THE ROLE OF SIGMA-1 RECEPTORS IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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ABSTRACT

Alzheimer's disease (AD) is an incurable disease characterized by a slow, progressive decline in cognitive functions as well as the presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles. Interestingly, two thirds of AD patients are women who have a faster disease progression. Despite this clinical profile, sex differences in AD pathophysiology are largely ignored at the basic and clinical levels. Current therapies provide only mild to moderate improvement in patient symptoms. There is, therefore, an urgent need to expand our understanding of the underlying pathophysiology of AD, and to obtain alternative hypotheses and therapeutics. A recent and promising development involves the sigma-1 receptor (Sig1R), a protein regulated by steroid hormones, which has been implicated in AD. Most interestingly, Sig1R agonists have been shown to ameliorate cognitive deficits in an AD mouse model. Here, we investigated the role of Sig1Rs in an $A\beta_{25-35}$-infusion mouse model of AD, using behavioural paradigms. Previous studies employing this model have demonstrated Aβ-induced impairments in learning and memory in young male rodents, while no work has been done on females. We examined cognitive function following $A\beta_{25-35}$ infusion in wild-type and knock-out Sig1R adult male and female mice using the Morris water maze, spontaneous alternation in the Y-maze, and forced alternation in the Y-maze tasks. Overall, the data unexpectedly shows that genotype, $A\beta_{25-35}$-treatment, and sex had no effect on cognitive functions. These results suggest that additional efforts are required to obtain a working $A\beta_{25-35}$-infusion model in our Sig1R mice and behavioural tasks. Future experiments will hopefully shed some light on the link between Sig1Rs and AD, which could lead to the development of therapeutics and disease prevention.

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LIST OF ABBREVIATIONS

ACVS	Animal Care and Veterinary Services
AD	Alzheimer's disease
ANOVA	Analysis of variance
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
Aβ	Amyloid-beta
AβO	Amyloid-beta oligomer
EOAD	Early-onset Alzheimer's disease
ER	Endoplasmic reticulum
Fisher's LSD	Fisher's least significant difference
hAPP	Human amyloid precursor protein
HET	Heterozygous
ICV	Intracerebroventricular injection
KO	Knock-out
LOAD	Late-onset Alzheimer's disease
MAM	Mitochondria-associated endoplasmic reticulum membrane
MWM	Morris water maze
NFT	Neurofibrillary tangle
NMDAR	N-methyl-D-aspartate receptor
PCR	Polymerase chain reaction
PS1	Presenilin 1
PS2	Presenilin 2
REV	Reverse
SEM	Standard error of the mean
Sig1R	Sigma-1 receptor
SIGMAR1	Sigma-1 receptor gene

SKF-10,047	N-allylnormetazocine
Tg	Transgenic
WT	Wild-type

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1. INTRODUCTION

1.1 Alzheimer's Disease

1.1.1 Background

Dementia is a general term for major cognitive impairments severe enough to interfere with social or occupational activities and is associated with a diminished quality of life (Ankarcrona et al., 2016). Underlying pathological issues are often responsible for a decline in independent functioning from a certain premorbid state (Henderson, 2014). The most common form of dementia in the elderly is Alzheimer's disease (AD), first described in 1906 by the German psychiatrist Alois Alzheimer (Alzheimer et al., 1995; Maurer et al., 1997; Möller and Graeber, 1998; Reitz et al., 2011; Scheltens et al., 2016). AD has a socio-economic impact measured in the billions, with considerable suffering experienced not only by the patients but also by their caregivers and loved ones. With over 35 million people suffering from dementia worldwide, this growing social and economic burden on society is in part due to the ever-increasing aging of the worldwide population (Mota et al., 2014; Prince et al., 2013; Wimo et al., 2010). Despite decades of intense basic and clinical research, there are presently no cures for AD and only limited therapeutic interventions are available to manage the devastating symptoms.

AD is a progressive and fatal neurodegenerative disorder that is characterized by deterioration of cognitive functions as well as multiple behavioural disturbances and neuropsychiatric symptoms (Cummings, 2004; Mota et al., 2014; Puzzo et al., 2015). AD also gradually impairs regular skills such as reasoning, language, and abstraction, with female AD patients having relatively greater difficulty with naming, verbal fluency and episodic memory (Henderson and Buckwalter, 1994; Ripich et al., 1995; Selkoe, 2011; Small et al., 2000). Evidence suggests that AD is a disease of synapses in which dysfunction of neuronal networks is manifested as episodic

memory loss in early stages of the disorder (Jacobsen et al., 2006; Ondrejcak et al., 2010; Roy et al., 2016; Terry et al., 1991). Loss of synapses in the brain occur prior to neuronal loss and is highly correlated with the severity of dementia in AD (DeKosky and Scheff, 1990; Shankar and Walsh, 2009). Progressive loss of synapses and nerve cells first start in the hippocampus, a brain region critical for learning and memory, and adjacent structures of the medial temporal lobes (Braak and Braak, 1991, 1997; Henderson, 2014; Isik, 2010; Vorhees and Williams, 2014). As the disease progresses, association areas of the cerebral cortex are increasingly affected resulting in the progressive loss of short-term/working memory (also referred as visuospatial memory), cognitive flexibility, and other cognitive abilities (Carlesimo and Oscar-Berman, 1992; Diamond, 2014; Mucke and Selkoe, 2012; Twamley et al., 2006).

There are two forms of AD: a rare familial form (early-onset; EOAD) caused by autosomal dominant mutations, with an onset at an age younger than 60 years, and a common sporadic form (late-onset; LOAD), with an onset after 60 years of age (Brouwers et al., 2008; Kukull et al., 2002; Reitz and Mayeux, 2014). EOAD is associated with mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) genes, all of which are linked to excessive production, accumulation, or deposition of amyloid-beta (Aβ) peptides in the brain (Cummings, 2004; Goate, 2006; Lannfelt et al., 2014; Selkoe, 1989). Despite tremendous research focused on elucidating the complex molecular mechanisms of LOAD, its environmental and genetic components are not fully understood (Balin and Hudson, 2014; Zou et al., 2014).

1.1.2 Hallmarks

The microscopic hallmarks characteristic of AD include neurofibrillary tangles (NFTs) and neuritic plaques (Ballenger, 2006; Blennow et al., 2006; Selkoe, 2011). These NFTs involve intracellular filaments capable of self-assembly and are composed of hyperphosphorylated tau, a

protein involved in the stabilization of microtubules (Binder et al., 2005; Claeysen et al., 2012; Mucke and Selkoe, 2012). NFTs have been shown to be located within cell bodies of affected neurons in the cerebral cortex and brain stem (Braak and Braak, 1991; Henderson, 2014). In neuritic plaques, the main component is Aβ, a peptide derived from the cleavage of APP (Mucke and Selkoe, 2012; Puzzo et al., 2015; Zetterberg and Mattsson, 2014). By assuming a β-pleated configuration, Aβ peptides are capable of aggregating and forming soluble oligomers as well as insoluble amyloid sheets. These insoluble sheets of Aβ aggregates accumulate in the extracellular space between neurons, and over time associate with astrocytes, microglia, and dystrophic neurites (Selkoe, 1989, 2011; Toyn and Ahlijanian, 2014). Neuritic plaques first appear in the basal isocortex and spread through the entire isocortex structure as the disease advances. In the late stages of AD, neuritic plaques are found throughout the brain, with the isocortex demonstrating the largest neuritic plaque load (Braak and Braak, 1991).

In recent years, increasing evidence has shown that soluble Aβ oligomers (AβOs) are the molecules responsible for producing severe cytotoxicity and for causing detrimental effects on synaptic function and neuronal viability (Goate, 2006; Lambert et al., 1998; Lannfelt et al., 2014; Mucke and Selkoe, 2012; Oda et al., 1994).

1.1.3 Sex differences in pathology

While the concept of sex differences is well known, understanding the role of sex in AD pathology and treatment is considerably under-valued and under-studied (Cahill and Aswad, 2015; Zucker and Beery, 2010). Epidemiological and observational studies suggest a higher incidence and prevalence of AD in women compared to men (Bachman et al., 1992; Herrmann et al., 2015; Jorm and Jolley, 2000; Mielke et al., 2014; Prince et al., 2013; Ruitenberg et al., 2001). Additional gender differences were also found when looking at various aspects of the disease.

Strikingly, two thirds of AD patients are women, who frequently experience a more rapid decline in cognition than their male counterparts (Farrer, 1997; Herrmann et al., 2015; Mielke et al., 2014; Riedel et al., 2016; Sinforiani et al., 2010; Snyder et al., 2016a). Notably, it has been shown that synaptic structure and physiology can be altered by steroid hormones in the hippocampus and that these hormones can also be neuroprotective against various insults (Brann et al., 2007; Engler-Chiurazzi et al., 2016; Frick et al., 2015; Srivastava et al., 2013). Also of note, a decline of hormone levels, an event that takes place during menopause, affects hippocampal function and cognition (Adams and Morrison, 2003). Therefore, hormonal changes have the potential to influence processes associated with AD symptoms and pathogenesis and may partially explain the more rapid progression of AD in women.

From a histopathological angle, research shows women exhibit greater Aβ deposition and NFT production as well as faster cerebral atrophy (Barnes et al., 2005; Corder et al., 2004; Skup et al., 2011). Findings from human studies have also been corroborated by data obtained from animal research performed on mice. Consistent findings were observed, which exhibited more pronounced learning and memory impairments as well as higher levels of Aβ and tau aggregates in female compared to male mice (Callahan et al., 2001; Carroll et al., 2010; Grueninger et al., 2010; Hirata-Fukae et al., 2008; King et al., 1999; Lewis, 2001; Song et al., 2015; Yue et al., 2011). Therefore, it is imperative that the scope of AD research be broadened to include the role of sex in AD pathology and treatment.

1.1.4 Risk factors

Sporadic AD (or LOAD), which represents more than 90% of disease cases, is caused by a complex interaction between environmental and genetic risk factors, most of which are currently unknown. It is further complicated by the possible sex differences in pathophysiology,

reinforcing the heterogeneity of this disease. Population studies have identified levels of physical activity, smoking, obesity, and alcohol consumption as potential environmental risk factors (Graves et al., 1991; Rovio et al., 2005). However, the most common risk factor for AD is advancing age (Blennow et al., 2006; Ferreira et al., 2015; Jagust, 2013; Prince et al., 2013).

When examining genetic factors, the most prevalent is a mutation in the apolipoprotein E (ApoE) gene on chromosome 19, and the inheritance of ApoE ε4 alleles, which are associated with higher Aβ load in the brain (Andreasson et al., 2014; Selkoe, 1994; Strittmatter et al., 1993). Interestingly, this polymorphism has been shown to increase the risk of sporadic AD more so in women than in men (Bretsky et al., 1999; Farrer, 1997; Mielke et al., 2014; Reitz and Mayeux, 2014).

Finally, as mentioned above, hormonal changes inherent to menopause impact neuronal processes involved in cognition and may be implicated in the pathological processes linked to AD. This drop in sex steroid hormones has been suggested to be a risk factor in AD (Pike, 2017; Vest and Pike, 2013).

1.1.5 Amyloid cascade hypothesis

Despite this heterogeneous complexity, the dominant theory in AD research thus far has been the amyloid cascade hypothesis (depicted in **Figure 1**). First postulated in the early 1990s, this theory suggests that production and aggregation of Aβ into plaques initiates a toxic cascade that leads to cellular dysfunction and ultimate neuronal death (Glenner and Wong, 1984; Hardy and Allsop, 1991; Hardy and Higgins, 1992; Hardy and Selkoe, 2002; Karran et al., 2011; Selkoe, 1991). These plaques were believed to play a role in synaptic and neuronal loss, which promoted cerebral metabolism decline, brain inflammation, cognitive impairment and brain atrophy, with

the hippocampus showing the greatest decline in activity (Ball et al., 1985; Licastro et al., 2017). However, recent modifications to the hypothesis have suggested that the primary toxic agent responsible for instigating major facets of AD neuropathology is the soluble oligomeric Aβ species themselves, rather than the plaques (Bitan et al., 2003; Frackowiak et al., 1994; Glabe and Kayed, 2006; Lue et al., 1999; McLean et al., 1999; Paola et al., 2000; Walsh and Teplow, 2012; Walsh et al., 2002; Wang et al., 1999). Nonetheless, this hypothesis still views Aβ as the main driving force in AD pathogenesis with other pathological hallmarks, including tau accumulation and neurodegeneration, as downstream effects.

1.1.5.1 Amyloid-beta peptide

As shown in **Figure 1**, the Aβ peptide is produced through the sequential proteolytic cleavage of APP (Hamley, 2012; Kopan and Ilagan, 2004). Interestingly, APP, a highly conserved type-1 transmembrane glycoprotein located on chromosome 21 in humans, has been suggested to be essential for normal brain development as well as brain plasticity in adults (Selkoe, 1994; Shariati and De Strooper, 2013). APP undergoes proteolytic cleavage that is initially catalyzed by either α- or β-secretases. While the α-secretases produce soluble extracellular domains (αAPP) and 83 amino acid carboxy-terminal fragments (C83) at one site, the β-secretase cleavage produces βAPP and C99 peptides at another cleavage site (Cummings, 2004; Puzzo et al., 2015; Selkoe, 2011). A large multiprotein complex, known as γ-secretase (of which PS1 and PS2 are subcomponents), then acts on these initial cleavage products. Most notably, a 40 or 42 amino acid Aβ peptide, $A\beta_{1\text{-}40}$ or $A\beta_{1\text{-}42}$, is generated from subsequent cleavage of βAPP peptides by γ-secretase (Kopan and Ilagan, 2004; Selkoe, 1994). Due to the exposure of two hydrophobic residues (alanine and isoleucine), $A\beta_{1\text{-}42}$ molecules are able to bind additional

monomers and self-aggregate into toxic AβOs (Mucke and Selkoe, 2012; Toyn and Ahlijanian, 2014; Weiner et al., 2013).

