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Outclimbing Cognitive Decline: Age, Western Diet, Resistance Exercise, and the Brain.

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Abstract

Increased age and obesity diminish motivation, perseverance, and spatial memory function. Aerobic exercise interventions have successfully rescued some of these processes. However, in older and heavier populations aerobic exercise is not as sustainable due to high risk of injury. Resistance exercise consists of physical activity where maximum oxygen consumption is not increased and has been proposed as a safe and effective intervention for this population. Here, we used touchscreen-based cognitive testing to elucidate the influence of resistance exercise on motivation and spatial memory in aged, diet-induced obese mice, using a water restriction procedure I develop in Chapter 2. Mice underwent a ladder-based resistance exercise intervention. Obese and exercised mice were significantly more motivated in the progressive ratio touchscreen test of motivation than were non-exercised obese mice. Furthermore, exercised mice performed significantly better on a test of spatial memory. These findings suggest that resistance exercise is effective at rescuing cognition in older, overweight mice.

Keywords Spatial memory and learning, resistance exercise, motivation, perseverance, obesity, Western diet

Lay Summary

Our population is becoming older and heavier. Both increased age and adiposity act as risk factors for many chronic illnesses and present a heavy strain on the healthcare system. In the Western world, structural changes in our physical environment exacerbate the risk of increased adiposity and obesity. Our widespread consumption of what has been coined the "Western diet" – a diet high in processed sugars and fats – has been associated with many negative health outcomes. Aerobic exercise, like running and cycling, has been shown to decrease adiposity and decrease incidence of chronic illnesses. However, high impact exercise such as running and cycling is not always sustainable in this population since they are at a higher risk of falls and heart attacks. Resistance exercise is a lower impact alternative to aerobic exercise. It increases functional strength, bone density, and has led to reduction of some cognitive impairments in recent studies. We wanted to test whether a resistance exercise intervention was able to reduce impairment in motivation, perseverance, and spatial memory and learning in a population of middle-aged mice with diet induced obesity. The cognitive testing was completed in Bussey-Saksida touchscreen chambers that motivate mice to engage in tests of cognition through food restriction and appetitive rewards. Food restriction is not feasible in a model of diet-induced obesity; therefore, we established a water manipulation protocol using 2% citric acid water that reliably motivated completion of a test of learning and memory in groups consuming both standard chow and high-fat, high-sugar chow. The mice that completed an 8-week ladder-based exercise intervention were more motivated, had greater perseverance, and had improved spatial memory function than their non-exercised counterparts.

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List of abbreviations

ABETT – Animal Behaviour Environment Test

ACVS – Animal Care and Veterinary Services

ADHD – Attention-Deficit/Hyperactivity Disorder

ATP – Adenosine Triphosphate

BDNF – Brain Derived Neurotrophic Factor

BMI – Body Mass Index

CA – Citric Acid

CCAC – Canadian Council of Animal Care

CLGI – Chronic Low-Grade Inflammation

CRP – C-Reactive Protein

D2 – Discrimination Ratio

DIO – Diet Induced Obesity

F-R – Food Restricted

FR – Fixed Ratio

HFD – High Fat Diet

HFHS – High Fat, High Sugar

ITI – Inter-trial Interval

MWM – Morris Water Maze

NOR – Novel Object Recognition

PP1 – Protein Phosphatase 1

PR – Progressive Ratio

PVD – Pairwise Visual Discrimination

RES – Ladder-Based Resistance Exercise

SEM – Standard Error of the Mean

SIRT1 – Sirtuin 1

SLR – Spontaneous Location Recognition

WD – Western Diet

Chapter 1: General introduction

Humans' physiological health and cognitive function are largely shaped by their immediate physical and social environments (Malik et al., 2013). Rapid urbanization stemming from economic growth and globalization in many countries has resulted in an increase in consumption of sugar-sweetened beverages per capita, expansion of fast-food chains, and reductions in physical activity (Malik et al, 2013), resulting in increased adiposity (Kopp 2022). Urbanization and policy changes directly affect factors such as the affordability and accessibility of fresh fruits and vegetables (Cutlet et al., 2003). In addition to reduced availability of affordable healthy food, urbanization can result in limited opportunities for outdoor leisurely physical activity and increased reliance on motorized transportation systems instead of primary transportation by bicycle or foot (Malik et al., 2013). Childhood experiences in these types of environment further shape lifelong social behaviours that continue through adulthood and old age, such as the normalization of more time spent sedentary at home watching television, decrease in sleep duration and quality due to noise pollution, and increased consumption of obesogenic foods (Siegal et al., 2008).

Individuals in high stress, high pace cities and occupations may be more prone to consuming low nutritional density, high calorie diets (Ruigrok et al., 2021). Stress has long been associated with overeating in both males and females (Van Strien et al., 1986), especially in seeking highly palatable foods (Ruigrok et al., 2021). Western-style diet (WD) is defined as being high in saturated fats and refined sugars, and low in insoluble fiber and vitamins (Drewnowski, 2007). WD is highly accessible since it is easy to manufacture, ship, and store as well as being energy

dense and flavourful (Malik et al., 2013). Consumption of a WD is associated with a dramatic increase in insulin resistance, type 2 diabetes, hypertension, cancers, and mental illnesses in comparison with a more balanced diet (Lofterød et al., 2021; O'Dea et al., 1988; Kopp, W., 2020; Kopp, W., 2022; Firth et al., 2018). In rodent models consuming a high fat high sugar WD, a causal relationship has been established between WD and anxiety-like behaviour (Tsan et al., 2021), depression (Yu et al., 2021), and learning and memory deficits (Yeomans, 2017). Many of these conditions have also been associated with obesity (Yeomans, M., 2017). WD has widely been causally connected with obesity in humans (Ruigrok et al., 2021; Malik et al, 2013; Misra et al., 2009) and has been used to induce an accurate model of obesity in rodent studies that aim to further uncover the whole body and brain effects of obesity (Park et al., 2010; Attuquayefio et al., 2016; Stranahan et al., 2008; Mota et al., 2023) through a model called diet-induced obesity (DIO).

1.1 Factors in the development of obesity

Obesity is a metabolic disorder defined, in humans, as having a body mass index (BMI) > 30kg/m2 (Bortolin et al., 2018). BMI is calculated by dividing an individual's weight (kg) by height (m2) (Motil et al., 2011). Besides BMI, another reliable measurement of obesity is abdominal obesity which is quantified through measurements of waist circumference in humans (Fang et al., 2018) and visceral adiposity in rodents (Schipper et al., 2018). Obesity is a primary risk factor for many chronic diseases such as type 2 diabetes mellitus, various cardiovascular diseases, stroke, and some neurogenerative disorders (Terzo et al., 2021) which comprise a heavy

load on the healthcare system (Rimes-Dias et al., 2022). Visceral obesity is a major risk for insulin resistance, especially in aged human populations (Huffman and Barzilai, 2009).

Insulin resistance occurs due to a decrease in sensitivity to insulin in the body and is associated with dementia (Sripetchwandee et al., 2018), depression (Kan et al., 2013), schizophrenia (Guest, P., 2019), hypertension and dyslipidemia (Hardy et al., 2012), among other conditions. Sensitivity to insulin is important due to its vital role in regulating glucose levels within the body (Petersen et al., 2018). Insulin acts directly in the skeletal muscle, promoting glucose usage and synthesis of glycogen; in the liver, increasing gene expression of factors implicated in the conversion of dietary fatty acids into fat storage; and in the white adipose tissue, supressing metabolism of stored fats and increasing synthesis of new fat storage (Petersen et al., 2018). When an individual is insulin resistant, the decreased sensitivity to insulin means that higher circulating levels of insulin are necessary to successfully manage levels of glucose within the blood (Petersen et al., 2018). The actions of insulin in the body provide a compelling explanation of the primary physiological hallmarks of insulin resistance in obesity, such as increased abdominal adiposity and non-alcoholic fatty liver syndrome (Petersen et al., 2018). This increase of baseline insulin throughout the body presents a greater load on the β-cells that are responsible to produce insulin in the pancreas, which can result in deterioration of the pancreas and eventual development of type 2 diabetes (Petersen et al., 2018). This cluster of conditions, including insulin resistance, excess abdominal adiposity, and high blood sugar is called metabolic syndrome (Samson et al., 2014). Metabolic syndrome is often referred to as the “perfect storm” of physiological risk factors for the development of cardiovascular disease, diabetes, and stroke (Samon et al., 2014). Many factors can lead to insulin resistance, metabolic syndrome, and

ultimately obesity; however, it is commonly associated with unhealthy diet and lack of physical activity (Petersen et al., 2018; Cronier et al., 2008).

1.1.1 Diet and obesity

Food is an integral part of life, and it exists as much more than a simple source of calories to power the body. Following a rate of obesity that almost doubled from 2002 to 2013 (Gomes et al., 2019), Brazil published a groundbreaking dietary guideline document that actively moved away from the common food pyramid structure that simply illustrates the number of servings per each food group that is widely used in other countries (PAHO, 2014). Instead, this new and updated food guide included recommendations such: as avoid consumption of ultra-processed foods; develop, exercise, and share cooking skills; eat in the company of others; and devote time towards making cooking and eating an important part of life (PAHO, 2014). This set of recommendations was one of the first to recognize that both the biochemical makeup of the diet and the context within which it is consumed matters (PAHO, 2014).

In 2018, Statistics Canada reported that 7.3 million Canadian adults were obese (BMI >30), and 9.9 million were overweight (BMI 25-29.9) (Stats Can, 2018). The center for disease control and prevention in the USA reported that 41.9% of adult Americans were obese (CDC, 2017). The USA and Canada share a similar fast-paced lifestyle culture as well as heightened consumption of their namesake diet, the WD. Previous work showed that high intakes of saturated fatty acids, but not total fat, in individuals 65 years and older over a four-year period led to increased risk of Alzheimer's disease and mild cognitive impairment (Kalmijn et al., 2004). A subsequent study

investigating the effect of sucrose consumption in addition to standard chow in young rats demonstrated impairments in learning and memory on a novel object recognition task (Jurdak and Kanarek, 2009). Additionally, several studies demonstrated that even very short-term consumption of WD impaired spatial and working memory. For example, Murray et al (2009) fed mice WD for only 9 days and observed significantly more spatial memory errors in a radial arm maze task in comparison to controls. Kanoski and Davidson (2010) found that mice that consumed WD for only 72 hours made significantly more working memory errors when the radial maze task required learning about spatial cues, but not when spatial cues were not required.

WD has commonly been associated with the detrimental impacts of obesity in humans (Terzo et al., 2021; Hardy et al., 2012; Kopp, W., 2022; Firth et al., 2018); however, historically, adherence to diet and inaccurate self-reporting have been major hurdles in studies investigating the effects of diet (Johnson, S., 1992; Martin et al., 2000; Corral et al., 2009). In addition to hurdles in adherence and accurate reporting, understanding a physiological condition as integrated in the body and the brain at a biomolecular level is not possible without an accurate animal model. As a result, diet-induced models of obesity (DIO) have been created in rodents and have been validated utilizing measurements mirroring those used in humans to ensure translatability such as quantification of blood glucose and HbA1c (Preguiça et al., 2020). Historically, obesity based on increased body weight and body adiposity was induced in rodents through the administration of a high fat diet (HFD) (Turner et al., 2013; Preguiça et al., 2020). However, subsequent experimental evidence in the field of obesity research demonstrated that sugar consumption had become one of the primary drivers of the obesity epidemic in humans (Preguiça et al., 2020). The

effects of sugar became important due to both the number of calories it provided and the specific interactions of the macromolecules in the system, and as a result high fat-high sugar (HFHS) diet became a prominent nutritional strategy in establishing translatable DIO in mice (Morenzo-Fernandez et al., 2018; Preguiça et al., 2020). A HFHS diet is made up of calories mainly coming from fats and carbohydrates (Masi et al., 2018; Preguiça et al., 2020). HFHS models have successfully induced metabolic syndrome (Moreno-Fernandez et al., 2018), polycystic ovary syndrome (Roberts et al., 2017), impairments in spatial and social memory (Tran et al., 2017; Reichelt et al., 2019), and anxiety (Baker et al., 2016) in rodents.

1.1.2 Physical activity and obesity

Ultimately, in most instances obesity and high levels of adiposity originate due to one reason: overnutrition. Overnutrition is a nutritional imbalance that occurs from excessive consumption of nutrients that results in an unhealthy accumulation of body fat which ultimately negatively impacts health (Mathur and Pillai, 2019). Overnutrition has become commonplace due to the increase in accessibility of very calorie-dense foods and an increase in portion sizes (Mathur and Pillai, 2019). Decreases in physical activity due to increased television viewing (>3 h/day), motorized transportation, reduced time devoted to outdoor activities, and the increased mechanization of daily tasks were all associated with obesity in a study of individuals in India (Mathur and Pillai, 2019).

To combat overnutrition, caloric intake needs to be reduced to the amount required by the organism to function at a healthy level (Rooney and Ozanne, 2011). One avenue for reaching this

energy balance is by expending excess calories through physical activity (Rooney and Ozanne, 2011). The solution of simply expending excess calories may seem like a quick and easy solution; however, there are many interacting factors that complicate it. For example, due to the aforementioned alterations in the structure of our living environments, physical activity is no longer a necessity of life to get to work, school, or the store (Malik et al., 2013). Reliance on motorized transportation and the degradation of bike and walkways has changed physical activity from a daily fact of life to a luxury that is pursued during leisure time (Malik et al., 2013).

Seabra et al (2008) discuss genetic and environmental factors on propensity to engage in physical activity and found that individual's habits were often shaped by those of their spouses or siblings. A study of maternal exercise in mice found that offspring of mothers that exercised while pregnant were more protected from the detrimental physiological effects of a sustained high fat diet and developing obesity (Wasiniki et al., 2015). Exercise during pregnancy also promotes activation of genes that protect cardiac health (Chung et al., 2017) and upregulates mitochondrial gene expression (Ching et al., 2017) in offspring. Fathers play an important role in their offspring's future adiposity and exercise habits too: McPherson et al. (2015) established that the deleterious effects that female offspring of obese paternal rodents, such as insulin resistance and increased white adipose tissue, can be mitigated through targeted lifestyle interventions in the obese father. These findings have been replicated (Guo et al., 2018; Stanford et al., 2015) and illustrate an interesting a complex relationship between parental physical activity and offspring obesity.

Physical activity as a treatment for mitigating pre-existing obesity has been studied in both humans and rodents, especially in relation to reducing the risk of cardiac infarct and chronic pain (Okay et al., 2009; McInnis, K., 2000; Wiklund, P., 2016; Freitag et al., 2021; Wasser et al., 2017). Although diet interventions are generally more effective for weight loss, exercise is more beneficial for developing functional strength through muscle development (Vreede et al., 2004) and increasing bone density (Benedetti et al., 2018), among other benefits. Laing et al. (2016) demonstrated that voluntary wheel running enhanced insulin sensitivity and hypothalamic function in obese mice. In addition, several studies have demonstrated that voluntary access to running wheels reliably reduces body mass and body fat in obese rodents (Kelly et al., 2006), prevents weight gain in mice consuming high-fat diet by altering the gut microbiota (Evans et al., 2014), and improves obesity-related lymphatic dysfunction (Hespe et al., 2016).

Despite the long history of exercise research in models of obesity, several hurdles are still present in understanding its efficacy and applicability. Farrance et al (2016) demonstrated the importance of fostering a sense of community and support when prescribing exercise interventions in older adults. Similarly, cancer patients had a higher chance of adherence to the exercise intervention if they had high intrinsic motivation, family and group support, and proximity to the rehabilitation centre (Ormel et al., 2017). Effective exercise interventions are often shown to require a welcoming social setting and proximity to individuals partaking, however there are few studies investigating rates of adherence to independent exercise outside of a class setting (Toft et al., 2006). Additionally, most exercise intervention studies target a specific subgroup of individuals, such as the elderly and patients with severe illnesses, so it is not an accurate representation of the average population that is employed, commutes to their workplace, has children, and most likely

has less leisurely time to commute to an exercise center for a scheduled class. However, due to the heterogeneity in individual's resting fitness state, definitions of maximum physical exertion, and knowledge of fitness, it can be necessary to have the studies take place where individuals can be observed and guided in their physical activity (Toft et al., 2006).

1.2 Obesity and cognition

1.2.1 Obesity and cognition in humans

Obese adults (BMI >30) have been shown to have cognitive impairments across most cognitive domains (Prickett and Brennan, 2015). Previously, the consensus was that obesity in middle-age should be avoided mainly because it resulted in an increased chance of dementia later in life; however, it has also been associated with reduced cognitive performance during middle age even if individuals are otherwise healthy (Gunstad et al., 2007).

Isolating the effects on cognitive performance simply to obesity is a challenge since it is a condition with many comorbidities, including but not limited to: type 2 diabetes, depression, and hypertension (Prickett and Brennan, 2015). The aforementioned comorbidities all have impacts on cognition; however, studies have shown that cognition is negatively affected even in obese individuals that are otherwise healthy and do not have these comorbidities (Gunstad et al., 2007; Volkow et al., 2009). Making this distinction is important due to the variability in appropriate intervention for each condition; a weight loss plan may be effective in managing obesity, but an anti-depressant therapy may be more useful for treating depression. Distilling the effects on

cognition down to one factor is a challenge in human studies with so many interacting factors (Prickett and Brennan, 2015).

Individuals who are obese often have impaired executive function (Boeka and Lokken, 2008; Pignatti et al., 2006; Loeber et al., 2012). These impairments result in obesity and cognition having a bidirectional relationship; individuals who are obese have altered cognition, which can then increase the likelihood that they will continue to make detrimental lifestyle choices that can then continue the weight gain (Spyrirdaki et al., 2016). One theory regarding the deterioration of cognition in obese individuals is that it is a result of chronic low-grade inflammation (CLGI) (Spyridaki et al., 2016). This theory suggests that obesity activates CLGI, as quantified by C-reactive protein (CRP) and inflammatory cytokines and chemokines IL-6 and TNF-α (Das, 2006). A study investigated this relationship in a population of middle-aged Greek individuals and established an inverse association between cognitive performance and BMI (Spyridaki et al., 2016). Obese individuals had significantly worse performance on measurements of non-verbal logical reasoning and fluid intelligence, while also having elevated markers of CLGI (Spyridaki et al., 2016). This inflammation can affect various brain areas, including the hippocampus, cerebral cortex, brain stem, and amygdala (Gómez-Apo et al., 2021). Inflammation resulting from obesity can occur in the periphery, leading to insulin resistance, and in the brain as neuroinflammation that is capable of deteriorating the integrity of the blood brain barrier (Novo and Batista, 2017).

Cognitive deficits have even been demonstrated in obese children (Smith et al., 2011). Obese children were found to consistently underperform in test of cognitive function, such as the digit span, continuous performance task, and switching attention (Lee et al., 2009). Additionally, they demonstrated reduced short-term memory and visuospatial organization (Li et al., 2016). A study specifically targeting preschool children aged 4 to 8 years in Bavaria demonstrated that obese girls had greater issues with perseverance, specifically the ability to maintain focused attention during an examination, than their normal-weight female counterparts (Mond et al., 2007).

In addition to changes in cognition, brain imagining studies have proposed a relationship between obesity and neural atrophy (Dye et al., 2017). Obese BMI, independent of age, has been associated with a reduction of gray matter in the inferior frontal gyri, right insula, left and right precentral gyri, left middle temporal gyrus, left amygdala, and left cerebellar hemisphere (Gómez-Apo et al., 2021). Reduced amounts of white matter have also been demonstrated in obese individuals (Gómez-Apo et al., 2021). Interestingly, changes are observed in the white matter fibre tracts that link limbic structures with prefrontal areas of the brain, possibly providing a structural explanation of the common cognitive impairments and increased risk for dementia in obese individuals (Cai, D., 2013). Imaging studies provide a compelling picture of the structural changes occurring, and cognitive tests provide an interesting insight into changes in brain function. However, to thoroughly elucidate the physiological changes occurring throughout the body and observe changes in cognition in a controlled environment, rodent models are necessary.

1.2.2 Obesity and cognition in rodents

1.2.2.1 Motivation

Motivation is so important because it is necessary in most aspects of life (Braver et al., 2014). Goal-directed motivation leads to effort being invested in an action in order to achieve desired outcomes (Braver et al., 2014). Deficiencies in motivation have been partly implicated in the cognitive deficits present in schizophrenia, depression, and attention deficit/hyperactivity disorder (Westbrook and Frank, 2018). Motivation functions in tandem with cognitive processes such as cognitive control, which is implicated in reasoning and inhibition (Westbrook and Frank, 2018). Through these pathways, motivation signalling has been shown to be mediated by dopamine signalling in the striatum in the brain (Westbrook and Frank, 2018).

In a DIO model in rodents, motivation on a progressive ratio task was impaired following weight gain (Blaisdell et al., 2014). In this study, rats were fed either refined or unrefined low-fat diets, and the rats receiving the refined low-fat diet demonstrated significant weight gain and impairments in cognition, thus suggesting that it is both the macromolecular components of the diet and the quality of the diet that contributes to the formation of obesity (Blaisdell et al., 2014). Here, the refined diet represented the types of nutrients that make-up the WD diet; since they are more highly processed, they are broken down into simpler, more digestible structures such as simple sugars and refined flour (Blaisdell et al., 2014). In contrast, an unrefined diet is characterized by foods such as whey, vegetables, and complex carbohydrates (Blaisdell et al., 2014).

A study proposed the hypothesis that individuals who are more susceptible to the motivational effects of food cues have a higher risk of becoming obese (Robinson et al., 2015). Differences exist between individuals in terms of the motivational value they place on certain rewards, and this may be related to stronger activation of the nucleus accumbens and caudate putamen in certain people (Robinson et al., 2015). These differences in activation have previously been demonstrated in obese people (Rothemund et al., 2007), even prior to the development of obesity (Stice et al., 2010). Rats that gained weight following consumption of a "junk food" WD showed enhanced motivation to obtain sucrose following development of obesity (Robinson et al., 2015). Motivation is heavily dependent on the striatum and dopamine signalling (Schoffelmeer et al., 2011). Insulin resistance stemming from obesity and consumption of a WD acts directly upon insulin receptors in the striatum that modulate motivation (Francis, 2013).

1.2.2.2 Spatial memory

Spatial memory, a function largely dependent on the hippocampus, refers to information stored within a spatiotemporal frame (O'Keefe and Nafel, 1978). The hippocampus is an area that is very susceptible to deterioration with increased age, obesity, and excessive consumption of HFHS diet (Reichelt et al., 2021). In addition to its vulnerabilities, the hippocampus is an incredibly important area of the brain regarding learning, memory, and adult neurogenesis (Reichelt et al., 2021). Spatial memory is often tested using apparatuses such as the T-maze, radial maze, and the Morris water maze (Sharma et al., 2010).

A study investigating DIO in rodents tested their spatial memory using the Morris water maze (MWM) and their working memory using the novel object recognition (NOR) test (Lewis et al.,

2019). The MWM tests animal's ability to learn and recall the location of a rest platform within a large tub of water, while the NOR test investigates the animal's ability to recognize that an object is different from the objects that had been used previously in testing phases (Lewis et al., 2019). The rats demonstrated a robust model of human obesity, exhibiting dyslipidaemia, inflammation, and impaired glucose tolerance (Lewis et al., 2019). They also exhibited impairment in the MWM and the NOR task; however, findings have been variable in the field using the MWM test apparatus due to the high level of stress inherent to the test and increased animal handling necessary to complete it (Lewis et al., 2019).

Even maternal obesity has been shown to negative effects on spatial learning and memory of offspring in their younger years (Tozuka et al., 2010). Offspring of obese mothers who had consumed high-fat diet during pregnancy had less brain-derived neurotrophic factor (BDNF) in their hippocampi following birth (Tozuka et al., 2010). BDNF is important due to its actions ensuring the survival, maintenance, and differentiation of certain types of neurons (Tozuka et al., 2010). Due to its important role in regulating the fates of neurons, BDNF is incredibly important in learning and the formation of new, lasting memories (Bekinschtein et al., 2014). These findings imply an obesity-dependent decrease in levels of BDNF and subsequent neurogenesis may explain some of the deficits seen in spatial memory in these models (Tozuka et al., 2010). In addition to alterations in levels of hippocampal BDNF following WD consumption, a subsequent study demonstrated that obese mice maintained on a WD showed impairments in novel object recognition, object location memory, and significant reductions in the mRNA levels of a variety of genes associated with hippocampus-dependent memory formation, (i.e. Sirtuin 1 (SIRT1) and protein phosphatase 1 (PP1)) (Heyward et al., 2012).

1.3 Behavioural tests

1.3.1 Spontaneous Location Recognition (SLR) Task

The spontaneous location recognition task tests spatial learning and memory (Reichelt et al., 2016). Spatial learning and memory are reliant on hippocampal function, and the hippocampus has been shown to be particularly vulnerable to the detrimental outcomes of consuming a HFHS diet (Hsu et al., 2015) and increased age (Bettio et al., 2017). Various conditions exist in the SLR test that provide varying degrees of difficulty in recognizing the novel object (Reichelt et al., 2020; Reichelt et al., 2016). Difficulty is controlled by increasing or decreasing the amount of space between two of the three objects in the sample phase, thus testing whether the mouse is capable of spatially differentiating and recalling which object is in a novel location (Reichelt et al., 2020). Most mice are capable of successfully completing the easier conditions (dSLR and sSLR) but may exhibit impairment in the most difficult condition (xSLR) (Reichelt et al., 2020). However, various interventions targeting hippocampus-specific mechanisms can impair function on less difficult stages as well. Such an effect was observed by Reichelt et al (2016) where they observed impairments in the middle difficulty phase, sSLR, in rats that were consuming increased levels of sucrose.

1.3.2 Translational touchscreen-based paradigms

As mentioned previously, variation exists in tasks that probe specific cognitive deficits in both rodents and humans. Rodent methods have mostly involved hand testing, including maze-based tests (Tozuka et al., 2010), quantifying the number of licks to an appetitive reinforcer (Robinson

et al., 2015), or number of lever presses. In contrast, human methods rely on methods such as CANTAB assessments (Smith et al., 2015).

Automated touchscreen operant chambers provide an attractive and reliable substitute to hand testing and self-reported data. Tasks tailored to the Bussey-Saksida touchscreens allow for a high level of translatability and control by administering virtually the same testing paradigm to both rodent and human subjects (Homer et al., 2013; Nithianantharajah et al., 2015). Many cognitive tasks that currently exist in rodent research have attempted to mirror tasks that were already widely used in a different form in humans (i.e. a maze task to mirror a path finding task done on pen and paper), but these touchscreen tasks were developed in the same systems, using the same physical hardware and program software, which removes most of the environmental variation and confound (Heath et al., 2019). The tasks utilized in the touchscreen allow a high level of specificity in regard to the brain area being targeted in the tests, through the usage of optogenetic, chemogenetic, surgical, or lifestyle interventions in tandem with behavioural tasks tailored to the question at hand.

Importantly, the reduction in handling and involvement of the researcher during the actual training and test phases has shown to decrease stress behaviours in the test subjects (Nithianantharajah et al., 2015). In addition to the existence of the same cognitive task tailored to both human and rodent, the existence of the Mousebytes database further strengthens the basis of these cognitive tests by providing preexisting findings in an accessible and shareable manner (Beraldo et al., 2019). The Mousebytes database exists as an international repository for all

touchscreen-based studies that have been completed and helps promote collaboration while reducing unnecessary replication of previously completed research (Beraldo et al., 2019; Sullivan et al., 2020).

1.3.2.1 Pairwise Visual Discrimination (PVD)

The pairwise visual discrimination touchscreen task has been widely used to test the effects of various interventions on learning, memory, and visual discrimination (Talpos et al., 2011). In this task, mice are trained to respond to a correct stimulus image (S+) that is presented on the touchscreen, and not to interact with an incorrect stimulus image (S-). Limited research has been conducted utilizing these testing paradigms in models of obesity simply due to the fact that most DIO rodents are already consuming appetitive diets high in fat, sugar, or both, and they may be less motivated to complete tasks for a food reward (Kim et al., 2015).

