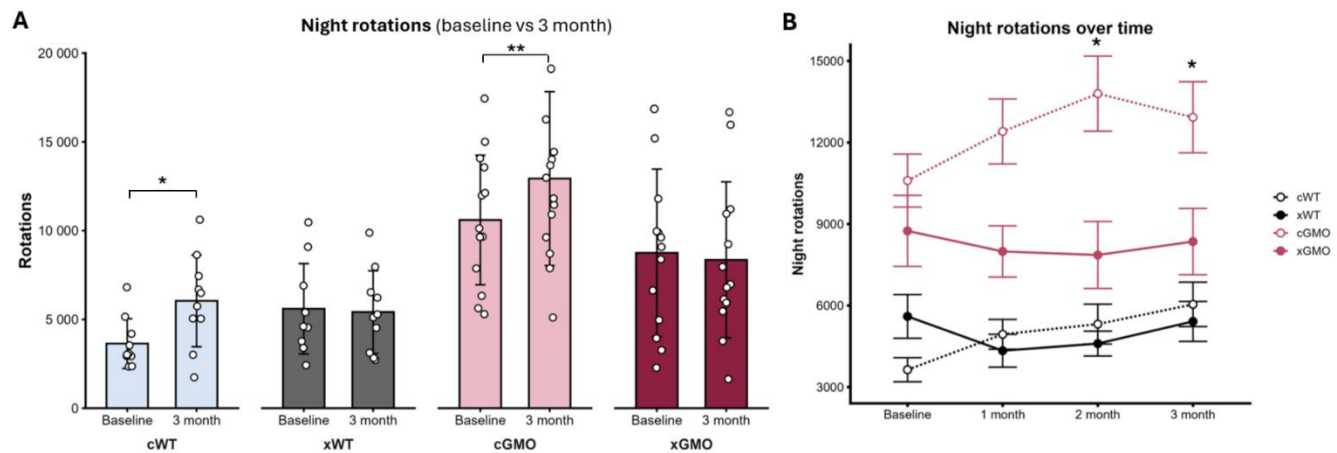


Figure 12. Night rotation (A) difference between baseline and third month. (B) Night rotation changes between groups throughout the study. Asterisks above the line plot indicate when a significant increase was first observed. A significant increase in night rotations was observed from the second month in the cGMO group ($p = 0.016$) and in the cWT group ($p = 0.029$) from the third month ($p < 0.001$). Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$.

Additionally, the interaction between genotype $\times$ diet $\times$ time was not significant for total, day or night rotations, indicating that the effect of diet was similar across all groups. No significant differences in changes between groups were observed throughout the experiment.

Mixed model analysis revealed significant effects of time ($F(3,129) = 5.364$, $p = 0.002$) and genotype ($F(1,43) = 59.719$, $p < 0.001$) on activity index. The interaction between time $\times$ diet was also significant ($p = 0.010$). However, the interaction between time $\times$ genotype $\times$ diet was not significant ($p = 0.734$). A significant decrease in the activity index was first observed from the first month in the xWT ($p = 0.025$) and xGMO ($p = 0.038$) groups. During the third month, the cGMO group had a higher activity index than the cWT ($p < 0.001$) and xGMO ($p = 0.008$) groups (Figure 13B). The activity index decreased in xWT group by 82.7 % ($p = 0.015$) and xGMO group by 44.5 % ($p = 0.012$) from baseline to the end of the study. (Figure 13A). No significant differences in changes between groups were observed throughout the experiment (Figure 13B).

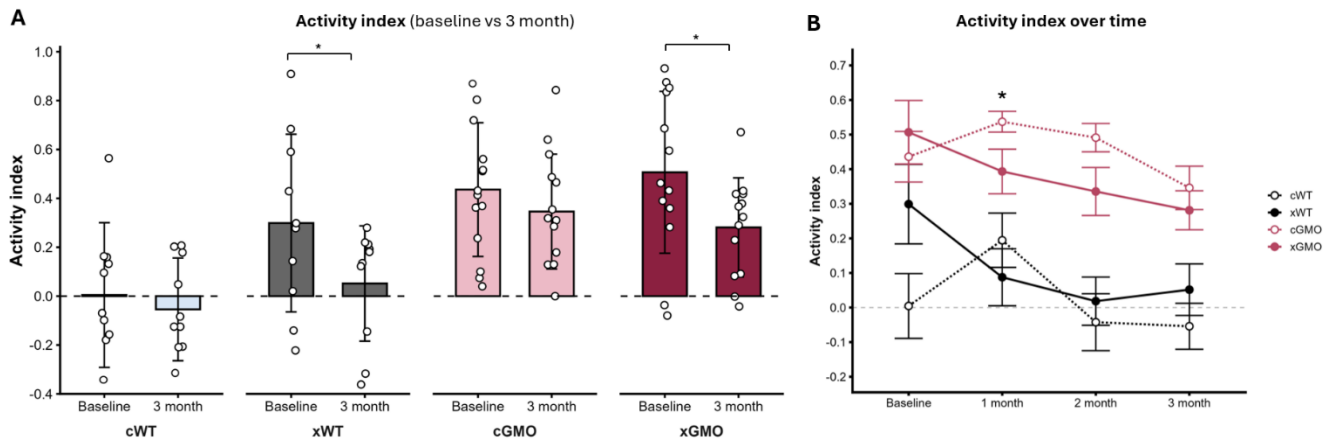


Figure 13. Activity index (A) difference between baseline and third month. (B) Activity index changes between groups throughout the study. Asterisks above the line plot indicate when a significant decrease was first observed. A significant decrease in activity index was observed from the first month in the xGMO ($p = 0.038$) and xWT ($p = 0.025$) groups. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$

In summary, at baseline, GMO mice had more rotations and had a higher activity index. During the last month of the experiment, cGMO and cWT groups had a significant increase in total, night and day rotations. In addition, xGMO group had a significant increase only in day rotations. The activity index decreased in the xWT and xGMO groups.

3.3. Y-maze test

One mouse from the cGMO group was excluded from further Y-maze test analysis due to having extreme data outliers (cWT $n=10$; xWT $n=10$; cGMO $n=13$; xGMO $n=13$). Baseline arm entries ($\chi^2(3) = 16.364$, $p < 0.001$) and arm visits ($\chi^2(3) = 13.636$, $p = 0.003$) differed significantly between groups. Post hoc analysis revealed that the xWT group had fewer entries than cGMO ($p = 0.010$) and xGMO ($p = 0.004$) (Figure 14A) groups and fewer visits than the xGMO group ($p = 0.002$) (Figure 14B).

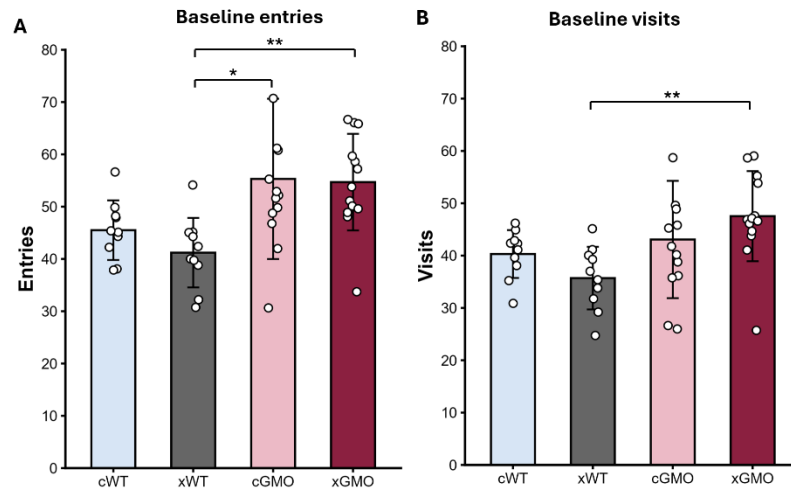


Figure 14. Baseline differences between groups in Y-maze arm entries (A) and visits (B). Data is presented as mean $\pm$ SD * $p < 0.05$, ** $p < 0.01$.

Baseline correct ($\chi^2(3) = 9.736$, $p = 0.021$) and incorrect ($\chi^2(3) = 10.656$, $p = 0.014$) alternations also differed significantly between groups. The xGMO group had significantly higher correct alternations than cWT group ($p = 0.039$) (Figure 15A). The xWT group had fewer incorrect alternations than the xGMO ($p = 0.021$) and cGMO ($p = 0.037$) groups (Figure 15B). Additionally, the baseline of spontaneous alternation differed between groups ($\chi^2(3) = 8.197$, $p = 0.04$). The xWT had a higher number of spontaneous alternations than the cGMO group ($p = 0.047$) (Figure 15C).

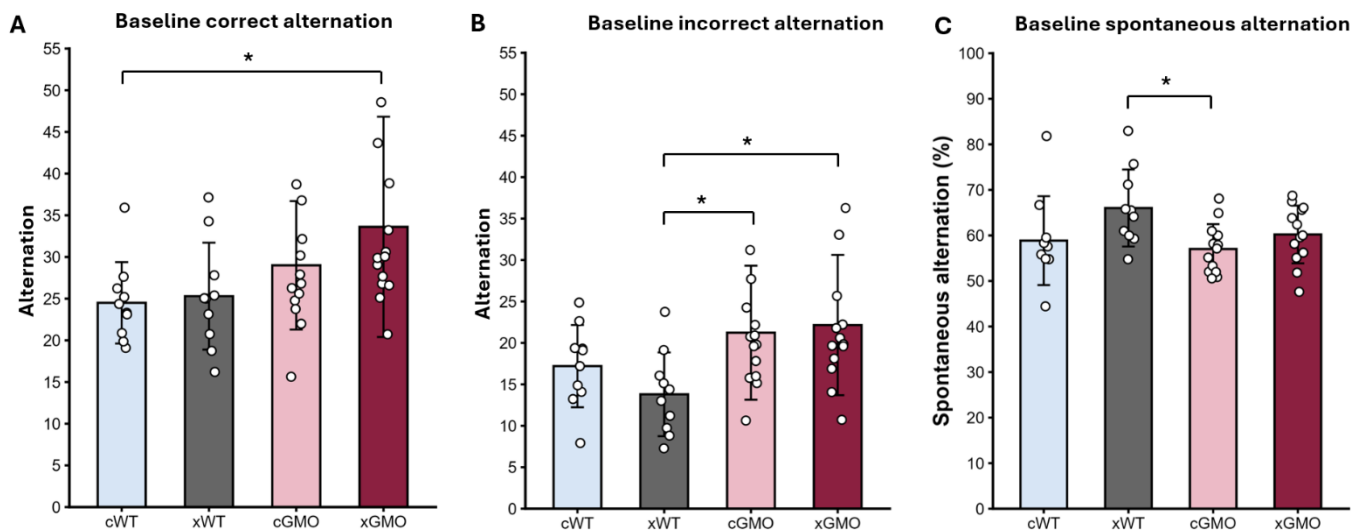


Figure 15. Baseline differences between groups in Y-maze correct (A), incorrect (B) and spontaneous (C) alternation. Data is presented as mean $\pm$ SD, * $p < 0.05$.

The linear mixed model revealed a significant effect of time on both entries ($F(3,124) = 13.834$, $p < 0.001$) and visits ($F(3,124) = 15.462$, $p < 0.001$). The interaction between genotype $\times$ time was also significant in both entries ($F(3,124) = 7.277$, $p = 0.001$) and visits ($F(3,124) = 10.079$, $p < 0.001$) (Figures 16B, D). Post hoc analysis showed that both GMO groups had a larger decrease in arm entries and visits than the WT groups ($p < 0.001$) from baseline to the last month (Figure 16A). A significant decrease in arm entries was first observed in the second month in xGMO ($p < 0.001$), cGMO ($p < 0.001$) and xWT ($p = 0.032$) groups (Figure 16B). Similarly, a decrease in arm visits was first observed during the second month of the study in both the cGMO ($p = 0.032$) and xGMO ($p < 0.001$) groups (Figure 16D). Within-group analysis showed that arm entries significantly decreased in cGMO by 37.6 % ($p < 0.001$) and xGMO by 31.2 % ($p < 0.001$) groups (Figure 16A). While arm visits decreased in the cGMO group by 30 % ($p < 0.001$) and xGMO group by 36.7 % ($p < 0.001$) from baseline to the end of the study (Figure 16C).