1.1.6 Clinical trials

As mentioned previously, the Aβ peptide is viewed as central to the pathogenesis of AD, both initiating and driving a cascade leading to neuronal dysfunction and eventually cognitive impairments (Gharibyan et al., 2007; Hamley, 2012; Lee et al., 2012; Pepys, 2006; Williams and Serpell, 2011). Some clinicopathological studies, however, have suggested that the relationship between cognitive dysfunctions and amyloid burden is unclear (Castellani and Smith, 2011; Castellani et al., 2009; Gustafson et al., 2007; Näslund, 2000; Vos et al., 2014). Furthermore, results from therapeutic approaches targeting Aβ production or Aβ clearance have been disappointing (Mangialasche et al., 2010). In recent clinical trials, a number of Aβ-targeting drug candidates such as secretase inhibitors and anti-Aβ antibodies have failed to improve patient outcomes (Barten et al., 2006; Doody et al., 2013, 2014; Forman et al., 2012; Hardy et al., 2014; Martenyi et al., 2012; Salloway et al., 2014).

Currently available AD therapies are not disease-modifying and provide only mild to moderate improvement in patient symptoms (Scheltens et al., 2016). Therefore, there is an urgent need to expand our understanding of the underlying molecular pathogenesis of AD and to obtain alternative hypotheses and therapeutic strategies. Recent and promising developments involve the sigma-1 receptor (Sig1R), which has been implicated in sporadic AD in a variety of ways.

1.2 Sigma-1 Receptor

1.2.1 Brief history

The sigma receptor was first discovered by Martin *et al.* in 1976, at which time it was described as a subtype of opioid receptors due to its binding properties with SKF-10,047 (N-allylnormetazocine) and related benzomorphans (Martin et al., 1976). This was later corrected and sigma receptors are now considered unique receptors (Hellewell and Bowen, 1990; Quirion et al., 1992; Su, 1982). Two subtypes of the sigma receptor, sigma-1 and sigma-2, were distinguished based on their drug selectivity patterns, molecular weights, and tissue distribution (Bowen et al., 1993; Hellewell et al., 1994; Torrence-Campbell and Bowen, 1996). The most characterized and well-studied of the sigma receptor subtypes is the Sig1R. Recently, Schmidt *et al.* succeeded in obtaining the crystal structure of the human Sig1R, which revealed a trimeric organization with each subunit containing only a single transmembrane domain (Schmidt et al., 2016).

1.2.2 Endogenous ligands

It has been suggested that some neurosteroids, in particular progesterone, act as endogenous ligands for these receptors (Bergeron et al., 1996; Ganapathy et al., 1999; Hanner et al., 1996; Klein et al., 1994; McCann and Su, 1991; Ramamoorthy et al., 1995; Su et al., 1988; Yamada et al., 1994). Other neurosteroids, such as pregnenolone sulfate, testosterone, and deoxycorticosterone, have also been considered as putative ligands (Debonnel et al., 1996a; Maurice et al., 1996a; McCann and Su, 1991; Su et al., 1988; Yamada et al., 1994). Furthermore, studies identify some ligands act as Sig1R agonists, such as pregnenolone, while others as antagonists, as is the case with progesterone (Bergeron et al., 1999; Maurice et al., 2001; Su et

al., 1988). Therefore, hormonal loss with aging is likely to affect Sig1R function differentially in males and females.

1.2.3 Anatomical distribution and cellular localization

The Sig1R is highly conserved and widely expressed throughout the brain as well as peripheral organs such as the heart, liver and spleen, suggesting they perform essential physiological functions (Jin et al., 2015; Novakova et al., 1995; Seth et al., 2002; Wolfe et al., 1997). The highest concentration of sigma receptors in the brain is found in the brainstem (Bouchard and Quirion, 1997; Gundlach et al., 1986; McLean and Weber, 1988). The limbic regions also display a significant level of these receptors (Bouchard and Quirion, 1997; Gundlach et al., 1986; McLean and Weber, 1988). Moreover, an enrichment of Sig1Rs is seen in the hippocampus, implicating it in learning and memory as well as the modulation of cognitive behaviours (Bouchard and Quirion, 1997).

Advances in cellular and molecular biology have allowed for significant progress in the search for valuable information on Sig1Rs, namely insights on its structure and function. In 1996, the Sig1R was successfully cloned in guinea pigs and estimated to be a 25-29kDa single polypeptide (Hanner et al., 1996). The human Sig1R was subsequently cloned by three separate laboratories, with a 93% amino acid sequence homology to the guinea pig Sig1R (Jbilo et al., 1997; Kekuda et al., 1996; Prasad et al., 1998). Thereafter, both rat and mouse Sig1Rs were cloned using homology screening (Mei and Pasternak, 2001; Pan et al., 1998; Seth et al., 1997, 2002). The human Sig1R contains an endoplasmic reticulum (ER) retention signal and therefore, not surprisingly, has been shown to localize to ER membranes and mitochondria-associated ER membranes (MAM) where it is believed to regulate cellular processes (Cagnotto et al., 1994;

DeHaven-Hudkins et al., 1994; Hayashi and Su, 2007; Itzhak et al., 1991; Jbilo et al., 1997; Wong et al., 2016).

1.2.4 Function

Sig1Rs are found throughout the body and are uniquely positioned in the central nervous system to regulate a variety of cellular processes (Hayashi et al., 2011; Kourrich et al., 2012; Maurice and Su, 2009; Su et al., 2010). Residing at the MAM, the Sig1R acts as an intracellular ER chaperone by regulating calcium signaling (Brent et al., 1996, 1997; Hayashi and Su, 2007; Hayashi et al., 1995, 2000; Nguyen et al., 2015; Vilner and Bowen, 2000). Sig1Rs modulate the activity of multiple kinases, receptors, and ion channels, which affect many cellular processes that directly influence neuronal health and survival (Kourrich et al., 2012; Morio et al., 1994; Wu et al., 1991). Sig1R ligands have been shown to modulate the activity and localization of the N-methyl-D-aspartate receptor (NMDAR), a receptor involved in synaptic plasticity, a cellular mechanism underlying learning and memory in the hippocampus (Bergeron et al., 1993, 1995, 1996, 1997; Debonnel et al., 1996b; Martina et al., 2007; Pabba et al., 2014). Moreover, it has been shown that Sig1Rs act on various voltage-gated potassium, sodium, and calcium channels (Aydar et al., 2002; Maurice and Su, 2009). It is believed that Sig1Rs are largely inactive under normal conditions but act as molecular chaperones under cellular stress (Cobos et al., 2008; Hayashi and Su, 2005). In such conditions, Sig1Rs become activated and exert neuroprotective properties by acting on ion channels and apoptotic pathways, which serve a vital role in maintaining calcium homeostasis and preventing apoptosis (Hayashi and Su, 2007; Maurice and Lockhart, 1997; Rousseaux and Greene, 2015).

1.2.5 Sigma-1 receptor knock-out

The Sig1R is a 223 amino acid protein that shows no homology to any other mammalian protein, suggesting that Sig1Rs have a fundamental function. Cloning of the Sig1R has not only allowed for the study of its structure and function, but also the development of the Sig1R knock-out (KO) mice (Langa et al., 2003). Sig1R KO mice are reported to exhibit lower densities of axons as well as impaired neurogenesis in the hippocampus (Sha et al., 2013, 2015; Tsai et al., 2015). Using electrophysiological techniques, our laboratory revealed mild synaptic plasticity deficits in Sig1R KO compared to WT male mice, with other aspects of basic cellular physiology showing no change (Snyder et al., 2016b).

Interestingly, these KO mice develop normally and show only subtle, but sex-specific, behavioural phenotypes in pain, depression, anxiety, and cognition paradigms. Sig1R KO male and female mice, six to nine weeks of age, have been reported to exhibit diminished responses to pain compared to WT mice using the tail-flick and paw withdrawal tasks in models of chemically-induced and neuropathic pain (Cendán et al., 2005; Entrena et al., 2009; Nieto et al., 2014; Puente et al., 2009). Several studies demonstrate depressive-like phenotypes in two to eight month old male, but not female, Sig1R KO compared to WT mice using the forced swim and tail suspension tasks (Chevallier et al., 2011; Sabino et al., 2009; Sha et al., 2015; Zhang et al., 2017). Furthermore, anxiety behaviours were detected in two month old Sig1R KO males using the elevated plus-maze, passive avoidance, and open-field tasks (Chevallier et al., 2011). The spontaneous alternation in the Y-maze, Morris water maze (MWM), and passive avoidance tasks revealed learning and memory deficits in two and twelve month old female, but not male, Sig1R KO compared to WT mice (Chevallier et al., 2011).

1.2.6 Sigma-1 receptor and Alzheimer's disease

Previous research has shown decreased Sig1R binding sites in ex vivo experiments and lower density of Sig1Rs in patients suffering from AD (Jansen et al., 1993; Mishina et al., 2008). Furthermore, a polymorphism in the Sig1R gene (*SIGMAR1*) has been associated with an increased risk of developing AD (Fehér et al., 2012; Jin et al., 2015). This mutation, found in the 5'-upstream region of *SIGMAR1*, reduces its transcriptional activity and thereby reduces Sig1R expression (Huang et al., 2011b; Miyatake et al., 2004).

Most interestingly, Sig1R agonists have been shown to attenuate mnemonic deficits induced in various rodent models of amnesia (Earley et al., 1991; Matsuno et al., 1994, 1997; Maurice and Privat, 1997; Maurice et al., 1994a; Senda et al., 1998; Zou et al., 2000). Moreover, Sig1R agonist treatment in males has been shown to be neuroprotective and anti-amnesic in AD animal models (Ishikawa and Hashimoto, 2010; Maurice and Su, 2009; Maurice et al., 1998, 2006; Meunier et al., 2006a; Nguyen et al., 2015; Urani et al., 2002).

Together, these studies suggest that loss of Sig1R function may predispose an individual to AD and potentially contribute, at least partially, to the progression of AD. Therefore, to fully elucidate how loss of Sig1Rs contribute to AD and cognitive decline, it is necessary to utilize an animal model of AD and assess cognitive function.

1.3 Mouse Models of Alzheimer's Disease

1.3.1 Transgenic and non-transgenic models

Pathological changes associated with AD such as neuritic plaque formation and cognitive dysfunctions can be observed in some longer living organisms including dogs, cats, sheep and

goat, and non-human primate species (Bons et al., 1993; Braak et al., 1994; Cummings et al., 1993, 1996; Gearing et al., 1994; Gunn-Moore et al., 2006; Head et al., 2005; Kimura et al., 2003; Rofina et al., 2006). However, this is not the case for the most widely used model organism of neuropharmacological studies: rodents (Van Dam and De Deyn, 2011). The development of transgenic (Tg) mice expressing AD-associated genes paved the way for modern AD research.

Based on the amyloid cascade hypothesis, initial attempts were made to create a Tg model overexpressing various mutant forms of the human APP (hAPP) (Higgins et al., 1994; Quon et al., 1991; Sandhu et al., 1991). In 1995, Games *et al.* developed the PDAPP mouse, expressing high levels of hAPP containing a familial AD-associated mutation. This mouse model recapitulated several aspects of AD: neuritic plaque formation, dystrophic neurites, apoptosis, and loss of synapses, which spread progressively from the hippocampus to the cortex (Games et al., 1995; Masliah et al., 1996). One year later, the Tg2576 mouse line was created by Hsiao *et al.* (Hsiao et al., 1996). This line expressed a hAPP isoform bearing a double mutation, referred to as the Swedish mutations. The result was a major overproduction of $A\beta_{1-40}$ and $A\beta_{1-42}$ as well as plaque formation in areas of the brain such as the frontal cortex, hippocampus, cerebellum, and entorhinal cortices. Combining the Tg2576 mouse line with mice expressing a mutant PS1 gene produced the APP/PS1 model (Gong et al., 2004, 2006; Puzzo et al., 2009; Trinchese et al., 2008). These mice present elevated soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ levels as well as robust age-dependent Aβ deposition (Holcomb et al., 1998; Kurt et al., 2001; Radde et al., 2006). Notably, female APP/PS1 mice produce Aβ deposits at a younger age compared to male mice (Wang et al., 2003b).