1.3.2.2 Fixed-Ratio/Progressive Ratio (FR/PR)

The fixed-ratio and progressive ratio tasks of motivation and perseverance have existed for decades (Rusted et al, 1998; Schneider et al., 2003; Ferguson et al., 1997). Instead of training mice to interact with various levers and requiring researchers to actively extend and retract those levers (Ferguson et al., 1997) the automated FR/PR task in the touchscreens reduces researcher labour, influence, and presence.

The fixed-ratio task teaches mice that at each stage, a certain number of nosepokes to the correct illuminated square on touchscreen apparatus results in an appetitive reward (Finger et al., 2010). Mice completed one session per day. Following completion of all of the stages of fixed-ratio, mice are trained on the progressive ratio task. The PR task requires continuously greater amounts of effort to complete each trial at each stage, and this increase in effort is based on a linear ramp dependent on the requirement of that stage. The task tests how much effort a mouse is willing to expend for a constant amount of reward.

Previously, various populations have shown impairment in this task. Individuals with a schizophrenia diagnosis demonstrated reduced willingness to expend the effort necessary to achieve a reward in a PR task due to condition related reward processing abnormalities (Strauss et al., 2016). Chronic cannabinoid treatment during the vulnerable phase of puberty in rats reduced the main measurement of motivation in the PR task, called breakpoint (Schneider and Koch, 2003). In a genetic model of ob/ob obese mice, administration of the anorectic drug fenfluramine motivated them to similar levels as their lean counterparts in the initial phases of the PR task but was insufficient to reach those levels in later stages (Finger et al., 2010).

1.3.2.3 Extinction

The extinction touchscreen task tests the willingness of mice to continue responding to a stimulus even without the presence of an appetitive reinforcer (Kim et al., 2020). Extinction is traditionally tested following animal training on a rewarded touchscreen cognitive task which ensures the expectation of reward while the animal is completing the task.

Extinction tests preservation and quantifies how long the animal is willing to continue engaging in the task without extended periods of inactivity, and the rate at which responses are made to the stimuli (responses) or the number of stimuli that are not interacted with (omissions).

Impairments in perseverance are prevalent in individuals that are more prone to impulsivity, such as individuals with gambling disorders (Mallorquí-Bagué et al., 2020), alcohol dependence (Nowakowska et al., 2008), and mild cognitive impairment (Rochat et al., 2013).

The inability to utilize such standardized, automated, and targeted tasks in diet-specific animal models presents a huge clinical population that is being disregarded. Touchscreen tasks widely require food restriction in animal models prior to testing commencement to motivate them to perform for the appetitive reinforcement that is provided following correct trials. In models utilizing HFHS diet induced obesity, specialized diets that mimic myelin degradation in conditions such as multiple sclerosis, or even in low carbohydrate diets, food restriction may reduce the impact of the diet intervention cognitively, and ultimately, the translatability of that model to human models of disease (Del Rio et al., 2016; Reichelt et al., 2019; Liu et al., 2020).

1.4 Water manipulation

An alternative to food restriction is necessary to motivate mice undergoing sensitive diet interventions in completing touchscreen tasks. Especially in studying DIO, limiting duration of access to diet, or restricting the amount of diet available for consumption to an amount that would result in weight loss makes it impossible to accurately model the snacking behaviours, large portion sizes, and excess in calories that are hallmarks of obesity in the Western world (Malik et al., 2013; Reichelt et al., 2019; Preguiça et al., 2020) and reduce the translatability on a physiological and cognitive level.

Obesity in mice has been linked to increased anxiety and depressive-like behaviours in mice (Del Rio et al., 2016). These symptoms manifest due to a reduction in dopamine (Fam et al., 2022), as well as serotonin secretion (Park et al., 2017). Vichaya et al (2019) suggest that chronic, low-grade inflammation, a condition exacerbated by consumption of HFHS diet as well as obesity (Novo and Batista, 2017), also contributes to the rodent depression phenotype. As a result of these symptoms, obese mice consuming WD commonly perform in a less motivated manner when completing conditioning paradigms (Park et al., 2017).

One alternative to utilizing food restriction to motivate mice in touchscreen cognitive tasks is water manipulation. There are several methods by which water can be manipulated: limiting the quantity of water (Guo et al., 2014), limiting the duration of access to water (Tucci et al., 2006), or making water less palatable (Urai et al., 2021).

Reducing the availability and amount of water that mice have access to has previously been an effective motivator in conditioning paradigms that offer a liquid reinforcement reward during testing (Goltstein et al., 2018), specifically in mouse models of obesity (Blaisdell et al., 2014). However, reducing water intake obviously increases the risk of negative health outcomes in the animals (Urai et al., 2021). Previous water restriction studies have demonstrated that mice on water restriction scored higher on measurements of discomfort and anxiety (Goltstein et al, 2018). The looming threat of water restriction studies is, of course, dehydration (Reinagel, 2018). Dehydration occurs quickly and is incredibly detrimental to overall physiological well-being (Bekkevold et al., 2013). In addition to increased risk of animal distress, sharp weight fluctuations, and additional health risks, water restriction protocols require many hours of researcher labour both in administration of water and monitoring of adverse health effects (Urai et al., 2020).

Water manipulation through the addition of a low concentration of citric acid results in water that is palatable but slightly sour (Urai et al., 2020). Mice readily consume citric acid water freely without negative health outcomes (Urai et al., 2020). This intervention has been presented as an attractive alternative to food or water restriction (Urai et al., 2020).

1.4.1 Citric acid water administration and applicability in touchscreen conditioning paradigms

Mice are slowly acclimated to 2% citric acid water by first exposing them to 1% citric acid water and subsequently increasing the concentration (Reinagel, 2018). The citric acid water solution is

made by dissolving 2g of powdered citric acid/100mL tap water in regular mouse water bottles (Urai et al., 2020). Weight changes are monitored to ensure that mice are within a healthy range and are not decreasing food consumption as a result of decreased fluid consumption (Reinagel, 2018).

Usage of citric acid water as a motivating factor in behavioural testing has not been widely studied at this time; however, the existing literature is promising. When administered to young C57BL/6J mice, they maintained a healthy weight and water intake and performed successfully on visual decision-making tasks (Urai et al, 2020). In a cohort of older C57BL/6J mice that only had their health monitored, there were no negative health outcomes (Urai et al., 2020).

Rats that consumed citric acid water also presented no adverse health outcomes, with no additional water supplements required to maintain a healthy weight (Reinagel, 2018). In addition, these rats performed similarly to their regular water consuming counterparts on a self-paced random dot motion discrimination task (Reinagel, 2018).

The research pertaining to the efficacy of citric acid water as a motivator suggests an exciting opportunity for motivation in animal models that are unable to undergo food restriction. Utilizing water manipulation to motivate mice to complete touchscreen-based tasks as opposed to food restriction would allow a more accurate model of the cognitive impairments of diet-induced obesity stemming from free feeding of high fat, high sugar WD, furthering the understanding of the biochemical, neurological, and cognitive changes that occur and how these hurdles can be addressed within human populations.

1.5 Exercise interventions and the cognitive consequences of obesity

Besides dietary roots of obesity, sedentary lifestyles are a primary driver in the development of obesity (Trovato et al., 2018). Most people do not reach the minimum suggested requirements for physical activity (Guess, 2012). Various types of exercise have demonstrated efficacy in not only expending calories, but also in reducing anxiety (Uysal et al., 2018), inhibiting neuroinflammation (Liu et al., 2020), increasing bone density in osteoporotic patients (Benedetti et al., 2018), and reducing cardiometabolic risk (Gaesser et al., 2011), among other benefits.

Exercise has been widely shown to be effective in reversing whole body consequences of obesity and high fat, high sugar diet consumption (Evans et al., 2014; Johnson et a., 2009; Janney et al., 2010), however, recently interest has piqued in the applicability of exercise interventions in rescuing cognitive deficits that stem from obesity, as well as furthering the understanding of how exactly this occurs in the brain (Kim et al., 2015).

1.5.1 Aerobic exercise and cognition

Special interest has been devoted to aerobic exercise interventions in various populations. Aerobic exercise increases heart rate and relies on aerobic metabolism to extract energy (Patel et al., 2017) . Additionally, it is an exercise that relies on large muscle groups and is maintained continuously and rhythmic in nature (Bourbeau et al., 2023). This type of exercise is measured through peak oxygen consumption (VO2 max) (Patel et al., 2017). Examples of aerobic exercise

include hiking, jogging, walking, and swimming (Patel et al., 2017). It is a type of activity that is easily integrated into a daily commute, leisurely time, or sports and community-based activities.

Aerobic exercise has previously been shown to rescue cognitive deficits in adults with a variety of neurological disorders; including mild cognitive impairment (Baker et al., 2010), senile dementia (Kwak et al., 2008), brain injury (Grealy et al., 1999), schizophrenia (Falkai et al., 2017), and stroke (Quaney et al., 2009). In addition, it has enhanced cognitive flexibility in older adults (Masley et al., 2009), improved depressive symptoms in individuals with major depressive disorder (Knochel et al., 2014), and improved cognitive performance in adolescents with ADHD (Van Riper et al., 2023).

Although many studies rely on measurements such as the Mini-Mental State Exam, research in recent years has turned towards combining these measurements with brain imaging to provide a more nuanced understanding of both the condition and the treatment. One such study investigated walking and dancing as interventions to reduce white matter deterioration, a condition that happens naturally with age but is accelerated in individuals with Alzheimer's disease (Mendez Calmenares et al., 2021). This intervention successfully improved episodic memory performance and reduced levels of white matter deterioration (Mendez et al., 2021). In addition, rodent work using swimming and running wheels has furthered the understanding of what is occurring in the brain when cognition is improved by exercise (Duman et al., 2008). Chronic voluntary running has also been shown to improve depression and anxiety-like behaviours in rodents (Duman et al., 2008; Castilla-Ortega et al., 2013; Cunha et al., 2013).

The discovery of brain-derived neurotrophic factor (BDNF) and its effects throughout the body and the brain following exercise interventions changed the field of exercise research. BDNF functions as a modulator of neuronal structure and function (Bramham et al., 2005). It is highly expressed in the brain and is a key player in neuroplasticity within the brain (Lu et al., 2014). Levels of circulating BDNF are reliably increased following physical activity (Griffin et al., 2011). The discovery of BDNF and its role in conditions from depression to brain cancer (Colucci-D'Amato et al., 2020) encouraged the development and usage of rodent-based models of exercise that allow further understanding of the neurological basis of exercise-related cognitive improvements.

Despite its demonstrated benefits, aerobic exercise is not always the ideal solution for all populations. Individuals with higher BMIs are more prone to musculoskeletal injuries, joint pain, and illness (Janney and Jakicic, 2010). Obese individuals are at a higher risk of certain fractures and less than ideal bone health (Gkastaris et al., 2020). To achieve the levels of activity required to sustainably reduce body fat in amounts that reverse the detrimental effects of obesity, high intensity interventions are necessary (Julian et al., 2022). High intensity programs are not always sustainable, especially in older, obese individuals. A study by Neri et al (2020) demonstrated that individuals that are 60 years old or older and obese have a much higher risk of falls compared with non-obese, age matched controls. In addition, intensive aerobic exercise is not always accessible to older, obese individuals due to cost barriers related to gyms and aquatic centers, poor weather conditions, and lack of transportation from urban centers to exercise facilities (Sallis et al., 1990; McAuley et al., 2003).

1.5.2 Resistance exercise and cognition

Resistance exercise is less widely studied than aerobic exercise, but curiosity regarding its efficacy in many populations has increased in recent years. Resistance exercise, often also referred to as anaerobic exercise or strength training, consists of intense, short bouts of physical activity that uses energy stores within the contracting muscles (Patel et al., 2017). This type of exercise is called anaerobic exercise since its energy metabolism does not rely on inhaled oxygen; instead, it depends on glycolysis and fermentation for ATP production (Patel, 2017).

Resistance exercise has commonly been studied in older individuals. Dunstan et al (2002) investigated the efficacy of high-intensity resistance training in improving glycemic control in sedentary and overweight people aged 60-80 years who were diagnosed with type 2 diabetes. Dunstan and his colleagues found that this intervention improved glycemic control, improved muscular strength, had no negative health outcomes, and resulted in moderate weight loss. Subsequent studies demonstrated the usefulness of resistance exercise in improving body composition by increasing skeletal muscle and decreasing body fat (Deschenes and Kraemer, 2002), reducing blood pressure (Vincent et al., 2003), combatting insulin resistance (Davidson et al., 2009) and promoting arterial elasticity (Jefferson et al., 2015).

Resistance exercise is especially effective at improving functional capacity in age and obesity related declines of muscle and bone strength. De Oliveira Silva and colleagues (2022) investigated whether a resistance exercise intervention would be capable of rescuing functional capability in elderly women with obesity induced sarcopenia (muscle loss). In their study, they

were able to establish that a 16-week intervention was effective in rescuing some functional capacity and reducing adiposity (De Oliveira Silva et al., 2022). Cassilhas et al (2007) were interested in whether a 24-week resistance exercise treatment would be sufficient to positively impact cognitive function in a group of elderly individuals. Follow the 24 weeks, elderly participants completing either moderate or high intensity resistance exercise had better performance on the digit span forward task, Corsi's block tapping task, Osterrieth complex figure immediate recall, and improvements in measurements of anxiety and depression in comparison to control individuals who completed stretching classes (Cassilhas et al., 2007).

Liu-Ambrose et al (2008) investigated the effects of resistance exercise on executive function through tasks targeting set shifting, updating, and response inhibition. They established that the individuals in the exercise intervention scored significantly higher on response inhibition, a cognitive ability that has also been closely associated with fall prevention in older individuals at risk for falls (Verghese et al., 2002; Liu-Ambrose et al., 2008).

Currently, animal model-based research in the neural mechanisms of resistance exercise is lacking. A ladder-based resistance training intervention using incremental carrying load increases has been validated as effective in eliciting morphological nerve changes in the forelimb and hindlimb of young rats (Neto et al., 2021), increasing skeletal muscle (Lourenço et al., 2020), and promoting recovery rates of bones (Song et al., 2018).

Suijo et al (2016) found that mice undergoing voluntary resistance exercise over 14 days demonstrated improved spatial memory function as shown in the Morris water maze and increased concentrations of hippocampal BDNF. Cassilhas et al (2012) compared the efficacy of aerobic and resistance exercise interventions on spatial memory and found that both groups had similar improvements in spatial memory; however, there were variations in both signalling pathways and proteins in the hippocampus. A subsequent study attempted to elucidate the molecular explanation regarding reductions in neuroinflammation following resistance exercise training (Kelty et al., 2019). Kelty et al established that chronic resistance training was effective in ameliorating lipopolysaccharide levels after they had been injected in the dentate gyrus to increase inflammation, and that this process may occur through the IGF-1 signalling pathway.

1.6 Rationale

Increased adiposity and advanced age have reliably been shown to result in a plethora of cognitive impairments, such as decreased motivation, impaired spatial learning and memory, and depression (Fam et al., 2020). Aerobic exercise interventions have previously been effective in ameliorating some of these deficits (Colucci-D'Amato et al., 2020); however, this mode of exercise is not always sustainable for the older, heavier population.

Resistance exercise and its capacity to rescue cognitive function in the elderly and the obese is an exciting new field of study. Due to the increase in prevalence of obesity, high levels of consumption of WD, and increasing mean age in Canada, it is important to further understand and validate effective interventions. However, research is lacking and the mechanisms by which

these interventions function are still unclear. Rodent models of obesity and exercise are necessary to provide a sufficient level of control and adherence to both the treatment (HFHS diet) and the intervention (resistance exercise). To ensure the translatability of the findings from this model to humans, touchscreen conditioning paradigms can be used. The progressive ratio task can be used to elucidate alterations in motivation, and the extinction task can be used to assess perseverance in the population of interest. Additionally, a test of spatial memory such as SLR will allow for a clearer picture of the interacting impairments that increased age and WD induced obesity generate.

However, before touchscreen tasks can effectively be used in this obese population, an alternative to food restriction must be validated as a sufficient motivator. Therefore, we evaluated citric acid water as a possible motivator across various rodent diets to ensure that it is reliable and effective. To test its efficacy in touchscreen tasks, the progressive ratio task touchscreen task was completed to demonstrate that there were no significant differences in motivation to complete the task between groups. Additionally, a task requiring a higher cognitive load such as the pairwise visual discrimination task was completed to demonstrate that 2% citric acid water is effective at motivating free fed mice in tasks of learning and memory as well.

Given previous research, we predicted that 2% CA water could sufficiently motivate free-fed mice to complete touchscreen cognitive tasks without any serious adverse health effects. In addition, we predicted that a consistent exercise regime effectively rescued impairments in motivation and spatial learning and memory that are caused by increased age and WD induced obesity.

Chapter 2: Water manipulation experiment

2.1 Introduction

The incidence of obesity worldwide has been classed a pandemic and the blame has been laid on inexpensive calorie-dense food, community structures that reduce physical activity, and inexpensive nonphysical entertainment (Meldrum et al., 2017). Obesity has made a name for itself as the primary risk factor for many illnesses with high mortality rates and even higher load on the healthcare system, such as type 2 diabetes mellitus (Malik et al., 2012), cancer (World Health Organization, 2009), and Alzheimer's (Terzo et al., 2021). Recently, interest in the association between obesity and cognitive function has skyrocketed. Studies have established associations between obesity and various dementias (Terzo et al., 2021), with special focus on the effects of inflammation, insulin resistance, and changes in gray matter volume (Fernandez-Andujar et al., 2021). Additional work has identified the connection between obesity and impaired episodic and working memory, decision making, attention, executive function, and neuroplasticity (Dye et al., 2017). To achieve a deeper understanding of the mechanisms behind this complex, whole body condition, an animal model of human obesity can be used to uncover the neuropathophysiology of obesity-related cognitive dysfunction as well as developing sustainable and effective treatments to combat this issue (Wali et al., 2023).

Examining cognition in pre-clinical models of obesity requires behavioural assays that are translatable as human-health relevant findings. The formal design of the pre-clinical assays used in obesity – the water maze, radial arm maze, fear conditioning, etc. – do not measure behaviours in mice in an identical manner to those in humans (Cassilhas et al., 2012; Heyward et al., 2012;

Klein et al., 2016). In clinical settings, computerized test batteries and automated touchscreen systems (e.g. the Cambridge Neuropsychological Test Automated Battery (CANTAB), CogState, Mindstreams, Cognitive Performance Testing Services) are increasingly utilized to test cognition (Meo et al., 2019). Using similar principles, rodent touchscreen operant chambers robustly bridge the gap to human behavioural assessments.

Rodent touchscreen operant chambers are more desirable than traditional methods used in the study of animal cognition for several reasons. Touchscreen chambers have experimental conditions, such as the testing environment, stimuli presentation, and apparatus, that stay consistent between trials, paradigms, and animals (Kim et al., 2017). Additionally, they offer a wide battery of tasks specific to various cognitive domains, including attention, spatial memory, motivation, and learning (Kim et al., 2017) that can be delivered to both humans and rodents in a nearly identical manner (Sullivan et al., 2020).

However, there is a significant limitation to using touchscreen conditioning paradigms when testing models of obesity. Touchscreen tasks usually utilize an appetitive reinforcer, such as strawberry milkshake or condensed milk (Sullivan et al., 2020) which motivates mice to complete the task. A reward's desirability can be enhanced and maintained by food restriction – a protocol in which food access is limited by duration or quantity (Goltstein et al., 2018; Lattal and Williams, 1997; Kwak et al., 2015; Mallien et al., 2016). Previous work by Kant et al. (1988) demonstrated this concept by comparing the speed at which food-restricted and unrestricted mice completed a maze task. As hypothesized, food-restricted mice receiving a food reward learned to

complete a maze task more quickly than unrestricted mice receiving the same reward (Kant et al., 1988). These findings have been directly demonstrated in touchscreens as well, with Yang et al. (2019) showing that rats interacted with the touchscreens less when they were no longer food restricted.

Food restriction is not an appropriate motivational protocol in studies utilizing specific diet interventions or HFHS diet induced models of obesity (DIO) (Reichelt et al., 2019). Food restriction maintains mice at a bodyweight that is 85-90% of baseline free-feeding weight (Heath et al., 2016), however, DIO require free-feeding of HFHS diet to accurately model human obesity (Moura e Dias et al., 2021). Several studies looking at cognition in diet-induced obesity using touchscreen have noted this limitation (Dumont et al., 2021; Harb & Almeida, 2014; Miles et al., 2022). Furthermore, obesity is linked with anxiety and depressive-like behaviour in mice (Del Rio et al., 2016; Park et al., 2017), resulting in mice that are less motivated to perform in conditioning paradigms (Del Rio et al., 2016; Park et al., 2017). Based on these findings, it is necessary to validate alternatives to food restriction paradigms so that obese mice can be motivated to perform touchscreen tasks of cognition in this model.

One such possible alternative to food restriction is water restriction. Water restriction has previously been applied through two different methods: limiting the quantity of water available (Guo et al., 2014) or limiting the duration of access to water (Tucci et al., 2006). Such water restriction protocols have previously effectively increased motivation in mouse models of obesity in conditioning paradigms (Blaisdell et al., 2014). Restricting the amount and availability of

water presents greater health risks and discomfort in mice than restricting the amount and availability of food (Gollstein et al., 2018). In addition to discomfort, the risk of serious outcomes such as dehydration and unsafe weight loss requires rigorous monitoring to ensure the well-being of the mice (Urai et al., 2020).

An alternative method of water restriction exists that does not limit the quantity or availability of water – water with the addition of citric acid (Urai et al., 2021). The addition of a small amount of citric acid powder into water creates a mild citric acid solution that has a sour taste (Reinagel et al., 2018). Due to the sour taste, rodents consumed less of the 2% citric acid water when it was provided *ad libitum* (Reinagel et al., 2018; Urai et al., 2021). This reduction of water consumption was sufficient to motivate rodents to complete conditioning paradigms when presented with water rewards (Urai et al., 2021). In addition to sufficiently motivating mice, the risk of dehydration is reduced in comparison to other water restriction protocols since the 2% citric acid water is administered *ad libitum* (Reinagel, 2018; Urai et al., 2021). Ultimately, water manipulation using citric acid is non-labor-intensive and a low-error option that benefits animal health without hindering behavioral training progress (Reinagel, 2018; Urai et al., 2021).

Citric acid water has demonstrated its efficacy and reliability in motivating behavioural task performance in mice (Urai et al., 2021). However, research regarding its applicability in touchscreen studies, especially those requiring specialized diets, is lacking. This study aimed to answer two questions: 1) are mice undergoing a water manipulation protocol using citric acid water as motivated as food restricted mice to perform and learn on touchscreen tasks? and 2) are

mice consuming high-fat, high sugar (HFHS) diet as motivated as those consuming standard chow to complete touchscreen tasks while on the citric acid water manipulation protocol?

2.2 Methods

2.2.1 Animal housing and care

All experiments were conducted in compliance with the standards set by the Canadian Council of Animal Care and under direct veterinary supervision at the University of Western Ontario. Seventy-two C57BI/6J mice (36/sex, The Jackson Laboratory, US) arrived aged 8-12 weeks old. Mice were separated by sex and housed in groups of 4/cage (28 x 18 cm plastic cages with wire tops) (Figure 1) in a temperature (23 ± 1°C) and humidity (50 ± 1%) controlled room under a reverse 12h light/dark cycle (lights off at 9:00).

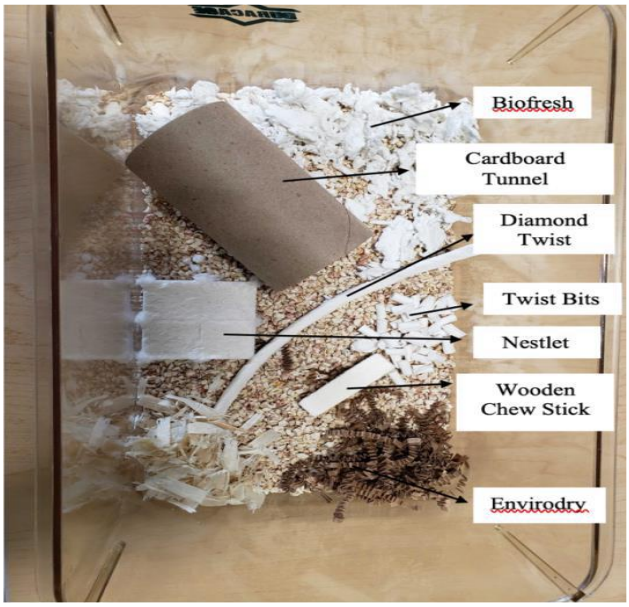


Figure 1. Example mouse home cage with environmental enrichment.

Levels of enrichment were kept even between cages to avoid discrepancies in effect of enrichment. Extra chewing enrichment was included in all cages to reduce grinding of high-fat, high-sugar diet by mice.

Upon arrival, mice were acclimatized to their cages for 5 days with *ad libitum* access to standard diet and untreated water. Mice were randomly assigned to standard diet (Teklad Envigo 7913, 18% protein rodent diet, 3.1 kcal/g, 5% fat, 5% fibre), (N = 20/sex) or high fat high sugar (HFHS) chow (Bioserv F6724, 4.57 kcal/g), (N = 16/sex). All mice had *ad libitum* access to their respective food and untreated water for three weeks. Following three weeks of diet acclimatization, baseline weights were calculated as the average of their bodyweight over three days.

2.2.2. Food restriction and manipulation

Food restriction and water manipulation protocols were then implemented. Sixteen mice (n=8 per sex) on standard diet were randomly chosen to undergo food restriction to 85-90% of their baseline weight. All mice receiving *ad libitum* standard diet (n = 24) and HFHS diet (n = 32) underwent 2% citric acid water manipulation *ad libitum*. Two grams of CA (citric acid anhydrous, Thermofisher Scientific, USA) were dissolved in 100mL of tap water to produce 2% CA water.

2.2.3 Health monitoring

Mice were weighed daily, with a healthy weight defined as 85-90% of baseline weight in food restricted mice, whereas mice on 2% CA water had no upper boundary of acceptable body weight. Food restricted mice found to be under a healthy weight were provided additional diet; water manipulated mice found to be under a healthy weight were provided one hour of access to

0.5% CA water to encourage water consumption. Water-manipulated mice found to be underweight for three consecutive days were provided one hour of access to regular tap water. In addition to weight monitoring, all water manipulated mice were monitored for signs of dehydration. Daily scores were assigned based on their activity, posture and grooming, eating and drinking, and dehydration levels using a health scoring system (table 1)

2.2.4 Touchscreen apparatus

All touchscreen behavioural testing was completed using standard Bussey-Saksida mouse touchscreen chambers (model 80614, Lafayette Instrument Company, Lafayette IN – see Figure 2) as described elsewhere (Mar et al., 2013). Briefly, the apparatus consists of a trapezoidal chamber with a

Figure 2. The Bussey-Saksida mouse touchscreen chamber. An automated touchscreen chamber with interchangeable masks and tests of cognition. Multiple measurements can be recorded. Operated through ABET II touchscreen software.

touchscreen on one end and a reward magazine tray on the other side. In front of the touchscreen there is a space in which removable masks specialized to each of the cognitive tests can be inserted. The chamber is insulated from environmental noise and light. A built-in light and

camera above the chamber allow real-time observation of behaviour as well as recording. The reward tray illuminates and delivers a liquid milkshake reward upon trial completion.

2.2.5 Touchscreen habituation

Mice were then provided with a sample of Neilson strawberry milkshake (H1P1X8, Saputo Inc., Montreal, Canada) mixed with their diet and added to their home cage. Mice were habituated to milkshake for three days. Once mice were habituated to the milkshake reward, initial touchscreen training began. All experiments were conducted using Bussey-Saksida Touchscreen Chamber systems (Campden Instruments Ltd., Loughborough, UK) and ABET II Touchscreen Software (Lafayette Instrument, Lafayette, USA).

Habituation to the chambers occurred in two stages. The first stage was a 10-minute habituation in which mice were left alone in the touchscreen chamber without any stimulus presentation or reward. The second stage, on the subsequent day, was a 20-minute habituation that began with a 3kHz tone lasting for 1 second, after which the LED light was illuminated and ~150 µL strawberry milkshake was released into the reward tray (feeder pulse time: 6000 ms). Following collection of the reward, the light turned off and the tone and light were presented again after a 1 second delay; however, in this instance only 20 µL of milkshake was dispensed (feeder pulse time: 800ms). This process continued for the remainder of the 20-minute habituation. A successful habituation session was defined as the mice collecting all the reward from the reward dispenser. If successful, the third session on the subsequent day consisted of a 40-minute habituation with parameters identical to day 2.