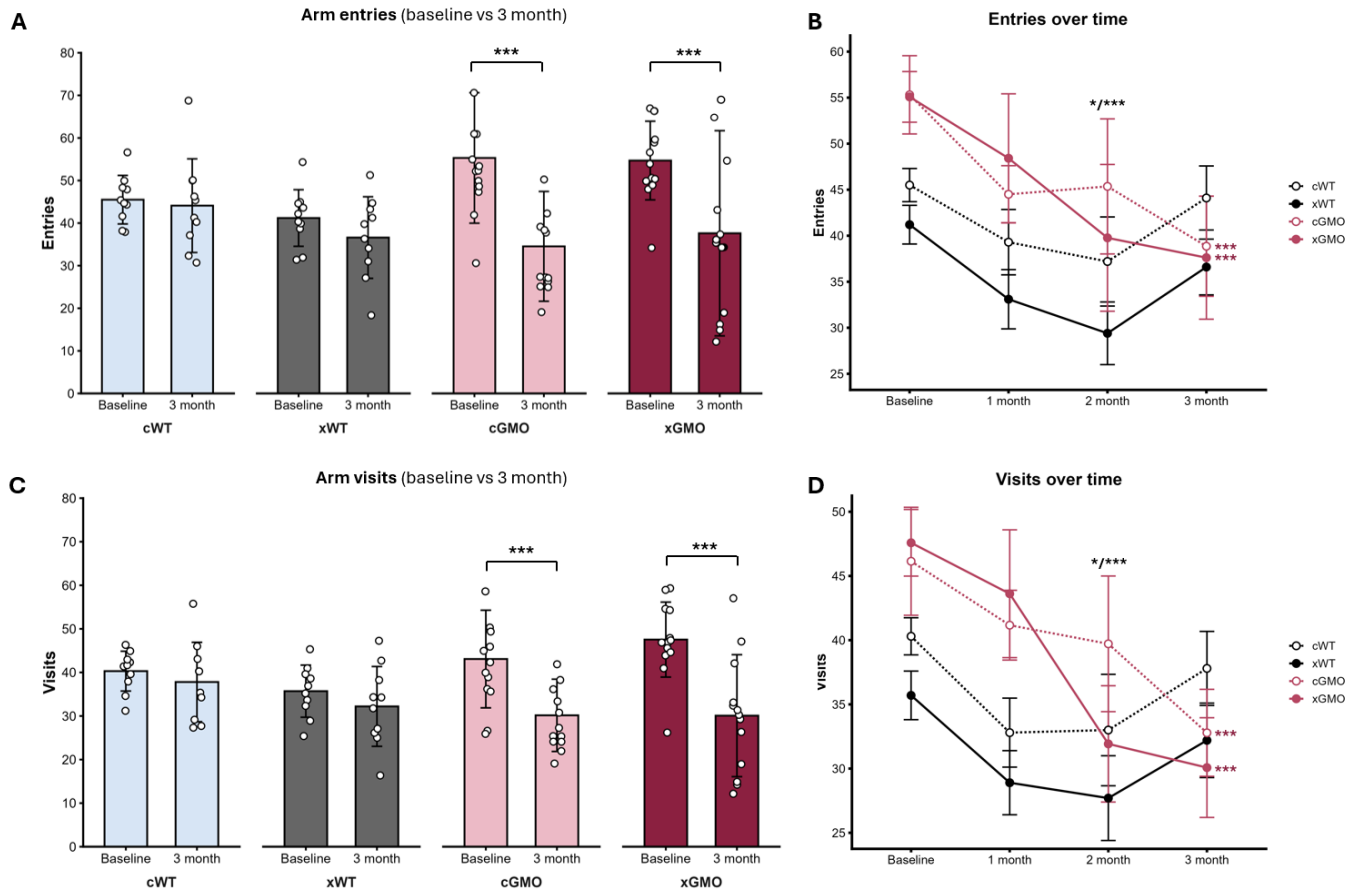


Figure 16. (A) Arm entry difference between baseline and the third month. (B) Arm entry changes between groups throughout the study. A significant decrease in arm entry was observed from the second month in the xGMO ($p < 0.001$), cGMO ($p < 0.001$) and xWT ($p = 0.032$) groups. (C) Arm visit difference between baseline and the third month. (D) Arm visit changes between groups throughout the study. A significant decrease in arm visits was observed from the second month in the xGMO ($p < 0.001$) and cGMO ($p = 0.032$) groups. Both GMO groups showed significant changes in arm entries (B) and visits (D) compared with the WT group ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. While asterisks on the side indicate which groups significantly changed from the baseline. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, *** $p < 0.001$.

A significant effect of time was found in correct ($F(3,124) = 11.102$, $p < 0.001$) and incorrect ($F(3,124) = 19.819$, $p < 0.001$) alternations. Interaction between time $\times$ genotype was found in both correct ($F(3,124) = 4.992$, $p = 0.002$) and incorrect ($F(3,124) = 8.051$, $p < 0.001$) alternations. A significant decrease in correct alternations was first detected in the first month in both GMO groups ($p < 0.001$) (Figure 17B). Similarly, incorrect alternations first decreased from the second month of the experiment in cGMO ($p = 0.016$) and xGMO ($p < 0.001$) groups (Figure 17D). Additionally, both GMO groups showed a larger decrease in correct and incorrect alternations from baseline to the third month than the WT groups ($p < 0.001$) (Figure 17B, D). Correct alternations significantly decreased in cGMO group by 27.1 % ($p = 0.002$) and xGMO group by 39.4 % ($p < 0.001$) (Figure 17A). Similarly, incorrect alternations decreased in the cGMO by 51.8 % ($p < 0.001$) and xGMO by 37.8 % ($p < 0.001$) groups from baseline to the end of the study (Figure 17B).

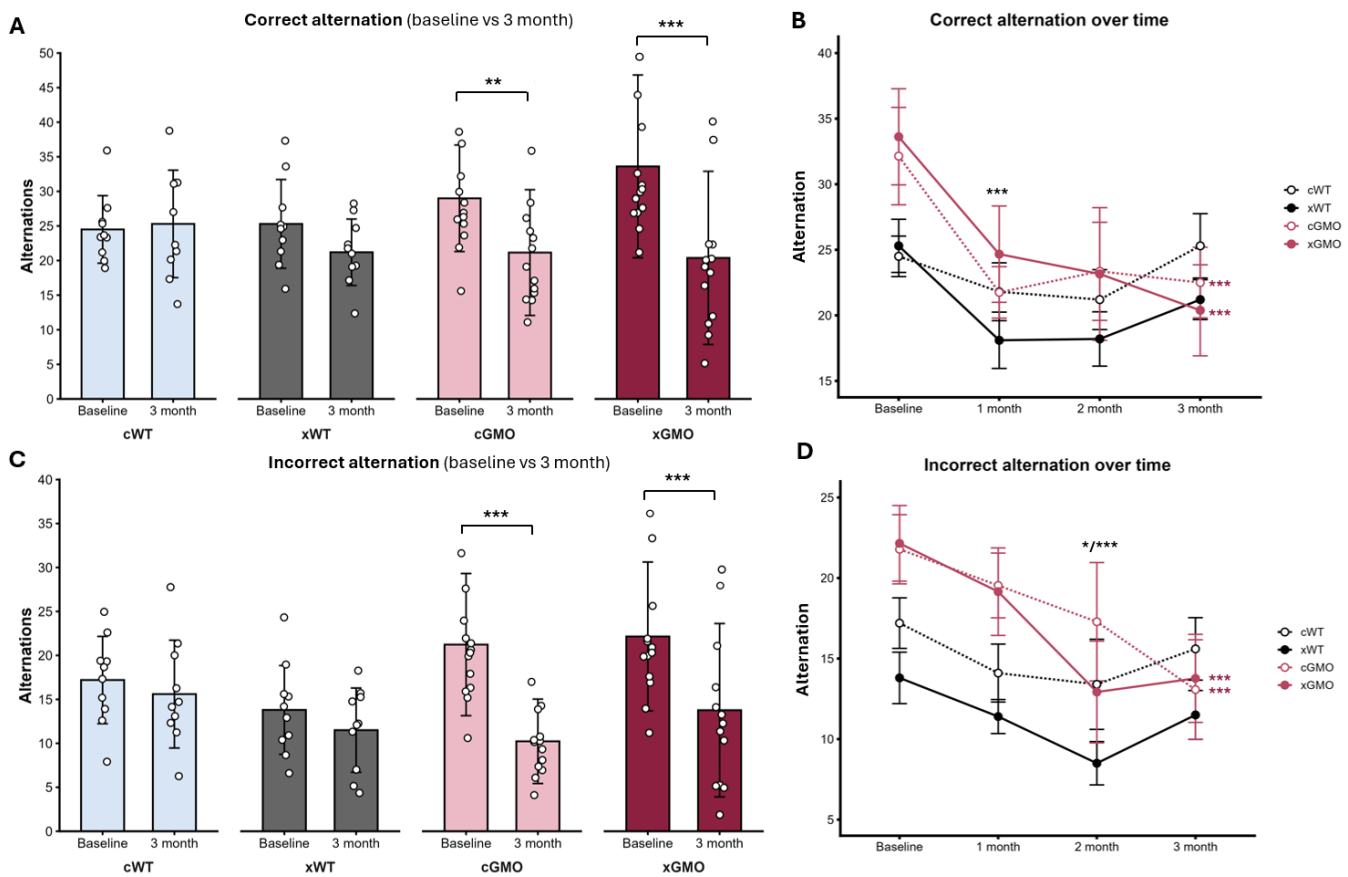


Figure 17. (A) Correct alternation difference between baseline and third month. (B) Correct alternation changes between groups throughout the study. A significant decrease in correct alternation was first observed from the first month in the xGMO ($p < 0.001$) and cGMO groups ($p < 0.001$). (C) Incorrect alternation difference between baseline and the third month. (D) Incorrect alternation changes between groups throughout the study. A significant decrease in incorrect alternation was observed from the second month in the xGMO ($p < 0.001$) and cGMO ($p = 0.016$) groups. Both the GMO groups showed a larger decrease in correct (B) and incorrect (D) alternation than the WT group ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. While asterisks on the side indicate which groups significantly changed from the baseline. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Spontaneous alternation had a significant effect of time ($F(3,125) = 5.321$, $p = 0.001$). A significant increase in spontaneous alternation was first observed in the cGMO group ($p = 0.016$) during the third month. However, no significant differences in changes between groups were observed throughout the experiment (Figure 18B). Spontaneous alternation increased only in the cGMO group by 17.8% ($p = 0.002$) (Figure 18A).

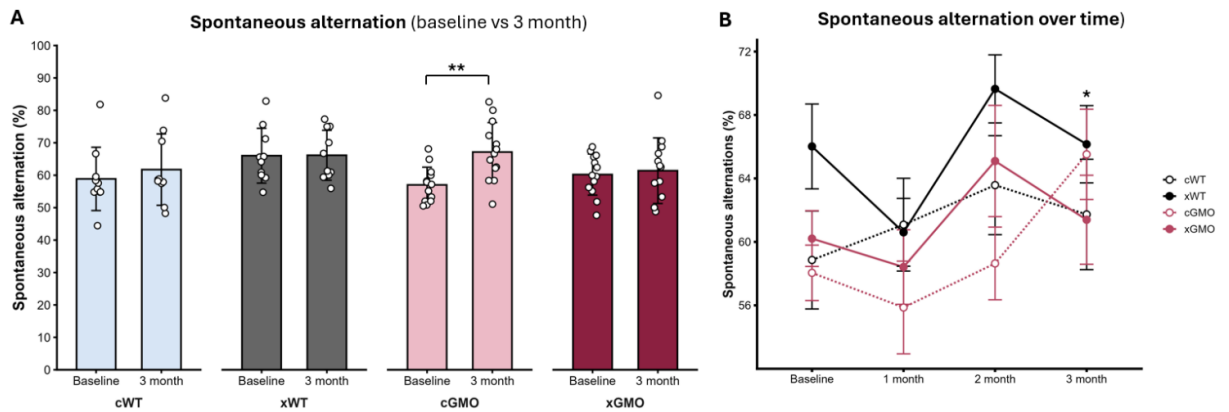


Figure 18. (A) Spontaneous alternation difference between baseline and third month. (B) Spontaneous alternation changes between groups throughout the study. A significant increase in spontaneous alternation was first observed in the cGMO group during the third month ($p = 0.016$). Asterisks above the line plot indicate when a significant decrease was first observed. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$.

In summary, xWT had significantly fewer baseline arm entries, visits and incorrect alternation than the GMO groups. Similarly, the cWT group had fewer correct alternations than the xGMO group. Throughout the experiment, both GMO groups showed the largest decrease in arm entries and visits, and in correct and incorrect alternation, compared to WT groups. During the third month, only the cGMO group had a significant increase in spontaneous alternation.

3.4. Novel object recognition test

3.4.1. Habituation phase

The novel object recognition test had a few extreme outliers. Five mice (1 cWT, 2 cGMO and 2 xGMO) were excluded from further analysis. Final mouse group sizes were cWT $n=9$; xWT $n=10$; cGMO $n=12$; xGMO $n=11$.