A triple-Tg model of AD was introduced in 2003, expressing the mutant human tau protein, the hAPP with Swedish mutations, and the mutant PS1 (Oddo et al., 2003). This 3xTgAD model develops increased Aβ production and tau hyperphosphorylation, as well as cognitive deficits, anxiety, circadian changes, and restlessness (Billings et al., 2005; Guzman-Ramos et al., 2012; Kazim et al., 2014; Mastrangelo and Bowers, 2008; Oddo et al., 2003, 2006, Sterniczuk et al., 2010a, 2010b; Stevens and Brown, 2015). As alluded to earlier, decreased levels of sex hormones are thought to be a significant risk factor for AD in post-menopausal women. Interestingly, ovariectomy procedures, which induce oestrogen depletion, have been shown to exacerbate Aβ accumulation and learning impairments in female 3xTgAD mice (Carroll et al., 2007). Finally, in 2006, the 5xFAD model was developed, containing three APP and two PS1 mutations, which resulted in enhanced amyloidogenic Aβ production and neuronal loss in the cortex (Jawhar et al., 2012; Oakley et al., 2006).

The use of these Tg mouse models is complimented by non-genetically modified, or non-Tg, animals. While Tg mice reflect more genetic forms of the disease since they overexpress EOAD-associated mutations, non-Tg mouse models better recapitulate the process of sporadic AD, or LOAD, which accounts for the vast majority of human cases (Bird, 2008; Puzzo et al., 2014). Most non-Tg models are obtained by injecting toxins, such as Aβ peptides or tau, directly into the brain by intracerebroventricular (ICV) or intrahippocampal infusions (Puzzo et al., 2014). Research shows that synthetic and AD brain-derived AβOs share similar structures and toxicologies (De Felice et al., 2008; Gong et al., 2003; Kayed et al., 2003; Klyubin et al., 2012). When infused with AβOs, animals show Aβ accumulation, synaptic dysfunction, impaired brain metabolism, neuritic plaque formation, and cognitive impairments (Chang et al., 2003; Cleary et al., 2005; Guo and Lee, 2011; Lacor et al., 2004; Walsh et al., 2002). Therefore, these non-Tg

infusion models also allow researchers to investigate Aβ-induced impairments in areas such as synaptic and memory dysfunctions, which are crucial when designing new pharmacotherapeutic strategies.

1.3.2 $A\beta_{25-35}$-infusion mouse model of Alzheimer's disease

In 1996, Maurice *et al.* induced an AD-type amnesia in young male mice by ICV injections of aggregated $A\beta_{25-35}$ peptides (Maurice et al., 1996b). Importantly, $A\beta_{25-35}$, the highly amyloidogenic region of the Aβ peptide, has similar toxic properties to $A\beta_{1-42}$ and is found in brains of AD patients (Gruden et al., 2007; Kubo et al., 2002; Peters et al., 2016). Extensive research using this paradigm demonstrates that $A\beta_{25-35}$ peptide infusion recapitulates the main characteristics of AD such as impairments in learning and memory, cell loss in the hippocampus and cortex, tau hyperphosphorylation, and Aβ plaque deposit (Delobette et al., 1997; Klementiev et al., 2007; Lu et al., 2009; Maurice et al., 1996b, 1996c, 1998, Stepanichev et al., 2003, 2004, 2005, 2006, Zussy et al., 2011, 2013). Spatial learning and reference memory deficits were observed in four to seven week old male mice and rats using the MWM task (Chen et al., 1996; Delobette et al., 1997; Maurice et al., 1996b; Wang et al., 2003a; Zussy et al., 2011, 2013). Furthermore, the delayed alternation in the T-maze, radial arm maze, and spontaneous alternation in the Y-maze tasks revealed Aβ-induced spatial shot-term and working memory impairments in four to nine week old male rodents (Maurice et al., 1996b, 1996c, 1998, Stepanichev et al., 2003, 2004, 2005, 2006, Villard et al., 2009, 2011, Zussy et al., 2013, 2011). Aβ-induced memory deficits were also detected in three to nine week old male rodents using the passive avoidance, cued/contextual fear conditioning, and social recognition tasks (Klementiev et al., 2007; Lu et al., 2009; Maurice et al., 1996b, 1996c, 1998, Villard et al., 2009, 2011; Wang et

al., 2003a). Of note, these studies exclusively utilized young rodents and omitted the use of both sexes when establishing this model, producing male-specific findings.

Despite the progress made with EOAD, research into the genetic causes of LOAD, accounting for the vast majority of cases, is lagging. Most animal models and therapeutic strategies developed thus far have been based on knowledge gained from rare, familial forms of the disease. A large part of this is due to the complexity of sporadic AD, with a multitude of genetic and non-genetic risk factors interacting to cause the disease. Although no animal model can perfectly recapitulate all aspects of a clinical disease, they provide the best tool to assess behavioural outcomes and cognitive deficits, which are at the core of AD.

1.4 Behavioural Tasks

1.4.1 Morris water maze

The MWM (**Figure 2 A-C**) is currently one of the most frequently used behavioural paradigms to evaluate spatial learning and reference memory, the ability to learn, store, and retrieve visuospatial information to navigate the surroundings (D'Hooge and De Deyn, 2001; Morellini, 2013; Patil et al., 2009; Sharma et al., 2010; Webster et al., 2014). Originally designed to examine spatial learning in rats (Morris, 1981), it has since been modified slightly for use in mice (Crawley et al., 1997; Owen et al., 1997; Upchurch and Wehner, 1989; Wehner and Silva, 1996). Extensive research has been done to substantiate its validity as a measure of hippocampal-dependent spatial learning and long-term memory (Eichenbaum et al., 1990; Fallis, 2013; Morris et al., 1982, 1986; O'Keefe et al., 1975; Olton et al., 1978; Schenk and Morris, 1985; Sutherland et al., 1983; Vorhees and Williams, 2006). By creating spatial maps of the environment, rodents

are able to discriminate spatial locations within the pool solely based on distal extra-maze visual cues located around the room (Fallis, 2013; Morris, 1981; Schenk and Morris, 1985). In the absence of audible or visual proximal cues, these rodents manage to navigate from random starting positions around the perimeter of an open swimming arena and locate a hidden platform. Escaping from the water environment and onto a safe platform is the positive reinforcement (Cravens, 1974; Hodges, 1996).

Spatial learning is assessed across repeated trials for multiple consecutive days of training, while reference memory is assessed in the probe trial during which the platform is removed. Evidence of spatial learning and reference memory are detected using escape latencies during the training session and the percent time spent in each quadrant during the probe trial. Impairments in learning and memory are positively correlated to latency to find the platform and negatively correlated to percent time spent in the probe quadrant (quadrant in which the platform was previously located). Therefore, a longer escape latency and a lower percent time spent in the probe quadrant suggest deficits in cognition. Navigational search strategies (spatial, systematic non-spatial, repetitive looping, and floating) can also be analyzed over the course of the acquisition phase in order to assess behavioural flexibility associated with cognitive reserve (Granger et al., 2016). A common and frequently informative addition to the classic spatial navigation task is the reversal training in which the platform is relocated to the opposite quadrant and rodents are trained to learn the new location. These reversal training trials enhance the detection of spatial impairments by assessing the ability of an animal to extinguish their initial spatial learning and adapt to changed contingencies, referred to as cognitive flexibility (Morris et al., 1986; Vorhees and Williams, 2006; Whishaw and Tomie, 1996).

1.4.2 Spontaneous alternation in the Y-maze

Another key paradigm used extensively when testing AD models is the spontaneous alternation in the Y-maze task (**Figure 2 D**), which assesses working memory, the ability to store transitory information to plan and carry out an action or behaviour (Morellini, 2013; Webster et al., 2014). This task involves many parts of the brain such as the hippocampus, septum, basal forebrain and prefrontal cortex and centers on the fact that rodents have an innate preference to alternate arms when exploring new environments (Chevallier et al., 2011; Jackson, 1943; Maurice et al., 1994b, 1996b; Prior et al., 2013; Sarnyai et al., 2000; Swonger and Rech, 1972). Testing occurs in a Y-shaped maze with three opaque arms at a 120° angle from each other. The animal is allowed to freely explore the three arms. Over the course of multiple arm entries, rodents should show a tendency to enter the less recently visited arm. The number of arm entries and sequence of entries are recorded in order to measure the exploratory behaviour and alternation percentage. A high alternation percentage score is indicative of sustained cognition, while a low alternation percentage score suggests impaired working memory.

1.4.3 Forced alternation in the Y-maze

A variation of the Y-maze task, which occurs in the same testing apparatus as the spontaneous version, is the forced alternation in the Y-maze (**Figure 2 E,F**). First described by Wolf *et al.* (2006), this behaviour task assesses spatial working memory and exploratory behaviour using various proximal and distal visual cues. This task measures correct entry and percentage of time spent in the novel arm (arm blocked off in the first part of the task). Incorrect entry into the novel arm and a decrease in time spent in the novel arm are indicative of impaired spatial working memory.

2. OBJECTIVES

Sig1Rs are implicated in AD in a variety of ways. Taken together, previous studies have suggested that the loss of Sig1Rs may predispose an individual to AD, speed up disease progression, and increase symptom severity. However, so far, no report examines the fundamental question of whether Sig1R loss potentially contributes to cognitive deficits in AD.

Therefore, this study aims to explore Sig1R wild-type (WT) and KO mice at the behavioural level in an $A\beta_{25-35}$-infusion mouse model of AD. We also investigate the role of Sig1Rs and sex differences using both mature adult male and female mice.

3. HYPOTHESIS

We hypothesize that both Sig1R WT and KO mice treated with $A\beta_{25-35}$ will show decreased performance on all behavioural paradigms. Furthermore, we expect that the loss of Sig1R function will exacerbate cognitive deficits following Aβ-infusion compared to WT mice. Additionally, we suspect that Sig1R loss will increase vulnerability of female mice to Aβ-infusion, and therefore result in more pronounced behavioural phenotypes, compared to male mice.

4. MATERIALS AND METHODS

4.1 Animals

All procedures were approved and performed in accordance with the Canadian Council of Animal Care and the University of Ottawa Animal Care and Veterinary Services (ACVS) guidelines. Male and female Sig1R WT and homozygous Sig1R$^{-/-}$ KO C57BL/6J x 129s/SvEv mice (Jackson Laboratory, Maine, USA) were bred in the Roger Guindon facilities of the University of Ottawa and housed in the step-down facility. Mice were group housed (unless otherwise specified) in plastic cages in a regulated environment (20^{o}C ± 2^{o}C, 20-40% humidity) until they reached three or six months of age, at which point they were transferred to the main core facility where they underwent surgery followed by behavioural testing. All female mice were ovariectomized one week prior to ICV injections (described below). This procedure, executed by trained ACVS technicians, was performed in order to remove the contribution of circulating hormones and to create a hormonal state more similar to the AD post-menopausal female patient population. A different strain of mice, C57BL/6J, was also used for certain behavioural experiments (Jackson Laboratory, Maine, USA). The step-down facility had a light/dark cycle of 14 hr on/10 hr off, while the main core facility had a 12 hr light/dark cycle. These facilities allowed mice free access to water and standard laboratory food. Behaviour experiments were carried out in air-regulated, sound-proof laboratory rooms to which mice were habituated for at least 30 min prior to each test.

4.2 Weaning and transfer

At post-natal day 21, mice were segregated by gender and placed into new cages. Some male and female Sig1R WT and KO mice were occasionally kept for breeding, though the majority of mice were evenly mixed between cages reserved for future behaviour experiments. A maximum

of five mice were placed in each cage. All cohort cages were transferred from the step-down facility to the Behaviour Core within the main facility of Roger Guindon at least three weeks before the start of behaviour experiments.

4.3 Genotyping

To verify genotypes, ear samples were obtained from mice during weaning. A DNA extraction was first performed by adding 75 µL of 50 mM NaOH to each ear sample and incubating these at 90^{o}C for 45 min in a polymerase chain reaction (PCR) thermocycler (Epppendorf AG, Brinkmann Instruments, Westbury NY). Tubes containing these samples were subsequently flicked until the solution became cloudy. Samples were stored at 4^{o}C after reaching room temperature. Previously extracted DNA samples were amplified using two sets of primers and a PCR thermocycler (Epppendorf AG, Brinkmann Instruments, Westbury NY). One set of primers consisted of 0989-5' (TCTGAGTACGTGCTGCTCTTCG) and LTR-rev (ATAAACCCTCTTGCAGTTGCATC) primers. This set was responsible for amplifying the KO sequence (233bp). The second set consisted of 0989-5' (TCTGAGTACGTGCTGCTCTTCG) and 0989-3' (CAGAAATCTCAGCCCAGTATCG) primers. This set of PCR primers was responsible for amplifying the WT sequence (209bp). Both sets of primers (Sigma-Aldrich, St. Louis, USA) were used with each DNA sample in order to determine whether the mice were WT, KO or heterozygous (HET). The touchdown PCR reaction parameters used are as follows: 94°C (15 sec) - 65°C (30 sec) - 72°C (40 sec) for 10 cycles followed by 94°C (15 sec) - 55°C (30 sec) - 72°C (40 sec) for 30 cycles. Agarose gels (2.5-3%) were used to visualize PCR results. TAE buffer (40mM Tris-Acetate, 0.1mM EDTA) and agarose (FroggaBio, North York, ON, CAN) were heated with occasional mixing. This agarose gel solution was cooled for approximately two min after which 5µL of ethidium bromide (ThermoScientific, IL, USA) was

added. This solution was then allowed to solidify in a mold with combs for 20-30 min. These gels were loaded with 5µL of 100-bp (50µg/500µL) DNA ladder (GeneDireX, LA, USA) as well as 15µL of each DNA sample in a chamber filled with 1X TAE buffer. Agarose gel electrophoresis was carried out at 60-70V for 60 min. Visualization of gels was performed at 302 nm using AlphaImager (Biocompare, CA, USA).