2.2.6 Initial operant training

Following successful completion of all three habituation sessions, each mouse underwent a 60-minute operant conditioning session on the subsequent day. In this session, a white illuminated square stimulus was presented in the center of a five-square panel on the touchscreen (see Figure 3). The stimulus was present on the screen for 30 seconds, after which the stimulus was removed, and the trial was terminated – a process which was signaled by a 1 second long 3 kHz tone, light illumination, and administration of 20 µL of reward into the reward tray. The light turned off and a 4.5 second delay was presented following reward collection. After this initial trial, if the mouse touched the illuminated white square with its nose, that trial was classed as a correct trial and resulted in removal of the stimulus, illumination of the reward dispenser, presentation of the same tone, and release of 60 µL of reward. Initial operant training was successfully completed once the mouse achieved 30 correct trials within 60 minutes. If the mouse failed to achieve these 30 correct trials in 60 minutes, this session would be repeated on subsequent days until the session was successfully completed.

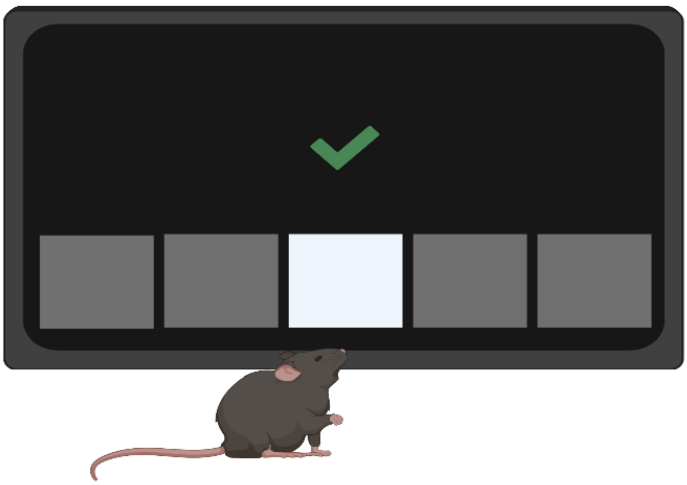


Figure 3. The touchscreen chamber configuration used in the FR, PR, and extinction tasks of motivation and perseverance. The 5-square mask is utilized in these cognitive tasks.

2.2.7 Fixed-ratio (FR) touchscreen task

Once initial operant training was completed, the mice began with the fixed-ratio (FR) touchscreen task. This task was compromised of several stages with similar parameters to the previous session; however, correct trial completion was dependent on mice reaching a defined number of nose pokes to the stimulus.

FR1 required that the mice achieve 30 trials in a 60-minute session, making one nose-poke per trial to receive reward. The subsequent stage, FR2, required that the mice interact with the stimulus twice in each trial to receive reward. Following the nose poke to the initial stimulus, the stimulus was removed for 0.5 seconds and an audible "click" noise was presented after which the stimulus reappeared. FR2 required that mice achieve 30 trials in 60 minutes in this manner. FR2 progressed to 3 responses/trial (FR3), and 5 responses/trial (FR5) for a reward in subsequent sessions. All the other parameters were identical to FR1 and sessions lasted 60 min. The criterion to advance to the next FR session was the completion of at least 30 trials/session. Following FR5 training, the animals progressed to two sessions of unrestricted FR5 (FR5-UC) with no maximum trial limit across each 60 min session.

2.2.8 Progressive ratio (PR) touchscreen task

Following the completion of both sessions of FR5, the progressive ratio touchscreen task began. The mice performed unlimited trials in 60-minute sessions, one session per day for six days. PR task parameters were similar to those in the FR task, differing only in the number of interactions required to complete a trial. The number of interactions required to complete a trial increased in a

linear + n manner (i.e. the responses required for reward in the PR4 stage were as follows; 1, 5, 9, etc.) following completion of each trial. Following PR4, mice completed PR8 and PR12 on subsequent days, adhering to the same parameters for trial completion.

This task was used to assess motivation, which was possible by calculating breakpoint. Breakpoint was defined as the number of responses performed in the last successfully completed trial. Sessions terminated either when mice stopped interacting with the touchscreen for five minutes or when the 60 minutes had elapsed. In addition to breakpoint, the number of trials successfully completed, reward latency, and blank touches to the screen were recorded.

2.2.9 Pairwise visual discrimination (PVD)

In the pairwise visual discrimination task, mice learned to respond to a correct stimulus image (S+) and not interact with an incorrect stimulus image (S-) when both were displayed on either side of the touchscreen with presentation sides for the images pseudo randomly varied across each trial (see Figure 4). A correct trial, defined as a nose poke to the S+, resulted in 20 µL reward release into the reward tray and tray light and tone identical to those described previously. An incorrect trial, defined as a nose poke to the S-, resulted in a 5-second time-out during which the chamber was illuminated by the house light. Following the time out, a correction trial began. In a correction trial, the stimuli were presented in the same configuration as the previous trial. The correction trial was repeated until the mouse successfully made a correct response. Each session consisted of 30 trials, with each session ending when those trials were completed, or 60 minutes had elapsed. If a session ended without 30 trials completed, the session was continued the subsequent day until 30 trials were completed. Correction trials are not counted towards

session completion. Mice completed a total of 10 sessions of 30 trials each. To assess the learning of the discrimination rule, the accuracy (% correct responses) and number of correction trials were determined for each session.

Figure 4. The touchscreen mask used in the PVD test of learning and memory.

2.3 Data analysis

All data were analyzed using GraphPad Prism (Version 10.0.0 – 153). Three-way ANOVAs were utilized, with sex and restriction protocol or diet as the between-subject factors and day, week, or session as the within-subject repeated factors. Tukey post-hoc analyses were used as required. In instances where sphericity assumptions were violated (Mauchly's test), a Greenhouse-Geisser correction was applied. The homogeneity of the between-subjects variable was tested with Levene's equality of variance test. Data are presented as mean ± standard error (SEM). Significance was set at $\alpha < 0.05$.

Analyses were executed by comparing each dependent variable in two sets: 1) restriction protocol and sex within the standard diet group and 2) diet and sex within citric acid groups. Both analyses included the same standard diet (non-food-restricted) mice on citric acid, since including a group of mice consuming HFHS diet while having their consumption restricted

would be an ineffective model of diet-induced obesity. By eliminating irrelevant comparisons using these two sets of analysis, we reduced type 2 error. This study design allowed us to answer 1) whether CA water and food restriction motivate mice consuming standard chow in a similar manner, and 2) whether CA water can motivate HFHS diet mice to a similar extent as standard chow diet mice.

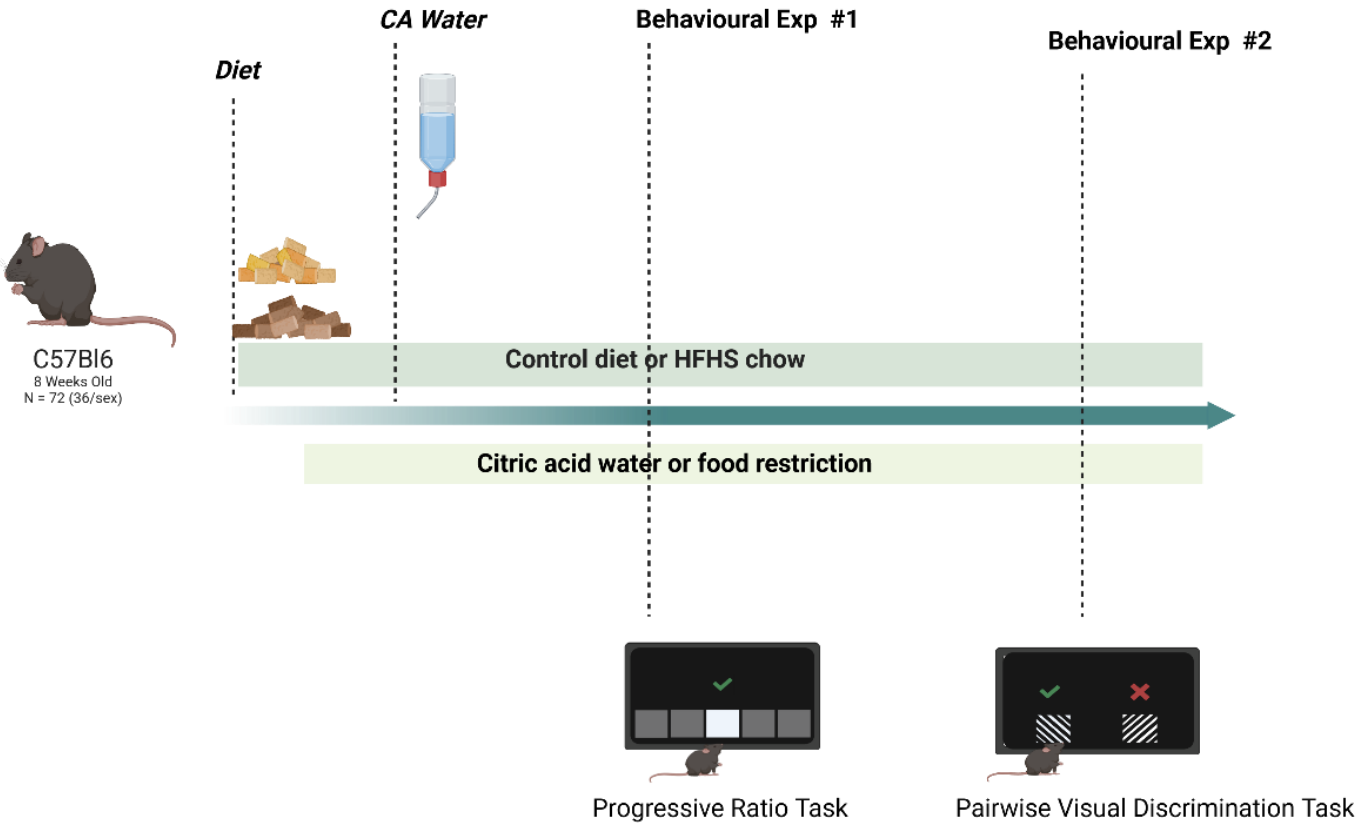


Figure 5. Experimental timeline for the water manipulation experiment.

2.4 Results

2.4.1. Diet and water manipulation health outcomes

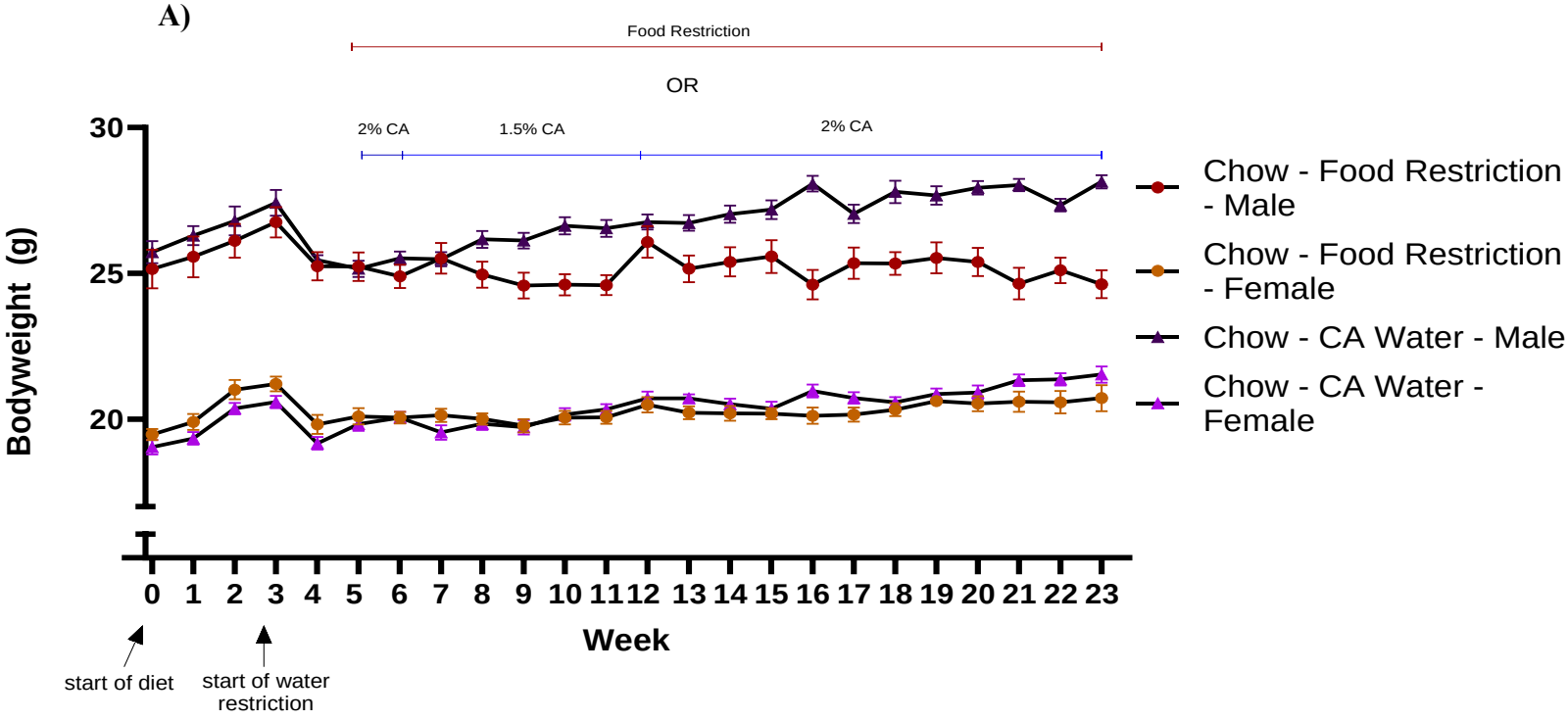
Health monitoring consisted of measuring body weight, activity, grooming, and if the animal was eating and drinking regularly. Following the first week of 2% citric acid (CA) water administration in the water restricted groups, a sharp decrease in bodyweight was observed. Due to the rapid nature of this weight loss, the percentage of citric acid in the water was reduced to 1.5% as a safety measure. Following the move to 1.5% CA, weight loss stopped, and the animals eventually maintained at a healthy 85% of their baseline free-fed body weight or greater. Following several days stabilized at this weight, mice were then returned to 2% CA water. Mice consuming HFHS diet and 2% CA water had reduced fecal output when compared to standard chow fed mice, earning a score of 1 (Table 1); however, this was independent of CA water administration. Additionally, a small number of mice received a score of 1 for posture and grooming within the initial days of CA water administration (Table 1). Body weight (g $\pm$ SEM, figure 6) in standard chow fed mice receiving either CA water (6A) or food restriction (6B) across 20 weeks showed main effects of week ($F(23, 960) = 9.757$, $p<0.001$), restriction ($F(3,960) = 2959$, $p<0.0001$), and sex $F(1,960) = 7984$, $p<0.0001$) (as shown in figure 6). There was a significant within-subjects interaction between week and restriction ($F(23,1224) = 3.862$, $p<0.0001$), with standard chow 2% male mice showing significantly greater bodyweights from week 9 onwards ($p<0.05$). Mice in all groups decreased in weight to a stabilized weight of 85-90% of pre-restriction weight before the beginning of behavioural testing (figure 2-1A-B). Following a post-hoc investigation, female mice had significantly lower bodyweight in

comparison to male mice, regardless of restriction type (food restriction $p<0.0001$; standard chow 2% CA water $p<0.0001$; HFHS diet 2% CA water $p<0.0001$). In addition, male standard chow fed mice receiving CA water had significantly higher bodyweights than male standard chow fed mice on food restriction ($p=0.038$).

Activity	**Score**
Moves around the cage	0
Moves slowly around the cage	1
Moves only when touched	2
Does not move	3
Posture and Grooming	
Normal posture and smooth fur	0
Hunched posture or ruffled fur	1
Hunched posture and slightly ruffled fur	2
Hunched posture and all fur ruffled	3
Signs of Eating and Drinking	
Feces and urine observed	0
Minimal fecal and/or urine	1
No signs or feces and/or urine	2
Signs of Dehydration	
Skin does not tent when scuffed	0
Skin tents briefly but returns to normal	1
Skin tents and takes more than 2 seconds	2
Skin tents and stays tented	3
Total Scores Any animal that has a score: • ≤ 4 cumulatively or ≤ 1in any one category should be monitored but no action required • ≥ 2 in any one category or cumulatively to ≥ 5 required veterinary support to monitor the animal	

Table 1. Health scoring system used to assess activity levels and hydration of mice on citric acid water. All mice receiving water manipulation treatment were monitored using this table 6 times per week.

A)


Food Restriction
OR
2% CA
1.5% CA
2% CA
Bodyweight (g)
30
25
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Week
start of diet
start of water restriction
Chow - Food Restriction - Male
Chow - Food Restriction - Female
Chow - CA Water - Male
Chow - CA Water - Female

B)

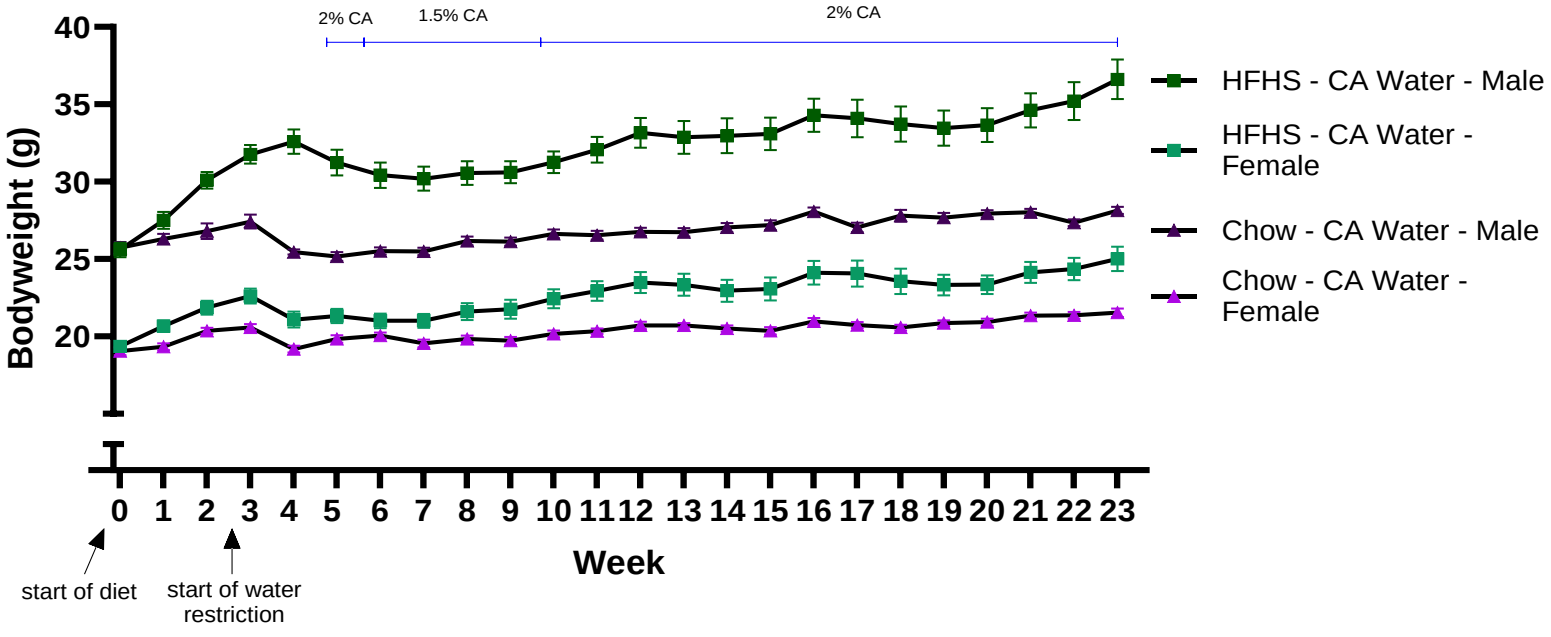

2% CA
1.5% CA
2% CA
Bodyweight (g)
40
35
30
25
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Week
start of diet
start of water restriction
HFHS - CA Water - Male
HFHS - CA Water - Female
Chow - CA Water - Male
Chow - CA Water - Female

Figure 6. Average bodyweight maintained from baseline and from the start of (A) restriction type on standard diet and (B) water manipulation. Mice began receiving either standard chow or HFHS diet *ad libitum* from timepoint week 0 (baseline) to week three and were weighed three times per week. Week 0 was established based on the three consecutive weights record prior to the start of week 0. On week 3, chow-fed mice were food restricted (n=8/sex per group) **or** started receiving 2% CA water (n=12/sex per group), and HFHS diet mice started receiving 2% CA water (n=16 per group) *ad libitum.* The weight recorded at week 3 functioned as a baseline weight. Mice were initially provided 2% CA water at week four, but in an effort to prevent rapid bodyweight loss, mice were moved to 1.5% CA water from week 5 to week 8 to stabilize weight, after which they were returned to 2% CA water from week 8 until the end of the study. Following the commencement of food restriction or water manipulation, mice were weighed and had their health monitored 6 times a week. Data presented as mean±SEM.

2.4.2 Water manipulation and diet modulates motivation in obese mice in the fixed-ratio and progressive ratio tasks.

No differences were found in sessions to criterion for the FR/PR tasks between standard chow diet food restricted and standard chow diet with 1.5% CA water mice ($p>0.05$, figure 7A). Conversely, following the switch to 2% CA water, only obese male mice on HFHS diet and 2% CA water were slower to train in comparison with male mice on standard chow diet and 2% CA water (main effect of diet $F(3, 51) = 0.7029$, $p<0.01$, figure 7A, 7B) and female mice on standard chow and 2% CA water (post hoc; $p<0.05$). A comparison of the rate of response during the fixed-ratio (FR) task showed a main effect of stage (figure 7C, $F(2.024, 72.86) = 107.5$, $p<0.0001$) when comparing diet groups, since each stage of FR requires greater responses/minute to complete. In the water manipulated groups, there was once again a main effect of stage (figure 7D, $F(1.859, 94.18) = 125.6$, $= p<0.0001$), as well as a main effect of diet ($F(3, 152) = 5.548$, $p<0.01$), with mice consuming standard chow responding more/minute, and an interaction of stage x diet ($F(3, 152) = 5.549$, $p<0.01$).

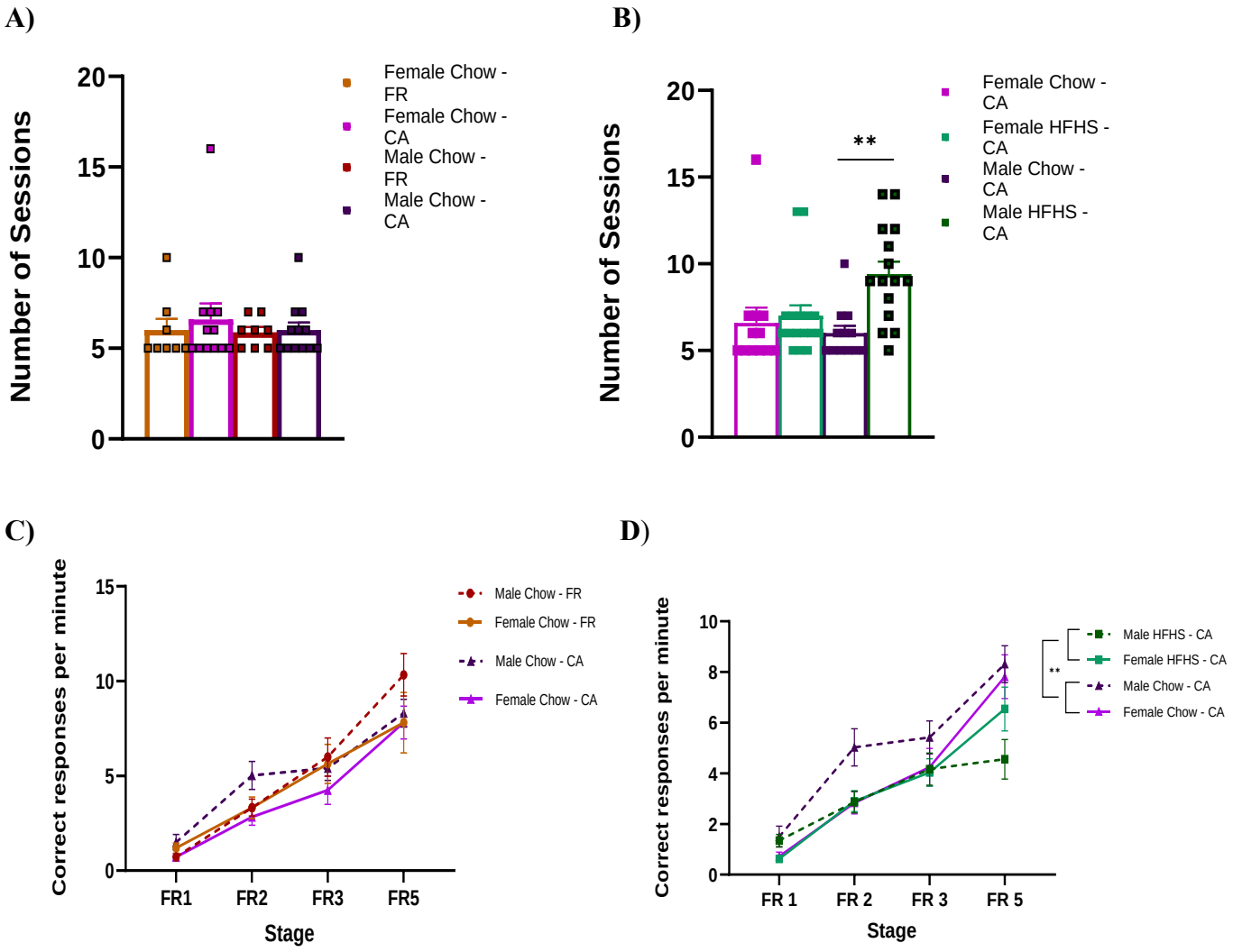


Figure 7. Effects of restriction type on standard diet or water manipulation on days to train and response rate in the fixed-ratio touchscreen task. Days to training encompassed habituation to the touchscreen chambers, learning to interact with the touchscreens, and learning to retrieve reward. The groups did not differ significantly in the days required to train on the touchscreen task across restriction type on standard diet (A). They did differ across groups in the water manipulation (B) groups, with obese HFHS males taking significantly longer to train than standard chow males. The FR task trained the mice to make a certain number of responses at each stage to successfully receive the reinforcement reward (strawberry milkshake). There was an effect of stage in both restriction type on standard diet (C) and water manipulation (D). There was also an effect of diet, and an interaction of stage x diet in (D). Performance within each group is further broken down by sex. Data presented as mean±SEM, ** $p < 0.01$.

On the progressive ratio task itself, mice performed it once while CA water mice were on 1.5% CA water, and then again when they returned to 2% citric acid water. These two runs are analyzed separately.

Breakpoint, an important marker of motivation, was defined as the maximum number of responses that were given at each stage of the PR task. Comparing 1.5% citric acid to food restriction, mice had lower breakpoints at PR4 than at PR8 or PR12 ($p<0.001$; main effect of stage, $F(1.569, 56.5)=11.962$, $p<0.001$, figure 8A). There were, however, no significant differences between restriction types or diets ($p>0.05$). The resulting total rewards collected showed a similar pattern, with a main effect of stage ($F(1.37, 49.316)=373.203$, $p<0.0001$, figure 8E) with PR4 producing many more rewards than PR8 or PR12 ($p<0.0001$) and PR12 resulting in slightly more rewards than PR8 ($p<0.05$). Notably, food restricted mice received more rewards than 1.5% CA water mice ($F(1,36)=4.327$, $p<0.05$, figure 8E). Responses to non-illuminated windows (i.e. blank touches) differed by sex ($F(1,36)=9.009$, $p<0.01$, figure 8C; males more than females) and stage ($F(2, 72)=7.517$, $p<0.01$, figure 8C), with stage significantly interacting with sex ($F(2,72)=3.523$, $p<0.05$) and restriction type ($F(2,72)=5.515$, $p<0.01$). Notably, male mice made significantly more blank touches in PR8 than did female mice ($p<0.01$).