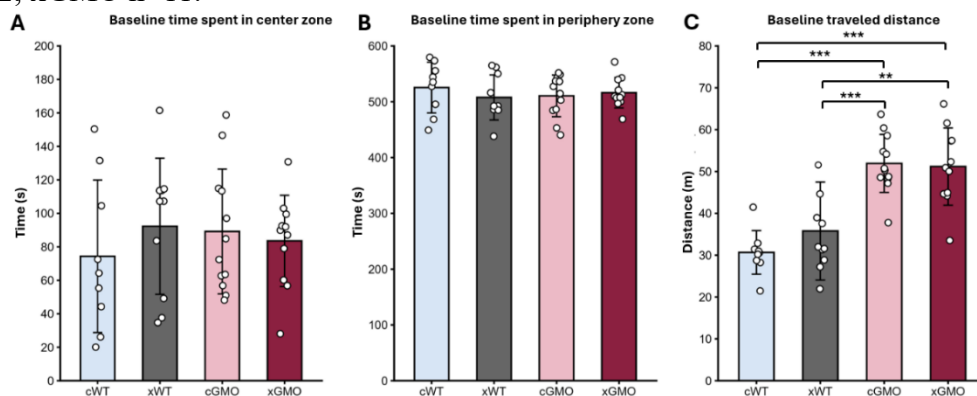


Figure 19. Time spent in the center (A) and periphery (B) zones and traveled distance (C) differences during the habituation test between baseline and the third month. Data are presented as mean $\pm$ SD, ** $p < 0.01$, *** $p < 0.001$

Baseline time spent in the center ($p = 0.742$) and periphery ($p = 0.748$) zones did not differ between groups (Figure 19A, B). A significant difference in traveled distance was found between groups ($F(3,38) = 16.01$, $p < 0.001$). Post hoc analysis revealed that the WT groups had a significantly shorter traveled distance than the GMO groups ($p < 0.001$) (Figure 19C).

Linear mixed model analysis revealed a significant effect of time on time spent in the center ($F(3,114) = 23.955$, $p < 0.001$) and periphery ($F(3,114) = 25.619$, $p < 0.001$) zones. Post hoc analysis showed that cWT spent more time in the center ($p = 0.021$) and less time in the peripheral ($p = 0.029$) zones than the xGMO group. A significant decrease in time spent in the center zone was first observed during the second month, while an increase in the periphery zone was observed in cGMO, xWT, and xGMO groups ($p < 0.001$) (Figure 20B, D). Within-group analysis showed that time spent in the center zone significantly decreased in xWT group by 48.37 % ($p = 0.002$), cGMO group by 55.8 % ($p = 0.001$) and xGMO group by 69.5 % ($p < 0.001$) (Figure 20A). In contrast, time spent in the periphery increased in the xWT by 9.7 % ($p = 0.001$), cGMO by 9.8 % ($p = 0.001$) and xGMO by 11.9 % ($p < 0.001$) groups from baseline to the end of the study (Figure 20C). Additionally, no significant differences in changes between groups were observed throughout the experiment (Figure 20B, D).

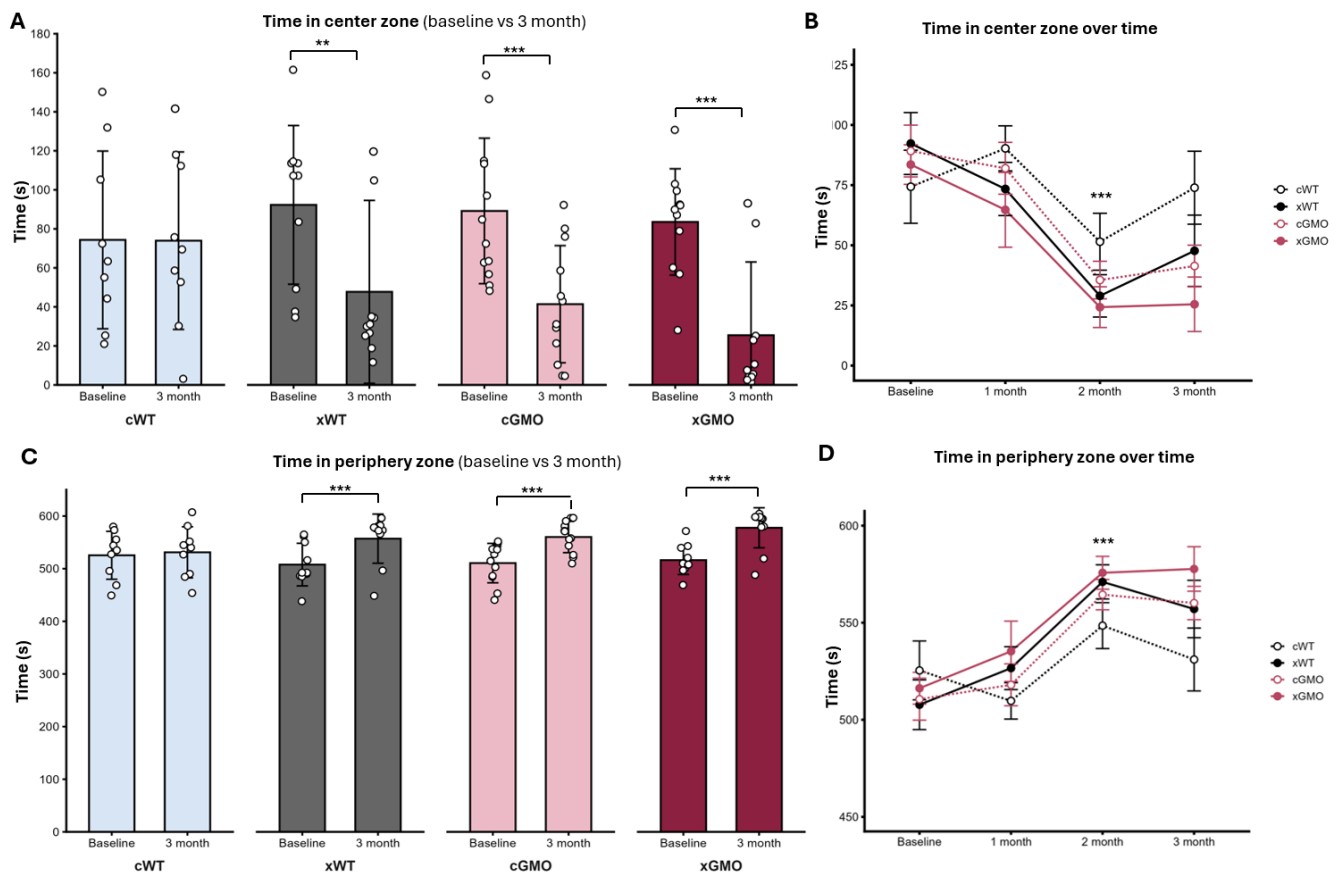


Figure 20. (A) Time in the center zone difference between baseline and the third month. (B) Time in the center zone changes between groups throughout the study. A significant decrease in time in the center zone was first observed from the second month in the xGMO, cGMO, xWT groups ($p < 0.001$). (C) Time in the periphery zone difference between baseline and the third month. (D) Time in the periphery zone changes between groups throughout the study. A significant increase in time in the periphery zones was first observed from the second month in the cGMO, xGMO and xWT groups ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), ** $p < 0.01$, *** $p < 0.001$

A significant effect of time ($F(3,114) = 191.323$, $p < 0.001$) and genotype ($F(1,38) = 26.599$, $p < 0.001$) was observed in traveled distance. The interaction between diet $\times$ time ($p < 0.001$) and between genotype $\times$ diet ($p < 0.001$) was also significant. Additionally, a trend toward genotype $\times$ time interaction was observed ($p = 0.066$). Both GMO groups had a larger decrease in traveled distance than the WT groups throughout the experiment ($p < 0.001$). A significant decrease in all groups was first observed during the first month ($p < 0.001$) (Figure 21B). The largest decrease was observed in the xGMO group with 63.3 % decrease, followed by cGMO (by 60%), xWT (by 50.9%) and cWT (by 36.1%) (Figure 21A).

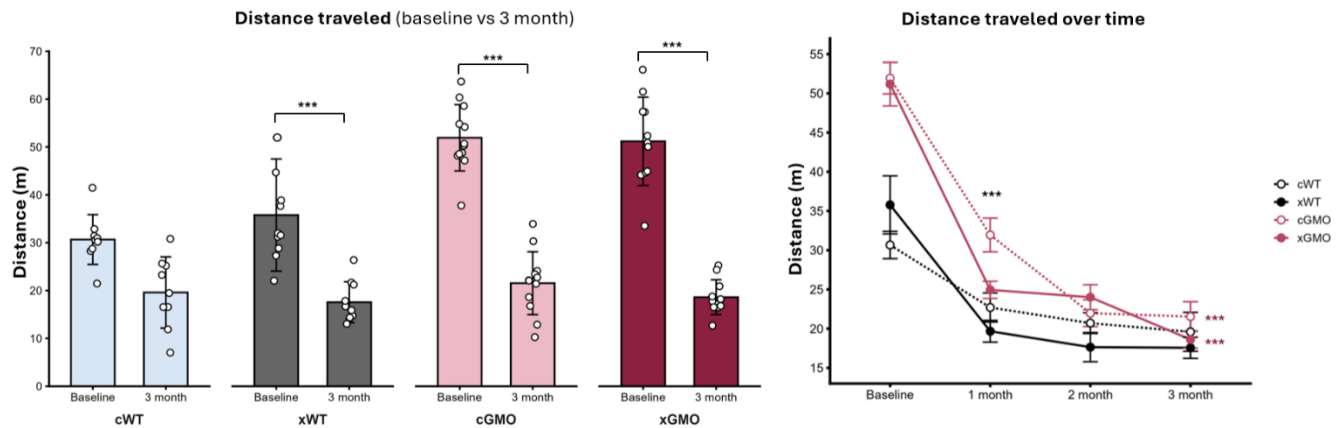


Figure 21. (A) Traveled distance difference between baseline and third month. (B) Traveled distance changes between groups throughout the study. A significant decrease in traveled distance was first observed during the first month in all groups ($p < 0.001$). Both GMO groups had a greater decrease than the WT groups throughout the experiment ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), *** $p < 0.001$.

3.4.2. Learning and testing phase

During the learning phase, no significant side preference was observed, indicating similar exploration of both objects throughout the experiment. Due to missing data, 3 mice from each of the cGMO and xGMO groups at the third month and 1 mouse from the xWT group were excluded. Additionally, 1 mouse from the cGMO group at 2 months was excluded from further analysis.

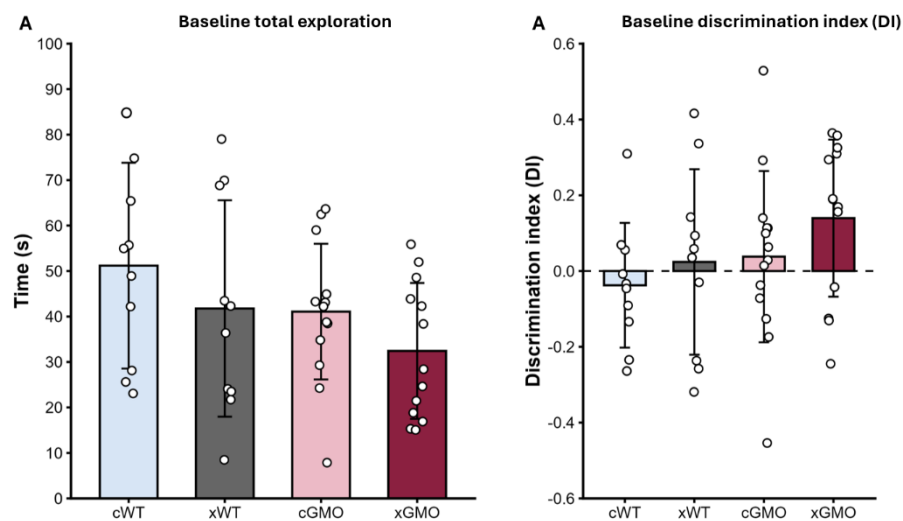


Figure 22. Baseline differences in total exploration and discrimination index between groups. Data is presented as mean $\pm$ SD.

Baseline total ($p = 0.148$) object exploration did not differ significantly between groups. A trend towards familiar object exploration was observed in the xGMO compared to the cWT group ($p = 0.068$) (Figure 22A). Similarly, no significant differences were observed in the discrimination index across groups (Figure 22B).

Linear mixed model analysis revealed a significant effect of time ($F(3,121) = 24.455$, $p < 0.01$) and genotype ($F(1,43) = 9.787$, $p = 0.003$) on total object exploration. All groups had a significant decrease in total exploration from baseline to the third month ($p < 0.001$), with no differences between groups ($p > 0.05$) (Figure 23A-B).