4.4 $A\beta_{25-35}$-infusion mouse model

The $A\beta_{35-25}$ (reverse; REV) control peptides and $A\beta_{25-35}$ (AB) peptides (Sigma, St. Louis, MO, USA) were incubated in sterile distilled water at 37^{o}C for 4 days. Mice were anesthetized with isofluorane USP (PPC, Fresenius Kabi, Canada) and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The ICV injections (-0.4 mm AP, +1.0 mm ML, -3.0 mm DV) were performed at a rate of 1 µL/min using a 33-gauge needle. The mice were either infused with 3 µL of REV or aggregated Aβ peptides (9 nmol). Two weeks post-surgery (unless otherwise stated), cohorts were ready to be tested using the behavioural paradigms described below.

4.5 Morris water maze task

The MWM apparatus consisted of a circular, blue-colored plastic pool with a diameter of 132 cm. White, non-toxic Liquid Tempera paint (My Scholar's, Educator Supplies Limited) was used to render the water opaque. The water temperature was maintained at 20 ± 1^{o}C with a bath heater. Two distal visual cues, a large black square and a large black "X", were placed on two separate walls of the testing room (**Figure 2 A**). The clear plastic platform, 10 cm in diameter, was submerged 1 cm below the water surface. A video camera was mounted on the ceiling above the centre of the pool and the image was relayed to a computer, located behind a white blind, equipped with Ethovision XT version 10 software (Noldus Information Technologies). The pool

was divided into four quadrants (arbitrarily designated as back right (BR), back left (BL), front left (FL), and front right (FR)) by creating an imaginary "+" using two perpendicular lines bisecting the maze. The platform remained fixed in the BR quadrant for the duration of the training (acquisition) trials. The spatial acquisition phase consisted of four training trials per day for each mouse, and seven or ten consecutive days of training with an inter-trial interval of 20-30 min. Each mouse was randomly placed at one of four starting positions around the pool, with their heads facing the pool wall. The mice were allowed to swim for 60 sec without the presence of any local cues to indicate the position of the hidden platform. Rate of acquisition of escape behavior (or escape latencies) was recorded. Search strategy data (average and daily percent incidence) was also obtained using the MWM Visual (Granger et al., 2016). If the mice did not locate the platform after 60 sec, they were gently guided onto the platform and allowed to stay there for approximately 15 sec in order for them to learn the location of the hidden platform in relation to the distal visual cues. A probe trial was performed 24 hr after the last acquisition session; this involved removing the platform from the pool and measuring the percent time spent in each quadrant as well as thigmotaxis, a measure of time spent swimming around the circumference of the pool (**Figure 2 A**). Each mouse, starting at the halfway point between quadrants FR and FL, was given 60 sec to recall and find the location in the BR quadrant that previously contained the platform (probe quadrant) using the visual cues. Motivational and sensorimotor deficits may be misinterpreted as impairments in performance (Morris, 1984). Therefore, control procedures were introduced to ensure valid results. A visual test, consisting of four trials per mouse and each lasting 60 sec, was performed after the probe trial to ensure the elimination of any visually impaired mice from analyses. An escape latency exclusion cut-off was set at 25 sec. This test involves the use of a local cue, an object placed on top of the

platform, in the absence of distal wall cues (**Figure 2 B**). Thigmotaxis data obtained from the probe trials are also used as part of the control procedures, with an exclusion criterion set at 80% or higher of the total probe duration time. For certain cohorts, a harder version of the MWM (hard MWM) task was used which involved the use of only a single, small visual cue (black "x") placed on a wall of the testing room (**Figure 2 C**). Furthermore, this variation of the MWM task was also combined with three days of reversal training in which the platform was moved to the opposite quadrant, FL, allowing us to investigate cognitive flexibility. As in the acquisition session, each reversal training day consisted of four trials per mouse, each lasting 60 sec. A second probe trial was performed 24 hr after the last reversal training session.

4.6 Spontaneous alternation in the Y-maze task

Testing occurred in a black plastic Y-shaped maze with three identical arms having a length of 38.1 cm, a height of 12.7 cm and a width of 7.62 cm (**Figure 2 D**). The mice were allowed to freely explore all three arms of the Y-maze during the eight-minute test session. The series of arm entries, including possible returns into the same arm, were recorded with a video camera connected to a computer equipped with Ethovision XT version 10 software (Noldus Information Technologies). Arm entry was considered as complete when all four limbs had fully entered the arm. At the end of the test period, the mouse was returned to its home cage. The apparatus was then cleaned with an alcohol solution and allowed to dry between sessions. An alternation is defined as entries into all three arms on consecutive occasions and the number of maximum alternations as the total number of arm entries minus two. Therefore, the percentage of alternation was calculated as:

$$\frac{actual\ alternations}{maximum\ alternations} x100\%$$

For example, if the three arms were called A, B, C and the mouse performed ABCACBACCABCA, the number of arm entries would be 13, and the successful alternations: ABC, BCA, ACB, CBA, BAC, CAB, ABC, BCA. Therefore, the percent alternation would be:

$$\frac{8}{13-2} x100\% = 72.7\%$$

4.7 Forced alternation in the Y-maze task

Testing was performed in the same apparatus as the spontaneous alternation in the Y-maze task, with the addition of black and white square and triangle cues on the testing room walls as well as proximal cues at the end of each Y-maze arm (**Figure 2 E,F**). This task involved two 5 min trials, T1 and T2, separated by a 30 min inter-trial interval. During T1, one of the Y-maze arms was blocked and the mice were allowed to move freely between the other two arms. The mice were then returned to their home cage during the inter-trial interval. The apparatus was cleaned with an alcohol solution between trials. In T2, the mice were placed back into the maze and the first arm entry was recorded as either correctly or incorrectly entering the novel arm (arm previously blocked in T1 trial). Arm entry was considered as complete when all four limbs had fully entered the arm. The percent time spent in the novel arm in the first minute of the T2 trial was also recorded and calculated with a video camera connected to a computer equipped with Ethovision XT version 10 software (Noldus Information Technologies).

4.8 Statistical analysis

Group data is expressed as means ± standard error of the mean (SEM). Statistical analyses were performed using Prism 7 (GraphPad Software, CA, USA). Differences between means were analyzed using a standard unpaired t-test, one-way analysis of variance (ANOVA), or two-way

ANOVA followed by a Fisher's least significant difference (LSD) *post hoc* analysis. Results were deemed statistically significant when $P < 0.05$.

5. RESULTS

5.1 Learning and memory are unaffected by genotype and Aβ treatment in adult male and female mice

Unlike transgenic models, infusion mouse models better recapitulate the process of sporadic AD, which accounts for the vast majority of disease cases (Bird, 2008; Puzzo et al., 2014). Extensive research using the $A\beta_{25-35}$-infusion mouse model has shown that administration of aggregated $A\beta_{25-35}$ peptides results in Aβ plaque deposits, tau hyperphosphorylation, cell loss in the hippocampus and cortex, and impairments in learning and memory (Maurice et al., 1996b, 1998; Meunier et al., 2006b; Urani et al., 2002). However, none of these studies utilized the same background strain as our Sig1R mice. Since behavioural task performance is known to vary based on mouse strain differences (Brandeis et al., 1989; Clapcote et al., 2005; Crabbe, 1999; Crawley et al., 1997; Lipp and Wolfer, 1998; Upchurch and Wehner, 1988; Wahlsten et al., 2005; Wehner and Silva, 1996), we first set out to replicate $A\beta_{25-35}$-induced cognitive impairments in our younger adult (three month old) male Sig1R WT mice using the MWM task (**Figure 3 A,B; Figure 4 A-D**). This hippocampus-dependent task assessed spatial learning and long-term reference memory as well as cognitive reserve (D'Hooge and De Deyn, 2001; Vorhees and Williams, 2015).

As expected, our data showed a decrease in escape latency across training days in the MWM task in the three month old male Sig1R WT mice. However, Aβ treatment failed to impair spatial learning and memory in these mice (**Figure 3 A,B**). Furthermore, the average percent incidence (**Figure 4 A**) and individual daily percent incidence (**Figure 4 B-D**) of search strategies were unaffected by treatment. Therefore, we did not observe an effect of $A\beta_{25-35}$ on behavioural performance in three month old males. This is contrary to previously published studies utilizing

this model as well as electrophysiological data obtained from our laboratory, demonstrating Aβ- and sex-induced changes in synaptic physiology (data not shown). Based on these results, we decided to experiment on older adult (six month old) male mice, including both WTs and KOs, and to employ another behavioural paradigm used extensively in AD research, the spontaneous alternation in the Y-maze task. This task was used to measure short-term working memory (Diamond, 2014; Jackson, 1943; Prior et al., 2013; Sarnyai et al., 2000; Swonger and Rech, 1972). Exploratory behaviour and working memory were unaffected by genotype and treatment in six month old Sig1R WT and KO male mice (**Figure 5 A,B**).

Only a small portion of scientific studies utilize both sexes of animals when conducting their research and often neglect to examine the potential role of sex differences (McCarthy, 2015; Miller, 2014; Zucker and Beery, 2010). Moreover, although studies have established $A\beta_{25-35}$-induced behavioural task impairments in males, testing has yet to be done in females. As mentioned previously, Sig1Rs were found to be modulated by neurosteroids, suggesting the hormonal changes associated with aging may differentially affect Sig1R function in males and females. Therefore, since cognitive deficits were not detected in male mice, we set out to investigate the role of sex on genotype in our infusion model using adult female mice.

The MWM task was performed using six month old Sig1R WT and KO female mice (**Figure 6 A,B; Figure 7 A-D**). There were no effects of genotype or Aβ treatment on either spatial learning or reference memory (**Figure 6 A,B**). The percent incidences of search strategies employed was also unaffected by genotype and treatment (**Figure 7 A-D**). In order to obtain additional information and possibly detect mild cognitive deficits we performed a variation of the spontaneous alternation in the Y-maze paradigm, the forced alternation in the Y-maze task, which assesses spatial working memory (Diamond, 2014; Webster et al., 2014; Wolf et al.,

2016). No effect of genotype and treatment were observed on the spatial working memory of six month old female Sig1R WT and KO mice (**Figure 8 A,B**).

Through these experiments, we were unable to detect any impairments in cognition using the MWM, spontaneous alternation in the Y-maze, and the forced alternation in the Y-maze tasks in three and six month old Sig1R WT and KO male and female mice.

5.2 Reference memory and spatial working memory are impaired when implementing modifications to behavioural task and housing conditions

The behavioural tasks performed thus far failed to provide us with significant results. Therefore, modifications to the most widely used behavioural paradigm in AD research, the MWM task, were implemented to increase difficulty and sensitivity in an attempt to detect milder phenotypes between groups.

Reviewing data obtained from the MWM task so far, we observed a plateau in escape latency between day seven and ten, which suggested no further changes in spatial learning after day seven. Therefore, our first modification was to shorten the acquisition phase from ten to seven training days in order to prevent overtraining the mice and losing the ability to detect differences in the probe trial. Furthermore, the difficulty of the task was increased by reducing the number and size of distal cues around the testing room (hard MWM).

These modifications were applied and tested on a three month old male Sig1R WT cohort (**Figure 9 A,B; Figure 10 A-D**). Spatial learning was unaffected by Aβ treatment (**Figure 9 A**). However, a significant reference memory impairment was detected in Sig1R Aβ-treated WT mice compared to REV-infused mice (**Figure 9 B**; main group effect: $F_{3,48} = 4.853$, $P = 0.005$). Moreover, WT Aβ-treated mice used significantly less systematic non-spatial search strategies

on day one (main group effect: $F_{6,72} = 2.254$, $P = 0.0476$), as well as significantly more repetitive looping search strategies on day one and two (main group effect: $F_{6,72} = 8.561$, $P < 0.0001$) compared to WT REV mice (**Figure 10 C,D**).

To verify the validity of the above-mentioned results, the experiments were replicated using a different mouse strain, the C57BL/6J background, which is the most commonly used background strain in mouse models (**Figure 11 A,B; Figure 12 A-D**). As alluded to previously, behavioural task performances can vary widely between mouse strains and even certain substrains (Crabbe, 1999; D'Hooge and De Deyn, 2001; Owen et al., 1997; Wahlsten et al., 2005; Wehner and Silva, 1996). Furthermore, C57BL/6J mice are a commonly used and commercially available inbred strain that have been characterized on a large number of behavioural tasks (Clapcote et al., 2005; Crawley et al., 1997; Upchurch and Wehner, 1988). Moreover, we utilized an additional modification that involved individually housing mice. Previous studies have shown that individually housing rodents increases their response to added stress and has been suggested to affect males and females differently (Baker and Bielajew, 2007; Brain, 1975; Ferrari et al., 1998; Goldsmith et al., 1978; Kwak et al., 2009; Palanza et al., 2001; Võikar et al., 2005). Based on these studies, we believed individually housing our mice may have been required in order to observe Aβ-induced, and possibly genotype-specific, impairments in our model.