While on 1.5% citric acid, mice of both diets had lower breakpoints at PR4 than at PR8 or PR12 ($p<0.05$; main effect of stage, $F(1.523, 77.667)=8.507$, $p<0.01$, figure 8B). Chow fed mice had higher breakpoints than HFHS mice (main effect of diet, $F(1,51)=22.437$, $p<0.0001$, figure 8B), with no effect of sex ($p>0.05$). Similarly, chow fed mice received more rewards resulting from

their increased responding (main effect of diet, $F(1,51)=19.836$, $p<0.0001$, figure 8F), particularly at PR4 and PR12 ($p<0.05$; stage by diet interaction, $F(1.508,76.892)=11.643$, $p<0.001$, figure 8F). Here there was a significant three-way interaction ($F(1.508,76.892)=7.257$, $p<0.01$, figure 8F), but with no relevant significant pairwise comparisons. Number of blank touches differed by diet ($F(1,51)=7.823$, $p<0.01$, figure 8D), with diet interacting with reward contingency ($F(2,102)=8.06$, $p<0.001$) and sex ($F(1,51)=5.521$, $p<0.05$) alone and as a three-way interaction ($F(2,102)=3.206$, $p<0.05$, figure 8D). Notably, male chow fed mice made more blank touches than male HFHS mice ($p<0.01$), particularly at PR4 ($p<0.05$).

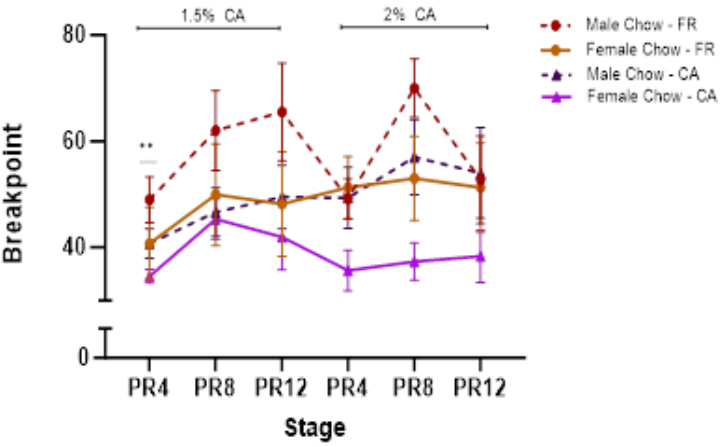
Following testing with CA mice on 1.5% CA water, mice were returned to 2% CA water and tested again on PR. In a comparison of breakpoint by restriction type on standard diet, there was a main effect of stage ($F(1.456, 52.401) = 7.931$, $p<0.01$, figure 8A). There were no main effects of treatment or restriction type ($p>0.05$, figure 8A). A significant sex by stage interaction was observed ($F(1.456, 52.401) = 5.227$, $p<0.05$) but no relevant and significant pairwise comparisons were found. There was a main effect of stage ($F(1.044, 37.596) = 31.632$, $p<0.0001$, figure 8E) in number of rewards collected, with more rewards received in PR4 than PR8 or PR12. The number of blank touches made significantly differed by stage ($F(2,72)=14.579$, $p<0.0001$, figure 8C), but not by sex or restriction type.

Importantly, in the water manipulated groups, a main effect of diet on breakpoint was present ($F(1, 51) = 5.51$, $p<0.05$, figure 8B) with standard chow mice outperforming HFHS diet mice. Once again, there was also a main effect of stage ($F(2, 102) = 4.463$), $p<0.05$) but no main effect

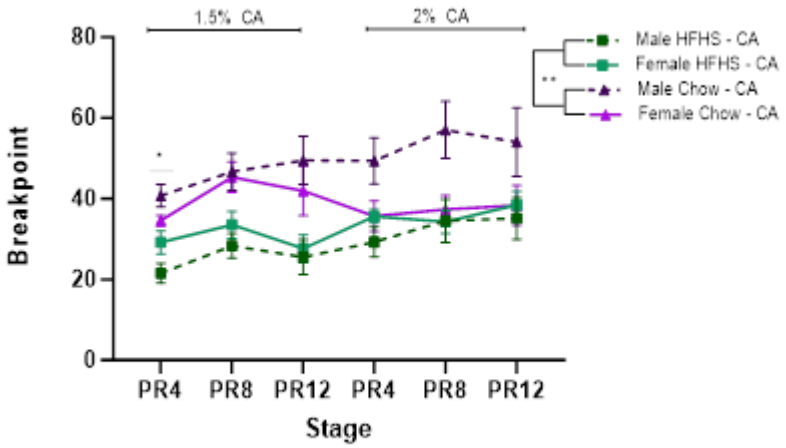
of sex ($p>0.05$). A diet by sex interaction was present ($F(1, 51) = 4.641$, $p<0.05$), with a significant difference between standard and HFHS fed male mice ($p<0.05$). This effect of diet carried over to the number of rewards collected ($F(1, 51) = 4.873$, $p<0.05$) with HFHS mice collecting fewer rewards. In this group there was also no effect of sex ($p>0.05$), but the number of rewards collected was higher in PR4 than in PR8 or PR12 ($p<0.0001$; main effect of stage ($F(1.069, 54.522) = 45.952$, $p<0.0001$, figure 8F). Blank touches differed across stage ($F(1.768, 90.17) =4.9$, $p<0.05$, 8D), with no further main effects ($p>0.05$). Finally, there was a three-way interaction ($F(1.768, 90.17) = 6.059$, $p<0.01$) with no significant pairwise comparisons.

These findings provide important insight into the applicability of 2% CA water as a motivator by suggesting that it can be used effectively in studies that compare performance across groups of obese animals. While CA water may not motivate standard-fed mice to a similar level as food restriction in this sensitive test of motivation, the results of this experiment provide compelling evidence that CA water can successfully motivate obese mice to perform touchscreen tasks. Thus, we found changes in responding during PR which is highly sensitive to changes in motivation. However, we were interested in the practical application of the CA intervention in tests of cognition. Therefore, in the next experiment we tested the applicability of water manipulation in a test of learning and memory, the pairwise visual discrimination (PVD) task.

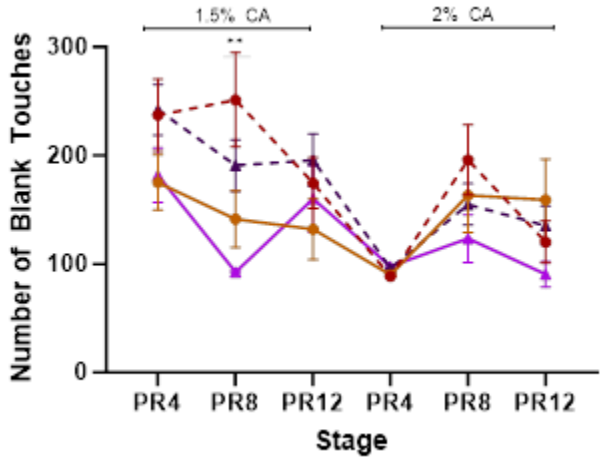
A)


1.5% CA
2% CA
Male Chow - FR
Female Chow - FR
Male Chow - CA
Female Chow - CA
Breakpoint
80
60
40
0
**
PR4
PR8
PR12
PR4
PR8
PR12
Stage

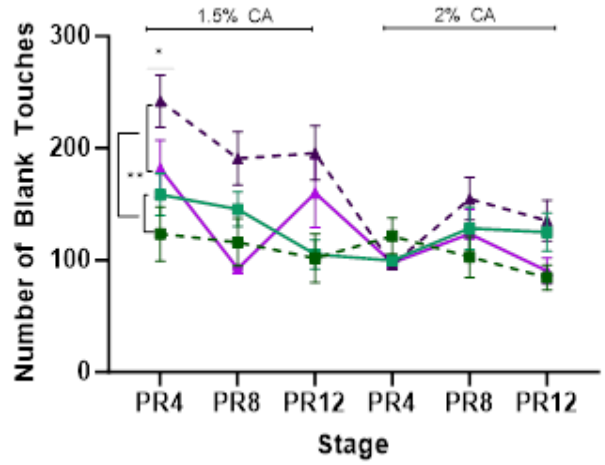
B)


1.5% CA
2% CA
Male HFHS - CA
Female HFHS - CA
Male Chow - CA
Female Chow - CA
**
*
Breakpoint
80
60
40
20
0
PR4
PR8
PR12
PR4
PR8
PR12
Stage

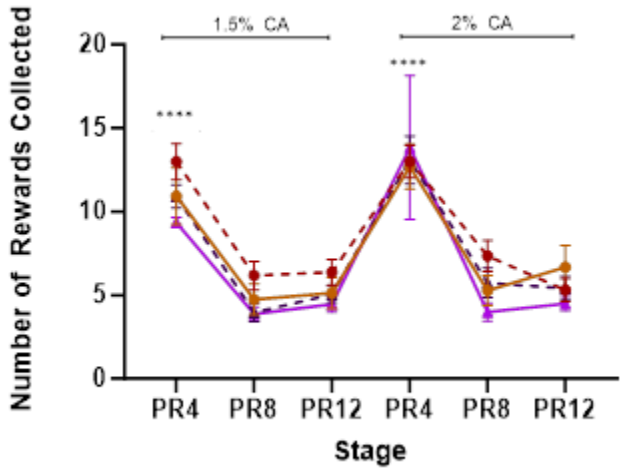
C)


1.5% CA
2% CA
**
Number of Blank Touches
300
200
100
0
PR4
PR8
PR12
PR4
PR8
PR12
Stage

D)


1.5% CA
2% CA
*
**
Number of Blank Touches
300
200
100
0
PR4
PR8
PR12
PR4
PR8
PR12
Stage

E)


1.5% CA
2% CA
****
****
Number of Rewards Collected
20
15
10
5
0
PR4
PR8
PR12
PR4
PR8
PR12
Stage

F)

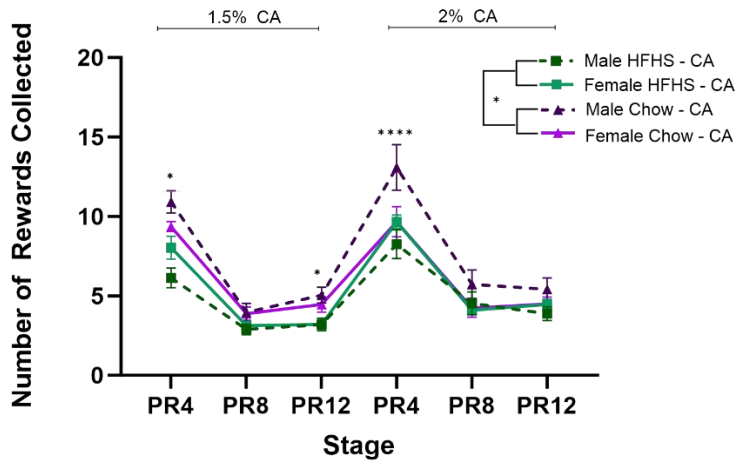

1.5% CA
2% CA
Male HFHS - CA
Female HFHS - CA
Male Chow - CA
Female Chow - CA
*
*
*
****
Number of Rewards Collected
20
15
10
5
0
PR4
PR8
PR12
PR4
PR8
PR12
Stage

Figure 8. Levels of motivation within the progressive ratio touchscreen task vary based on group.

In the first round of PR, 1.5% CA chow mice and F-R mice had lower breakpoints at PR4 in comparison to PR8 and PR12 (A). These same effects were observed in number of total rewards collected, and FR mice collected more rewards (E). F-R mice completed more blank touches, and male mice had significantly more than females (C). 1.5% chow vs. 1.5% HFHS diet also had lower breakpoints at PRR4 than in PR8 or PR12, and chow had higher breakpoints than HFHS (B). Chow fed also collected more trials, especially at PR4 and PR12. (F) Male chow mice made more blank touches than male HFHS mice (D).

In the second round of PR, 2% CA chow and F-R mice had similar breakpoints (A), blank touches (E), and rewards collected (C). More rewards were collected in PR4 than in PR8 or PR12 (C). Water manipulated groups showed greater breakpoint in chow mice than in HFHS mice, and an interaction demonstrated a significant difference between male chow and male HFHS mice (B). Rewards collected also differed between diets with chow mice collecting more rewards, and both groups collecting more rewards in PR4 than in PR8 or PR12 (F). Groups performed similarly in blank touches (D). Data presented as mean±SEM, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

2.4.3 Groups consuming 2% CA water performed similarly in the pairwise visual discrimination task.

In contrast to the clear variation between groups in performance observed during PR, there were no clear differences between groups in PVD (figure 9). There was a main effect of session when comparing groups by restriction type (figure 9A, $F(5.692, 204.898) = 46.704$, $p<0.0001$), as well as an interaction of session x sex x restriction type ($F(5.692, 204.898) = 2.402$, $p<0.05$; no statistically significant and relevant pairwise comparisons, $p>0.05$), but no effect of sex or restriction type ($p>0.05$). A main effect of session (figure 9B, $F=(6.041, 302.054) = 75.67$, $p<0.0001$) was also present when investigating percent correct between water manipulated groups. Additionally, here a main effect of sex ($F(1, 50)=5.057$, $p<0.05$) which demonstrated a greater percent correct in females than in males. Importantly, there was no significant difference

between groups at session 10 (p>0.05). Once again, there was no significant difference between water manipulated groups (p>0.05).

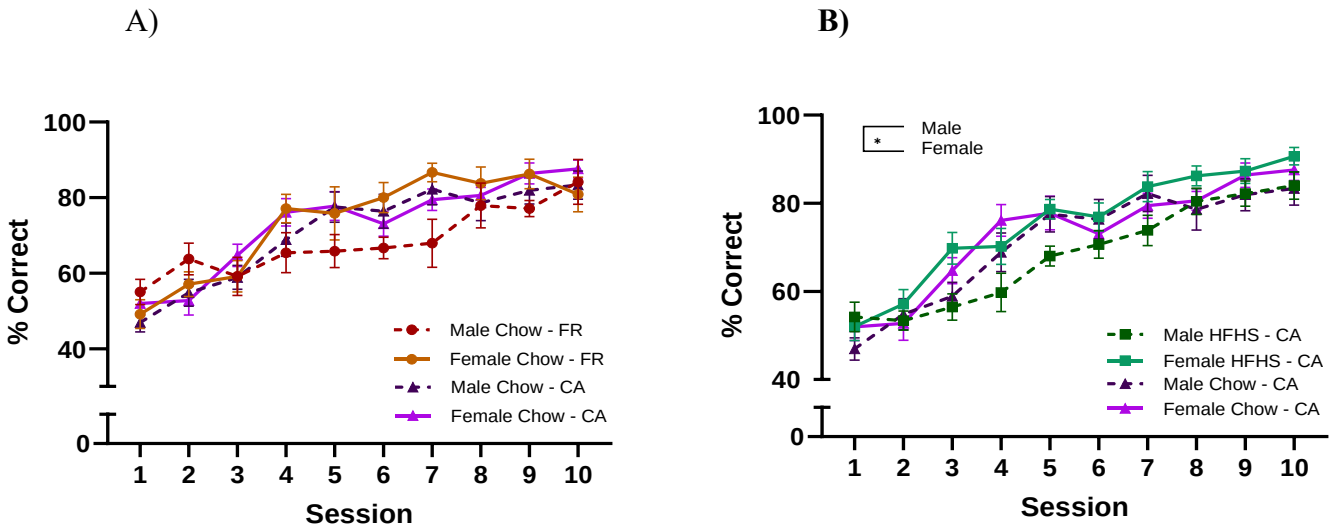


Figure 9. Percentage correct trials across 10 sessions of the pairwise visual discrimination task. Percentage of correct trials in the PVD task by restriction type (A) and water manipulation (B) both demonstrated variation based on session. (A) showed an interaction of session x sex x restriction type. In (B) an effect of sex was observed. Data presented as mean±SEM, *$p<0.05$.

Additionally, there were some differences in correction trials between groups (figure 10A-B). Correction trials occurred following an incorrect trial response and repeated until the animal made a correct response. Correction trials did not count towards total trial count during the session. There was a main effect when comparing restriction groups by session (figure 10A, $F(5.116, 184.177) = 24.369$, $p<0.0001$), as well as a main effect of sex ($F(1, 36) = 6.64$, $p<0.05$; males made more correction trials than females), but no effect of restriction type (p>0.05). When probing performance across water-manipulated groups (figure 10B), effects differed from those observed across restriction groups. Here, there was a main effect of session ($F(5.187, 259.346) = 77.39$), $p<0.0001$), a main effect of diet ($F(1, 50) = 13.32$, $p<0.0001$) that demonstrated a greater

number of correction trials in chow fed mice than in HFHS fed mice. Additionally, a main effect of sex was present ($F(1, 50) = 8.85$, $p<0.01$) which showed that males completed more correction trials than females.

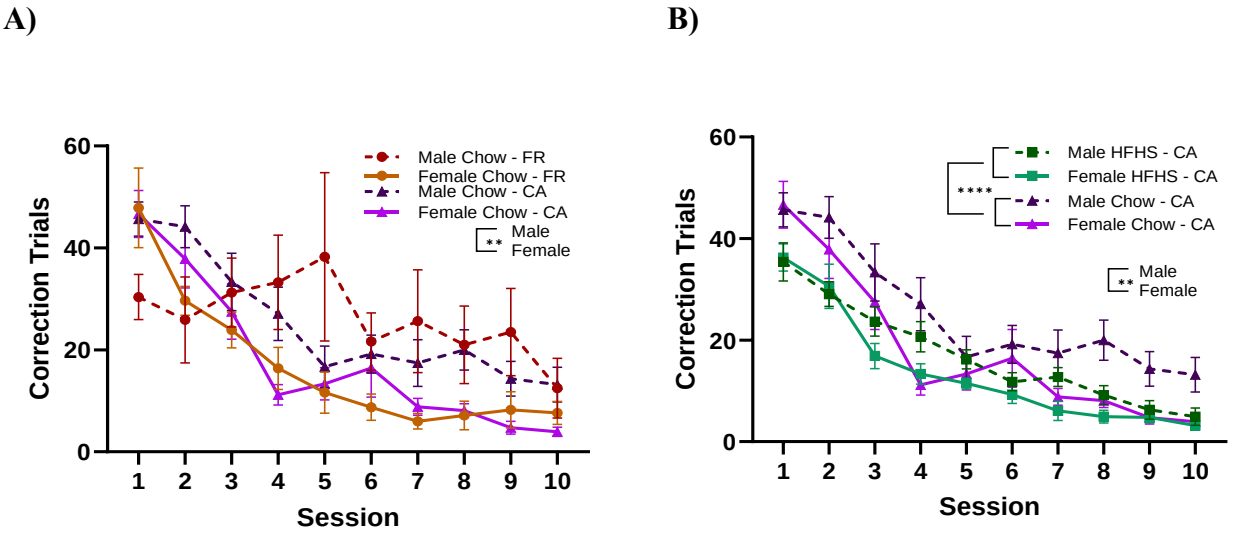


Figure 10. Number of correction trials across 10 sessions of the pairwise visual discrimination task. Number of correction trials in the PVD task by restriction type (A) and water manipulation (B) both demonstrated variation based on session. In (A) there was also a main effect of sex, but no effect of restriction type. In contrast, (B) demonstrated a main effect of diet, sex, and session. Performance within each group is further broken down by sex. Data presented as mean±SEM, ****$p < 0.0001$.

To further contextualize these findings, the perseverance index (PI) was calculated. The PI is a ratio of the number of correction trials to each incorrect trial, representing the number of corrections required for the mouse to complete the trial correctly. There was no main effect of restriction type (figure 11A, $p<0.05$), however there was a main effect of session ($F(0, 359) = 7.730$, $p<0.0001$). In addition, there was a main effect of sex ($F(1, 359)$ 15.77, $p<0.0001$) with males exhibiting a higher PI than females. Similar effects were seen when investigating performance across water-manipulated groups. There was no significant difference in PI between diet groups (figure 11B, $p<0.05$) but once again there was a main effect of session ($F(9. 496) =$

5.058, p<0.0001). There was also a main effect of sex (F(1, 496) = 19.19, p<0.0001) with males completing the task with a greater PI than females.

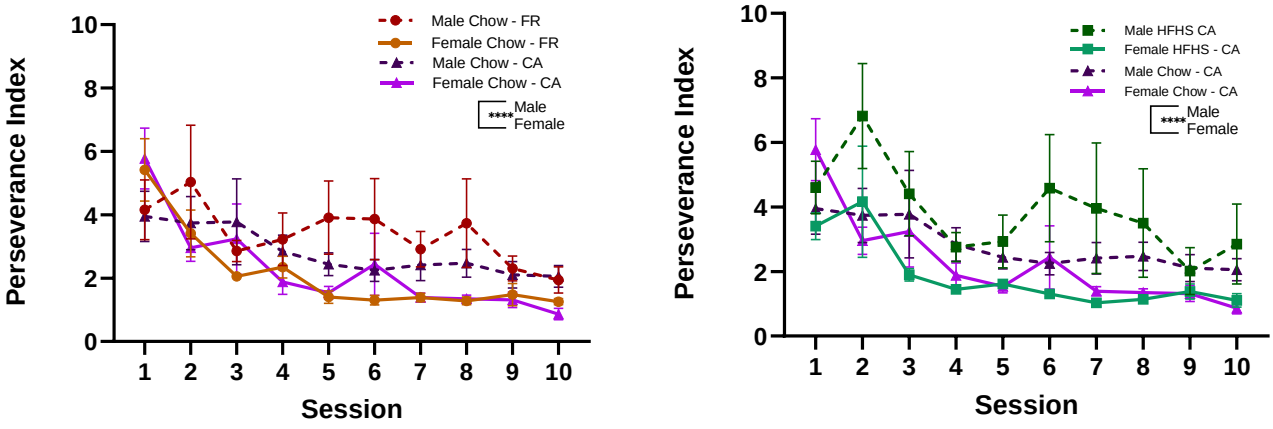


Figure 11. Perseverance index across 10 sessions of the pairwise visual discrimination task. Perseverance index in the PVD task by restriction type (A) and water manipulation (B) both demonstrated a main effect of session and sex. No main effect of restriction type (A) or diet (B) was present. Performance within each group is further broken down by sex. Data presented as mean±SEM, ****$p < 0.0001$.

Interestingly, these findings show that the variations in performance between standard chow and food restricted mice in the progressive ratio task are not observed during the pairwise visual discrimination task. This homogeneity in performance across groups in PVD suggests that in tasks that the use of 2% CA water intervention functions as a reliable and effective motivator in touchscreen behavioural tasks that are not specifically probing motivation.

Chapter 3: Resistance exercise experiment

3.1 Introduction

High prevalence, complex origins, and interacting health complications make obesity a difficult condition to address in a clinical setting (Wharton et al., 2020). Sustained obesity, characterized by excessive adiposity, is often casually associated with chronic conditions such as type 2 diabetes, nonalcoholic fatty liver disease, cardiovascular disease, and various cancers (Wharton et al., 2020). In addition to physiological impacts, obesity has also been associated with various cognitive impairments (Buie et al., 2019). Obesity exerts its effects on cognitive performance by promoting inflammation, dysfunction in insulin sensitivity, and gut homeostasis which in turn results in impaired endothelial function, oxidative stress, and mitochondrial dysfunction (Buie et al., 2019; de Mello et al., 2018; Sripetchwandee et al., 2018). Clinical studies have suggested that mid-life obesity (45-65 years old) is associated with worse cognitive outcomes and more adverse effects on cognition (Smith et al., 2011; Dye et al., 2017). Mid-life obesity has been correlated with lower scores on the Mini-Mental State Examination (MMSE) (Bischof et al., 2015), worse performance on testing involving organization, attention and visuomotor speed, executive function (Wolf et al., 2007), and memory (Gunstad et al., 2009).

The growing rate of obesity and its detrimental effects have largely been attributed to excessive consumption of "western diet" which is characterized by high levels of sugar and fat (Rakhra et al., 2020). Consumption of WD is pushing incidence of obesity into even younger age brackets, with type 2 diabetes now commonly presenting in childhood and adolescence instead of in adulthood (Rakhra et al., 2020). WD is used widely in diet-induced models of obesity in rodents

(Buie et al., 2019). WD induces cognitive impairment in these models (Darling et al., 2013), especially spatial, working, and recognition memory as investigated through performance on several established behavioural tests such as the Morris water maze (Gladding et al., 2018), y-maze (Labouesse et al., 2018), and novel object recognition test (Cordner and Tamashiro, 2015), respectively. During the early stages of diet-induced obesity, rats demonstrated profound deficits in cognitive tasks requiring the prefrontal cortex as well as reduced numbers of dendritic spines and structural alterations to microglia in the PFC (Bocarsly et al., 2015). Using preclinical models of diet-induced obesity in rodents allows a greater level of control in domains that affect the manifestation of obesity such as age, activity level, and type of diet consumed.

In combatting obesity, the most recommended interventions consist of a change of diet or an increase in physical activity levels (Sharma, 2007). For many, a change of diet away from a WD is cost-prohibitive or inaccessible (Malik et al., 2013). Rapid urbanization resulting in densely populated areas with minimal outdoor recreational spaces, reliance on motorized transportation systems, and the increasing rate of employment in sedentary work sectors limits the possibility and probability of engaging in physical activity (Malik et al., 2013). However, improvements in physiological markers of health (Maffiuletti et al., 2005; Nelson et al., 2006; Kennedy et al., 2005) and cognitive function (Peven et al., 2020) were observed when sustainable exercise regimes were implemented.

Previously, aerobic exercise interventions have been exceedingly popular and effective in alleviating inflammation in children and adolescents with obesity (Calcaterra et al., 2022) as well

as cognitive dysfunction in mice consuming WD (He et al., 2023). 13-week treadmill exercise led to an improvement in visuospatial working memory capacity in 12 overweight participants (Russo et al., 2018). Short term memory and spatial memory, as tested by performance in the Y-maze, were improved in obese mice following 12 weeks of treadmill exercise (Kim et al., 2016). Park et al (2019) found similar effects in the performance of obese mice in Morris Water Maze following 12 weeks of treadmill exercise. Subsequent studies investigating the variation in effect dependent on the level of intensity of the aerobic intervention found that significant improvements in spatial and working memory occurred only in the high intensity exercise group, not in the low or medium intensity groups (Kim et al., 2020; Mora-Gonzalez et al., 2019).

Despite the demonstrated efficacy of aerobic exercise interventions in alleviating many of the deleterious effects of obesity, it is not sustainable or safe in certain populations. Obesity increases the risk of falls and multiple falls in individuals aged 60 years or more (Neri et al., 2020), and due to alterations in body composition that often lead to sarcopenia or osteoporosis, falls are more likely to result in fractures (Scott et al., 2016). In older populations, a fracture can result in a prolonged hospital stay, an increased risk of subsequent injury, and ultimately a loss of independence (Sabesan et al., 2015). Since aerobic exercise interventions commonly consist of running, jumping, or other high intensity activities, it may not be as useful in older, obese individuals. Instead, resistance exercise presents itself as an attractive alternative. Resistance exercise interventions increase functional strength more than aerobic exercise, allowing older individuals to maintain independence and self-sufficiency (Villareal et al., 2017). Resistance exercise prioritizes growth of skeletal muscle as opposed to simple fat loss, although it reliably reduces visceral and subcutaneous adiposity in the abdominal region (Strausser et al., 2011).

Abdominal adiposity is especially dangerous in models of obesity as it is a major risk factor for metabolic disorders (Strausser et al., 2011). In addition to improvements in physiological markers of obesity in older populations, they also demonstrated improved performance on tests such as the digit span forward task and Rey-Osterrieth complex figure immediate recall when compared to age-matched individuals that did not participate in any resistance exercise (Cassilhas et al., 2007). Preclinical rodent models have demonstrated that a consistent resistance exercise regime inhibits neuroinflammation and attenuates neuropathological changes in Alzheimer's model mice (Liu et al., 2020), significantly increases strength (Kim et al., 2015), improves performance in the Y-maze in sarcopenic, obese mice (Lim et al., 2022), and reduces hepatic insulin resistance in obese mice (da Crus Rodrigues et al., 2021). Most pre-clinical reports studying resistance exercise utilize a weighted repeated ladder-climbing task to model the exercise accurately (Liu et al., 2020; Kim et al., 2015; Lim et al., 2022). However, investigation into the capability of a resistance exercise intervention to improve specific cognitive functions that have been previously shown to be affected by increased age and obesity, such as spatial memory and motivation (Wolf et al., 2007), is lacking.