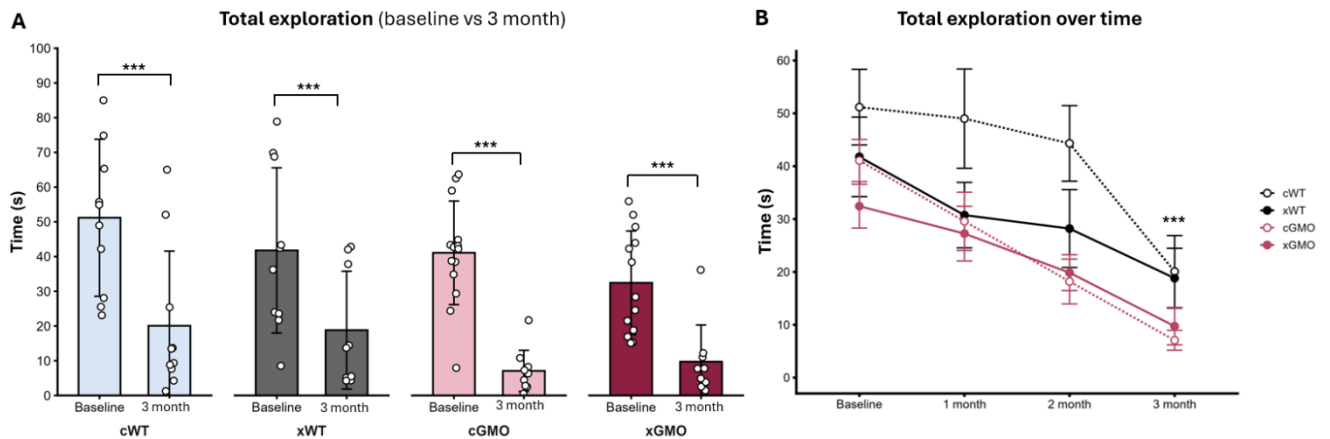


Figure 23. (A) Total exploration difference between baseline and the third month. (B) Total exploration changes between groups throughout the study. A significant decrease in total exploration was first observed during the third month in all groups ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), *** $p < 0.001$.

Significant effects of time ($F(3,163) = 5.567$, $p = 0.001$) and interaction between time $\times$ diet ($F(3,162) = 5.383$, $p = 0.001$) were observed for the discrimination index. A significant increase in cWT was first observed in the second month, which then significantly decreased. However, no significant differences were found in within or between-group comparisons (Figure 24A, B).

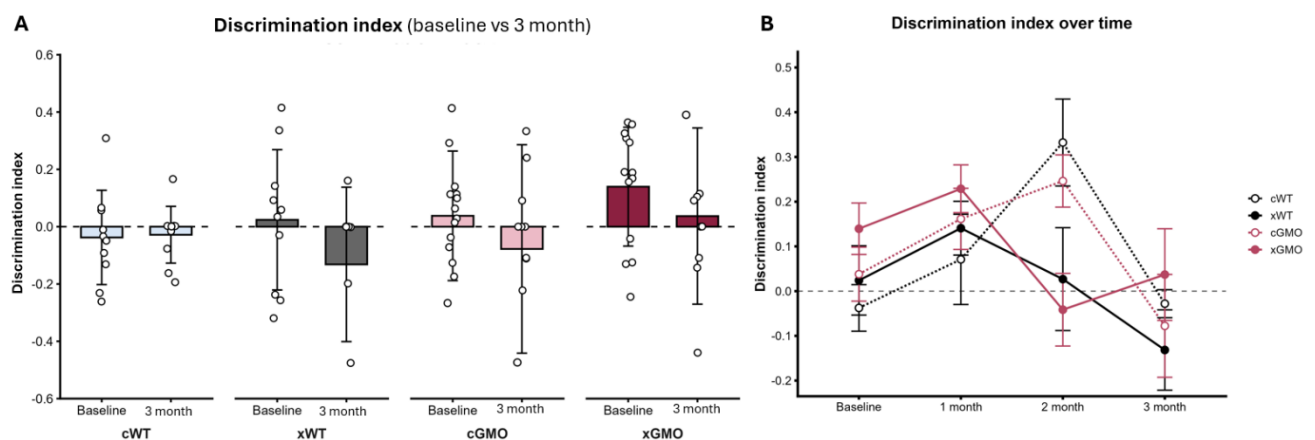


Figure 24. (A) Traveled distance difference between baseline and third month. (B) Traveled distance changes between groups throughout the study. A significant decrease in traveled distance was first observed during the first month in all groups ($p < 0.001$). Both GMO groups had a greater decrease than the WT groups throughout the experiment ($p < 0.001$). Asterisks above the line plot indicate when a significant decrease was first observed. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), *** $p < 0.001$.

In summary, at the baseline habituation phase, time spent in the periphery and center zones was similar. Only the GMO groups had a larger traveled distance than the WT groups. During the third month, xWT, cGMO and xGMO had a significant decrease in time spent in the center zones, while time spent in the periphery zone increased. Similarly, all groups had a significant decrease in traveled distance compared to baseline. Both GMO groups had the largest decrease in traveled distance. Additionally, at the baseline test phase, no difference between groups in the total exploration and discrimination index was found. During the third month, the total exploration decreased in all groups. No difference was found in the discrimination index.

3.5. Barnes maze

3.5.1. Learning phase

Extreme outliers were not detected during the learning phase of the Barnes maze test. However, 1 mouse from the cGMO group was excluded from the fourth day of the first month and 4 cGMO mice were excluded from the third and fourth days of the second month due to a lack of active search.

On the third day, baseline primary latency ($\chi^2(3) = 10.6$, $p < 0.014$) and primary errors ($\chi^2(3) = 11.8$, $p < 0.008$) differed significantly between groups. Post hoc analysis showed that the cWT group had significantly lower primary latency than the cGMO ($p = 0.031$) and xGMO ($p = 0.021$) groups during the third learning day (Figure 25A). Similarly, during the third learning day, the cWT group had fewer primary errors than the cGMO ($p = 0.029$) and xGMO ($p = 0.013$) groups (Figure 25B).

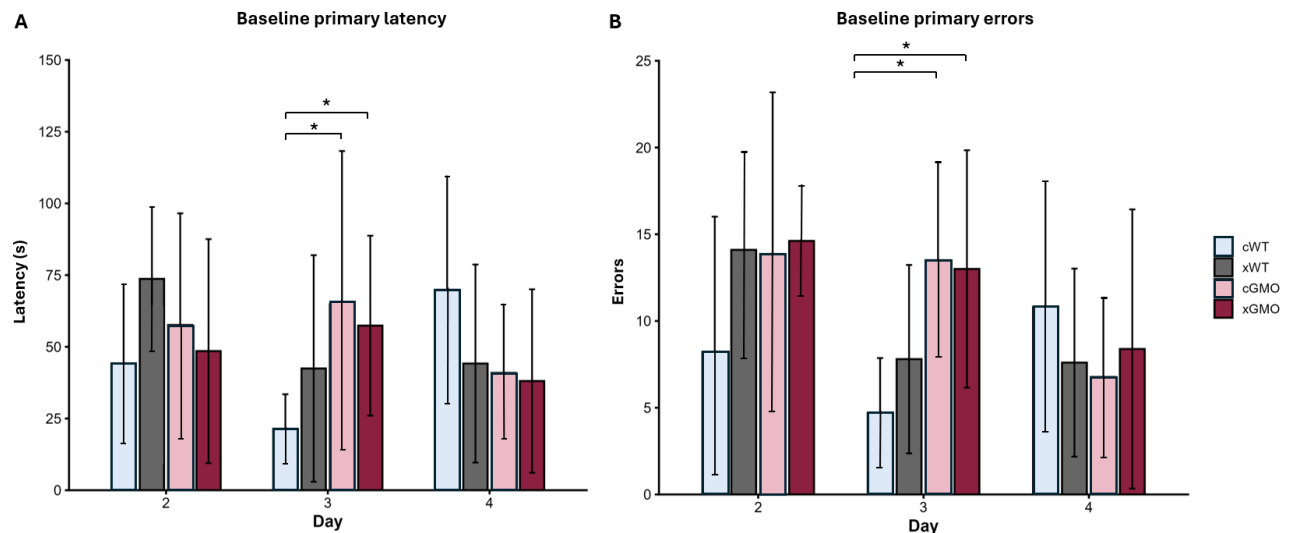


Figure 25. Baseline differences between primary latency (A) and primary errors (B) between groups. Data is presented as mean $\pm$ SD * $p < 0.05$.

Linear mixed model analysis revealed a significant effect of learning days on primary latency ($F(2.468) = 5.106$, $p < 0.006$). Post hoc analysis showed that primary latency significantly increased on the third day of the first month of learning in the cWT group compared to baseline ($p = 0.001$) but later decreased significantly after the second ($p = 0.012$) and third ($p = 0.004$) months (Figure 23B). Within-group analysis showed that on the fourth day of learning, primary latency in the third month

increased significantly compared to baseline ($p = 0.037$). In contrast, in cGMO, primary latency increased significantly on the second day compared to baseline ($p = 0.013$). During the third month, primary latency was longer in the cGMO group than in the cWT ($p = 0.031$), xWT ($p = 0.041$), and xGMO ($p = 0.010$) groups (Figure 26A). All groups changed similarly over the months (Figure 26B).

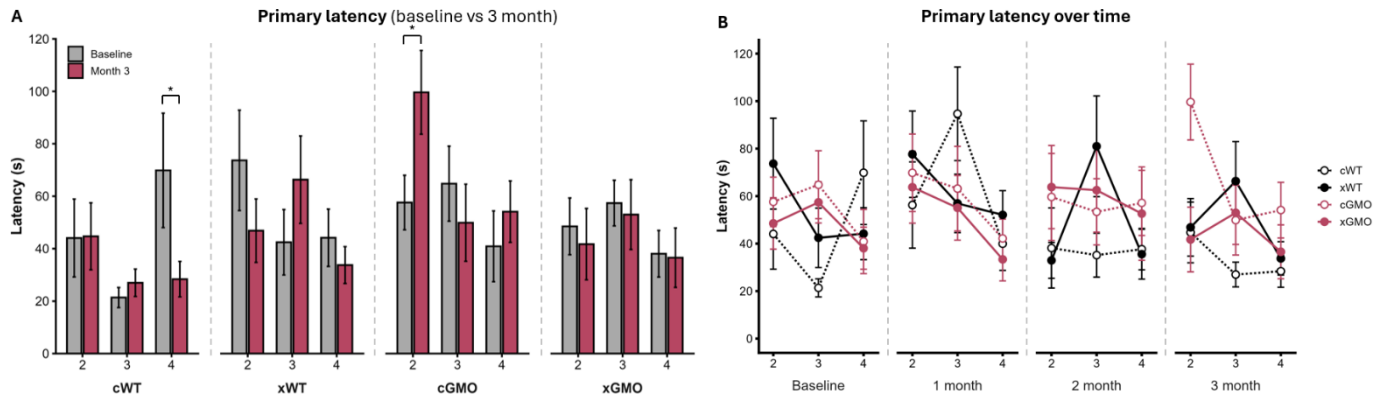


Figure 26. (A) Primary latency difference between baseline and the third month. (B) Primary latency changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), *** $p < 0.001$.

A significant effect of time was observed in total latency ($F(3,468) = 63.133$, $p < 0.001$) and in learning days ($F(2,468) = 9.121$, $p < 0.001$). Post hoc analysis showed that, in most groups, total latency significantly decreased from baseline to later months. During the second of learning, a significant decrease was observed in the cWT group ($p = 0.025$) after the first month and in the cGMO group ($p < 0.001$) after the second month. During the third learning day, a significant decrease was found in the cWT ($p < 0.001$), cGMO ($p = 0.020$), and xGMO ($p = 0.035$) groups after the third month. On the fourth day, total latency significantly decreased in the cWT group ($p < 0.001$) after the first month and in the cGMO ($p = 0.005$), xWT ($p = 0.026$), and xGMO ($p = 0.011$) groups after the third month (Figure 24B). Within-group analysis revealed that all groups had significantly lower total latency on all learning days in the third month than at baseline ($p < 0.001 - p = 0.026$) (Figure 27A). During the last month, on the second day of learning, total latency was significantly higher in the cGMO group compared to the cWT group ($p = 0.036$). All groups changed similarly over the months (Figure 27B).

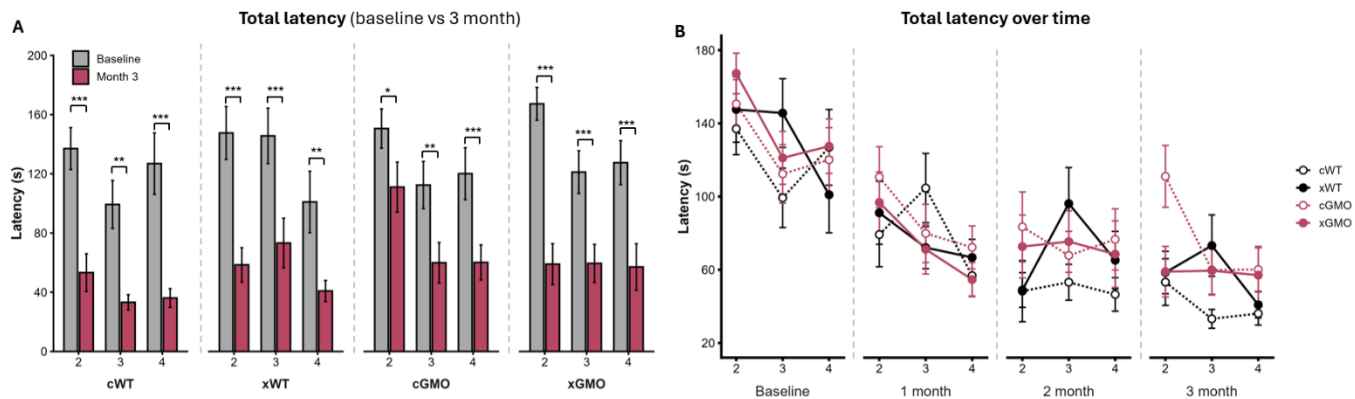


Figure 27. (A) Total latency difference between baseline and the third month. (B) Total latency changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

No significant effects of genotype, diet, time or learning days were observed on primary errors. A trend was observed for an interaction between genotype $\times$ time ($F(3,467) = 2.125$, $p = 0.096$) (Figure 28A, B).