It was found that spatial learning in the MWM task was unaffected by treatment and housing conditions in three month old C57BL/6J male mice (**Figure 11 A**). Reference memory, however, was significantly impaired in Aβ-treated individually housed mice compared to all three other groups (**Figure 11 B**; main group effect: $F_{9,96} = 4.788$, $P < 0.001$). No significant differences were found in the percent incidences of search strategies used between all four experimental groups (**Figure 12 A-D**).

This C57BL/6J background strain was also used to test the effect of housing conditions on behavioural performance in the forced alternation in the Y-maze task (**Figure 13 A,B**). Correct entry into the novel arm was unaffected by treatment and housing conditions (**Figure 13 A**). Spatial working memory was found to be significantly impaired by housing conditions but not by Aβ treatment (**Figure 13 B**; main group effect: $F_{3,21} = 7.624$, $P = 0.0012$).

Taken together, these findings clearly show that the combination of a shorter and harder MWM task as well as individual housing conditions resulted in Aβ-induced reference memory deficits in two different mouse strains. Furthermore, Aβ-induced effects in the search strategies utilized were detected in our Sig1R mice. Moreover, individually housing mice resulted in a spatial working memory impairment.

5.3 Impairments in learning and memory failed to be detected in adult mice despite behavioural paradigm modifications

Altogether, these modifications led to the establishment of an effective behavioural paradigm which was used, moving forward, with our Sig1R WT and KO male and female mice. In order to obtain additional information, a reversal training phase was added at the end of the MWM task. This allowed for the enhanced detection of spatial impairments and cognitive flexibility.

Spatial learning deficits were observed on the first two acquisition days of the MWM task in individually housed six month old KO Aβ-treated compared to WT Aβ-treated male mice (**Figure 14 A**; main group effect: $F_{18,384} = 1.779$, $P = 0.0259$). However, reference memory (**Figure 14 B,D**), cognitive flexibility (**Figure 14 C**), and search strategies (**Figure 15 A-D**) were unaffected by genotype and Aβ treatment.

Pilot studies were performed using the spontaneous alternation in the Y-maze task, in which short-term working memory was unaffected by genotype and Aβ treatment in individually housed six month old Sig1R WT and KO male and female mice. Therefore, based on the preliminary data obtained we decided to employ the forced alternation version of the task. Genotype and treatment had no effect on spatial working memory in individually housed six month old Sig1R WT and KO male mice in the forced alternation in the Y-maze task (**Figure 16 A,B**).

Spatial learning (**Figure 17 A**), reference memory (**Figure 17 B,D**), cognitive flexibility (**Figure 17 C**) and search strategies (**Figure 18 A-D**) were unaffected by genotype and Aβ treatment in individually housed six month old Sig1R WT and KO female mice in the MWM task. Furthermore, genotype and treatment had no effect on spatial working memory in the forced alternation in the Y-maze task (**Figure 19 A,B**).

Therefore, these results suggest that overall the newly established behavioural paradigm, implementing various modifications, was ineffective in detecting genotype or treatment effects in older adult male and female Sig1R WT and KO mice, with the exception of a mild genotype-induced spatial learning impairment in Aβ-infused male mice. Furthermore, a sex analysis performed on the above-mentioned MWM and forced alternation in the Y-maze task data revealed no significant differences between males and females (data not shown).

6. DISCUSSION

This thesis studied whether Sig1R function could be involved, at least in part, in the underlying etiology of AD. More specifically, we set out to determine whether loss of Sig1Rs sex-specifically increases vulnerability to AD in an $A\beta_{25-35}$-infusion mouse model. To investigate this, we utilized Sig1R WT and KO male and female mice and three behavioural tasks used extensively in AD research: the MWM, spontaneous alternation in the Y-maze and forced alternation in the Y-maze. Our main findings suggest that, in order to more accurately address these objectives, further infusion and behaviour paradigm modifications are needed to acquire a reliable AD model in our Sig1R mice.

$A\beta_{25-35}$-infusion model

The results of several studies demonstrate that $A\beta_{25-35}$-infusion in rodents recapitulates characteristics of sporadic AD such as impairments in learning and memory, Aβ plaque deposits, tau hyperphosphorylation, as well as cell loss in the cortex and hippocampus (Delobette et al., 1997; Lu et al., 2009; Maurice et al., 1996b; Stepanichev et al., 2003, 2004, 2006, Zussy et al., 2011, 2013). Through our behavioural task experiments, our laboratory was initially unable to reproduce Aβ-induced spatial learning, reference memory and spatial working memory deficits in Sig1R WT and KO mice. By implementing various experimental modifications, we demonstrated reference memory and spatial working memory impairments in three month old male mice, which are aspects of cognition affected in AD patients. Using these modifications, we attempted to examine whether similar results could be revealed in our six month old Sig1R WT and KO males and females. Cognitive deficits failed to be detected in these mice.

Background strain

While our laboratory did attain $A\beta_{25-35}$-induced cognitive impairments in certain cohorts, many factors may have been involved in the inability to reproduce consistent behavioural deficits with our infusion model. Previous studies characterizing this $A\beta_{25-35}$-infusion mouse model have primarily utilized two background strains, the Swiss and ICR mice (Chen et al., 2010; Choi et al., 2014; Fang and Liu, 2006; Kim et al., 2008; Kwon et al., 2011; Maurice and Lockhart, 1997; Maurice et al., 1996b, 1996c, 1998; Mazzola et al., 2003; Reggiani et al., 2016; Um et al., 2009). It has been shown that mouse strains can differentially affect not only behavioural performance but also their response to drugs and clearance of Aβ in AD mouse models (Carlson et al., 1997; Gerlai, 1996, 2001; Hall and Roberson, 2012; Lehman et al., 2003; Qosa and Kaddoumi, 2016; Salomons et al., 2012; Sunyer et al., 2007; Võikar et al., 2001; Weitzner et al., 2015; Wolfer and Muller, 1997). These studies suggest strain differences could affect the ability of mouse models to be successfully developed and consistently recapitulate phenotypic characteristics of AD. Therefore, this may explain our difficulty in obtaining a reliable expression of pathological AD phenotype in our infusion model.

Dose of $A\beta_{25-35}$ peptides

A possible solution for achieving dependable results from this AD infusion model could be increasing the dose of $A\beta_{25-35}$ peptides (9nmol) infused in Sig1R mice. Doses employed by various researchers range, in general, from 3-16 nmol, with one rat study reporting 45 nmol (Fang and Liu, 2006; Kim et al., 2008; Liu et al., 2009, 2013, Maurice et al., 1996b, 1996c, 1998; Mazzola et al., 2003; Stepanichev et al., 2005, 2006, 2003, 2004, Zussy et al., 2011, 2013). Consistent impairments in learning and memory may be achieved using a higher $A\beta_{25-35}$ dosage in our mice. Additionally, bilateral and/or chronic infusion of Aβ peptides, could be employed

independently or in conjunction with the higher Aβ infusion dose (Burgos-Ramos et al., 2007; Chen et al., 1996; Stepanichev et al., 2005).

Time post-injection

The time between ICV injections and behaviour experiment testing (post-ICV time) could also be increased in order to allow more time for $A\beta_{25-35}$-infusions to exert a cascade of neurotoxic effects. Several studies using this model report testing mice starting the day after surgery up to 13 days post-ICV, with the most common post-ICV time being seven days (Ahn et al., 2006; Chavant et al., 2010; Detrait et al., 2014; Maurice et al., 1996b, 1996c, 1998; Nisha and Devi, 2017; Sun and Alkon, 2002; Zhang et al., 2016). Despite the range of time utilized in these studies, they repeatedly demonstrated impairments in their mice. Interestingly, cognitive deficits were observed in our mice when tested one week, but not two weeks, post-ICV. These results were unexpected since considerable evidence, although in rats, has shown Aβ-induced impairments persisting for up to six months after ICV injections (Stepanichev et al., 2003, 2004, 2005, 2006, Zussy et al., 2011, 2013). As alluded to earlier, research suggests that differences in background strains may affect susceptibility to Aβ and ultimately our ability to recapitulate AD characteristics.

Age of animals

The majority of studies employing the $A\beta_{25-35}$-infusion model use young male rodents, approximately three to nine weeks of age (Ahn et al., 2006; Chavant et al., 2010; Chen et al., 2010; Choi et al., 2014; Delobette et al., 1997; Detrait et al., 2014; Klementiev et al., 2007; Kwon et al., 2011; Lu et al., 2009; Maurice et al., 1996c, 1998, 1996b; Nisha and Devi, 2017; Reggiani et al., 2016; Stepanichev et al., 2003, 2004, 2005, 2006; Um et al., 2009; Zussy et al.,

2011, 2013). To more accurately address LOAD, which is a version of the disease more prevalent in the elderly, our study utilized adult mice (three and six months of age) rather than young mice. Moreover, *in vitro* results from electrophysiological experiments performed in parallel in our laboratory have revealed alterations in synaptic physiology using age-matched animals. Intriguingly, impairments in behavioural performance were not only observed at a short, rather than longer, post-ICV time but also in our younger, rather than older, adult mice. This is contrary to what we would predict, since age-related impaired cellular processes are well documented in aging mice and would lead us to expect a greater, if not equal, vulnerability to Aβ in older compared to younger adult mice (Ishihara et al., 1999; Jackson et al., 2017; Johnson et al., 1999, 2013; Kokoszka et al., 2001; López-Otín et al., 2013; Mattson and Magnus, 2006; Van Meer and Raber, 2005; Poon et al., 2006; Shoji et al., 2016; De Strooper and Karran, 2016; Tower, 2015). It is likely that multiple environmental and genetic factors influence whether or not exposure to neurotoxins results in disease recapitulation in mice. Therefore, it could be informative to investigate not only various post-ICV times but also ages of mice, such as aged or old males and females, in our background strain.

Housing

The duration of individual housing prior to the start of behaviour testing could be explored. Both acute, as short as 72 hr, and long-term, up to four months, social isolation times have been reported to increase levels of stress and affect cognition in rodents (Ali et al., 2017; Chen et al., 2016; Goldsmith et al., 1978; Huang et al., 2011a; Ieraci et al., 2016; Kamal et al., 2014; Palanza et al., 2001; Takatsu-Coleman et al., 2013; Võikar et al., 2005). Thus, an investigation into the most optimal individual housing time that can promote the additional stress required to obtain Aβ-induced impairments could be performed in our mouse strain.

Other potential pitfalls

In contrast to previous studies reporting the use of the $A\beta_{25-35}$-infusion model, our results showed that $A\beta_{25-35}$ peptide infusion unreliably induced an AD-type amnesia in Sig1R mice. A potential explanation for these findings could be variability in the aggregation process of the Aβ peptides prior to ICV injections. Furthermore, inconsistencies in peptide aggregation could lead to varying concentrations or quantity of aggregated $A\beta_{25-35}$ in each infusion. Despite initially optimizing the brain injection coordinates, it is plausible that slight deviations in the infusion site occurred between mice and behaviour cohorts. One possible future consideration may be to perform histological analyses and examine neuritic plaque formation and cell death in Aβ-infused mice. It is probable that the combination of small cohort sizes and inherent biological variability between mice, especially of a mixed background strain, has led to the discrepancies observed in our results. Implementing group sizes of 15-20 mice in all future behaviour experiments would likely help control for these factors and minimize the probability of detecting group differences that are not biologically significant.

Sig1R KO mice

While there may be concerns with the mouse model, another factor to consider is the role of the Sig1R in our mice. Previous works, such as the study conducted by Chevallier *et al.* (2011), have investigated the behavioural phenotype of Sig1R KO mice. They observed learning and memory impairments in female Sig1R KO compared to WT mice. Using similar behavioural tasks, our laboratory was unable to reproduce these results. A possible explanation for this may be inherent differences in testing between laboratories. Despite standardization of tests across laboratories, performance of rodents in behaviour tasks has been repeatedly reported to be influenced by the

laboratory environment (Balcombe, 2006; Crabbe, 1999; Tucci et al., 2006; Wahlsten et al., 2007). Experiments characterizing mutants, such as effects of a gene knockout, could therefore produce varied results due to influences of environmental conditions specific to individual laboratories (Crawley, 2003; Enserink, 1999; Kafkafi et al., 2005; Van der Staay and Steckler, 2001, 2002, Wahlsten et al., 2003a, 2003b; Würbel, 2002). These environmental factors may be responsible for hiding, or diluting, the mild but sex-specific Sig1R KO phenotypes observed in other studies.

Alternative avenues

Altogether, it was not possible to conclusively determine the sex-specific role of Sig1Rs in our $A\beta_{25-35}$-infusion mouse model of AD using the MWM, spontaneous and forced alternation in the Y-maze tasks. Further alternative avenues could be explored to more effectively address our objectives and validate our hypotheses. Implementation of minor changes to current behavioural tasks could lead to enhanced detection of phenotypes. Possible modifications may include room lighting intensity, room and MWM water temperature, positioning and type of cues employed, and overall duration of tasks. Slight variations in these aspects of our behaviour experiments may allow us to reveal exciting findings in our Sig1R mice. Future work could also involve two additional behavioural paradigms, the passive avoidance and novel object recognition tasks, which are also extensively used in AD research. These tasks address other areas of cognition affected in AD, contextual long-term memory and novelty response/memory, respectively. Additionally, rodents are naturally nocturnal animals (Arakawa et al., 2007; Laviola et al., 1994; McLennan and Taylor-Jeffs, 2004; Panksepp et al., 2007; Refinetti, 2004; Terranova et al., 1998). Therefore, switching to a reverse light cycle and running these behaviour experiments in

the dark phase may enable us to detect otherwise subtle phenotypes in our mice (Hossain et al., 2004; Roedel et al., 2006).