Resistance exercise has been presented as an exciting, sustainable, and effective intervention in mitigating some of the negative effects of obesity in older individuals as an alternative to aerobic exercise (Cassilhas et al., 2007). However, maintaining homogeneity in diet, age-related factors, and exercise intensity between human participants is challenging. Although a reliable resistance exercise preclinical model exists (Liu et al., 2020; Kim et al., 2015), it has not been used to thoroughly elucidate the effects of resistance exercise on motivation, perseverance, and spatial memory in DIO mice. A reliable and safe manner to answer these questions is through the usage

of the already validated weighted ladder-climbing task in conjunction with touchscreen behavioural testing paradigms. This study will further the understanding of the effects of a WD on spatial memory, motivation, and adiposity in older mice, and whether a resistance exercise intervention is sufficient to rescue these effects.

3.2 Methods

3.2.1 Animal housing and care

All experiments were conducted in compliance with the standards set by the Canadian Council of Animal Care and in collaboration with the veterinary staff at the University of Western Ontario with an approved animal protocol (2021-082). Forty-eight C57BI/6J mice (24/sex, The Jackson Laboratory, US) arrived aged 8-12 weeks old. Mice were separated by sex and housed in groups of 4/cage (28 x 18 cm plastic cages with wire tops) in a temperature (23 ± 1°C) and humidity (50 ± 1%) controlled room under a reverse 12h light/dark cycle (lights off at 9:00). Upon arrival, mice were acclimatized to their cages for 5 days with *ad libitum* access to standard diet and untreated water. Mice were aged to 13 months of age with regular handling and standard chow (Teklad Envigo 7913, 18% protein rodent diet, 3.1 kcal/g, 5% fat, 5% fibre). At 13 months of age, all mice began consuming high fat high sugar (HFHS) chow (Bioserv F6724, 4.57 kcal/g). All mice had *ad libitum* access to water and their respective food. Following 1 week of habituation to HFHS chow, mice were then given 2% citric acid (CA) water as described in chapter 1. Following one week of acclimation to 2% CA water, mice randomly assigned to ladder-based resistance exercise (RES), (N =12/sex) or non-exercise (non-RES), (N=12/sex) intervention group.

3.2.2 Exercise paradigm

Following acclimatization to diet and CA water, all mice were habituated to the ladder resistance exercise apparatus (see Figure 12). The apparatus was made up of a 1m long plexiglass ladder with 1.5cm grids and a dark box positioned at the top. The angle of the ladder was adjustable. The RES condition group was habituated to the ladder while it was set at an 85-degree angle, while the non-RES group was habituated to the ladder while it was set at a 0-degree angle. Mice were habituated to the apparatus for 15 minutes/day for 3 days. Mice were placed at the bottom of the ladder and climbed to the top. After reaching the dark box at the top of the ladder, they were given 120 seconds before beginning the next trial. A successful trial was defined as the mouse climbing from the bottom of the ladder to the top and entering the dark box. Following habituation, the mice were tested for 5 trials/day, 5 days/week, for 8 weeks. The first resistance day consisted of the mice climbing to the top of the ladder carrying only their bodyweight. Once the bodyweight trial was successfully completed 5 times on the same day, the equivalent of 10% of the mouse's body weight was added to the base of its tail. The weights utilized for this experiment were 2- and 15-mL falcon tubes that were filled with varying amounts of water. The weights were attached using a coastlock snap swivel and Scotch 23 rubber tape. Following 5 successful trials (on the same testing day) with the 10% of their bodyweight added, an additional 2g of weight was added. The mice were required to complete 5 successful trials (on the same testing day) at each addition of weight before another 2g was added. The load weight was increased until the mouse was either unable to complete 5 successful trials at a certain weight, or they reached 100% of their starting body weight which was defined as their bodyweight following one week of diet intervention.

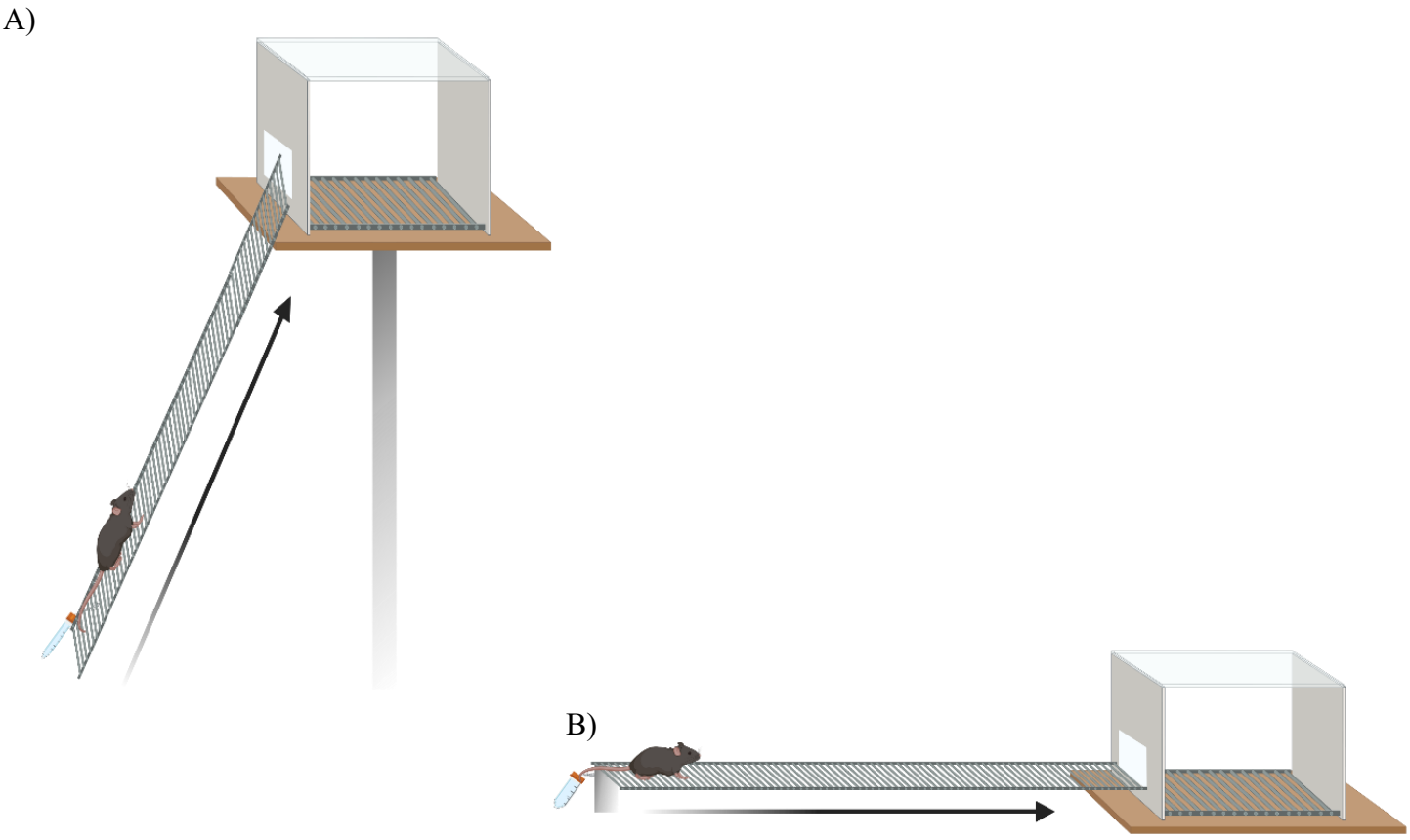


Figure 12. The resistance exercise ladder apparatus. The ladder exercise apparatus (A) in RES position at 85 degrees and (B) non-RES position at 0 degrees. The ladder is 1 metre tall with 1.5cm acrylic steps and a square opaque rest enclosure on the top.

3.2.3 Fixed-Ratio and Progressive Ratio (FR/PR)

Fixed-ratio (FR) and progressive ratio (PR) touchscreen testing were completed in Bussey-Saksida touchscreen operant chambers (Lafayette Instruments) as described in Chapter 1. Progressive ratio was completed twice and then averaged to control for variation in exercise level and borderline effects.

3.2.4 Extinction

Following PR, mice returned to FR1 training followed by an extinction protocol. In the extinction task, stimuli were presented in the same location on the screen but disappeared following a nose poke (defined as a response) or after 10s of presentation with no interaction from the mouse (omission). No reinforcement was provided for either responses or omissions. Each session of the extinction task consisted of 30 trials, with a pause of 4.5 seconds between each trial. The extinction task was performed over 10 sessions.

3.2.5 Spontaneous Location Recognition (SLR)

The spontaneous location recognition (SLR) task tests the subject's ability to distinguish between two similar locations in memory (Reichelt et al., 2021).

SLR Apparatus

The SLR apparatus consisted of a large, circular tub made of black plastic that was 47cm in diameter and 30cm deep This tub functioned as the open field and was placed within a black, rectangular enclosure (61cm x 61cm x 123cm). This enclosure functioned as a source of distal cues, with a unique black and white image attached to three out of the four walls, and the fourth wall functioned as a door. The floor of the field was covered with standard mouse bedding (Bed-o'Cobs Combination bedding, The Andersons Inc., Delphi IN). The top of the enclosure was open with a camera positioned above that allowed for recording of sessions. ANY-Maze behavioural tracking software (Stoelting Company, Wood Dale IL) was utilized in tandem with the camera to track the animal's location and movement.

Various objects made of plastic or glass were used as visual stimuli and were disinfected with 5% ethanol between each trial along with the walls of the open field.

SLR habituation

Habituation to the open field-testing area in the absence of stimulus objects was necessary to reduce any stress stemming from a novel environment. The animals were placed in the empty field and allowed to explore for 10 minutes per day for 3 days.

SLR test

The SLR test phase began the day following habituation. Mice first completed a sample phase, followed by a 3hr delay, and then completed the test phase each day. In the sample phase, the

mice were exposed to three identical objects (referred to as A1, A2, A3) in a triangular formation for 10 minutes. The configuration of these objects (i.e. the angle at which they were placed in relation to one another) defined the condition of SLR testing the mouse was completing (Figure 13).

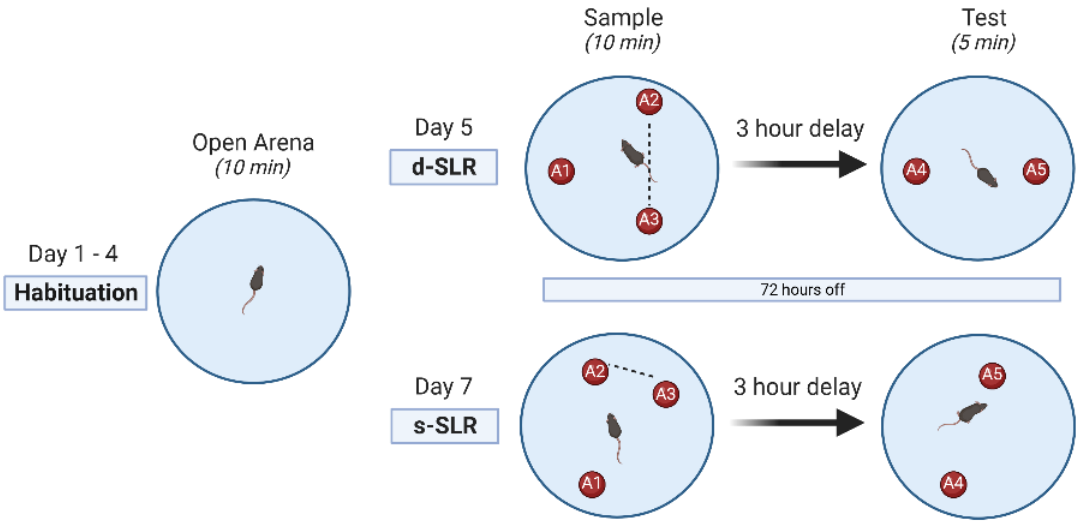


Figure 13. The spontaneous location recognition (SLR) task. Illustrated are the varying object configurations based on condition, d-SLR and s-SLR in sample phase and test phase.

The dissimilar condition (d-SLR), in which there was a larger amount of separation between objects, required all three objects to be 108° apart from each other. The similar condition (s-SLR), where there was a smaller amount of separation between objects A2 and A3, required objects A2 and A3 to be placed 72° apart from one another, and object A1 to be equidistant from A2 and A3. All objects were placed approximately 5cm away from the walls of the open field. A reduction in amount of separation between objects results in an increase in difficulty in the task; this is why the s-SLR phase is more challenging than the d-SLR phase.

Mice then underwent a delay of three hours during which they were returned to their home-cage. Following the delay, the mice completed the test phase. In the test phase, two of the identical objects used during the sample phase were presented in two locations (A4 and A5). The A4 object location was the exact same as the A1 location during the sample phase. The A5 location was novel, defined as the position exactly in between objects A2 and A3 in the sample phase. Each mouse completed both the d-SLR condition and the s-SLR condition with a 72-hour period separating each test.

Objects and condition order were randomized, and all mice were presented with different objects for each condition to increase novelty and therefore exploration. Both sample and test phases were recorded and manually assessed for exploration time and exploratory behaviour towards each of the objects. Exploration was defined as the mouse having its nose clearly orientated towards the object with a distance of <1 cm.

3.2.6 Strength measurement

Grip strength was measured using a force transducer that was attached to an angled wire grid which the mouse gripped onto. The mouse, held by the base of its tail, was lowered towards the grid until it gripped the grid with both front paws. The torso of the mouse was held parallel to the grid. This position was held for 2 seconds before the experimenter gently pulled the mouse backwards until it let go of the grid. This measurement was repeated 3 times with a break of 60 seconds between each measurement. The grip strength measurement was recorded the day before the start of the exercise intervention, and once a month following with the final test less than 1 week prior to perfusion. The grip strength apparatus was connected to a computer to ensure accuracy and reduce the number of trials required. Furthermore, grip strength was recorded on days that mice were not undergoing resistance exercise to avoid fatigue.

3.2.7 Tissue collection

Mice were anesthetized using isoflurane and transcardially perfused with 4% paraformaldehyde in PBS. All white adipose tissue deposits from both sides of the animal were removed and weighed. In addition, the entire liver was also removed and weighed.

3.3 Data Analysis

All data were analyzed using GraphPad Prism (Version 10.0.0 – 153). Two-way ANOVAs were used to identify main effects of group, session, or group-by-session interactions. One sample t-test was used to evaluate between-group differences. Tukey post hoc analyses were used as required. In instances where sphericity assumptions were violated (Mauchly's test), a Greenhouse-Geisser correction was applied. Data are presented as mean ± standard error (SEM). Significance was set at $\alpha < 0.05$

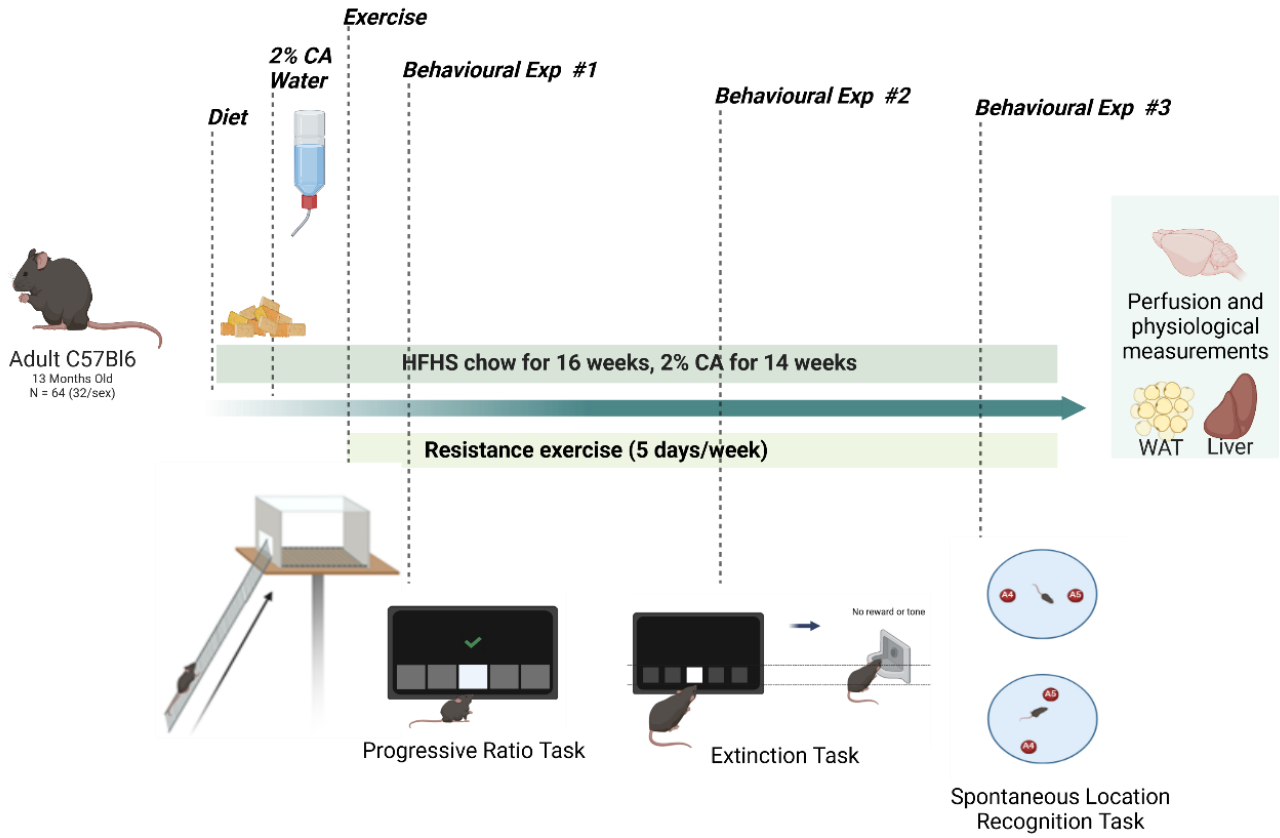


Figure 14. Experimental timeline of resistance exercise experiment.

3.4 Results

3.4.1 Diet and exercise intervention outcomes following resistance exercise intervention in aged, obese mice.

In this experiment, all mice consumed HFHS diet and 2% CA water. Mice began consuming HFHS at 13 months of age, and following two weeks of diet habituation they began receiving 2% CA water. There were significant differences in bodyweight between intervention groups (figure 15, main effect of exercise $F(1, 704) = 648.2$, $p<0.0001$. Additionally, there was a main effect of time point ($F(15, 704) = 23.82$, $p<0.0001$) and sex ($F(1, 44) = 51.51$, $p<0.0001$). There were significant interactions of timepoint x sex ($F(15, 660) = 2.980$, $p<0.001$), and week x

exercise (F(15, 660) = 7.843, p<0.0001). These results demonstrated that non-exercise (non-RES) mice weighed significantly more than exercise (RES) mice starting at week 13 of diet, and male mice weighed significantly more than female mice.

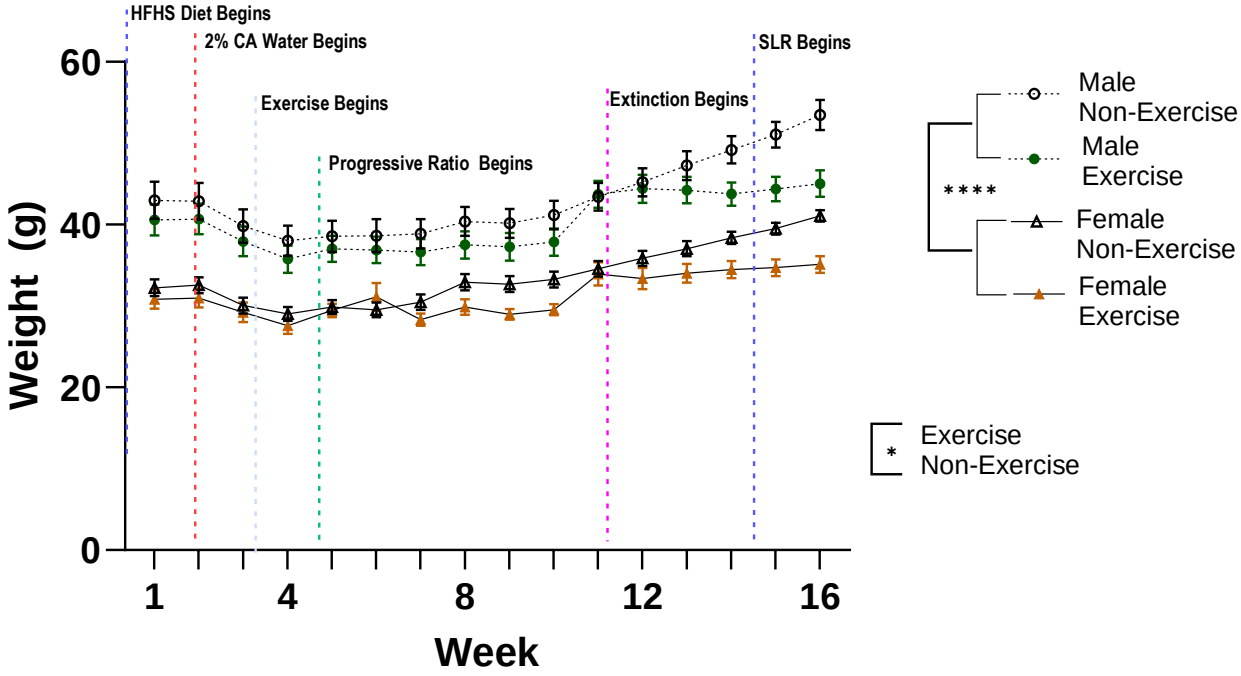


Figure 15. Body weight of each group over 16 weeks. At 13 months, all mice (n=24/sex) began consuming HFHS diet. Half (n=12/sex) underwent an exercise intervention. Mice undergoing the exercise intervention had reduced bodyweights compared to non-exercise mice. Female mice had reduced bodyweights across intervention, and an effect of week time point was also present. Data presented as mean±SEM, *$p < 0.05$, ****$p<0.0001$.

However, these differences in bodyweight were not due to a noticeable difference in diet consumption. Across the 16 weeks, the non-RES and RES males consumed a similar amount of HFHS chow, as did the non-RES and RES females (figure 16). There were no significant differences in consumption between RES and non-RES males (p>0.05) or RES and non-RES females (p>0.05), but there was a main effect of sex (F(1, 8) = 108.5, p<0.0001) with males

consuming more than females. Levels of HFHS diet consumption were of interest in this study as the mice were exposed to high levels of handling and novel exercise interventions that can lead to stress in mice, often characterized by a reduction in eating (Preez et al., 2021).

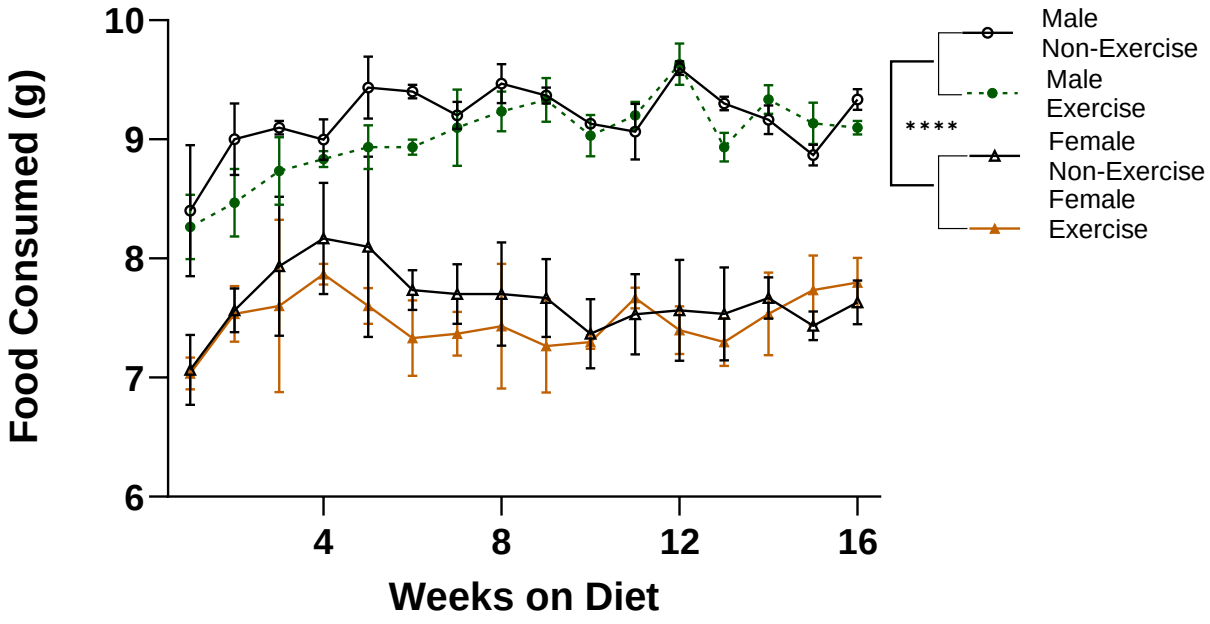


Figure 16. HFHS diet consumption by each group over 16 weeks. All mice consumed HFHS diet. There was no effect of exercise intervention, but there was an effect of sex on the average amount of diet consumed per week. Data presented as mean±SEM, ****$p < 0.0001$.

Following the resistance exercise intervention, significant differences were observed in our measure of strength in the mice, grip strength (figure 17, main effect of exercise $F(1, 44) = 89.36$, $p<0.0001$; a main effect of week ($F(2.351, 103.5) = 109.0$, $p<0.0001$), and an interaction between exercise x week $F(4, 176) = 67.70$, $p<0.001$). Surprisingly, no main effect of sex was observed ($p>0.05$). A post-hoc analysis showed that RES mice had significantly greater grip strength at 4 ($p<0.0001$), 6 ($p<0.0001$), and 8 ($p<0.01$) weeks.

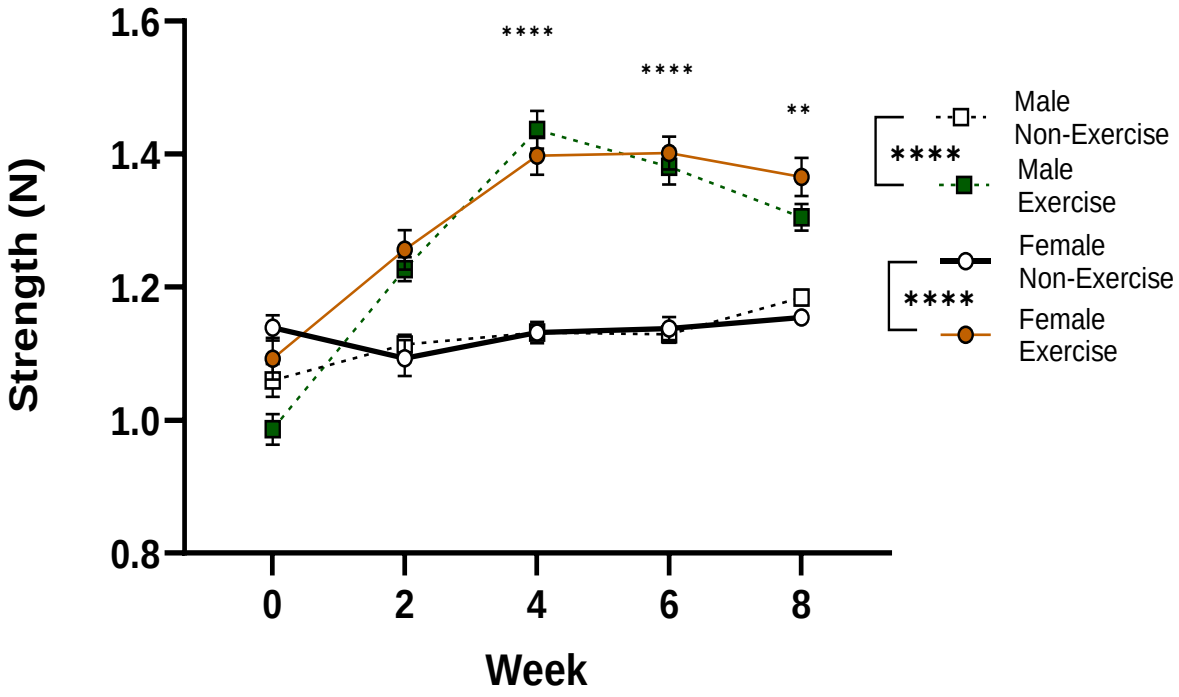


Figure 17. Grip strength of each group at baseline, 2-, 4-, 6-, and 8-weeks of exercise intervention. Differences were observed based on exercise intervention, week, and exercise x week. Data presented as mean±SEM, ***p<0.01*, ****p < 0.0001.*

3.4.2 Resistance exercise increases motivation in aged, obese mice in the progressive ratio touchscreen task.

In terms of days to reach training criteria, there were no significant differences between the RES and non-RES groups (figure 18A, p>0.05). During the fixed-ratio task, there were also no significant differences observed in the response rate which measures correct responses per minute (figure 18B, p>0.05).