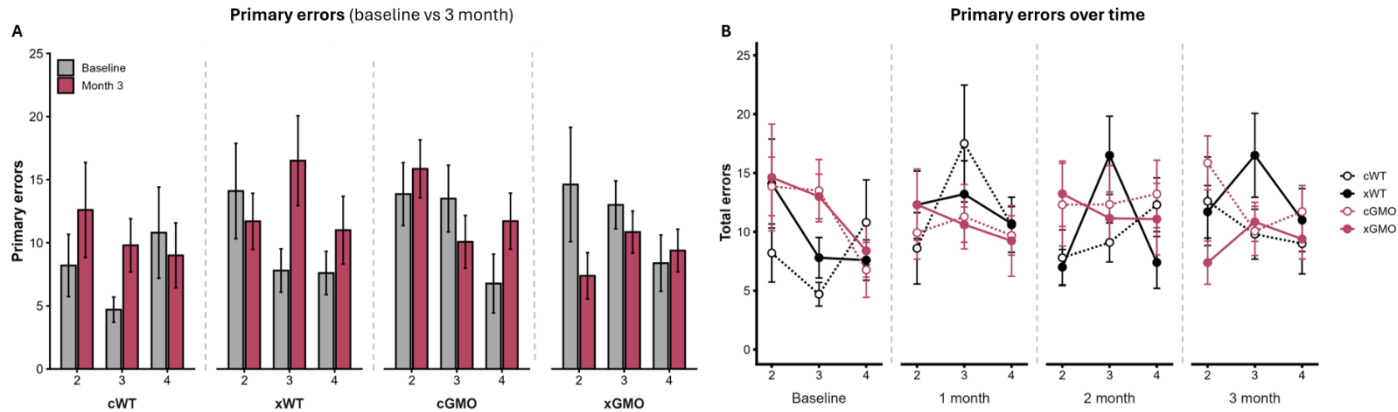


Figure 28. (A) Primary error difference between baseline and the third month. (B) Primary error changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B).

A significant effect of time ($F(3,467) = 34.309$, $p < 0.001$) and learning days ($F(2,467) = 7.637$, $p < 0.001$) were observed on the total number of errors. The interaction between time $\times$ learning days was also significant ($F(6,467) = 4.159$, $p < 0.001$). Additionally, a significant decrease in errors from baseline was observed in all groups. The cWT ($p = 0.002$) and cGMO ($p < 0.001$) groups had fewer errors from the first month. The xWT group showed a decrease from the second month, while xGMO showed a decrease from the first month (Figure 29B). Within-groups analysis revealed a decrease in total errors in all groups after the third month on the second learning day ($p < 0.001$). During the third learning day, a significant decrease was found in both cGMO ($p = 0.011$) and xGMO ($p = 0.004$) groups. No significant changes were observed during the fourth day in all groups. Only a trend in error decrease was observed in the xGMO group ($p = 0.068$). All groups changed similarly over the months (Figure 29B).

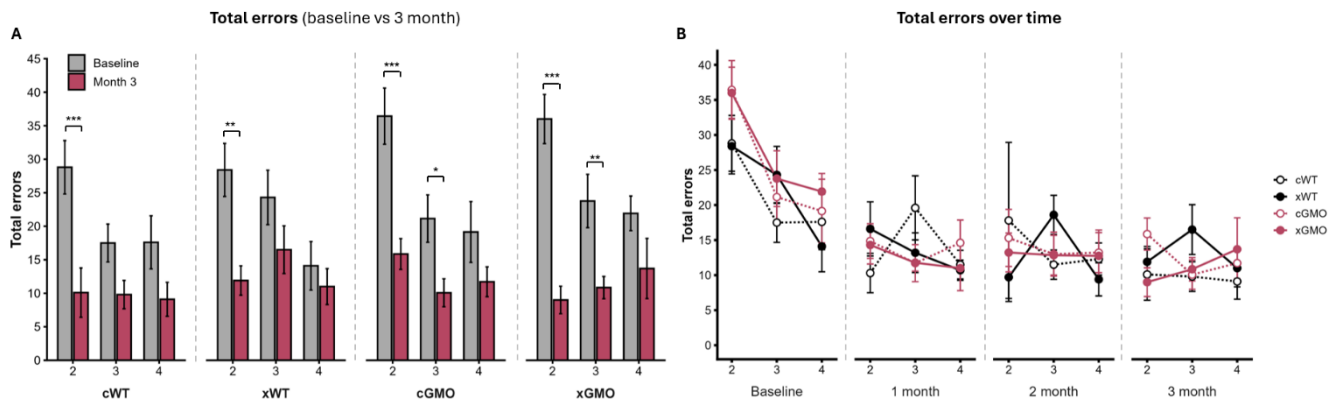


Figure 29. (A) Total error difference between baseline and the third month. (B) Total error changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Search strategy changes throughout the experiment were searched individually. There was a significant change between groups: in cWT ($p = 0.009$), xWT ($p < 0.001$), cGMO ($p = 0.005$) and xGMO ($p = 0.005$). In cWT group, during baseline measurements, the most common search strategy was random, which was used by 43.33 % of mice. During the third month, it changed to a serial strategy, used by 53.3 % mice (Figure 30). Both serial 2 and 3 were used the most, both by 16.7 % of mice, with 7-10 / 11-15 holes in sequence before the target hole. More detailed search strategy see supplementary material (Figure S3)

In xWT during baseline measurements, the most common search strategy was correction, which was used by 60 % of mice. During the third month, it changed to a serial strategy, used by 40 % mice (Figure 30). Serial 3 was mostly used by 13.3 %, with 11-15 holes in sequence before the target hole (Figure 30).

Additionally, in cGMO during baseline measurements, the most common search strategy was random, which was used by 47.6 % of mice. During the third month, the most common strategies were random (42.9 %) and serial (47.6 %) (Figure 30). Serial 3 was mostly used by 16.7 %, with 11-15 holes in sequence before the target hole (Figure 30).

In xGMO during baseline measurements, the most common search strategy was random, which was used by 43.6 % of mice. During the third month it changed to serial strategy, used by 59 % mice (Figure 30). The most used serial strategy was short serial by 20.5 %, with 3-4 holes in sequence before the target hole (Figure 30).

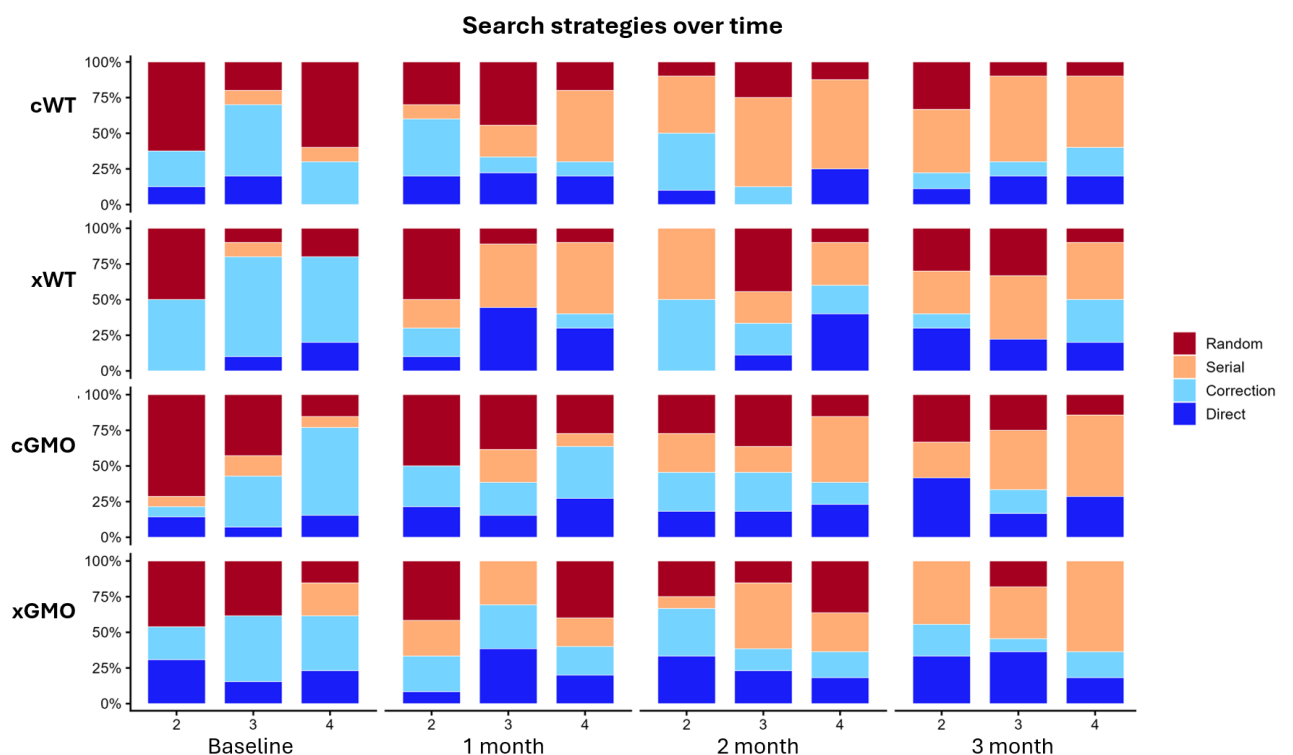


Figure 30. Search strategies between groups during the probe phase of Barnes maze

3.5.2. Probe phase

Extreme outliers were not detected in the probe phase of the Barnes maze test. However, 1 mouse from the cGMO group was excluded from the baseline, and 1 mouse from the xGMO group was excluded from 1 month. Additionally, 1 mouse from the cGMO and 2 mice from xGMO group were excluded at 2 months, and 1 mouse from the cGMO group at 3 months. These mice were excluded from further analysis due to a lack of active search and staying in one place.

No significant differences were found between groups in baseline primary latency, primary errors, time spent in the target quadrant, total errors and visits to the target hole. Linear mixed model analysis revealed no significant differences between groups in both primary latency and primary errors. A significant increase in primary errors was observed during the first month in the xGMO group ($p = 0.014$) (Figure 31D). All groups changed similarly over time, with no significant differences in within-group or between-group analyses (Figure 31A-D).

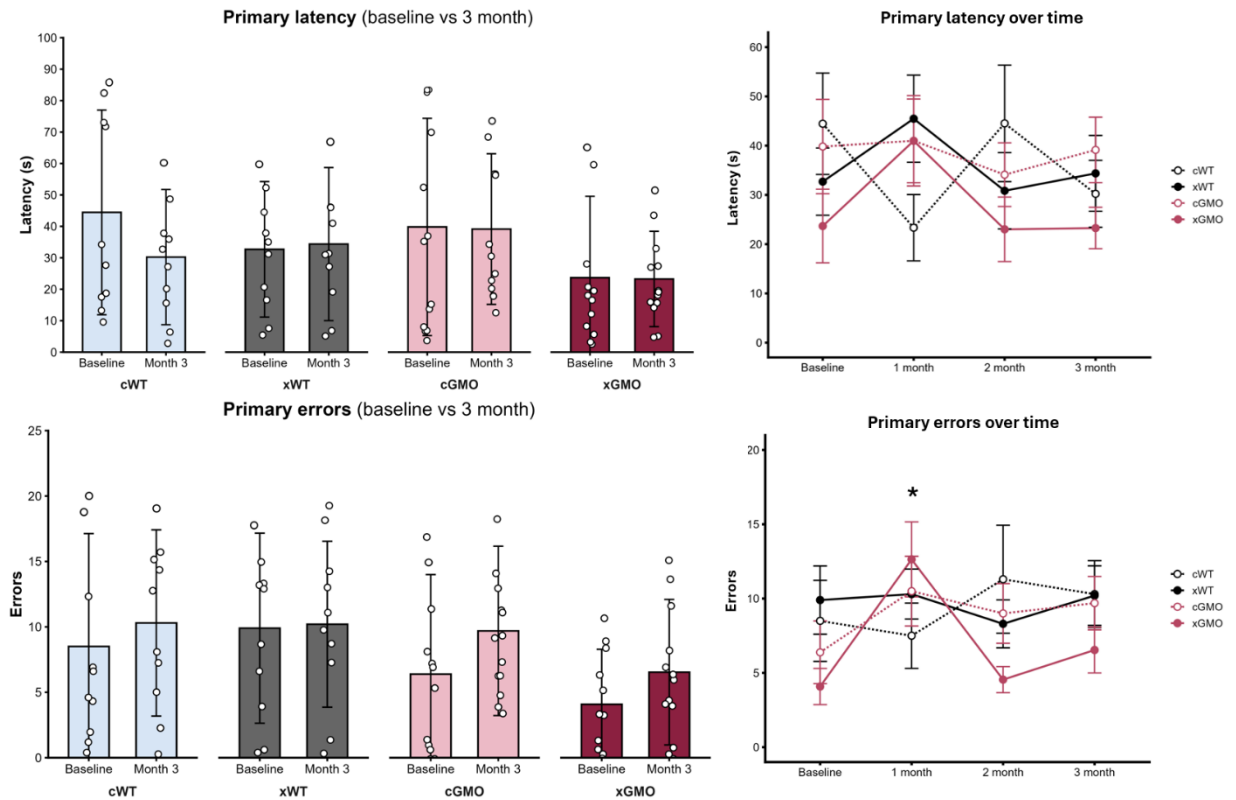


Figure 31. (A) Primary latency difference between baseline and the third month. (B) Primary latency changes between groups throughout the study. (C) Primary error difference between baseline and the third month. (D) Primary error changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), * $p < 0.05$.