3xTgAD mouse model

Another possible avenue to consider in order to explore the role of Sig1R in the pathophysiology of AD could be the use of a different model of AD, such as the 3xTgAD mouse model. Previous studies using this model have demonstrated an increase in Aβ production and deposition, tau hyperphosphorylation, and cognitive deficits in male and female mice (Billings et al., 2005; Carroll et al., 2007, 2010; Guzman-Ramos et al., 2012; Kazim et al., 2014; Mastrangelo and Bowers, 2008; Oddo et al., 2003, 2006, Sterniczuk et al., 2010a, 2010b; Stevens and Brown, 2015). Furthermore, utilizing the 3xTgAD model would not only remove the chance of error with ICV injections and aggregation of $A\beta_{25-35}$ peptides, but is also regarded as having a high face validity with AD when it comes to transgenic mouse models. Treating 3xTgAD mice with a Sig1R agonist, such as (+)-pentazocine, would allow us to investigate the role of Sig1R activation on behavioural performance in this AD mouse model.

Summary

This study aimed to elucidate the role of Sig1Rs in AD using the $A\beta_{25-35}$-infusion mouse model. As discussed, an AD-type amnesia was observed in some, but not all, cohorts. These above-mentioned modifications and future behaviour experiments will hopefully allow us to efficiently and accurately address our objectives and hypotheses. We are confident that future results from this project will provide important information for clinical studies, which could help treat AD patients and lead to a better understanding of potential sex-specific therapeutic interventions.

7. CONCLUSION

This research project aimed to determine the role of Sig1Rs using behavioural tasks in an animal model for AD. AD is a progressive and fatal neurodegenerative disorder, which leads to cerebral cortex and hippocampus shrinkage as well as cognitive deficits and behavioural disturbances. Although research has focused on the Aβ cascade for decades, little progress has been made in developing new effective therapies. A way to prevent, delay or slow the progression of the disease is desperately needed. A promising alternative research avenue is the Sig1R, which has been implicated in AD in a variety of ways. In our study, the overall results obtained indicate that the mouse model of AD utilized failed to enable us to verify our hypothesis. However, promising significant results were obtained upon implementation of certain modifications to the behavioural paradigms. Additional efforts are therefore necessary in order to obtain a consistent working infusion model of AD in our mouse strain. Future experiments will hopefully shed some light on the link between Sig1Rs and AD, which could lead to the development of novel therapeutics and preventative measures for AD.

8. FIGURES

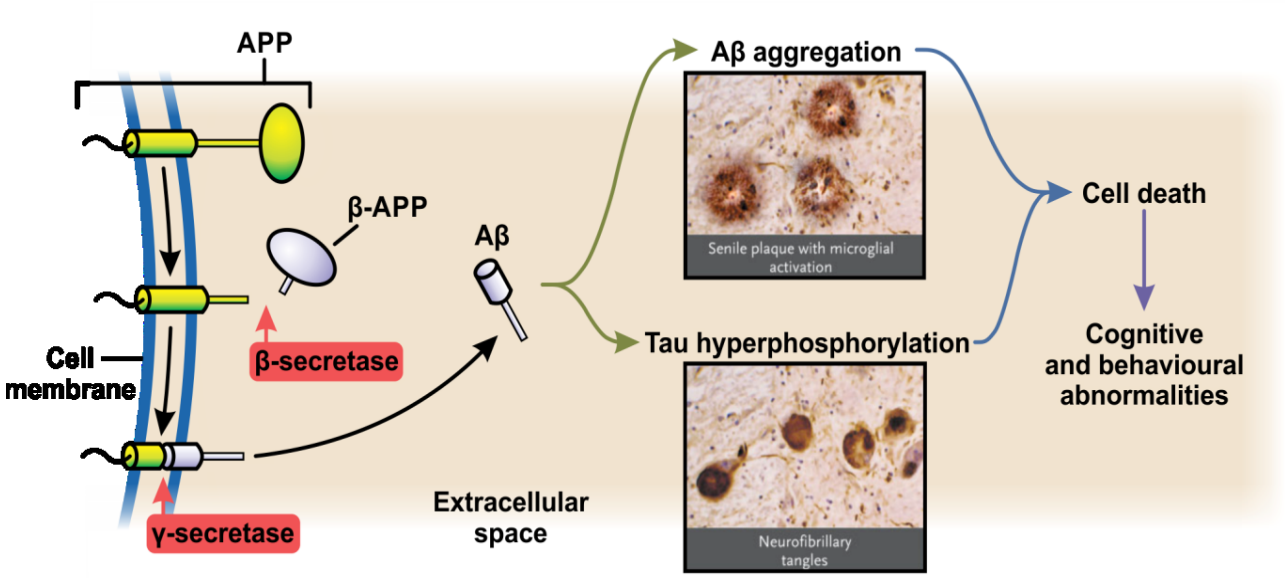

Figure 1. Putative amyloid-beta cascade.
The cleavage of the amyloid precursor protein (APP) progresses into the generation of amyloid-beta (Aβ) peptides which, through multiple secondary mechanisms, leads to cell death as well as cognitive and behavioural abnormalities. This hypothesis formed the basis for recent emerging options in the treatment of Alzheimer's disease. Adapted from Cummings, 2004. Created by Wissam Nassrallah.

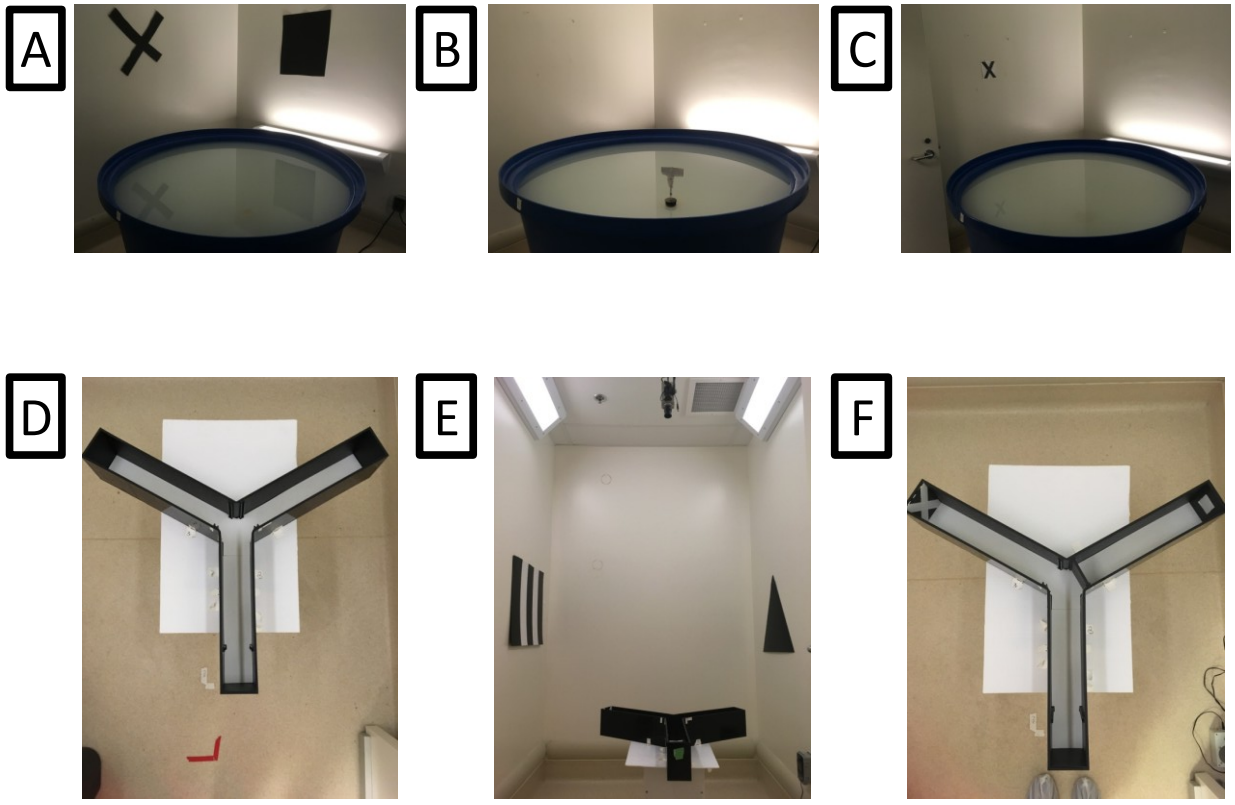

Figure 2. Behavioural testing equipment and set-up.
The Morris water maze (MWM) test equipment and set-up during the acquisition phase and probe trial (**A**), visual test (**B**), and hard MWM variation (**C**). Test equipment and set-up for the spontaneous (**D**) and forced (**E,F**) alternation in the Y-maze.

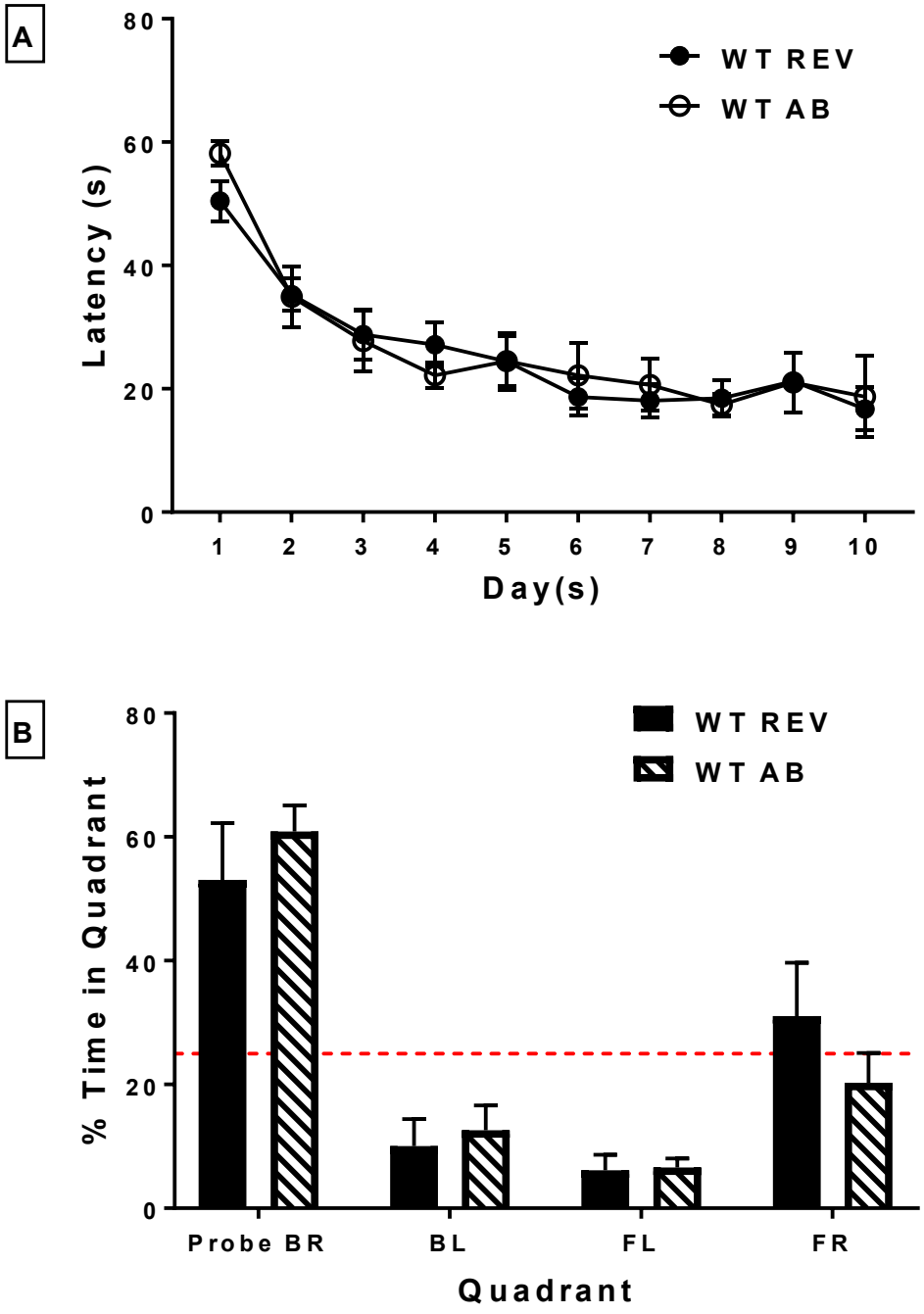

Figure 3. Spatial learning and reference memory in the MWM task in three month old Sig1R WT males.

Average latencies to reach the platform across training days (acquisition phase; **A**) and average percent times spent in each quadrant (probe trial; **B**). WT REV (n=5) and WT AB (n=5) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$.