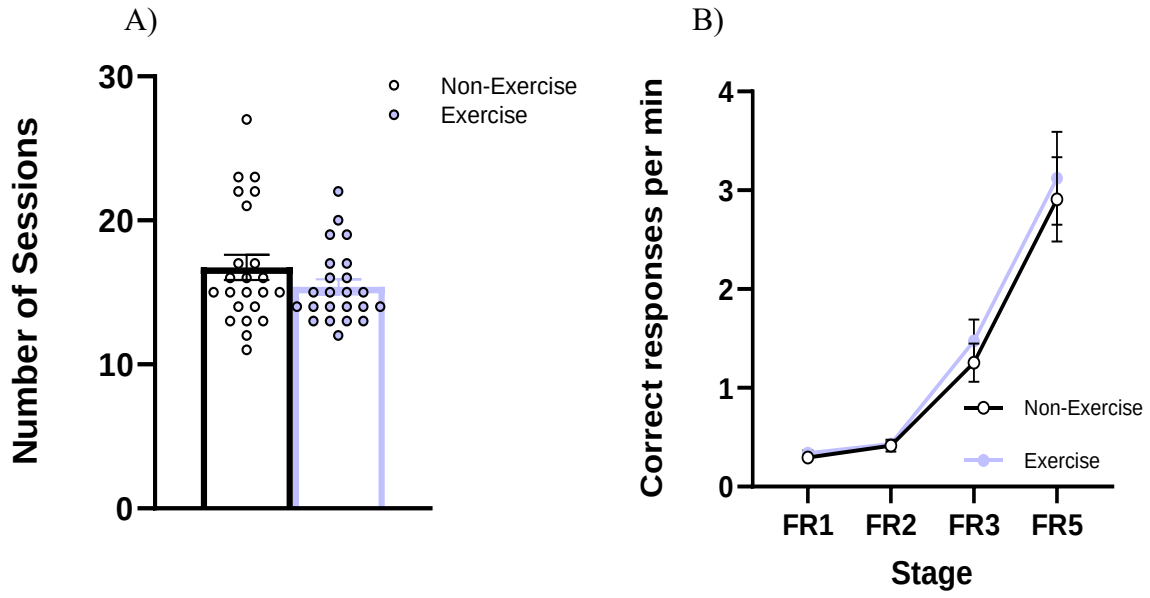


Figure 18. Effects of exercise on sessions to train and response rate in the fixed-ratio touchscreen task. Sessions to training encompassed habituation to the touchscreen chambers, their mechanisms, and the interactions required to successfully engage in the touchscreen task. The groups did not differ significantly in the sessions required to train on the touchscreen task based on exercise intervention (A). The fixed-ratio task trained the mice to complete a task that required a certain number of responses at each stage to successfully receive the reinforcement reward (strawberry milkshake). There were no differences in correct responses per minute in the fixed-ratio task based on exercise intervention (B). Data presented as mean±SEM.

There were no significant differences between the RES and non-RES groups in the number of days required to reach training criteria (figure 18A, $p>0.05$) or correct response rate in the FR task (figure 18B, $p>0.05$).

Despite the similarities between groups during the training phase of this task, the groups differed in multiple measures during the PR task. Mice in the RES group were willing to expend more effort across stages to receive a reward, according to analysis of breakpoint (figure 19A, main effect of exercise $F(5, 112) = 5.249$, $p=0.0002$). In addition to a higher breakpoint, the RES group also collected significantly more rewards in comparison to the non-RES mice (figure 19B,

main effect of exercise $F(5, 12) = 53.07$, $p<0.001$). The mice in the RES group showed greater levels of interaction with the touchscreen in the chamber even when their responses were to non-response windows, shown here by number of blank touches (figure 19C, main effect of exercise $F(5, 113) = 8.568$, $p<0.0001$). After identifying these differences in performance between groups, it was of interest whether this could be attributed to a difference in physical activity and speed in the chambers caused by fitness from the exercise intervention. Reward latency illustrates the amount of time each mouse takes to retrieve the milkshake reward following a successful trial. The lack of significant differences between groups in reward latency suggests that the variations observed in the progressive ratio task in this cohort cannot simply be attributed to global changes in locomotion (figure 19D, $p>0.05$).

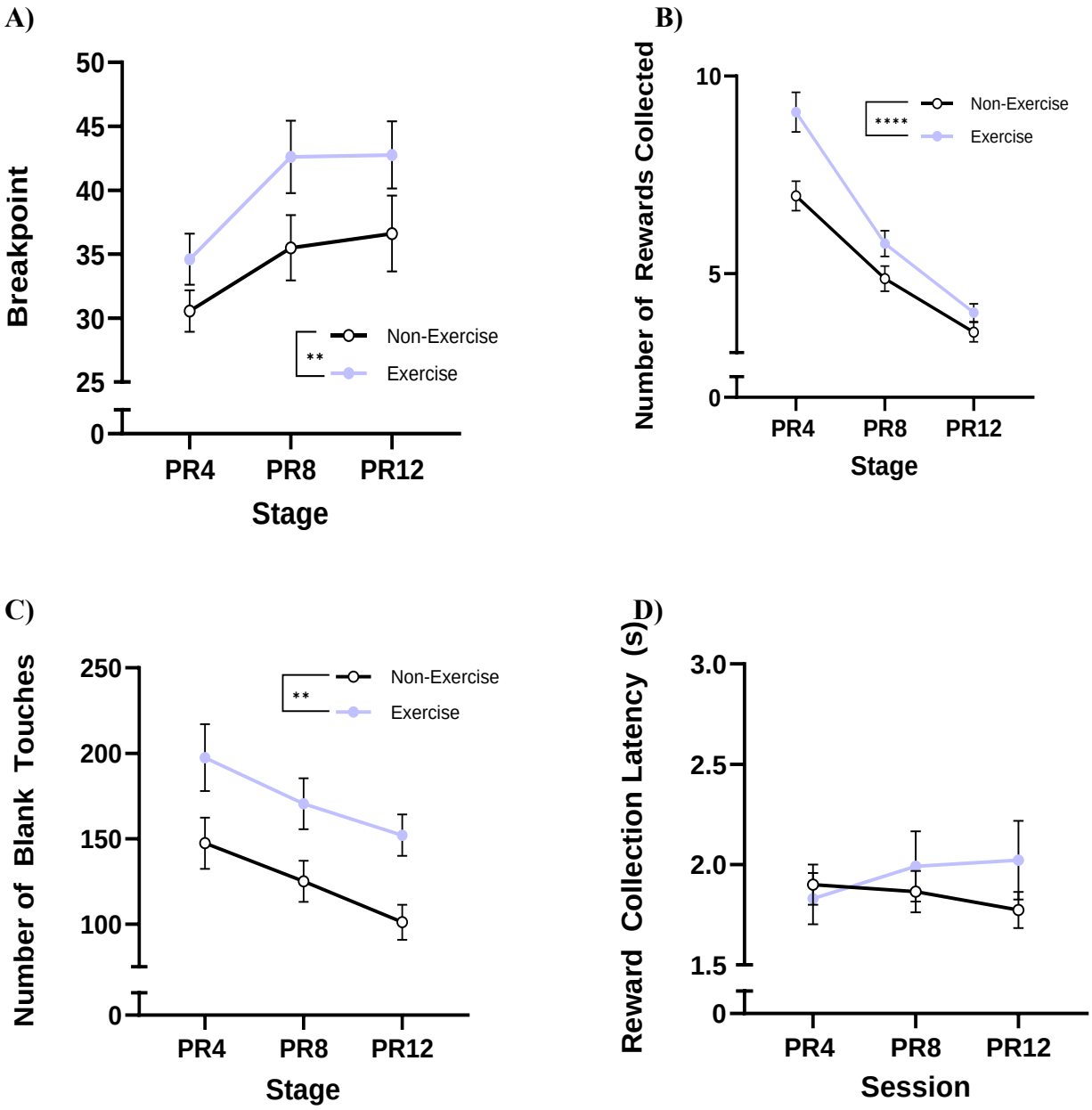
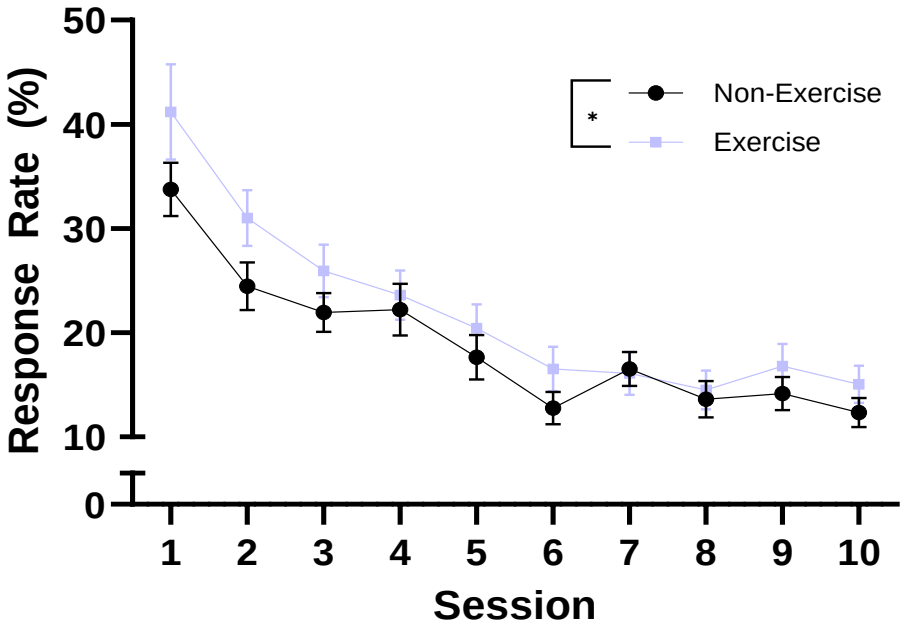


Figure 19. Resistance exercise intervention modulates performance during the progressive ratio touchscreen task (mean±SEM). A) Comparison of breakpoint during PR between older, overweight RES and non-RES mice. There was a significant main effect of exercise, with RES mice exerting more effort in responding. **B)** Number of rewards collected differed significantly between groups. RES mice successfully completed more trials and collected significantly more rewards. **C)** RES mice interacted significantly more with non-response windows during the task. **D)** There were no significant differences between groups in latency to collect reward following trial completion (p=0.7544). Data presented as mean±SEM, *** p < 0.01,* **** p<0.0001.*

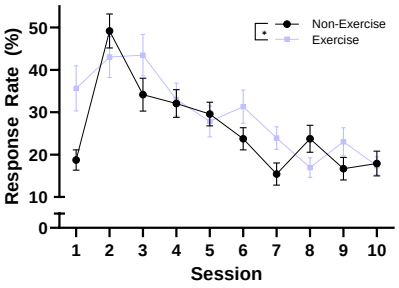
3.4.3 Resistance exercise intervention increases perseverance in aged, obese mice during the extinction touchscreen task.

Since differences in motivation were observed between groups in the progressive ratio task, it was of interest to investigate whether this perseverance would continue through the extinction touchscreen task in which responses resulted in no reward. Similarly, to the progressive ratio task, the RES group demonstrated a greater response rate than the non-RES group (figure 20A, main effect of group $F(1, 450) = 9.758$, $p=0.0019$). To further investigate whether there were any differences during different phases of the task, performance was split into an early, middle, and late phase for analysis. The early phase of the task represented the first ten trials of a session, the middle phase represented the middle ten, and the late phase represented the last ten trials. The aim with splitting the session up in such a manner was to further understand variation in responding within a session, as opposed to just comparing across sessions. Splitting the extinction performance into phases revealed some interesting effects. RES group mice demonstrated a significantly greater level of effort and motivation to complete the task, even in the absence of a reward, in the early (figure 20B, main effect of exercise $F(1, 450)= 4.908$, $p = 0.0272$) and late phases of the task (figure 20D, main effect of exercise $F(1, 450) = 7.659$, $p = 0.0059$). However, both groups performed at a similar level in the middle phase of the task with no significant differences observed (figure 20C, $p>0.05$). These findings suggest that the resistance exercise intervention motivates older, obese mice to respond during touchscreen tasks even in the absence of any reward.

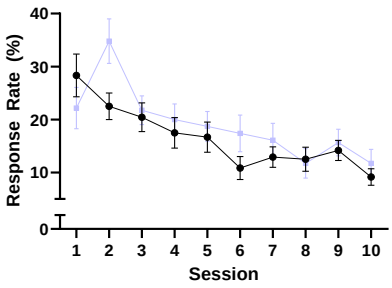
A)



B)



C)



D)

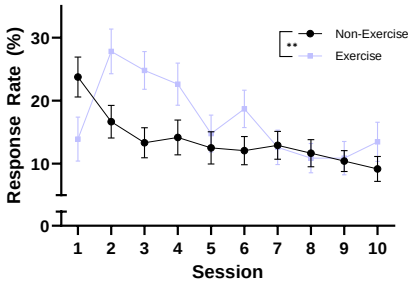


Figure 20. Performance on the extinction touchscreen task. Extinction responses rate (A), response rate in the early phase (B), middle phase (C), and late phase (D). The RES group had higher perseverance in the absence of a reward when responding to the extinction task. Specifically, rates of response were higher in the early and late phase of the task session, with no significant difference observed between groups in the middle phase. Data presented as mean±SEM. **p<0.01, **p<0.001.*

3.4.4 Resistance exercise intervention reduces impairments in aged, obese mice in the spontaneous location recognition task.

The last task that the mice completed was the spontaneous location recognition (SLR) task. Both groups of mice completed two conditions of the task that differed in difficulty: dissimilar (d-SLR) and similar (s-SLR). Performance on the task and spatial memory function was quantified through the discrimination ratio (d2) that was calculated by comparing the total amount of time the mouse spent investigating the novel object location versus the non-novel object location: *d2 = time(novel) − time(familiar)/time(novel + familiar)* (Reichelt et al., 2021).

Some level of impairment in the non-RES group was anticipated as consumption of HFHS diet and increased age has previously been shown to impair performance on cognitive tasks, including SLR (Reichelt et al., 2020; Yeomans, 2017; Murray et al., 2017). Of interest was whether the resistance exercise intervention would be sufficient to improve this performance. Here in the SLR task, performance on both conditions was significantly better in the RES mice (figure 21, unpaired t-test dSLR $t(36.56)=9.480$, $p<0.0001$; unpaired t-test sSLR $t(42.73)=5.573$, $p<0.0001$). Healthy rodents consuming standard chow are usually capable of completing both the d-SLR and s-SLR phases with limited impairment (Reichelt et al., 2016). Reichelt et al (2016) demonstrated that adolescent rats with daily access to sucrose were impaired during the s-SLR phase but not the d-SLR phase. Here, non-RES mice were impaired at both stages. These mice were matched on age, diet, and water manipulation, and the RES group performed significantly better at both stages.

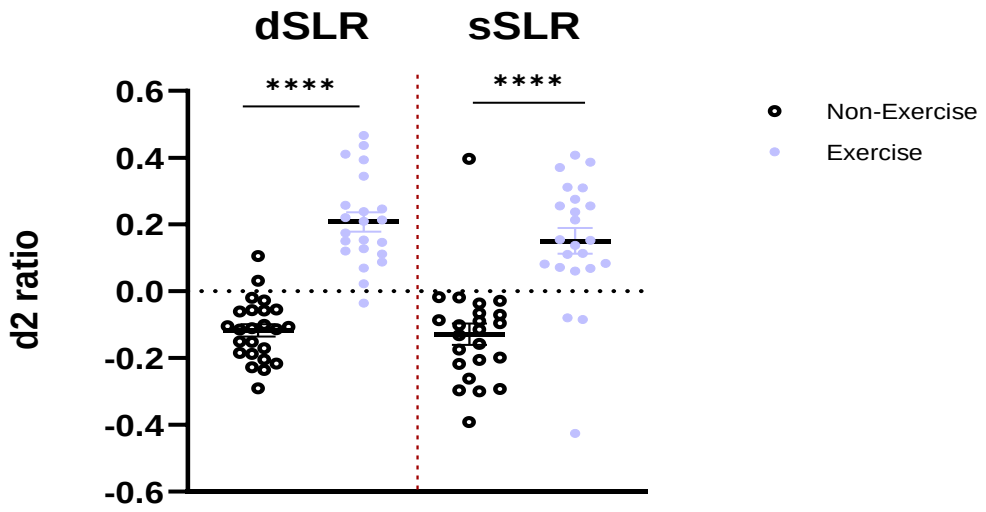


Figure 21. Performance on both the dSLR and sSLR conditions of the spontaneous location recognition task. A d2 ratio was calculated based on the amount of time each mouse spent investigating the novel object versus the familiar object within each condition of the SLR task. In both dSLR and sSLR, RES mice spent significantly more time investigating the novel object. Data presented as mean±SEM. ****$p<0.0001$

3.4.5 Physiological differences following resistance exercise intervention in aged, obese mice.

Following sacrifice, both liver weight and white adipose tissue (WAT) weight were recorded. Liver weight and WAT were of interest since they are clear biomarkers of obesity in diet-induced models of rodent obesity (Preguiça et al., 2020). RES mice had significantly less abdominal white adipose tissue than non-RES mice (figure 22A, main effect of exercise $F(3, 33) = 18.99$ $p<0.0001$). There was a main effect sex (figure 22A, $F(1, 44) = 33.91$, $p<0.0001$) and post hoc analysis showed non-exercised males had significantly greater amounts of WAT tissue than exercised males ($p<0.01$). The effect of the resistance exercise intervention was clearly demonstrated within the females, where exercised female mice had significantly decreased amounts of WAT in comparison to non-exercise females ($p<0.001$) . However, there was no significant interaction of treatment x sex ($p>0.05$).

Similar effects were observed in the liver weights. RES mice had significantly lighter livers than non-RES mice across sexes (figure 22B, main effect of exercise $F(3, 33) = 26.30$, $p<0.0001$). Males had significantly heavier livers than females (figure 22B, main effect of sex $F(1,44) = 12.07$, $p<0.01$), with no interaction effect ($p>0.05$). A post-hoc analysis revealed that non-RES males had significantly heavier livers than RES males ($p<0.05$) and non-exercise females ($p<0.0001$). Exercise males also had significantly heavier livers than exercise females at almost double the weight (male RES mean = 3.988; female RES mean = 1.918). Lastly, non-exercise females had significantly heaver livers than exercise females. These findings show that the 8-week resistance intervention was sufficient to decrease WAT amounts and fatty tissue within the liver.

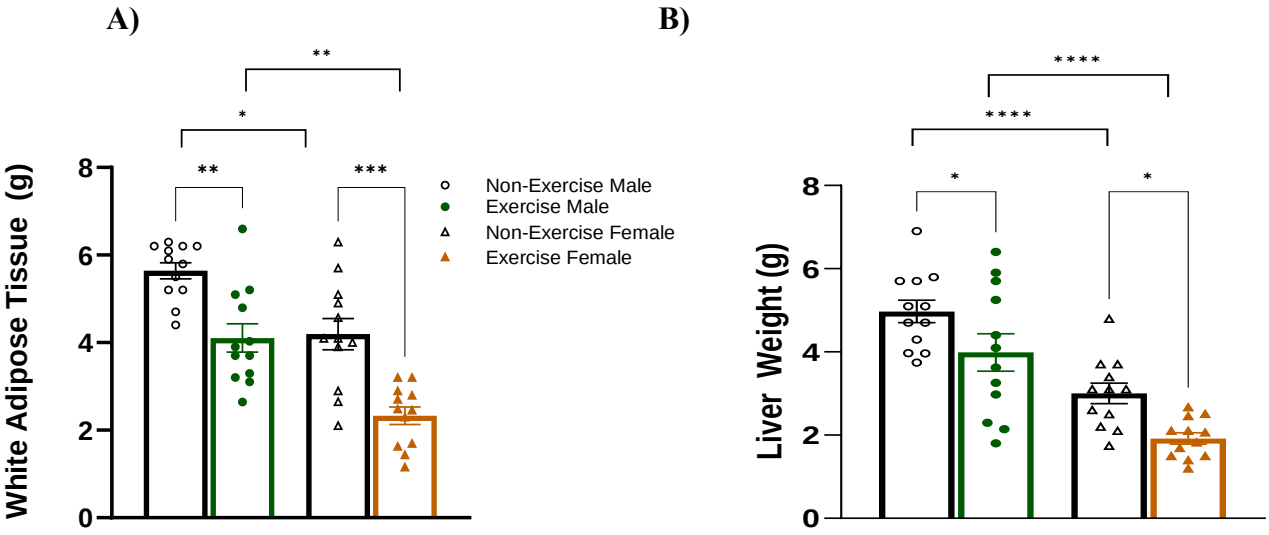


Figure 22. Mouse white adipose tissue weight by group (A) and liver weight by group (B) following an 8-week exercise intervention. Exercise intervention reliably reduced both markers of DIO and females had significantly less WAT and less heavy livers. Data presented as mean±SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Chapter 4: General Discussion

In this thesis I aimed to answer two questions:

1) is 2% CA water sufficient to motivate free fed mice to successfully complete touchscreen behavioural tasks?

2) is a consistent resistance exercise regime sufficient to rescue age and obesity-related cognitive deficits in motivation, perseverance, and spatial memory?

The first question had to be answered before the second could proceed; testing an exercised, WD-induced model of obesity in touchscreen tests of cognition would have been impossible if the efficacy of the 2% CA water were not validated first.

4.1 Applicability of CA water manipulation as a replacement for food restriction in motivating performance during touchscreen cognitive tasks.

In this study, we investigated the novel use of CA water as an alternative to food restriction as a motivator in touchscreen cognitive tasks. Establishing an alternative to food restriction was necessary since many models require free feeding of specialized diet to accurately study DIO and the related cognitive impairments. Research investigating the cognitive deficits in, for example, diet-induced obesity (HFHS diet) or demyelination (cuprizone diet) is not possible using food restriction.

We established that 2% citric acid water sufficiently motivated obese, free fed mice to complete touchscreen tasks at levels relatively comparable to those achieved by the food-restricted control group. Using a touchscreen-based test of motivation, the progressive ratio test, we established that all groups were sufficiently motivated to complete the task. Sex and diet interactions were

most commonly observed in the male mice consuming HFHS diet and 2% CA water, as this group of mice required more time to complete most tasks successfully in comparison to female mice consuming HFHS diet and 2% CA water. This subgroup of mice also increased in weight throughout the experiment.

In contrast, in a more cognitively engaging test of learning and memory, PVD, the 2% CA water sufficiently motivated the non-restricted, obese mice to complete the task without significant differences across groups or sexes. These findings suggest that when administering CA water, differences are present in a task that is specifically sensitive to motivation, but these variations in performance are not present in more general tests of cognition.

4.1.1 Administration of 2% citric acid water did not result in long-term adverse health outcomes.

Mice were closely monitored throughout the entirety of the study, with special attention devoted to measures of dehydration and stress. Following initial administration of 2% CA water, a steep decrease in bodyweight was observed. This decrease was sufficient to motivate a move back to 1.5% CA water as a safety measure, as it had less of a sour taste due to the reduced citric acid content. Once weight stabilized and mice were returned to 2% CA water, weight stayed relatively constant in all groups except for HFHS diet males that steadily increased in weight. In other measures of health, such as activity and grooming, all mice behaved normally.

This drop in weight following CA water administration is documented elsewhere (Urai et al., 2020) and is hypothesized to be an expected part of habituation to this treatment. Other studies

(Reinegal et al., 2018) habituated rats to the CA water more gradually with incremental increases in CA concentration to avoid this effect.

4.1.2 Water manipulation, sex, and diet modulates motivation in obese mice.

Performance on the progressive ratio task varied based on several factors. Across both 1.5% and 2% CA water manipulation, breakpoint was significantly different only between water manipulated groups; however, there were no significant differences in breakpoint between food restricted mice and 2% CA water chow mice. This finding establishes that with respect to breakpoint, 2% CA water is effective in motivating free-fed mice of both sexes consuming standard chow to perform at a level that is not significantly different to food-restricted mice. Past work established the efficacy of food restriction in motivating mice to complete tests of cognition, with Yang et al. (2019) demonstrating that mice interacted with touchscreens less when returned to free-feeding. Previously, work suggested that mice fed a HFHS diet could not be food restricted as a motivator for performance on cognitive tasks due to the fact that they would lose weight and that would be an inaccurate model of obesity (Liu et al., 2014). However, our findings suggest that this difference in performance on cognitive tasks may result from more than just differences in weight. Previous work has suggested that the composition of the diet, in addition to obesity, affects performance (Attuquayefio et al., 2016). Although both water-manipulated groups of male mice gained significantly more weight than the other groups during this experiment, only the HFHS fed mice were cognitively impaired in comparison to the food restricted mice. This discrepancy implies that the cognitive effects of the HFHS diet and its increased palatability in comparison to the standard chow diet may affect motivation more than

variations in weight. In a review by Cordner and Tamashiro (2015), WD or high-fat diet consumption led to a reliable impairment in the Morris water maze, radial arm maze, T-maze, and other tests (Cordner and Tamashiro, 2015). There was an additional interaction of diet x sex due to a significant difference in motivation between standard-chow males and HFHS males. Hwang et al (2012) also demonstrated a sex difference in performance and learning abilities in high-fat fed mice. They established that, not only did high-fat diet males gain significantly more weight despite consuming a similar amount of food to their female counterparts, but they were also more impaired on all tests of learning (Hwang et al., 2012). These authors confirmed that these variations were not due to impairments in sensorimotor ability stemming from obesity (Hwang et al., 2012).

4.1.3 No differences were present during the pairwise visual discrimination task regardless of diet or water manipulation.

The pairwise visual discrimination task tests visual learning and memory with a consistently correct image, and a consistently incorrect image, placed in inconsistent locations. When comparing the percentage of correct response trials, there were once again no significant differences in performance between food restricted mice and 2% CA water chow mice, regardless of sex. In contrast to the variation observed in the progressive ratio task, there were no differences based on diet between the water-manipulated groups. The perseverance index demonstrated a sex difference between both restriction types and diet types in PVD performance, but no effect of diet or restriction, Here, we have evidence that citric acid water can sufficiently motivate task completion in tasks of visual learning and memory without administration of food restriction and without introducing variations between groups, allowing further study in models

of diet-induced obesity, diet-induced disease through avenues such as the cuprizone diet, and investigations of the gut-brain axis.

4.2 Effectiveness of resistance exercise in reversing cognitive impairments due to age and diet-induced obesity.

In this study, I aimed to investigate the effects of diet -induced obesity in middle age on cognition, and to test whether a resistance exercise exercise regime would be sufficient to reverse or partially rescue the cognitive and physiological effects of obesity. This was tested through touchscreen cognitive tasks, strength testing, and post-mortem physiological measurements. Obesity in middle age is one of the greatest predictors of dementia later in life (Whitmer et al., 2005). Abdominal obesity especially is associated with arterial stiffness (Strasser et al., 2015), hypertension (Niskanen et al., 2004), and macular degeneration (Adams et al., 2011) in middle age. Most middle-aged individuals in North America lead relatively sedentary lives (Diaz et al., 2017; Blumel et al., 2015). Sedentary behaviours in middle age are associated with obesity, depressive symptoms, and hypertension (Blumel et al., 2015). In addition, there is a strong association between obesity and cognitive impairment in middle-age (Dye et al., 2017).