A significant effect of time was observed in time spent in the target quadrant ($F(3,124) = 3.164$, $p = 0.027$). Additionally, a trend was observed between time $\times$ diet interaction ($p = 0.065$). A significant decrease in time spent in the target quadrant was observed during the first month in the xGMO ($p = 0.008$) and xWT ($p = 0.004$) groups. However, no significant differences or changes were found between and within groups across the experiment (Figure 32A-D).

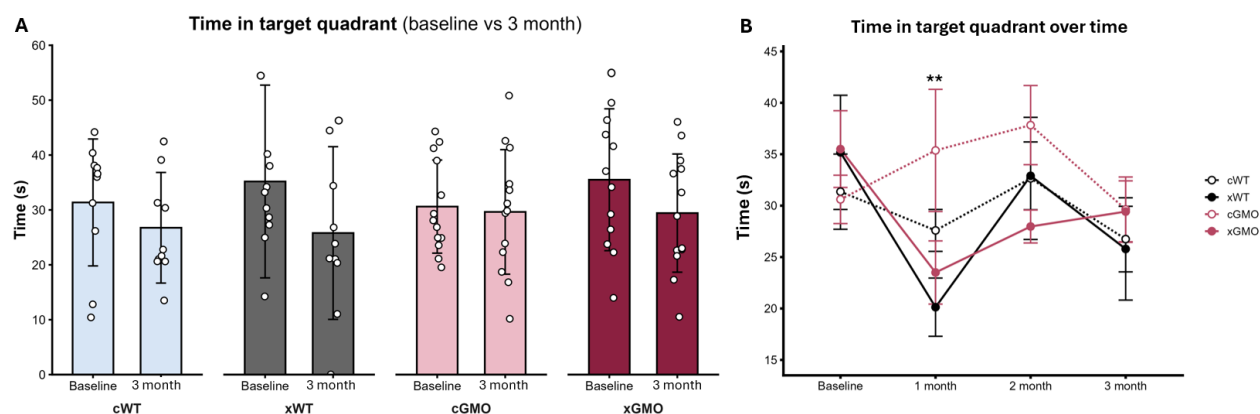


Figure 32. (A) Time spent in the target quadrant differences between baseline and the third month. (B) Time spent in the target quadrant changes between groups throughout the study. Data is presented as mean $\pm$ SD (A) and mean $\pm$ SEM (B), ** $p < 0.01$.

Strategies significantly changed only in the cGMO ($p = 0.011$) group. The use of the serial strategy increased by 53.8 % ($p = 0.001$), while the correction strategy decreased by 46.2 % ($p = 0.009$) from baseline to the last month. The most commonly used strategy was serial 1 (by 42.9 %) (Figure 29). For a more detailed search strategy, see supplementary materials (Figure 33). More detailed search strategy see supplementary material (Figure S4)

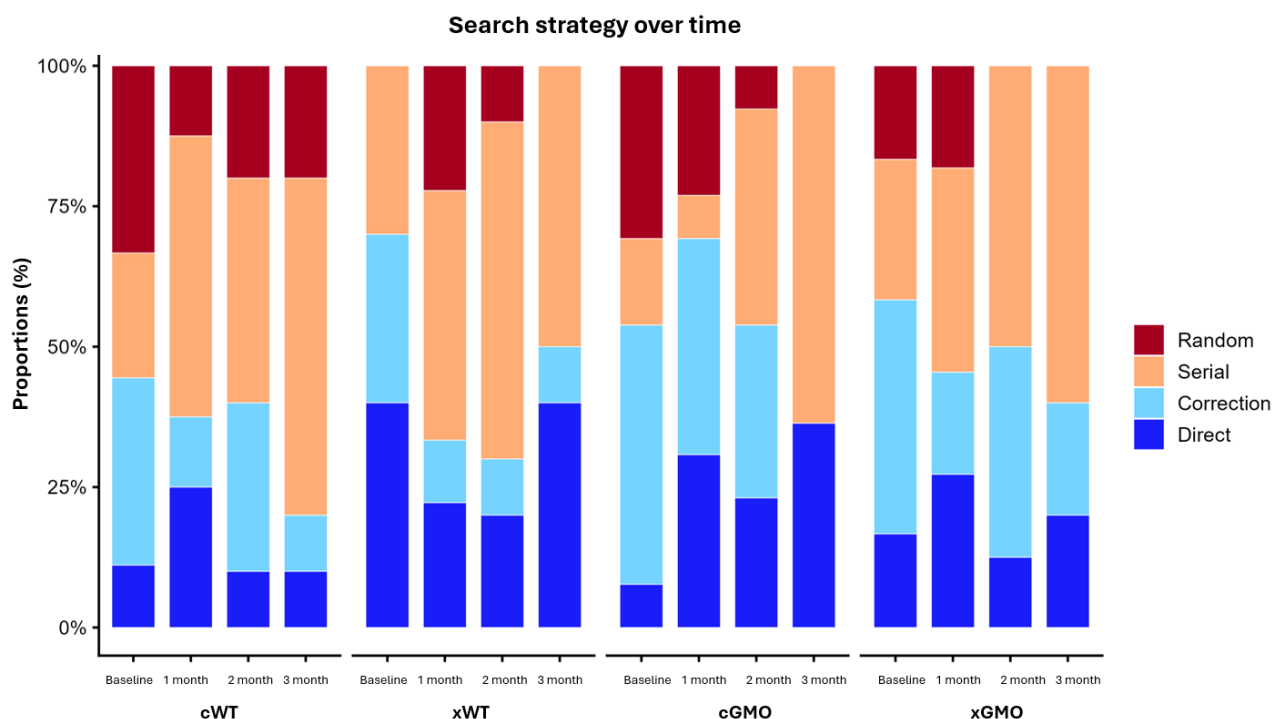


Figure 33. Search strategies between groups during the probe phase of Barnes maze.

In summary, both GMO groups had larger baseline primary latency during the third learning day than cWT group. During the third month on the fourth learning day, cWT had a significant decrease, while cGMO on the second learning day had an increase in primary latency. Similarly, total latency decreased in all groups throughout the experiment. Additionally, total errors significantly decreased in the WT groups on the second day, while in the GMO groups on the second and third days. Finally, during the probe phase, no differences were found in primary latency, errors and time spent in the target quadrant.

DISCUSSION

Dementia is the seventh leading cause of death worldwide. The most common form of dementia is Alzheimer's disease (AD), responsible for 60 – 80 % of all dementia cases (Wei et al., 2023). The development of AD is associated with the interaction of different modifiable and non-modifiable risk factors, including systemic inflammation, chronic health conditions, lifestyle and environmental factors (Guo et al., 2020).

In recent years, research has increasingly focused on the role of diet in the development and progression of AD. Suggesting that unhealthy dietary habits increase systemic inflammation, oxidative stress and dysbiosis, causing neuroinflammation leading to neurodegeneration (McGrattan et al., 2019). In contrast, diets enriched with antioxidants, B vitamins, vitamin E, polyunsaturated fatty acids and healthy fats have been shown to have neuroprotective effects (Stefaniak et al., 2022). Despite significant advances, the potential for an effective preventive approach remains unclear. Therefore, our longitudinal study aimed to investigate the effects of a functional diet on cognition and behaviour in the AD mouse model. The functional diet was similar in composition to the Mediterranean diet, as it was high in antioxidants, polyphenols and B vitamins.

Body weight was monitored throughout the study to assess the potential changes. At baseline, GMO mice were significantly lighter than the WT groups. However, during the third month, the functional diet significantly increased body weight in both the WT and GMO groups, resulting in greater weight gain throughout the study than in the control diet groups. Additionally, the GMO group fed a control diet had significant weight gain. Similarly, Jiang et al. found that their APP/PS1 group fed a control diet had an increased body weight, while the group that consumed Ketogenic diet had a reduced body weight (Jiang et al., 2023). This GMO mice weight gain may be associated with the development of metabolic syndrome, which increases adipose tissue, a common characteristic of the APP/PS1 mouse model (Webster et al., 2013). On the contrary, an experiment conducted by Connell et al. found that a Mediterranean inspired diet did not increase body weight in 5xFAD over a 12-week data intervention (Connell et al., 2026). In addition, Park et al. observed increased food intake in their APP/PS1 mouse model starting from the third week of the Mediterranean-ketogenic diet study (Park et al., 2024). Our study did not track food intake, which could be considered a limitation. Thus, it is difficult to determine whether the increase in body weight was caused by the functional food itself or by overconsumption.

To assess physical activity, a running wheel test was used. At baseline, GMO groups had more rotations than WT groups. Their activity index was also higher, indicating higher night activity, which is typical in rodents. The increased overall activity in APP/PS1 mice may be related to genotype, as this model often shows hyperactivity (Tulloch et al., 2021). Additionally, at the end of the study,

GMO mice had more total rotations than both WT groups. However, only the control fed diet groups had an increase in total rotations. Both control diet groups became significantly more active at night. Finally, both functional diet groups had a lower activity index at the end of the study, indicating a decrease in night rotations. Although the activity index decreased and body weight increased in the functional diet groups. Additional analysis revealed that body weight was not associated with behavioural changes or decreased activity. This change in activity could also be related to familiarity with the task, which could change overall activity patterns.

Moving on to the Y-maze, it was used to assess spatial working (short-term) memory. At baseline, the xWT group had fewer arm entries and fewer incorrect alternations than both GMO groups. Similarly, xGMO had more arm visits than the xWT group, and more correct alternations than cWT group. However, the xWT group had more spontaneous alternations, indicating better spatial working memory. During the third month, a significant decrease in arm entries and visits, with decreased correct and incorrect alternations, was observed in both GMO groups. This was the largest decrease among other groups. On the contrary, Jiang et al. found no differences in arm entries between dietary groups or genotype (Jiang et al., 2023). Additionally, a significant increase in spontaneous alternation was only observed in the control fed diet GMO group. Since spontaneous alternation reflects choices rather than overall activity, this increase may have been related to more accurate arm choices and a possible improvement in spatial working memory. However, no significant effect of diet was found. This result indicates that the control diet did not affect spatial working memory. The spontaneous alternation increase in cGMO could be related to the learning effect or habituation to the task. Similarly, Park et al. used the T – test to assess spatial working memory and found no effect of the Mediterranean-ketogenic diet on spontaneous alternation (Park et al., 2024). In contrast, Jiang et al. found that the ketogenic diet increased spontaneous alternation in the APP/PS1 group compared to the control diet APP/PS1 group (Jiang et al., 2023). Therefore, these results suggest that working memory deficits could be potentially reduced by a ketogenic diet alone, rather than by functional or Mediterranean-ketogenic diets.