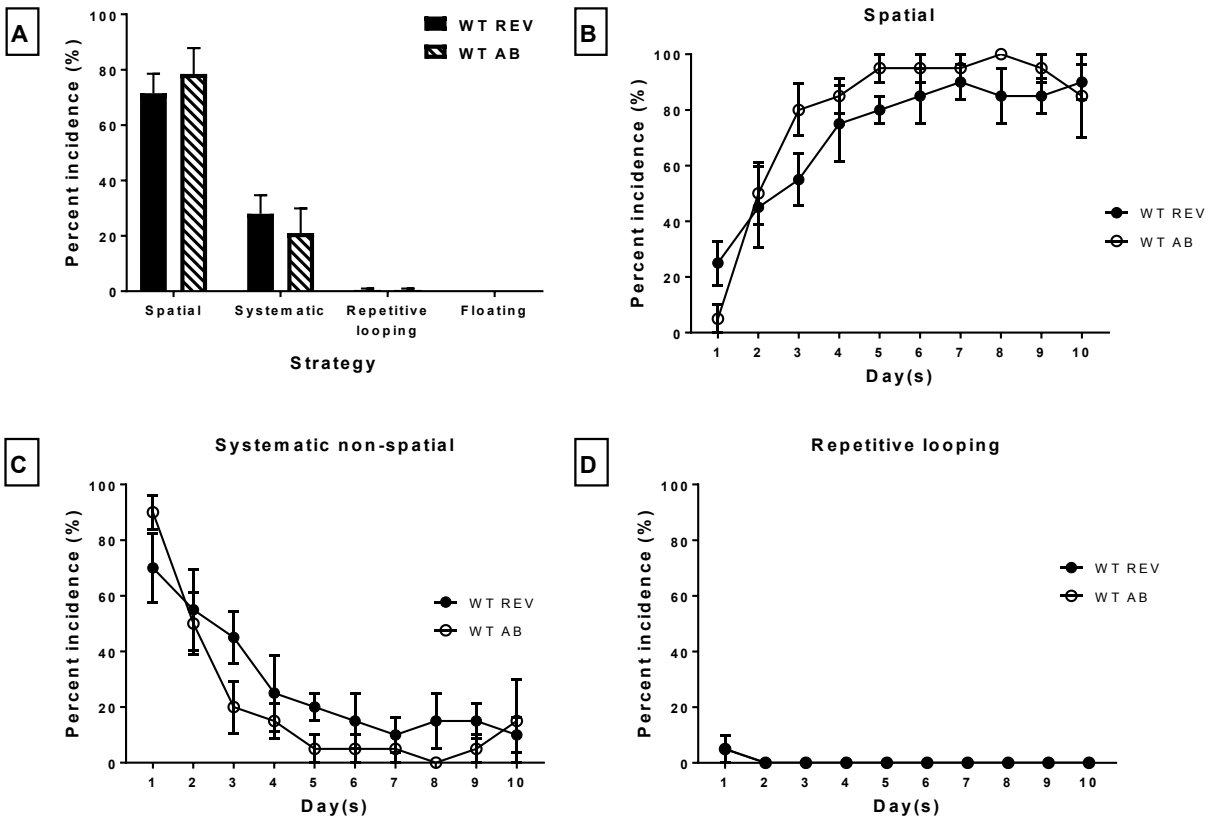

Figure 4. Search strategy incidences in the MWM task in three month old Sig1R WT males.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). WT REV (n=5) and WT AB (n=5) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$.

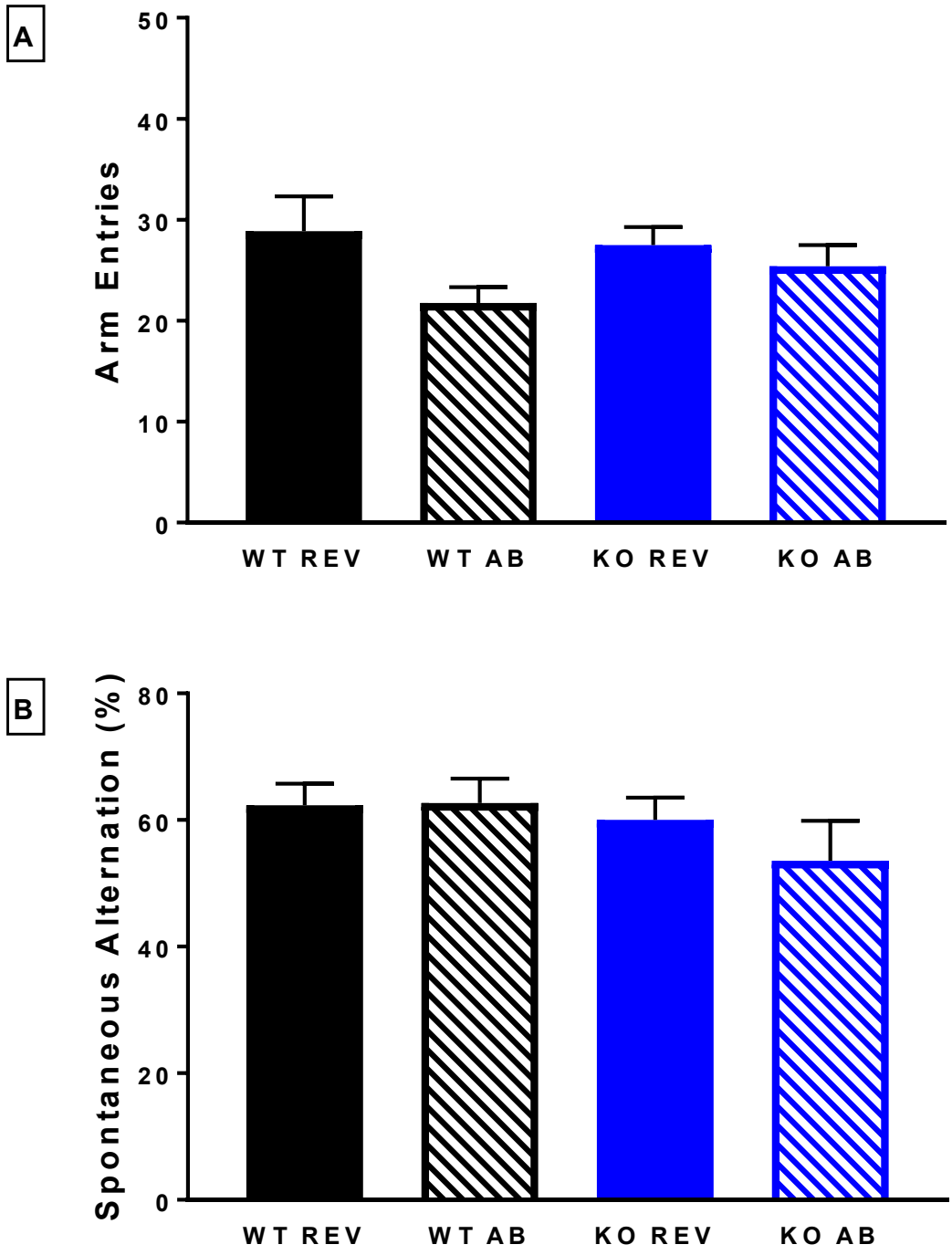

Figure 5. Working memory in the spontaneous alternation in the Y-maze task in six month old Sig1R WT and KO males.

Total number of arm entries (**A**) and spontaneous alternation percentage (**B**). WT REV (n=7), WT AB (n=8), KO REV (n=8) and KO AB (n=10) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$.

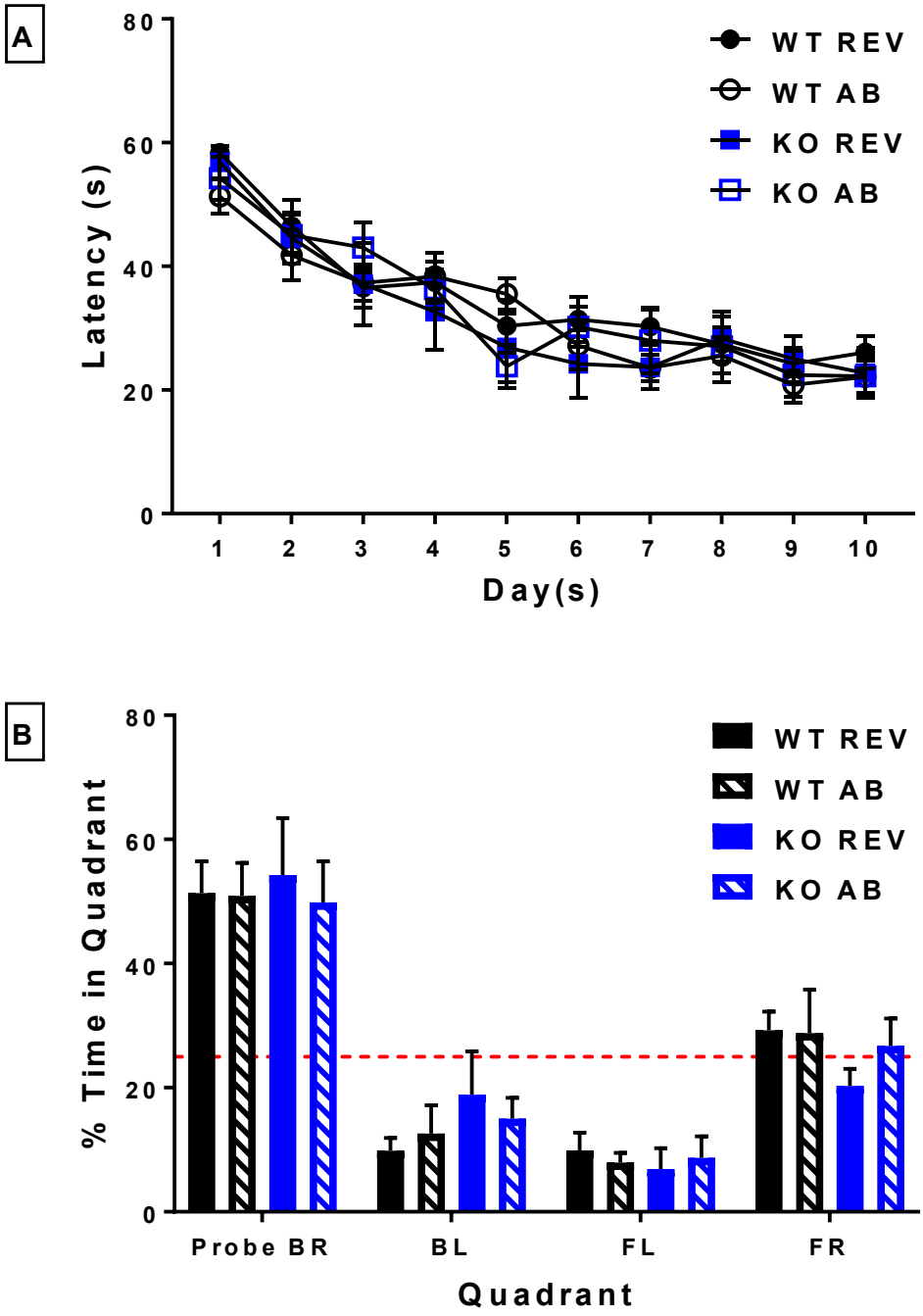

Figure 6. Spatial learning and reference memory in the MWM task in six month old Sig1R WT and KO females.

Average latencies to reach the platform across training days (acquisition phase; **A**) and average percent times spent in each quadrant (probe trial; **B**). WT REV (n=10), WT AB (n=9), KO REV (n=6) and KO AB (n=7) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$.

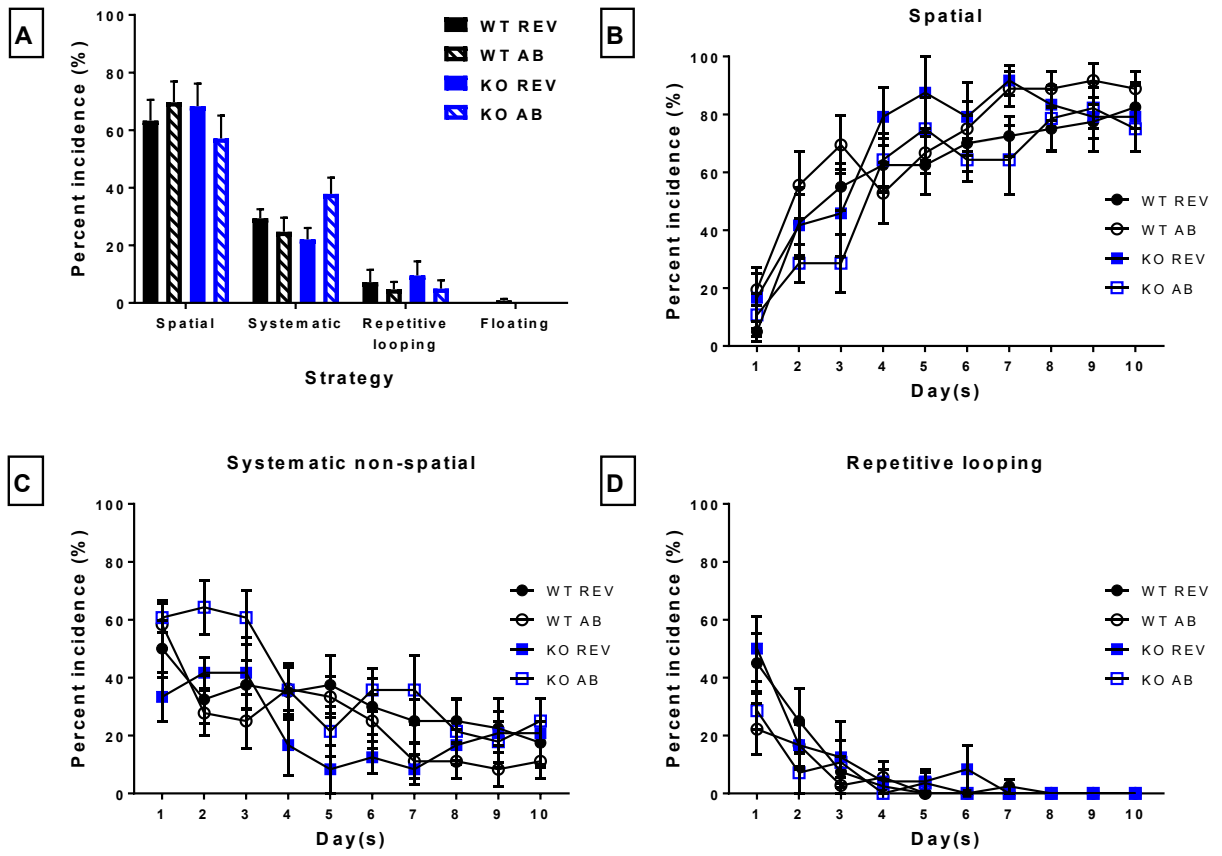

Figure 7. Search strategy incidences in the MWM task in six month old Sig1R WT and KO females.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). WT REV (n=10), WT AB (n=9), KO REV (n=6) and KO AB (n=7) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$.