4.2.1 Physiological characteristics confirm diet-induced model of obesity in 13-month-old rodents and demonstrate sex differences in presentation.

All mice were free fed standard chow for 12 months and started receiving HFHS diet 2 weeks prior to experiment commencement. Throughout the experiment there were no sharp drops in weight. Weight remained stable even after the beginning of 2% CA water administration, in contrast to experiment #1. All groups, including exercise groups, maintained a high weight

(mean bodyweight per group were as follows: non-exercise male = 43.20g; exercise male = 40.23g; non-exercise female = 33.69g; exercise female = 31.34g). Males were significantly heavier, and previous work has shown that males are more vulnerable than females in terms of weight gain resulting from a diet high in fat (Hwang et al., 2012).

Non-RES mice weighed significantly more than RES mice across sexes starting at 13 weeks, despite no significant differences in consumption between groups. Mort et al. (2023) found similar results in their mice that were consuming HFHS diet – despite a reduction in amount of diet consumed and calories consumed, body mass and fat deposition was increased. In our study, both non-RES and RES male mice consumed significantly more diet across 16 weeks than females did.

Following euthanasia, non-RES mice presented compelling markers of diet-induced obesity and metabolic syndrome as demonstrated by white adipose tissue content and liver weight. Consumption of WD is closely tied to increased white adipose tissue accumulation around the abdomen (Dye et al., 2017) as well as non-alcoholic fatty liver (Oddy et al., 2013). Both male and female non-RES mice had significantly more white adipose tissue than RES mice. However, there was a greater difference in adiposity between female RES and non-RES mice, implying that the resistance exercise intervention was more effective at reducing white adipose tissue in females than in males. This variation in lipolysis has been observed before, and Schmidt et al. (2014) demonstrated that this occurred due to sex difference in the adrenergic stimulation of lipolysis during exercise. In the present study, non-RES groups had significantly heavier livers

than RES groups as well. In addition, large differences were found within the exercised group, with RES males presenting with livers double the weight of RES females (male RES mean liver weight = 3.988g; female RES mean liver weight = 1.918g).

4.2.2 The 8-week resistance exercise regime was sufficient to induce changes in grip strength.

RES mice were significantly stronger than non-RES mice following four weeks of exercise intervention, with the effect lasting until the last week of the intervention. Interestingly, there were no significant sex differences in grip strength despite the discrepancy in size between sexes. Since exercise carrying load was based on percentage of body weight, males were carrying heavier loads while climbing the ladder and would therefore be expected to demonstrate greater grip strength. Ge et al., 2016 demonstrated interesting findings relating decreased grip strength as an early indicator of age-related decline due to age-related carpal and digital exostosis. Ge et al observed a decline in peak force starting at 12 months of age. The mice used in this experiment were 13 months old at the beginning of the study, so it is plausible that the lack of sex differences in grip strength may have to do with sex discrepancies in rates of aging in response to WD consumption.

4.2.3. Non-exercised mice were significantly less motivated in the progressive ratio touchscreen task and extinction task.

There were no significant differences in number of sessions to acquisition or response rate per minute during the FR training stage, implying that exercise had no effect on habituating to the touchscreen chambers or learning the rules of the task. However, RES mice scored significantly higher on all measurements of motivation during the probe trials. Due to the cohort's increased

age and adiposity, the apparent differences in responsiveness and motivation may have been due to differences in activity. However, reward collection latency, which is the time each mouse takes to retrieve the appetitive reinforcement following a correct trial, did not differ between non-RES and RES mice. These measurements suggest that the differences in performance between groups was not based on the speed at which they navigated around the touchscreen chamber or moved to the reward tray; it was based on differences in motivation.

RES mice also demonstrated greater levels of perseverance in the extinction task. Extinction engages the test subject to interact with the screen in the same manner as during the first phase of the FR task (FR1); however, no appetitive reinforcement is provided following a successful trial. When a trial passes without a response, that is counted as an omission, and a new trial commences after a pause of 4.5 seconds. To better understand when this perseverance was occurring during each session, performance was split into early (first 10 trials of the session), middle (middle 10 trials of the session) and late (last 10 trials of the session) stages. RES mice responded significantly more in the early and late stages, but responsiveness between groups was about equal in the middle phase. Perseverance is a characteristic often related to addictive, compulsive, and impulsive behaviours, in which the individual has difficulty resisting completing these behaviours (Volkow et al., 2013). Predisposition to impulsivity can be exacerbated by disruption in the neurobiological processes that control the brain's sensitivity to reward (Volkow et al., 2013). Here, we can compare the reward effect the appetitive milkshake reinforcement would have on a food-restricted mouse consuming standard chow in comparison to a mouse that is consuming HFHS diet *ad libitum*. The value of the reward becomes "re-baselined" following sustained consumption of HFHS diet, such that it may not be as satisfying and appetizing. The state of obesity is already a marker of the dysregulation in the brain that

results in an individual that is unable to stop engaging in eating behaviour despite no longer having hunger cues (Volkow et al., 2013).

4.2.4 Aged, obese mice consuming HFHS diet are impaired in the spontaneous location recognition task.

RES mice demonstrated improved performance on both the d-SLR and s-SLR conditions in the spontaneous location recognition task. The level of impairment demonstrated in the non-RES mice in suggests an impairment of spatial memory. Spatial memory impairments in a DIO model have been demonstrated widely (Heyward et al., 2012; Valladolid-Acebes et al., 2011; Underwood et al., 2016). In the SLR task, impairment usually does not present at the d-SLR stage (Reichelt et al., 2020). The reason impairment is usually not shown at the d-SLR stage is due to the fact that the s-SLR stage targets pattern separation specifically, whereas the d-SLR stage is dependent more generally on spatial memory (Reichelt et al., 2020). Pattern separation is a process within spatial memory, but it acts in a more highly specified manner (Reichelt et al., 2020). Pattern separation allows the subject to distinguish between highly similar inputs and to transform them into distinct representations (Goethem et al., 2018). However, since there was severe impairment in both stages in this experiment, it suggests that the impairment is at a more general level, impairing spatial memory.

Spatial memory and learning are largely reliant on hippocampal function (Ma et al., 2021). The hippocampus is very vulnerable to environmental insult, and a high-fat, high-sugar diet has previously been demonstrated by Reichelt et al., 2021; Kanoski et al., 2011; Attuquayefio et al., 2017. Kanoski et al. (2011) presented a compelling analysis of the detrimental effects of WD on

hippocampal function, which was correlated with impaired memory inhibition and other cognitive functions. They suggested that this impaired memory inhibition further encouraged the elicitation of appetitive behaviour based on food-related environmental cues (Kanoski et al., 2011). Attuquayefio et al. (2017) investigated these effects further in a human population and established that the experimental condition that consumed WD for four days showed significant impairments in hippocampal-dependent learning and memory. Reichelt et al. (2021) illustrated how HFHS diet affects the hippocampus and prefrontal cortex at the molecular level by modelling DIO in mice of various ages and quantifying alterations in neuronal density, perineuronal nets, and microglia. Reichelt et al. (2021) established that HFHS diet resulted in disturbances to parvalbumin neurons, perineuronal nets, and microglia most prominently in adult mice.

In contrast, RES mice had increased novel object location investigation in both conditions with and greater d2 scores in both conditions. This type of restoration in function has been demonstrated before by Ma et al., 2021 when they restored both hippocampal neurogenesis and learning and memory in obese mice by regulating the gut microbiota using metformin. The detrimental effect of chronic high fat diet consumption has also been shown to be prevented through a concurrent, long term aerobic exercise intervention on a running wheel (Klein et al., 2016). In that study the control, non-exercised HFD group demonstrated reduced immature neurons in adolescence and reduced performance on the Morris water maze test of spatial memory (Klein et al., 2016). Although previous research has shown that resistance exercise can rescue cognition, the present study provides a compelling first look at the possibilities of resistance exercise interventions in individuals that are both obese and middle-aged.

4.3 Study Limitations

Although this study has shown several exciting and novel findings, there are limitations as well. One limitation is the lack of histological measurements from the hippocampus and other tissue in both studies. The hippocampus has widely been shown to be vulnerable to detrimental effects following high fat high sugar diet consumption and the SLR task is heavily dependent on the hippocampus (Reichelt et al., 2020). In the water restriction study, it would have been interesting to explore whether citric acid administration had any effects on the adipose tissue. Previously, Abdel-Salam et al. (2014) established that citric acid administration significantly reduced levels of lipid peroxidation in the brain and liver following treatment with an endotoxin (lyophilized Escherichia coli) that was used to emulate a model of oxidative stress. The cognitive dysfunction associated with HFHS diet consumption has been linked to increased levels of oxidative stress resulting from greater levels of circulating free fatty acids resulting in systemic inflammation (Tan and Norhaizan, 2019). Increased oxidative stress induces lipid peroxidation, which in turn impairs adipogenesis and promotes insulin resistance (Soldo et al., 2022). Unfortunately, due to time and labour constraints, completing these measurements was not feasible. Future studies could repeat this study's parameters but delve deeper into investigating the relationship between several myokines and brain factors that have been suggested as the mediators of the beneficial effects of exercise, such as irisin and BDNF (Kim et al., 2015; Preciado et al., 2023), and their systemic and local action in various tissues. Previous work has demonstrated increases in serum BDNF in mice that showed improved hippocampal function following aerobic exercise (Griffin et al., 2011). Additionally, an increase in levels of irisin has been correlated with reduced anxiety

following aerobic exercise (Uysal et al., 2018) and improved muscle function in aging mice and humans (Kim et al., 2015).

Another limitation to the study is the lack of a matched, younger cohort to compare with the aged mice to further understand the interactions between age, obesity, and resistance exercise . It would be interesting to complete a similar study with a younger cohort as there may be differences in performance physiologically in the maximum load the mice are able to carry on the exercise ladder, variations in peak grip strength and strength maintenance, and levels of abdominal obesity. Previous work has demonstrated that older mice are more prone to muscle wasting (White et al., 2016), peak grip strength is lower in older mice and deteriorates more rapidly (Ge et al., 2016), and that middle-aged mice are more prone to diet-induced obesity than younger mice (Nishikawa et al., 2007).

In addition, it would be beneficial to repeat the exercise intervention in a cohort that is powered to detect sex differences in touchscreen tasks. Males develop diet-induced obesity at a greater and more rapid rate than females (Ge et al., 2016; Arpón et al., 2019). Sex differences in exercise efficacy have been established as well. Fagot et al. (2017) established that being sedentary had a greater impact on speed of processing in older aged females than older aged males. A systematic review completed by Barha et al (2017) demonstrated that aerobic exercise, resistance exercise, and multimodal training improved executive functions compared to control participants, with a greater effect seen in women. These sex differences may exist because of hormonal variations in level of circulating sex hormones that act on skeletal muscles (Aizawa et al., 2007) or even sex-

differences in BDNF signalling (Komulainen et al., 2008; Barha et al., 2017). Therefore, understanding and visualizing the effects between groups and between sexes would lend a great deal of strength to the conclusions taken from this study.

4.4 Conclusion

Together, these studies successfully established an aged, diet-induced model of obesity that can be safely and effectively exercised, while also being motivated, using CA water, to perform touchscreen based cognitive assessments without food restriction. The model we developed in this study allows for a much greater degree of translatability to the middle aged, obese human population through feeding habits as well as testing paradigms in comparison to studies using food restriction in touchscreen tests of cognition.

We found impaired motivation between water-restricted groups, with reduced responses from male HFHS-fed mice in comparison to male mice consuming standard chow. In contrast, this difference was not observed between chow and food restricted groups, which demonstrated that water manipulation through CA water is sufficient to motivate non-food restricted mice to perform at the same level as food restricted mice in a task of motivation. Additionally, no significant differences were observed between diet and water-manipulation groups in percentage of correct responses during a task of learning and memory. These findings suggest that variations in diet only affect performance in tasks specifically testing motivation, but they do not modulate performance in tasks more generally testing cognition. These findings help orient future studies where water manipulation is required for a variety of touchscreen tasks.

In addition, we found effects of increased age and obesity on motivation and perseverance, and an exceptionally large deficit in performance on the SLR task. However, we also established that

a consistent exercise regime is sufficient to rescue these effects. We established a safe and effective exercise intervention that reliably increased grip strength without any negative health outcomes.

The work completed in this thesis provides an exciting and novel next step in research investigating the various cognitive effects of diet-induced obesity, as well as the translation of this research to studies conducted in humans. In addition to uncovering the behavioural underpinnings of this cognitive model, this thesis also suggests a promising non-pharmacological therapeutic intervention to rescue these cognitive impairments through a sustainable and safe resistance exercise intervention.

References

Abbott, Kirsten N.; Arnott, Christopher K.; Westbrook, R. Frederick; Tran, Dominic M. D. (2019). The effect of high fat, high sugar, and combined high fat-high sugar diets on spatial learning and memory in rodents: A meta-analysis. *Neuroscience & Behavioral Reviews* (107), 399-421.

Adams, M.K., Simpson, J.A., Aung, K.Z., Makeyeva, G.A., Giles, G.G., English, D.R., Hopper,

J., Guymer, R.H., Baird, P.N.,Robman, L.D. (2011). Abdominal Obesity and Age-related Macular Degeneration. *American Journal of Epidemiology, 11*(173), 1246-1255.

Abdel-SalamOmar, M. E., YounessEman, R., MohammedNadia, A., Youssef, M. M., OmaraEnayat, A., & SleemAmany, A. (2014). Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. *Journal of medicinal food.*

Ansdell, P., Thomas, K., Hicks, K. M., Hunter, S. K., Howatson, G., & Goodall, S. (2020). Physiological sex differences affect the integrative response to exercise: acute and chronic implications. Experimental physiology, 105(12), 2007–2021. https://doi.org/10.1113/EP088548

Arpón, A., Milagro, F. I., Santos, J. L., García-Granero, M., Riezu-Boj, J. I., & Martínez, J. A. (2019). Interaction among sex, aging, and epigenetic processes concerning visceral fat, I nsulin resistance, and dyslipidaemia. *Frontiers in endocrinology*, 10, 496.

Attuquayefio, T., Stevenson, R. J., Boakes, R. A., Oaten, M. J., Yeomans, M. R., Mahmut, M., & Francis, H. M. (2016). A high-fat high-sugar diet predicts poorer hippocampal-related memory and a reduced ability to suppress wanting under satiety. *Journal of Experimental Psychology: Animal Learning and Cognition, 42*(4), 415.

Attuquayefio, T., Stevenson, R. J., Oaten, M. J., & Francis, H. M. (2017). A four-day Western-style dietary intervention causes reductions in hippocampal-dependent learning and memory and interoceptive sensitivity. *PLoS One, 12*(2), e0172645.

Barha, C. K., Davis, J. C., Falck, R. S., Nagamatsu, L. S., & Liu-Ambrose, T. (2017). Sex differences in exercise efficacy to improve cognition: A systematic review and meta-analysis of randomized controlled trials in older humans. Frontiers in neuroendocrinology, 46, 71–85.

Barha, C. K., & Liu-Ambrose, T. (2018). Exercise and the aging brain: considerations for sex

differences. Brain Plasticity, 4(1), 53-63.

Beilharz, J. E., Maniam, J., & Morris, M. J. (2014). Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain, behavior, and immunity*, 37, 134-141.

Bekkevold, C. M., Robertson, K. L., Reinhard, M. K., Battles, A. H., & Rowland, N. E. (2013). Dehydration parameters and standards for laboratory mice. *Journal of the American Association for Laboratory Animal Science, 5*2(3), 233-239.

Benedetti, M. G., Furlini, G., Zati, A., & Letizia Mauro, G. (2018). The effectiveness of physical exercise on bone density in osteoporotic patients. *BioMed research international*.

Bettio, L. E., Rajendran, L., & Gil-Mohapel, J. (2017). The effects of aging in the hippocampus and cognitive decline. *Neuroscience & Biobehavioral Reviews*, 79, 66-86

Blaisdell, A.P., Lau, Y.L.M., Telminova, E., Lim, H.C., Fan, B., Fast, C.D., Garlick, D., Pendergrass, D.C. (2014). Food quality and motivation: A refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats. *Physiology & Behavior* (128), 220-225.

Blümel Méndez, J., Chedraui, P., Aedo Monsalve, S., Fica, J., Mezones Holguín, E., Barón, G., Bencosme, A., Benítez, Z., Bravo, L., Calle, A.,Flores, D. (2015). Obesity and its relation to depressive symptoms and sedentary lifestyle in middle-aged women. Maturitas, 1(80), 100-105.

Botvinick, M., & Braver, T. (2015). Motivation and Cognitive Control: From Behavior to Neural Mechanism. Annual Review of Psychology, 1(66), 83-113.

Buie, J. J., Watson, L. S., Smith, C. J., & Sims-Robinson, C. (2019). Obesity-related cognitive impairment: The role of endothelial dysfunction. *Neurobiology of Disease*, 132, 104580.

Burokas, A., Martín-García, E., Espinosa-Carrasco, J., Erb, I., McDonald, J., Notredame, C.,

Dierssen, M., Maldonado, R., 2018.. (2018). Extinction and reinstatement of an operant responding maintained by food in different models of obesity. *Addiction Biology, 2*(23), 544-555.

Burokas, A., Martín-García, E., Espinosa-Carrasco, J., Erb, I., McDonald, J., Notredame, C., Dierssen, M., Maldonado, R.l. (2018). Extinction and reinstatement of an operant responding maintained by food in different models of obesity: Reinstatement in obesity. *Behaviouoral Brain Research* (418), 113-126.

C. Reichelt, Amy; D. Gibson, Gabrielle; N. Abbott, Kirsten; J. Hare, Dominic. (2019). A high-fat high-sugar diet in adolescent rats impairs social memory and alters chemical markers characteristic of atypical neuroplasticity and parvalbumin interneuron depletion in the medial prefrontal cortex. *Food&Function, 4*(10), 1985-1998.

Cassilhas, R. C., Viana, V. A., Grassmann, V., Santos, R. T., Santos, R. F., Tufik, S., & Mello, M. T. (2007). The impact of resistance exercise on the cognitive function of the elderly. Medicine & *Science in Sports & Exercise, 39*(8), 1401-1407.

Cassilhas, R.C.; Lee, K.S.; Fernandes, J.; Oliveira, M.G.M.; Tufik, S.; Meeusen, R.; De Mello, M.T. (2012). Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. *Neuroscience* (202), 309-317.

Chang, Y. K., Pan, C. Y., Chen, F. T., Tsai, C. L., & Huang, C. C. (2012). Effect of resistance-exercise training on cognitive function in healthy older adults: a review. *Journal of aging and physical activity, 20*(4), 497-517.

Colmenares, A.M., Voss, M.W., Fanning, J., Salerno, E.A., Gothe, N.P., Thomas, M.L., McAuley, E., Kramer, A.F., Burzynska, A.Z. (2021). White matter plasticity in healthy older adults: The effects of aerobic exercise. *NeuroImage* (239), 118-132.

Contu, Laura; Heath, Christopher J.; Hawkes, Cheryl A. (2022). Appetitive Motivation and Associated Neurobiology Change Differentially across the Life Course of Mouse Offspring Exposed to Peri- and Postnatal High Fat Feeding. *Nutrients, 23*(14), 51-61.

Cordner, Z. A., & Tamashiro, K. L. (2015). Effects of high-fat diet exposure on learning & memory. *Physiology & behavior 152*, 363-371.

Cornier, M.A., Dabelea, D., Hernandez, T.L., Lindstrom, R.C., Steig, A.J., Stob, N.R., Van Pelt, R.E., Wang, H., Eckel, R.H. (2008). The Metabolic Syndrome. *Endocrine Reviews, 7*(29), 777-822.

Creer, D. J., Romberg, C., Saksida, L. M., Van Praag, H., & Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proceedings of the national academy of sciences, 107*(5), 2367-2372.

Dantas, R. R., & Silva, G. A. P. D. (2019). The role of the obesogenic environment and parental lifestyles in infant feeding behavior. Revista Paulista de Pediatria, 37, 363-371.

Davidson, L.E., Hudson, R., Kilpatrick, K., Kuk, J.L., McMillan, K., Janiszewski, P.M., Lee, S., Lam, M., Ross, R. (2009). Effects of Exercise Modality on Insulin Resistance and Functional Limitation in Older Adults: A Randomized Controlled Trial. *Archives of Internal Medicine, 2*(169), 122-131.

de Macedo, I. C., de Freitas, J. S., & da Silva Torres, I. L.. (2016). The Influence of Palatable Diets in Reward System Activation: A Mini Review. *Advances in Pharmacological Sciences* (20), 44-49.

de Oliveira Silva, A., Dutra, M.T., de Moraes, W.M.A.M., Funghetto, S.S., Lopes de Farias, D., Dos Santos, P.H.F., Vieira, D.C.L., Nascimento, D.D.C., Orsano, V.S.M., Schoenfeld, B.J., Prestes, J. (2018). Resistance training-induced gains in muscle strength, body composition, and functional capacity are attenuated in elderly women with sarcopenic obesity. *Clinical Interventions in Aging*,(13), 411-417.

De Vreede, P. L., Samson, M. M., Van Meeteren, N. L., Duursma, S. A., & Verhaar, H. J. (2005).

Functional-task exercise versus resistance strength exercise to improve daily function in older women: a randomized, controlled trial. Journal of the American Geriatrics Society, 53(1), 2-10.

Del Corral, P., Chandler-Laney, P. C., Casazza, K., Gower, B. A., & Hunter, G. R. (2009). Effect of dietary adherence with or without exercise on weight loss: a mechanistic approach to a global problem. *The Journal of Clinical Endocrinology & Metabolism, 94*(5), 1602-1607.

Diaz, K.M., Howard, V.J., Hutto, B., Colabianchi, N., Vena, J.E., Safford, M.M., Blair, S.N., Hooker, S.P (2017). Patterns of Sedentary Behavior and Mortality in U.S. Middle-Aged and Older Adults. *Annals of Internal Medicine, 7*(167), 465-475.

Duman, C. H., Schlesinger, L., Russell, D. S., & Duman, R. S. (2008). Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain research, 1199*, 148-158.

Dye, L., Boyle, N. B., Champ, C., & Lawton, C. (2017). The relationship between obesity and cognitive health and decline. *Proceedings of the nutrition society, 76*(4), 443-454.

Evans, C.C., LePard, K.J., Kwak, J.W., Stancukas, M.C., Laskowski, S., Dougherty, J., Moulton, L., Glawe, A., Wang, Y., Leone, V.,Antonopoulos, D.A. (2014). Exercise Prevents Weight Gain and Alters the Gut Microbiota in a Mouse Model of High Fat Diet-Induced Obesity. *PLOS ONE, 3*(9), 921-926.

Fagot, D., Chicherio, C., Albinet, C. T., André, N., & Audiffren, M. (2019). The impact of physical activity and sex differences on intraindividual variability in inhibitory performance in older adults. Neuropsychology, development, and cognition. Section B, Aging, neuropsychology and cognition, 26(1), 1–23.

Farrance, C., Tsofliou, F., & Clark, C. (2016). Adherence to community based group exercise

interventions for older people: A mixed-methods systematic review. *Preventive Medicine*, 87, 155-166.

Finger, B. C., Dinan, T. G., & Cryan, J. F. (2010). Progressive ratio responding in an obese mouse model: Effects of fenfluramine. *Neuropharmacology, 59*(7-8), 619-626.

Finger, B. C., Dinan, T. G., & Cryan, J. F. (2012). Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology, 220*, 173-181.

Firth, J., Stubbs, B., Teasdale, S.B., Ward, P.B., Veronese, N., Shivappa, N., Hebert, J.R., Berk, M., Yung, A.R., Sarris, J. (2018). Diet as a hot topic in psychiatry: a population-scale study of nutritional intake and inflammatory potential in severe mental illness. World *Psychiatry, 3*(17), 365-367.

Francis, H., & Stevenson, R. (2013). The longer-term impacts of Western diet on human cognition and the brain. *Appetite, 63*, 119-128.

Gaesser, G. A., Angadi, S. S., & Sawyer, B. J. (2011). Exercise and diet, independent of weight loss, improve cardiometabolic risk profile in overweight and obese individuals. *The Physician and sportsmedicine, 39*(2), 87-97.

Ge, X., Cho, A., Ciol, M. A., Pettan-Brewer, C., Snyder, J., Rabinovitch, P., & Ladiges, W. (2016). Grip strength is potentially an early indicator of age-related decline in mice. *Pathobiology of Aging & Age-related Diseases,* 6(1), 32981.

Gomes, D. C. K., Sichieri, R., Junior, E. V., Boccolini, C. S., de Moura Souza, A., & Cunha, D. B. (2019). Trends in obesity prevalence among Brazilian adults from 2002 to 2013 by educational level. *BMC Public Health*, 19, 1-7.

Gómez-Apo, E., Mondragón-Maya, A., Ferrari-Díaz, M., & Silva-Pereyra, J. (2021). Structural brain changes associated with overweight and obesity. *Journal of obesity*.

Grealy, M. A., Johnson, D. A., & Rushton, S. K. (1999). Improving cognitive function after brain injury: the use of exercise and virtual reality. *Archives of physical medicine and rehabilitation, 80*(6), 661-667.

Griffin, É. W., Mullally, S., Foley, C., Warmington, S. A., O'Mara, S. M., & Kelly, Á. M. (2011). Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiology & behavior, 104(*5), 934-941.

Guest, P.C. (2019). Insulin Resistance in Schizophrenia. *Reviews on Biomarker Studies of Metabolic and Metabolism-Related Disorders*, 449-457.

Guo, Y., Wang, Z., Chen, L., Tang, L., Wen, S., Liu, Y., & Yuan, J. (2018). Diet induced maternal obesity affects offspring gut microbiota and persists into young adulthood. *Food & function, 9*(8), 4317-4327.

Hardy, O. T., Czech, M. P., & Corvera, S. (2012). What causes the insulin resistance underlying obesity?. *Current opinion in endocrinology, diabetes, and obesity, 19*(2), 81.

Hespe, G. E., Kataru, R. P., Savetsky, I. L., García Nores, G. D., Torrisi, J. S., Nitti, M. D., ... & Mehrara, B. J. (2016). Exercise training improves obesity-related lymphatic dysfunction. *The Journal of physiology, 594*(15), 4267-4282.

Heyward, F. D., Walton, R. G., Carle, M. S., Coleman, M. A., Garvey, W. T., & Sweatt, J. D. (2012). Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. *Neurobiology of learning and memory, 98*(1), 25-32.

Heyward, F. D., Walton, R. G., Carle, M. S., Coleman, M. A., Garvey, W. T., & Sweatt, J. D. (2012). Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. *Neurobiology of learning and memory, 98*(1), 25-32.

Hitchen, B., Norwood, K., Gault, V. A., & Leslie, J. C. (2021). Behavioural evaluation of mouse

models of type 2 diabetes. *Learning and Motivation*, 74, 101730.

Horton, A. L., Campbell, E. J., Aumann, T. D., O'Brien, K. R., Lawrence, A. J., & Brown, R. M. (2023). Addiction-like behaviour towards high-fat high-sugar food predicts relapse propensity in both obesity prone and obesity resistant C57BL/6 J mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 121, 110654.

Hsu, T. M., Konanur, V. R., Taing, L., Usui, R., Kayser, B. D., Goran, M. I., & Kanoski, S. E. (2015). Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus, 25*(2), 227-239.

Huffman, D. M., & Barzilai, N. (2009). Role of visceral adipose tissue in aging. *Biochimica et Biophysica Acta (BBA)-General Subjects, 179*0(10), 1117-1123.

Hwang, L.L., Wang, C.H., Li, T.L., Chang, S.D., Lin, L.C., Chen, C.P., Chen, C.T., Liang, K.C., Ho, I.K., Yang, W.S., Chiou, L.C. (2010). Sex Differences in High-fat Diet-induced Obesity, Metabolic Alterations and Learning, and Synaptic Plasticity Deficits in Mice. *Obesity, 18*(3), 463-469.