Long-term memory and object recognition were assessed using the novel object recognition test. During the baseline habituation phase, all groups spent similar amounts of time in the central and peripheral zones. In addition, the distance traveled differed significantly between the groups, with WT mice traveling a shorter distance than GMO mice. Similarly, Tulloch et al. observed in APP/PS1 significantly longer traveled distance than the control group, indicating hyperactive behaviour (Tulloch et al., 2021). During the third month, in xGMO, cGMO and xWT groups, time spent in the central zones significantly decreased, while time spent in the peripheral zones increased, indicating increased anxiety-like behaviour. In contrast, Park et al. found that in APP/PS1 the Mediterranean-ketogenic diet increased time spent in the center zones, indicating lower anxiety-like behaviour (Park

et al., 2024). This may be caused by components of the ketogenic diet that influence ketone production, which can be used as an alternative energy source in neurons when glucose metabolism is impaired during AD pathology. Additionally, the distance traveled significantly decreased in all groups. The largest decrease was observed in the GMO group fed the functional diet. A significant diet $\times$ time interaction indicates that the change in locomotor activity over time differed between the dietary groups. While a significant genotype $\times$ diet interaction indicates that the effect of the functional diet depended on genotype. These results suggest that the functional diet affected locomotor activity, with the effect depending on genotype. However, all groups had a decreased locomotor activity over time, which may be age related natural decrease in physical activity or habituation to the test. At the baseline testing phase, total exploration time and the discrimination index did not differ significantly between groups, indicating similar exploration behaviour and recognition memory. Although exploration decreased in all groups, the change was similar throughout the experiment. However, no difference was found in the discrimination index. Furthermore, no significant differences were found between groups, indicating that all groups changed similarly. In contrast, Park et al. found that the Mediterranean-ketogenic diet influenced exploratory behaviour compared to the control group (Park et al., 2024). This diet had a greater impact on exploration than our functional diet, which may be due to the diet containing ketogenic diet components that induce ketosis. Overall, our results indicate that object exploration decreased over time, with no effects of diet. These changes may be related to test habituation, reduced novelty effects and a general decrease in physical activity over time, which led mice to be less explorative towards objects, resulting in a reduced discrimination index.

The Barnes maze was used to assess spatial (long-term) memory and learning. At baseline learning phase the control diet fed WT group found the target hole significantly faster with fewer errors than the GMO groups. During the third month, latency to find the target hole was significantly longer in cGMO group than in the cWT, xWT, and xGMO groups. Additionally, total latency, defined as the time to enter the target hole, showed significant effects of time and days of learning, decreasing significantly in all groups, indicating that mice became increasingly quicker to enter the target hole. During each month, latency was highest on the second day of learning and decreased significantly on the third and fourth days, confirming a persistent learning effect throughout the study. In addition, a significant effect of time and days of learning was observed on the total number of errors, suggesting that mice may become more efficient at the task. The total number of errors decreased similarly across all groups, and no significant differences were observed between groups, indicating that all mice learned the task regardless of genotype or diet. At baseline probe phase, all groups were similar in primary latency and errors, and time spent in the target quadrant. During the third month, groups did not differ significantly in baseline primary latency, errors or time spent in the target quadrant,

indicating that memory was similar across all groups. On the contrary, in a study conducted by Kim et. Al, found that 5XFAD supplemented with theracurmin, a highly bioavailable formulation of natural polyphenol with potent antioxidant curcumin, spent significantly more time in the target quadrant, which indicated better spatial learning memory (J. Kim et al., 2019). Additionally, significant changes in strategies were detected only in the cGMO groups. In these mice, correction strategies were replaced by serial strategies over time, which may indicate a more organised and efficient search. These results show that all groups successfully learned the task, as latency and errors decreased during learning days throughout the study. All groups showed similar changes, indicating no clear functional food effect on cognition and behaviour in spatial learning and long-term memory.

All behavioural test parameters showed a time effect, indicating that each parameter changed over time regardless of group. Most parameters showed a genotype effect, indicating that WT and GMO genotypes differed. Additionally, the genotype $\times$ time interaction was present, indicating that genotypes changed differently over time. While interaction between diet $\times$ time indicated that each diet changed differently over time. However, the main genotype, diet and time interaction was not significant in any of the behavioural tests, suggesting that functional food did not affect behaviour and memory. Therefore, no visible neuroprotective effect of a functional diet on behaviour and cognition was observed. In contrast, a longitudinal experiment performed by Jiang et al. 2023, with similar behavioural tests and methodology, found that a ketogenic diet consumed for three months slowed down APP/PS1 learning and memory decline progression (Jiang et al., 2023). The lack of a significant effect of the functional diet in this study may be due to differences in the composition of the dietary intervention compared to previous studies. Different concentrations of biologically active components and their ratios may have been resulted in weaker or insufficient effects on cognitive function and behaviour. Additionally, it is possible that the APP/PS1 mice used in this study had not yet developed neuropathology, with behavioural and cognitive decline. As a result, the potential effect of the functional diet may have remained undetected. Although no significant changes were detected, the diet may have had a positive effect on AD molecular mechanisms, such as reducing neuroinflammation, oxidative stress or A β levels. Therefore, the combination of behavioural and molecular analysis would be more accurate in detecting the effect of diet on AD progression.

CONCLUSIONS

1. The functional diet increased body weight in both WT and GMO mice groups;
2. The functional diet reduced the physical activity index in both WT and GMO mice groups, indicating a shift toward increased daytime activity;
3. The functional diet did not affect spatial working (short-term) memory in WT and GMO mice groups;
4. The functional diet did not influence long-term memory and object recognition in WT and GMO mice groups;
5. The functional diet showed no impact on spatial (long-term) and learning in WT and GMO mice groups.

VILNIUS UNIVERSITY
LIFE SCIENCES CENTER

Karolina Krivičiūtė

Master thesis

**Effects of a Functional Diet on Cognitive Function and Behaviour in
Alzheimer's disease Mouse Model**

ABSTRACT

Dementia is one of the most severe clinical symptoms of neurodegeneration, consisted of 60 – 80 % of Alzheimer's disease patients. This disease is mostly characterised by extracellular accumulation of amyloid beta plaques, intracellular aggregation of neurofibrillary tangles, synaptic and neuronal loss, which cause memory loss, cognitive decline, language difficulties, behavioural changes, apathy and hallucinations. The development is multifactorial, caused by the interaction of genetic, environmental and lifestyle factors. Research suggests that nutrition plays a significant role in the development of neurodegenerative diseases. Specialised diets, enriched with antioxidants, B vitamins, vitamin E, polyunsaturated fatty acids, and healthy fats, may have neuroprotective effects, potentially slowing the progression of Alzheimer's disease.

Therefore, this study aimed to investigate the effects of a functional diet on cognitive function and behaviour in the Alzheimer's disease mouse model. The objectives of this thesis were to analyse the effect of a functional diet on mice body weight, physical activity (using the running wheel test), spatial working (short-term) memory (using the Y-maze), long-term memory and object recognition (using novel object recognition test) and spatial learning and memory (using Barnes maze).

Twenty wild type (WT) C57BL/6 and twenty-seven transgenic (GMO) APP/PS1 mice were used in this study. All mice were fed the same control diet till 7 months of age. Later, they were randomly assigned to dietary groups: Control wild type group receiving standard food (cWT, n = 10); Experimental wild type group receiving functional food (xWT, n = 10); Control transgenic group receiving standard food (cGMO, n = 14); Experimental transgenic group receiving functional food (xGMO, n = 13). Behavioral measurements were taken four times: at the end of each month. Mice behavior and physical activity were tested one month before and three months after dietary intervention. Mice body weight was monitored weekly throughout the study.

Overall, the results showed that the functional diet significantly increased body weight, while reducing the activity index in both the WT and the GMO groups. Additionally, functional diet did not have any significant effects on short-term working memory, spatial learning and long-term memory.

VILNIAUS UNIVERSITETAS
GYVYBĖS MOKSLŲ CENTRAS

Karolina Krivičiūtė

Magistro baigiamasis darbas

Funkcinės dietos poveikis pažintinėms funkcijoms ir elgesiui Alzheimerio ligos pelių modelyje

SANTRAUKA

Demencija yra viena iš sunkiausių neurodegeneracijos klinikinių simptomų, iš kurių 60 – 80 % sudaro Alzheimerio ligos atvejai. Šiai ligai būdingas amiloido plokštelių kaupimasis, neurofibrilinių raizginių agregacija ir sinapsių bei neuronų nykimas smegenyse, kurie sukelia atminties ir pažinimo sutrikimus, elgesio pokyčius, apatiją ir haliucinacijas. Alzheimerio liga išsivysto dėl daugybinių faktorių, genetinių aplinkos ir gyvenimo būdo veiksnių sąveikos. Tyrimai rodo, kad mityba vaidina svarbų vaidmenį neurodegeneracinių ligų vystymuisi. Specializuota dieta, praturtinta ankstioksidantais, B grupės vitaminais, vitaminu E, polinesočiosiomis riebalų rūgštimis ir sveikaisiais riebalais galimai turi neuroprotekcinį poveikį, galinti sumažinti Alzheimerio ligos progresavimą.

Tyrimo tikslas buvo ištirti funkcinės dietos poveikį pažintinėms funkcijoms ir elgesiui Alzheimerio ligos pelių modelyje. Iškelti darbo uždaviniai buvo skirti išanalizuoti funkcinės dietos poveikį kūno svorio pokyčiams, pelių fiziniam aktyvumui (naudojant bėgimo rato metodą), trumpalaikiai darbinei atminčiai (taikant Y-labirinto testą), erdviniam mokymuisi ir erdvinei atminčiai (taikant Barnes labirinto testą) bei ilgalaikiai atminčiai (taikant naujo objekto atpažinimo testą).

Šiame tyrime buvo panaudotos dvidešimt laukinio tipo (WT) C57BL/6 ir dvidešimt septynios transgeninės APP/PS1 (GMO) pelės. Visos pelės buvo maitinamos tuo pačiu kontroliniu pašaru iki 7 mėnesio amžiaus. Vėliau jos buvo atsitiktinai suskirstytos į mitybos grupes: kontrolinė laukinio tipo grupė gaunanti standartinę dietą (cWT, n = 10); eksperimentinė laukinio tipo grupė gaunanti funkcinę dietą (xWT, n = 10); kontrolinė transgeninė grupė gaunanti standartinę dietą (cGMO, n = 14), eksperimentinė transgeninė grupė gaunanti funkcinę dietą (xGMO, n = 13). Gyvūnų elgsenos tyrimai buvo atliekami keturis kartus: periodiškai vieną kartą per mėnesį. Pelių grupės buvo tiriamos vieną mėnesį prieš ir tris mėnesius po dietos pakeitimo. Pelių kūno svoris buvo fiksuojamas kas savaitę viso tyrimo metu.

Rezultatai parodė, kad funkcinė dieta reikšmingai padidino kūno svorį, tuo pačiu sumažindama aktyvumo indeksą tiek WT, tiek GMO pelių grupėse. Be to, funkcinė dieta neturėjo reikšmingo poveikio trumpalaikiai darbinei atminčiai, erdviniam mokymuisi ir ilgalaikiai atminčiai.

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SUPPLEMENTARY MATERIAL

Table S1. Nutritional list of a standard diet (C 1000 control diet for rats and mice)

Standard diet		
Metabolized energy		
Fat	457 (13%)	kcal/kg
Protein	691 (20%)	kcal/kg
Carbohydrates	2,358 (67%)	kcal/kg
Crude nutrients and moisture		
Moisture	82,540 (8.3%)	mg/kg
Crude ash	54,846 (5.5%)	mg/kg
Crude fiber	30,970 (3.1%)	mg/kg
Crude fat	50,830 (5.1%)	mg/kg
Crude protein	172,650 (17.3%)	mg/kg
Nitrogenfree extractives	608,164 (60.7%)	mg/kg
Carbohydrates		
Monosaccharides	0	mg/kg
Disaccharides	98,105	mg/kg
Polysaccharides	471,727	mg/kg
Minerals		
Calcium	9,311	mg/kg
Potassium	7,089	mg/kg
Magnesium	684	mg/kg
Sodium	2,488	mg/kg
Phosphorus	7,523	mg/kg
Trace elements		
Aluminium	3.71	mg/kg
Chlorine	3,630.00	mg/kg
Iron	178.58	mg/kg
Flourine	4.17	mg/kg
Iodine	0.51	mg/kg
Cobalt	0.15	mg/kg
Copper	5.75	mg/kg
Manganese	100.89	mg/kg
Molybdenum	0.20	mg/kg
Sulfur	2,791.54	mg/kg
Selenium	0.33	mg/kg
Zinc	29.30	mg/kg
Added vitamins		
Vitamin A	15,000	IU/kg
Vitamin D3	500	IU/kg
Vitamin E	180	mg/kg
Vitamin K3	10	mg/kg
Vitamin B1	20	mg/kg
Vitamin B2	20	mg/kg
Vitamin B6	15	mg/kg
Vitamin B12	41	µg/kg
Nicotinic acid	50	mg/kg
Pantothenic acid	50	mg/kg
Folic acid	10	mg/kg
Biotin	201	µg/kg
Choline chloride	1,012	mg/kg
Vitamin C	20	mg/kg
Amino acids		
Alanine	2,528	mg/kg
Arginine	9,829	mg/kg
Aspartic acid	3,583	mg/kg
Cystine	3,196	mg/kg
Glutaminc acid	23,675	mg/kg