Ishimoto, T., Lanaspa, M.A., Rivard, C.J., Roncal-Jimenez, C.A., Orlicky, D.J., Cicerchi, C., McMahan, R.H., Abdelmalek, M.F., Rosen, H.R., Jackman, M.R.,MacLean, P.S. (2013). High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. *Hepatology, 5*(58), 1632-1643.

Jefferson, M.E., Nicklas, B.J., Chmelo, E.A., Crotts, C.I., Shaltout, H.A., Diz, D.I., Marsh, A.P., Brinkley, T.E. (2016). Effects of Resistance Training With and Without Caloric Restriction on Arterial Stiffness in Overweight and Obese Older Adults. *American Journal of Hypertension, 4*(29), 494-500.

Johnson, N. A., Sachinwalla, T., Walton, D. W., Smith, K., Armstrong, A., Thompson, M. W., &

George, J. (2009). Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology, 50*(4), 1105-1112.

Johnson, S. B. (1992). Methodological Issues in Diabetes Research: Measuring adherence. *Diabetes Care, 11*(15), 1658-1667.

Joly, M., Ammersdörfer, S., Schmidtke, D., & Zimmermann, E. (2014). Touchscreen-based cognitive tasks reveal age-related impairment in a primate aging model, the grey mouse lemur (Microcebus murinus). *PLoS One, 9*(10), e109393.

Jurdak, N., Lichtenstein, A. H., & Kanarek, R. B. (2008). Diet-induced obesity and spatial cognition in young male rats. *Nutritional neuroscience, 11(*2), 48-54.

Kalmijn, S., Launer, L. J., Ott, A., Witteman, J. C., Hofman, A., & Breteler, M. M. (1997). Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Annals of neurology, 42*(5), 776-782.

Kan, C., Silva, N., Golden, S. H., Rajala, U., Timonen, M., Stahl, D., & Ismail, K. (2013). A systematic review and meta-analysis of the association between depression and insulin resistance. *Diabetes care, 36*(2), 480-489.

Kanoski, S. E., & Davidson, T. L. (2011). Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiology & behavior, 103*(1), 59-68.

Karlsson, R. M., Wang, A. S., Sonti, A. N., & Cameron, H. A. (2018). Adult neurogenesis affects motivation to obtain weak, but not strong, reward in operant tasks. *Hippocampus, 7*(28), 512-522.

Kelty, T.J., Schachtman, T.R., Mao, X., Grigsby, K.B., Childs, T.E., Olver, T.D., Michener, P.N., Richardson, R.A., Roberts, C.K.,Booth, F.W. (2019). Resistance-exercise training ameliorates LPS-induced cognitive impairment concurrent with molecular signaling changes in the rat dentate gyrus. *Journal of Applied Physiology, 1*(127), 254-263.

Kim, E., White, M.A., Phillips, B.U., Lopez-Cruz, L., Kim, H., Heath, C.J., Lee, J.E., Saksida,

L.M., Sreedharan, J., Bussey, T.J. (2020). Coexistence of perseveration and apathy in the TDP-43Q331K knock-in mouse model of ALS–FTD. *Transolational Psychiatry, 1*(10), 449-457.

Kim, E.W., Phillips, B.U., Heath, C.J., Cho, S.Y., Kim, H., Sreedharan, J., Song, H.T., Lee, J.E., Bussey, T.J., Kim, C.H., Kim, E., Saksida, Lisa M. (2017). Optimizing reproducibility of operant testing through reinforcer standardization: identification of key nutritional constituents determining reward strength in touchscreens. *Molecular Brain, 1*(10), 31.

Kim, H. J., So, B., Choi, M., Kang, D., & Song, W. (2015). Resistance exercise training increases the expression of irisin concomitant with improvement of muscle function in aging mice and humans. *Experimental Gerontology* (70).

Klein, C., Jonas, W., Iggena, D., Empl, L., Rivalan, M., Wiedmer, P., Spranger, J., Hellweg, R., Winter, Y., Steiner, B. (2016). Exercise prevents high-fat diet-induced impairment of flexible memory expression in the water maze and modulates adult hippocampal neurogenesis in mice. *Neurobiology of Learnring and Memory*, (131), 26-35.

Kopp, W. (2019). How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. *Diabetes, Metabolic Syndrome and Obesity* (12), 2221-2236.

Kopp, W. (2020). Development of obesity: the driver and the passenger. *Diabetes, Metabolic Syndrome and Obesity,* 4631-4642.

Lambert, C. P., & Evans, W. J. (2005). Adaptations to aerobic and resistance exercise in the elderly. *Reviews in Endocrine and Metabolic Disorders, 6*, 137-143.

Leigh, Sarah-Jane; Lee, Frances; Morris, Margaret J. (2018). Hyperpalatability and the Generation of Obesity: Roles of Environment, Stress Exposure and Individual Difference. *Current Obesity Reports, 1*(7), 45-53.

Lewis, A. R., Singh, S., & Youssef, F. F. (2019). Cafeteria-diet induced obesity results in impaired cognitive functioning in a rodent model. *Heliyon, 5*(3).

Li, Y., Dai, Q., Jackson, J. C., & Zhang, J. (2008). Overweight is associated with decreased cognitive functioning among school-age children and adolescents. *Obesity, 16*(8), 1809-1815.

Liu, C., Zhang, N., Zhang, R., Jin, L., Petridis, A.K., Loers, G., Zheng, X., Wang, Z., Siebert, H.C. (2020). Cuprizone-Induced Demyelination in Mouse Hippocampus Is Alleviated by Ketogenic Diet. *Journal of Aggricultural and Food Chemistry, 40*(68), 11215-112228.

Liu, Y., Chu, J. M. T., Yan, T., Zhang, Y., Chen, Y., Chang, R. C. C., & Wong, G. T. C. (2020). Short-term resistance exercise inhibits neuroinflammation and attenuates neuropathological changes in 3xTg Alzheimer's disease mice. *Journal of Neuroinflammation, 1*(17), 4.

Loeber, S.; Grosshans, M., Korucuoglu, O., Vollmert, C., Vollstädt-Klein, S., Schneider, S., Wiers, R. W., Mann, K., Kiefer, F. (2012). Impairment of inhibitory control in response to food-associated cues and attentional bias of obese participants and normal-weight controls. *International Journal of Obesity, 10*(36), 1334-1339.

Lofterød, T., Frydenberg, H., Veierød, M. B., Jenum, A. K., Reitan, J. B., Wist, E. A., & Thune, I. (2022). The influence of metabolic factors and ethnicity on breast cancer risk, treatment and survival: The Oslo ethnic breast cancer study. Acta *Oncologica, 61*(5), 649-657.

Lokken, K. L., Boeka, A. G., Austin, H. M., Gunstad, J., & Harmon, C. M. (2009). Evidence of executive dysfunction in extremely obese adolescents: a pilot study. *Surgery for Obesity and Related Diseases, 5*(5), 547-552.

Lu, B., Nagappan, G., Lu, Y. (2014). BDNF and Synaptic Plasticity, Cognitive Function, and Dysfunction. *Neurotrophic Factors*, 223-250.

Malik, V. S., Willett, W. C., & Hu, F. B. (2013). Global obesity: trends, risk factors and policy implications. *Nature reviews endocrinology, 9*(1), 13-27.

Mallien, A.S., Palme, R., Richetto, J., Muzzillo, C., Richter, S.H., Vogt, M.A., Inta, D., Riva,

M.A., Vollmayr, B., Gass, P., (2016). Daily exposure to a touchscreen-paradigm and associated food restriction evokes an increase in adrenocortical and neural activity in mice. *Hormones and Behavior* (81), 97-105.

Magaña, P., Mena-Moreno, T., Aymamí, N., Gómez-Peña, M., Del Pino-Gutiérrez, A., Mestre-Bach, G. (2019). Impulsivity and cognitive distortions in different clinical phenotypes of gambling disorder: Profiles and longitudinal prediction of treatment outcomes. *European Psychiatry* (61), 451-458.

Masley, S., Roetzheim, R., & Gualtieri, T. (2009). Aerobic exercise enhances cognitive flexibility. *Journal of clinical psychology in medical settings, 16*, 186-193.

McPherson, N. O., Owens, J. A., Fullston, T., & Lane, M. (2015). Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. *American Journal of Physiology-Endocrinology and Metabolism, 308*(9), E805-E821.

McTighe, S. M., Mar, A. C., Romberg, C., Bussey, T. J., & Saksida, L. M. (2009). A new touchscreen test of pattern separation: effect of hippocampal lesions. *Neuroreport, 20*(9), 881-885.

Meo, S. A., Altuwaym, A. A., Alfallaj, R. M., Alduraibi, K. A., Alhamoudi, A. M., Alghamdi, S. M., & Akram, A. (2019). Effect of obesity on cognitive function among school adolescents: a cross-sectional study. *Obesity facts*, *12*(2), 150-156.

Miles, B., Yang, W., Dezsi, G., Sokolenko, E., Gomes, F.M., Jupp, B., Hill, R., Hudson, M., Jones, N.C. (2022). High sucrose diet does not impact spatial cognition in rats using advanced touchscreen technology. *Behavioural Brain Research* (418), 102-117.

Misra, A., & Khurana, L. (2008). Obesity and the Metabolic Syndrome in Developing Countries. The *Journal of Clinical Endocrinology & Metabolism, 11(*93), 9-30.

Mobbs, O., Crépin, C., Thiéry, C., Golay, A., & Van der Linden, M. (2010). Obesity and the four facets of impulsivity. *Patient education and counseling, 79*(3), 372-377.

Mond, J. M., Stich, H., Hay, P. J., Kraemer, A.; Baune, B. T. (2007). Associations between obesity and developmental functioning in pre-school children: a population-based study. *International Journal of Obesit*y (201), 661-672.

Moreno-Fernández, S., Garcés-Rimón, M., Vera, G., Astier, J., Landrier, J. F., & Miguel, M. (2018). High fat/high glucose diet induces metabolic syndrome in an experimental rat model. *Nutrients, 10*(10), 1502.

Mota, B., Ramos, M., Marques, S.I., Silva, A., Pereira, P.A., Madeira, M.D., Mateus, N.,Cardoso, A. (2023). Effects of High-Fat and High-Fat High-Sugar Diets in the Anxiety, Learning and Memory, and in the Hippocampus Neurogenesis and Neuroinflammation of Aged Rats. *Nutrients, 6*(15), 1370.

Motil, K. J., & Grand, R. J. (1985). Nutritional management of inflammatory bowel disease. *Pediatric Clinics of North America, 32*(2), 447-469.

Murray, A. J., Knight, N. S., Cochlin, L. E., McAleese, S., Deacon, R. M., Rawlins, J. N. P., & Clarke, K. (2009). Deterioration of physical performance and cognitive function in rats with short-term high-fat feeding. *The FASEB Journal, 23*(12), 4353-4360.

Neto, W.K., Gama, E.F., de Assis Silva, W., de Oliveira, T.V.A., dos Santos Vilas Boas, A.E., Ciena, A.P., Anaruma, C.A., Caperuto, É.C. (2021). Ladder-based resistance training elicited similar ultrastructural adjustments in forelimb and hindlimb peripheral nerves of young adult Wistar rats. *Experimental Brain Research, 8*(239), 2583-2592.

Nishikawa, S., Yasoshima, A., Doi, K., Nakayama, H., & Uetsuka, K. (2007). Involvement of

sex, strain and age factors in high fat diet-induced obesity in C57BL/6J and BALB/cA mice. Experimental animals, 56(4), 263-272.

Niskanen, L., Laaksonen, D.E., Nyyssönen, K., Punnonen, K., Valkonen, V.P., Fuentes, R., Tuomainen, T.P., Salonen, R., Salonen, J.T. (2015). Inflammation, Abdominal Obesity, and Smoking as Predictors of Hypertension. *Hypertension, 6(*44), 859-865.

Oddy, W.H., Herbison, C.E., Jacoby, P., Ambrosini, G.L., O'sullivan, T.A., Ayonrinde, O.T., Olynyk, J.K., Black, L.J., Beilin, L.J., Mori, T.A., Hands, B.P. (2013). The Western Dietary Pattern Is Prospectively Associated With Nonalcoholic Fatty Liver Disease in Adolescence. *Official Journal of the American College of Gastroenterology, 5*(108), 778.

O'Dea, K., White, N. G., & Sinclair, A. J. (1988). An investigation of nutrition-related risk factors in an isolated Aboriginal community in Northern Australia: advantages of a traditionally-orientated life-style. *Medical Journal of Australia, 148*(4), 177-180.

Oertel-Knöchel, V., Mehler, P., Thiel, C., Steinbrecher, K., Malchow, B., Tesky, V., Ademmer, K., Prvulovic, D., Banzer, W., Zopf, Y., Schmitt, A. (2014). Effects of aerobic exercise on cognitive performance and individual psychopathology in depressive and schizophrenia patients. *European Archives of Psychiatry and Clinical Neuroscience, 7*(264), 589-604.

Ormel, H.l.; van der Schoot, G.g.f.; Sluiter, W.j.; Jalving, M.; Gietema, J.a.; Walenkamp, A.m.e. (2018). Predictors of adherence to exercise interventions during and after cancer treatment: A systematic review. *Psycho-Oncology, 3*(27), 713-724.

Park, H. R., Park, M., Choi, J., Park, K. Y., Chung, H. Y., & Lee, J. (2010). A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. *Neuroscience letters, 482*(3), 235-239.

Patel, H., Alkhawam, H., Madanieh, R., Shah, N., Kosmas, C. E., & Vittorio, T. J. (2017).

Aerobic vs anaerobic exercise training effects on the cardiovascular system. *World journal of cardiology, 9*(2), 134.

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiological reviews.*

Pontifex, M. B., Hillman, C. H., Fernhall, B. O., Thompson, K. M., & Valentini, T. A. (2009). The effect of acute aerobic and resistance exercise on working memory. *Medicine & Science in Sports & Exercise, 41*(4), 927-934.

Preguica, I., Alves, A., Nunes, S., Fernandes, R., Gomes, P., Viana, S. D., & Reis, F. (2020). Diet-induced rodent models of obesity-related metabolic disorders—a guide to a translational perspective. *Obesity Reviews, 21*(12), e13081.

Reichelt, A. C., Lemieux, C. A., Princz-Lebel, O., Singh, A., Bussey, T. J., & Saksida, L. M. (2021). Age-dependent and region-specific alteration of parvalbumin neurons, perineuronal nets and microglia in the mouse prefrontal cortex and hippocampus following obesogenic diet consumption. *Scientific reports, 11*(1), 5593.

Rimes-Dias, K. A., Costa, J. C., & Canella, D. S. (2022). Obesity and health service utilization in Brazil: data from the National Health Survey. *BMC Public Health, 22*(1), 1474.

Roberts, J.S., Perets, R.A., Sarfert, K.S., Bowman, J.J., Ozark, P.A., Whitworth, G.B., Blythe, S.N., Toporikova, N. (2017). High-fat high-sugar diet induces polycystic ovary syndrome in a rodent model. *Biology of Reproduction, 3*(96), 551-562.

Robinson, M.J., Burghardt, P.R., Patterson, C.M., Nobile, C.W., Akil, H., Watson, S.J., Berridge, K.C., Ferrario, C.R., (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology, 9*(40), 2113-2123.

Rochat, L., Billieux, J., Van der Linden, A. C. J., Annoni, J. M., Zekry, D., Gold, G., & Van der Linden, M. (2013). A multidimensional approach to impulsivity changes in mild Alzheimer's disease and control participants: Cognitive correlates. *Cortex, 49*(1), 90-100.

Roebuck, A. J., An, L., Marks, W. N., Sun, N., Snutch, T. P., & Howland, J. G. (2020). Cognitive impairments in touchscreen-based visual discrimination and reversal learning in genetic absence epilepsy rats from Strasbourg. *Neuroscience*, 430, 105-112.

Roebuck, A. J., Liu, M. C., Lins, B. R., Scott, G. A., & Howland, J. G. (2018). Acute stress, but not corticosterone, facilitates acquisition of paired associates learning in rats using touchscreen-equipped operant conditioning chambers. *Behavioural Brain Research,* 348, 139-149.

Rooney, K., Ozanne, S. E. (2011). Maternal over-nutrition and offspring obesity predisposition: targets for preventative interventions. *International Journal of Obesity, 7*(35), 883-890.

Rothemund, Y., Preuschhof, C., Bohner, G., Bauknecht, H. C., Klingebiel, R., Flor, H., & Klapp, B. F. (2007). Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. *Neuroimage, 37*(2), 410-421.

Ruigrok, S. R., Kotah, J. M., Kuindersma, J. E., Speijer, E., van Irsen, A. A. S., la Fleur, S. E., Korosi, A. (2021). Adult food choices depend on sex and exposure to early-life stress: Underlying brain circuitry, adipose tissue adaptations and metabolic responses. *Neurobiology of Stress* (15), 100-126.

Sallis, J F; Hovell, M F; Hofstetter, C R; Elder, J P; Hackley, M; Caspersen, C J; Powell, K E. (1990). Distance between homes and exercise facilities related to frequency of exercise among San Diego residents. *Public Health Reports, 2*(105), 179-185.

Samson, S.L.; Garber, A.J. (2014). Metabolic Syndrome. *Endocrinology and Metabolism Clinics,*

1(43), 449-462.

Schmidt, S. L., Bessesen, D. H., Stotz, S., Peelor III, F. F., Miller, B. F., & Horton, T. J. (2014). Adrenergic control of lipolysis in women compared with men. *Journal of Applied Physiology, 11*7(9), 1008-1019.

Schneider, M., & Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology, 28*(10), 1760-1769.

Seabra, A. F., Mendonça, D. M., Göring, H. H., Thomis, M. A., & Maia, J. A. (2008). Genetic and environmental factors in familial clustering in physical activity. *European journal of epidemiology*, 23, 205-211.

Sharma, S., Rakoczy, S., & Brown-Borg, H. (2010). Assessment of spatial memory in mice. *Life sciences, 87*(17-18), 521-536.

Sinclair, D., Purves-Tyson, T. D., Allen, K. M., & Weickert, C. S. (2014). Impacts of stress and sex hormones on dopamine neurotransmission in the adolescent brain. *Psychopharmacology, 8*(231), 1581-1599.

Small, L., Ehrlich, A., Iversen, J., Ashcroft, S.P., Trošt, K., Moritz, T., Hartmann, B., Holst, J.J., Treebak, J.T., Zierath, J.R., Barrès, R. (2022). Comparative analysis of oral and intraperitoneal glucose tolerance tests in mice. *Molecular Metabolism* (57), 101-132.

Soldo, A.M., Soldo, I., Karačić, A., Konjevod, M., Perkovic, M.N., Glavan, T.M., Luksic, M., Žarković, N., Jaganjac, M. (2022). Lipid Peroxidation in Obesity: Can Bariatric Surgery Help? *Antioxidants, 8*(11), 15-27.

Spyridaki, E. C., Avgoustinaki, P. D., & Margioris, A. N. (2016). Obesity, inflammation and cognition. *Current Opinion in Behavioral Sciences*, 9, 169-175.

Sripetchwandee, J., Chattipakorn, N., & Chattipakorn, S. C. (2018). Links between obesity-

induced brain insulin resistance, brain mitochondrial dysfunction, and dementia. *Frontiers in endocrinology*, 9, 496.

Stice, E., Yokum, S., Bohon, C., Marti, N., & Smolen, A. (2010). Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of DRD2 and DRD4. *Neuroimage, 50*(4), 1618-1625.

Stranahan, Alexis M., Norman, Eric D., Lee, Kim; Cutler, Roy G., Telljohann, Richard S., Egan, Josephine M., Mattson, Mark P. (2008). Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus, 11*(18), 1085-1088.

Strasser, B., Arvandi, M., Pasha, E. P., Haley, A. P., Stanforth, P., Tanaka, H. (2015). Abdominal obesity is associated with arterial stiffness in middle-aged adults. *Nutrition, Metabolism, and Cardiovascular Disease, 5*(25), 495-502.

Strauss, G. P., Whearty, K. M., Morra, L. F., Sullivan, S. K., Ossenfort, K. L., & Frost, K. H. (2016). Avolition in schizophrenia is associated with reduced willingness to expend effort for reward on a Progressive Ratio task. *Schizophrenia research, 170*(1), 198-204.

Sullivan, J.A., Dumont, J.R., Memar, S., Skirzewski, M., Wan, J., Mofrad, M.H., Ansari, H.Z., Li, Y., Muller, L., Prado, V.F., Prado, M.A. (2021). New frontiers in translational research: Touchscreens, open science, and the mouse translational research accelerator platform. *Genes, Brain and Behavior, 1(*20), 127-135.

Takayanagi, Y., Ishizuka, K., Laursen, T.M., Yukitake, H., Yang, K., Cascella, N.G., Ueda, S.,

Sumitomo, A., Narita, Z., Horiuchi, Y.,Niwa, M. (2021). From population to neuron: exploring common mediators for metabolic problems and mental illnesses. *Molecular Psychiatry, 8*(26), 3931-3942.

Terzo, S., Amato, A., & Mulè, F. (2021). From obesity to Alzheimer's disease through insulin resistance. *Journal of Diabetes and its Complications, 35*(11), 108026.

Toft, U. N., Kristoffersen, L. H., Aadahl, M., von Huth Smith, L., Pisinger, C., & Jørgensen, T. (2007). Diet and exercise intervention in a general population—mediators of participation and adherence: the Inter99 study. *European journal of public health, 17*(5), 455-463.

Tozuka, Y., Kumon, M., Wada, E., Onodera, M., Mochizuki, H., & Wada, K. (2010). Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. *Neurochemistry international, 57*(3), 235-247.

Tran, D. M., & Westbrook, R. F. (2017). A high-fat high-sugar diet-induced impairment in place-recognition memory is reversible and training-dependent. *Appetite*, 110, 61-71.

Trovato, F. M., Martines, G. F., & Catalano, D. (2018). Addressing Western dietary pattern in obesity and NAFLD. *Nutrire*, 43, 1-6.

Tsan, L., Décarie-Spain, L., Noble, E. E., & Kanoski, S. E. (2021). Western diet consumption during development: setting the stage for neurocognitive dysfunction. *Frontiers in Neuroscience*, 15, 632312.

Underwood, E. L., & Thompson, L. T. (2016). High-fat diet impairs spatial memory and hippocampal intrinsic excitability and sex-dependently alters circulating insulin and hippocampal insulin sensitivity. *Biology of sex differences,* 7, 1-15.

Uysal, N., Yuksel, O., Kizildag, S., Yuce, Z., Gumus, H., Karakilic, A.,

Guvendi, G., Koc, B., Kandis, S., Ates, M. (2018). Regular aerobic exercise correlates with reduced anxiety and incresed levels of irisin in brain and white adipose tissue. *Neuroscience Letters* (676), 92-97.

Valladolid-Acebes, I.., Stucchi, P., Cano, V., Fernández-Alfonso, M. S., Merino, B., Gil-Ortega, M., Fole, A., Morales, L., Ruiz-Gayo, M.., Olmo, N. (2011). High-fat diets impair spatial learning in the radial-arm maze in mice. *Neurobiology of Learning and Memorry, 1*(05), 80-85.

van den Heuvel, J. K.; Eggels, L.; van Rozen, A. J.; Luijendijk, M. C. M.; Fliers, E.; Kalsbeek, A.; Adan, R. A. H.; la Fleur, S. E. (2014). Neuropeptide Y and Leptin Sensitivity is Dependent on Diet Composition. *Journal of Neuroendocrinology, 6*(26), 377-385.

Vega-Torres, J. D., Reyes-Rivera, A. L., & Figueroa, J. D. (2019). Developmental regulation of fear memories by an obesogenic high-saturated fat/high-sugar diet. *Biorxiv*, 748079.

Vichaya, E.G., Laumet, G., Christian, D.L., Grossberg, A.J., Estrada, D.J., Heijnen, C.J., Kavelaars, A., Dantzer, R. (2019). Motivational changes that develop in a mouse model of inflammation-induced depression are independent of indoleamine 2,3 dioxygenase. *Neuropsychopharmacology*, *44*(2), 364-371.

Volkow, N. D., Wang, G. J., Tomasi, D., & Baler, R. D. (2013). The addictive dimensionality of obesity. *Biological psychiatry, 73*(9), 811-818.

Wang, H., Sun, N., Wang, X., Han, J., Zhang, Y., Huang, Y., & Zhou, W. (2022). A touchscreen-based paradigm to measure visual pattern separation and pattern completion in mice. *Frontiers in Neuroscience*, 16, 947742.

Wasinski, F., Bacurau, R.F.P., Estrela, G.R., Klempin, F., Arakaki, A.M., Batista, R.O., Mafra,

F.F.P., do Nascimento, L.F.R., Hiyane, M.I., Velloso, L.A., Câmara, N.O.S. (2015). Exercise during pregnancy protects adult mouse offspring from diet-induced obesity. *Nutrition & Metabolism, 1*(12), 56.

Wei, W., Pham, K., Gammons, J.W., Sutherland, D., Liu, Y., Smith, A., Kaczorowski, C.C., O'Connell, K.M. (2015). Diet composition, not calorie intake, rapidly alters intrinsic excitability of hypothalamic AgRP/NPY neurons in mice. *Scientific Reports*, (5), 168-178.

Westbrook, A., & Frank, M. (2018). Dopamine and proximity in motivation and cognitive control. *Current Opinion in Behavioral Sciences*, 22, 28-34.

Wharton, S., Lau, D.C., Vallis, M., Sharma, A.M., Biertho, L., Campbell-Scherer, D., Adamo, K., Alberga, A., Bell, R., Boulé, N., Boyling, E. (2020). Obesity in adults: a clinical practice guideline. *CMAJ, 31*(192), 875-891.

White, Z., Terrill, J., White, R. B., McMahon, C., Sheard, P., Grounds, M. D., & Shavlakadze, T. (2016). Voluntary resistance wheel exercise from mid-life prevents sarcopenia and increases markers of mitochondrial function and autophagy in muscles of old male and female C57BL/6J mice. Skeletal Muscle, 6(1), 1-21.

Whitmer, R. A., Gunderson, E. P., Barrett-Connor, E., Quesenberry, C. P., & Yaffe, K. (2005). Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. *Bmj, 330*(7504), 1360.

Wilckens, K.A., Stillman, C.M., Waiwood, A.M., Kang, C., Leckie, R.L., Peven, J.C., Foust, J.E., Fraundorf, S.H. and Erickson, K.I. (2021). Exercise interventions preserve hippocampal volume: A meta-analysis. *Hippocampus, 3*(31), 335-347.

Wong, D., Broberg, D.N., Doad, J., Umoh, J.U., Bellyou, M., Norley, C.J., Holdsworth, D.W.,

Montero-Odasso, M., Beauchet, O., Annweiler, C., Bartha, R. (2021). Effect of Memantine Treatment and Combination with Vitamin D Supplementation on Body Composition in the APP/PS1 Mouse Model of Alzheimer's Disease Following Chronic Vitamin D Deficiency. *Journal of Alzheimer's Disease 1*(81), 375-388.

Yeomans, M.R. (2017). Adverse effects of consuming high fat–sugar diets on cognition: implications for understanding obesity. *Proceedings of the Nutrition Society, 4*(76), 455-465.

Yu, H., Qin, X., Yu, Z., Chen, Y., Tang, L., & Shan, W. (2021). Effects of high-fat diet on the formation of depressive-like behavior in mice. *Food & Function, 12*(14), 6416-6431

Curriculum Vitae

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Education

2021- Present	Master of Science, Neuroscience Western University Thesis Supervisors: Dr. Lisa Saksida and Dr. Timothy Bussey Thesis Tite: Outclimbing Cognitive Decline: Age, western diet, resistance exercise, and the brain
2013- 2018	Bachelor of Science, Honours Health Studies (minors Psychology and Human Nutrition) University of Waterloo

Honours and Awards

2018	University of Waterloo Staff Association Award for Excellence in Volunteering
2017-2018	RWTH Aachen Research Internship Scholarship
2016-2018	University of Waterloo Dean Honour's List
2013	Applied Health Sciences Entrance Scholarship

Research Experience

2021- Present	Master's Student, Western Ontario
2017-2018	Neuroscience Research Assistant, RWTH University Aachen
2016-2017	Health Studies Research Assistant, University of Waterloo
2015-2017	Psychology Research Assistant, University of Waterloo

Presentations

2023	Outclimbing Cognitive Decline. International Touchscreen Symposium, Western University.
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Student Supervision

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