Amino acids		
Glycine	3,136	mg/kg
Histidine	5,276	mg/kg
Isoleucine	7,223	mg/kg
Leucine	14,763	mg/kg
Lysine	17,401	mg/kg
Methionine	7,223	mg/kg
Phenylalanine	7,172	mg/kg
Proline	12,763	mg/kg
Serine	5,268	mg/kg
Threonine	7,154	mg/kg
Tryptophan	1,977	mg/kg
Tyrosine	9,285	mg/kg
Valine	3,296	mg/kg
Fatty acid		
Arachidic acid C-20:0	50	mg/kg
Eicosanoic acid C-20:1	150	mg/kg
Alpha-Linolenic acid C-18:3	150	mg/kg
Linolenic acid C-18:2	28,500	mg/kg
Palmitic acid C-16:0	2,500	mg/kg
Stearic acid C-18:0	1,350	mg/kg
Oleic acid C-18:1	13,500	mg/kg

Table S2. Nutritional list of a functional diet

Functional diet		
Component name	Active substance, %	Biologically active compounds
Bluebell grass (<i>Bacopa monnieri</i>) dry extract (>40% bacosides)	40	Bacosides
Rosemary (<i>Rosmarinus officinalis</i>) CT cineol essential oil	30	1,8-cineole (eucalyptol)
Vitamin B12 (methylcobalamin)	97	Methylcobalamin
Vitamin B6 (pyridoxine hydrochloride)	82	Pyridoxine hydrochloride
Vitamin B9, folic acid (pteroylmonoglutamic acid)	96	Pteroylmonoglutamic acid
Rhodiola rosea root extract (>3% rosavins, >1% salidroside)	3/1	Rosavins/salidroside
Organic lion's mane (<i>Hericium erinaceus</i>) dried fruiting body extract 8:1 (15% beta-(1,3) (1,6)-glucans)	15	Beta glucans
Dry extract of Ginkgo biloba leaves (>24% flavanol glycosides and >6% terpene lactones)	24/6	Flavanol glycosides/terpene lactones
Choline bitartrate	100	Choline
Phosphatidylserine <70% (phosphatidylserine, emulsifier lecithin (E322 from soy), anticaking agent silicon dioxide (E551), antioxidant alpha tocopherol (E307))	70	Phosphatidylserine

The first four substances were nanoliposomized using the ethanol injection method by mixing with phospholipids, stabilised with the cryoprotectant sucrose and lyophilised.

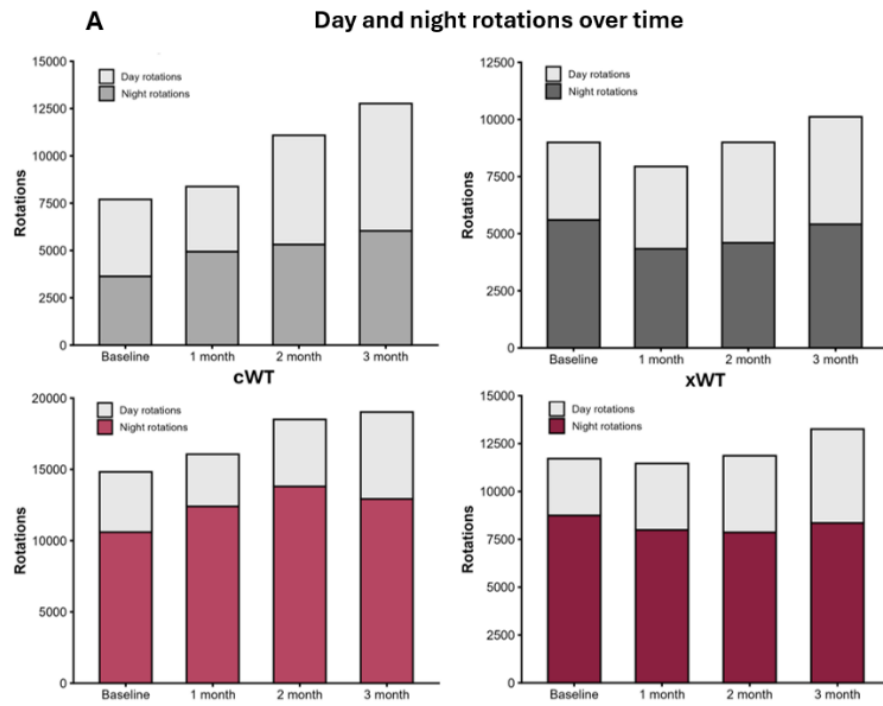


Figure S1. Running wheel total, night and day rotations

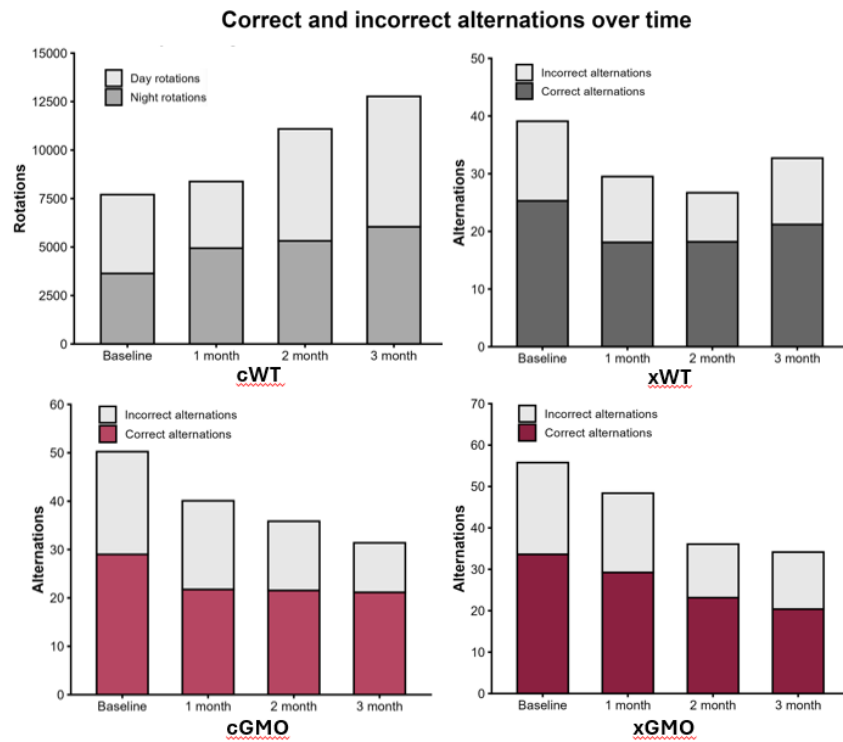


Figure S2. Y-maze total, correct and incorrect alternations

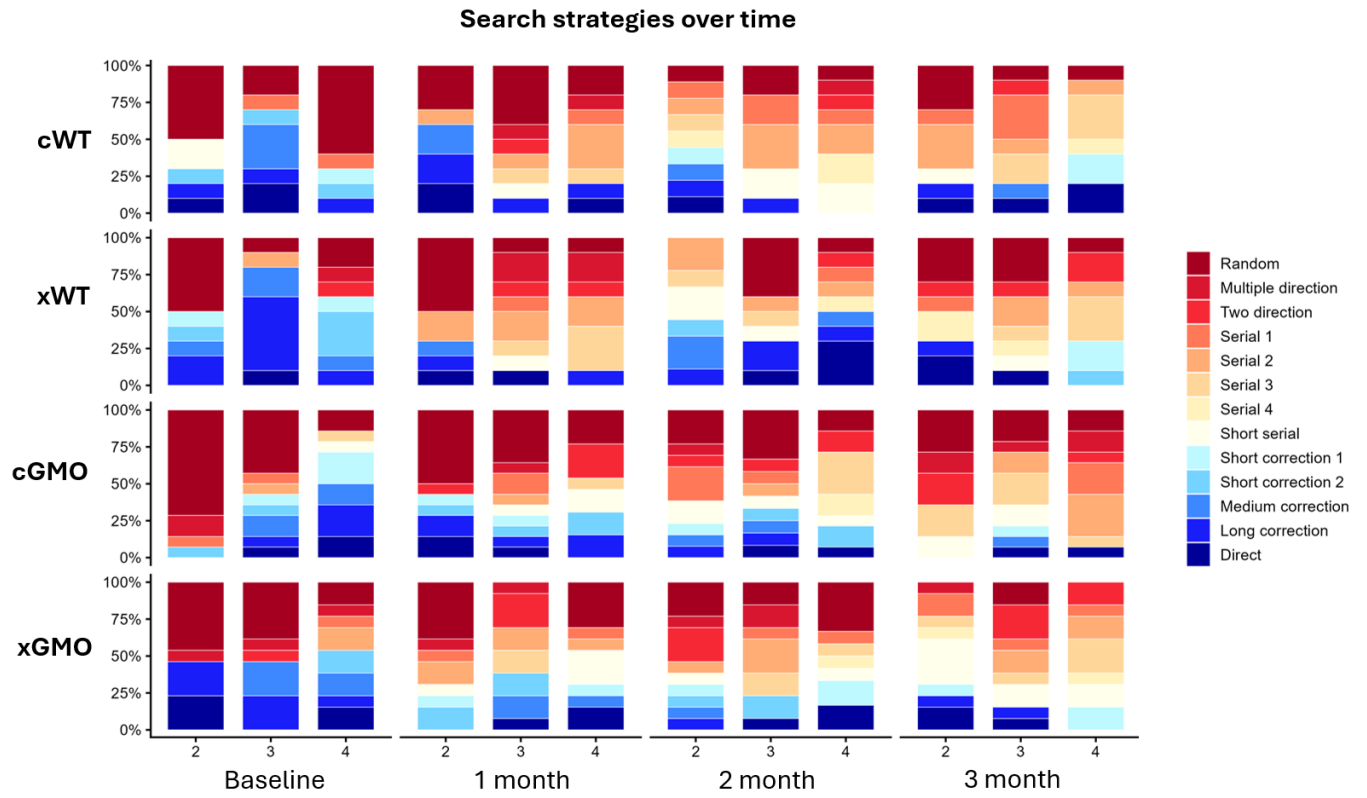


Figure S3. Detailed strategies over time between groups during learning days

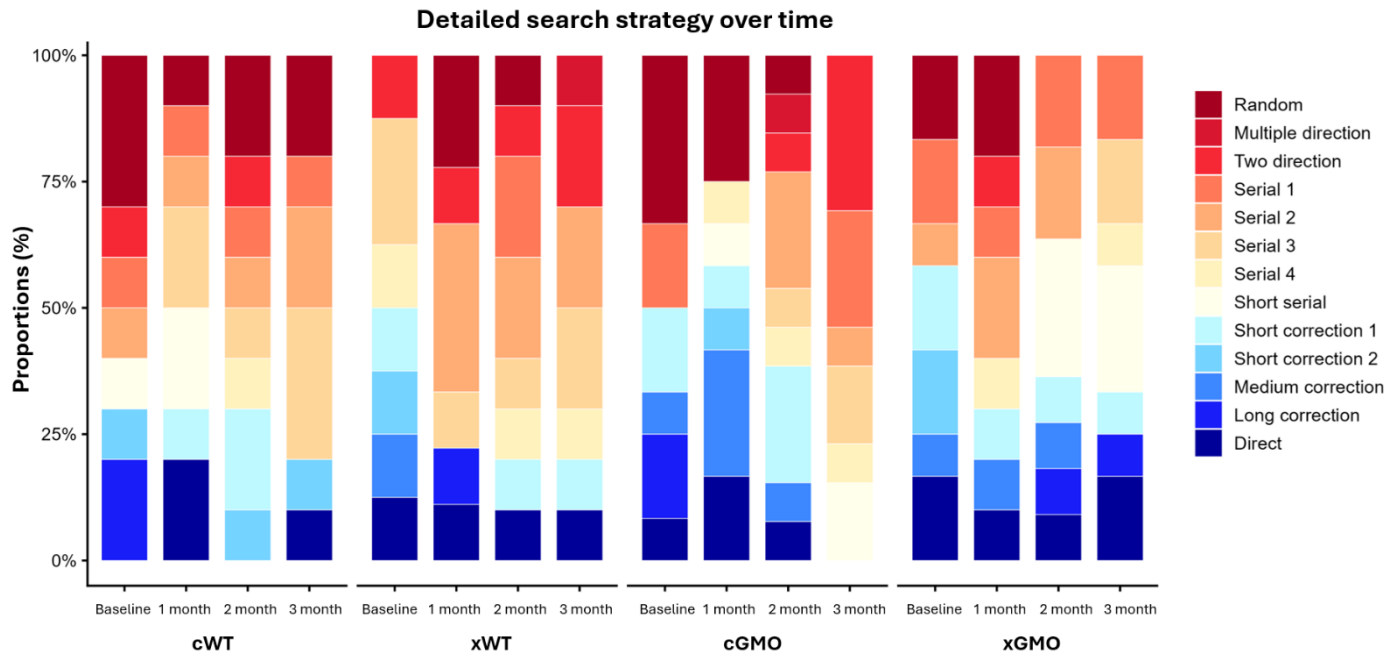


Figure S4. Detailed strategies over time between groups during probe phase