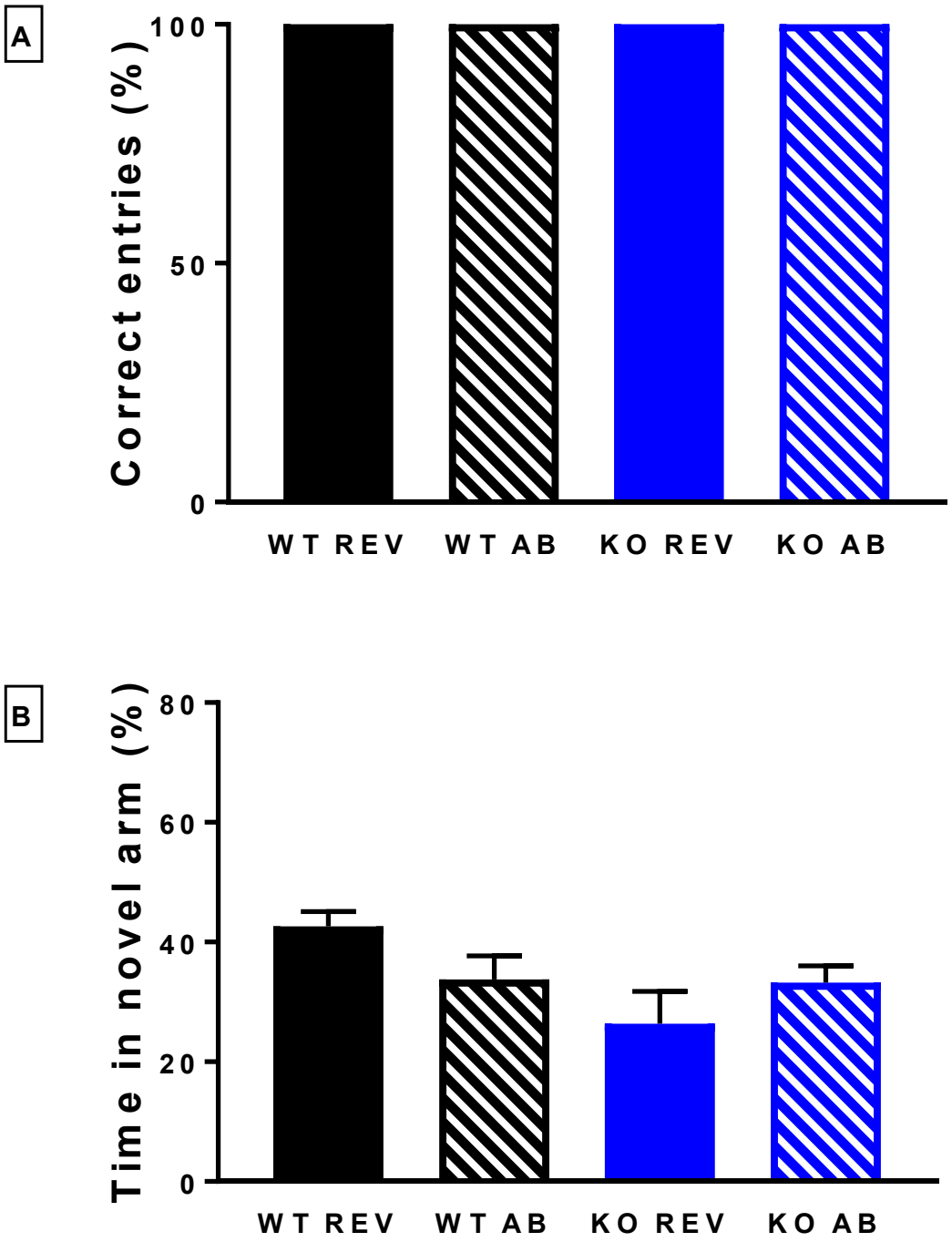

Figure 8. Spatial working memory in the forced alternation in the Y-maze task in six month old Sig1R WT and KO females.

Percentage of correct entries into the novel arm (**A**) and percent time spent in the novel arm in the first minute of the trial (**B**). WT REV (n=5), WT AB (n=6), KO REV (n=4) and KO AB (n=5) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$.

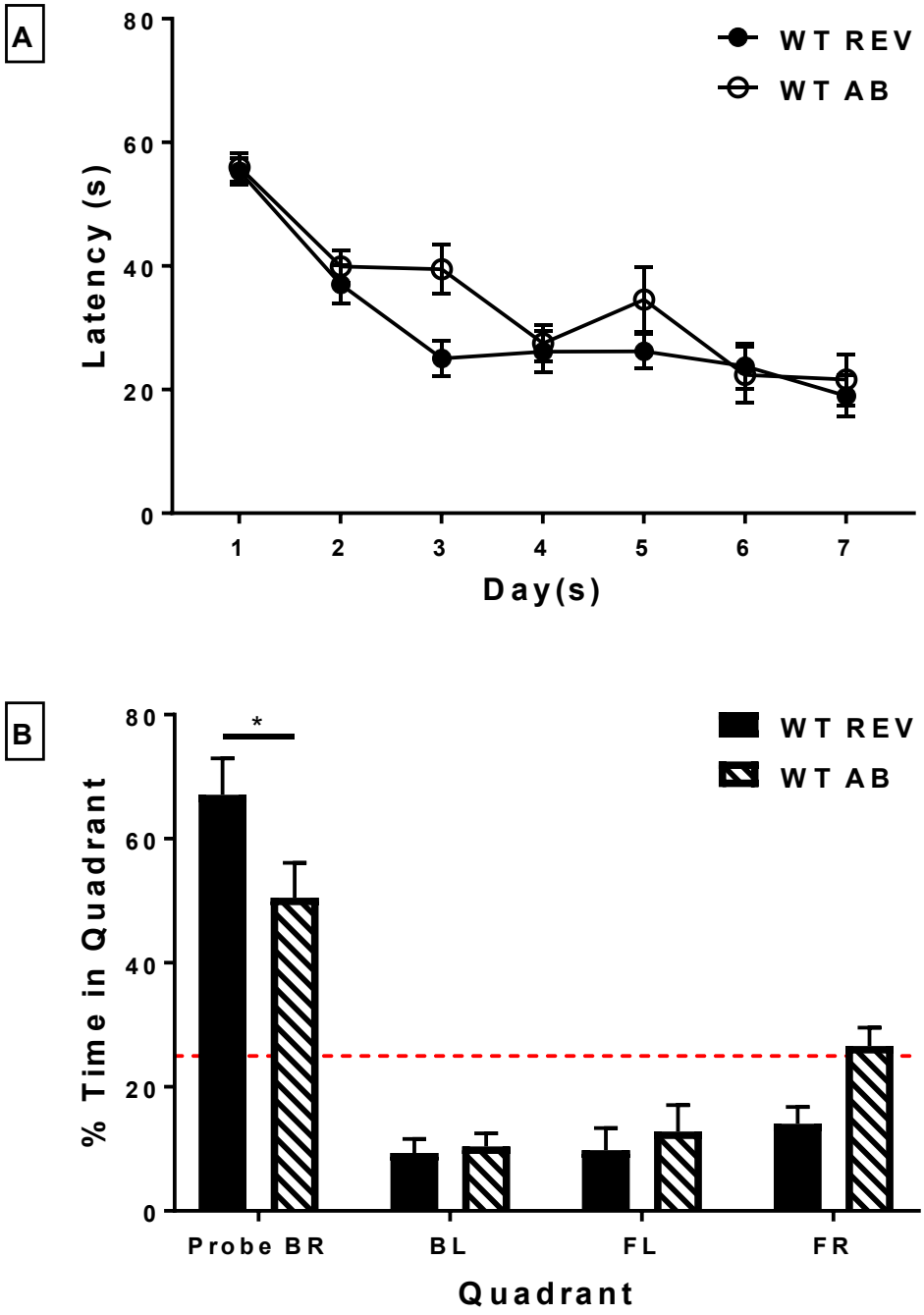

Figure 9. Spatial learning and reference memory in the hard MWM task in three month old Sig1R WT males tested one week post-ICV.

Average latencies to reach the platform across training days (acquisition phase; **A**) and average percent times spent in each quadrant (probe trial; **B**). WT REV (n=7) and WT AB (n=7) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$. *$p < 0.05$

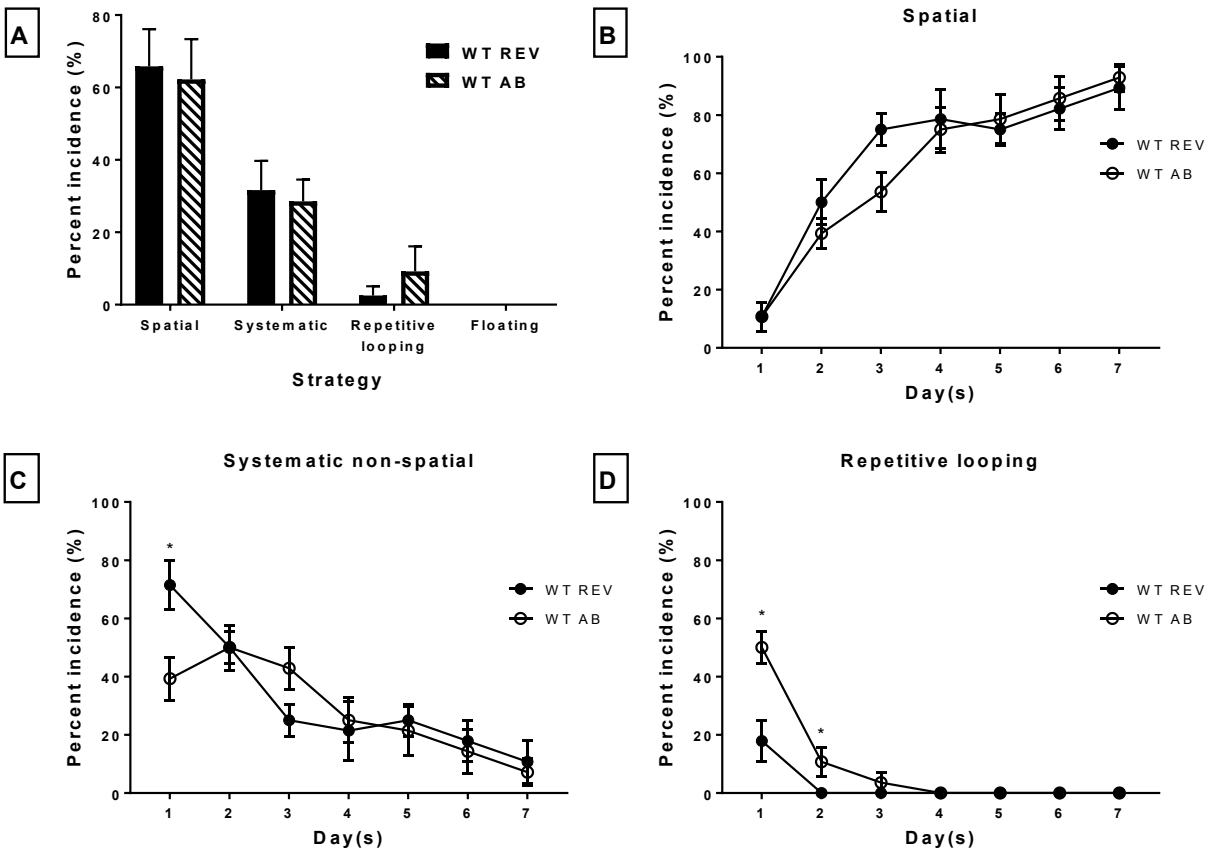

Figure 10. Search strategy incidences in the hard MWM task in three month old Sig1R WT males tested one week post-ICV.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). WT REV (n=7) and WT AB (n=7) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$. *$p < 0.05$

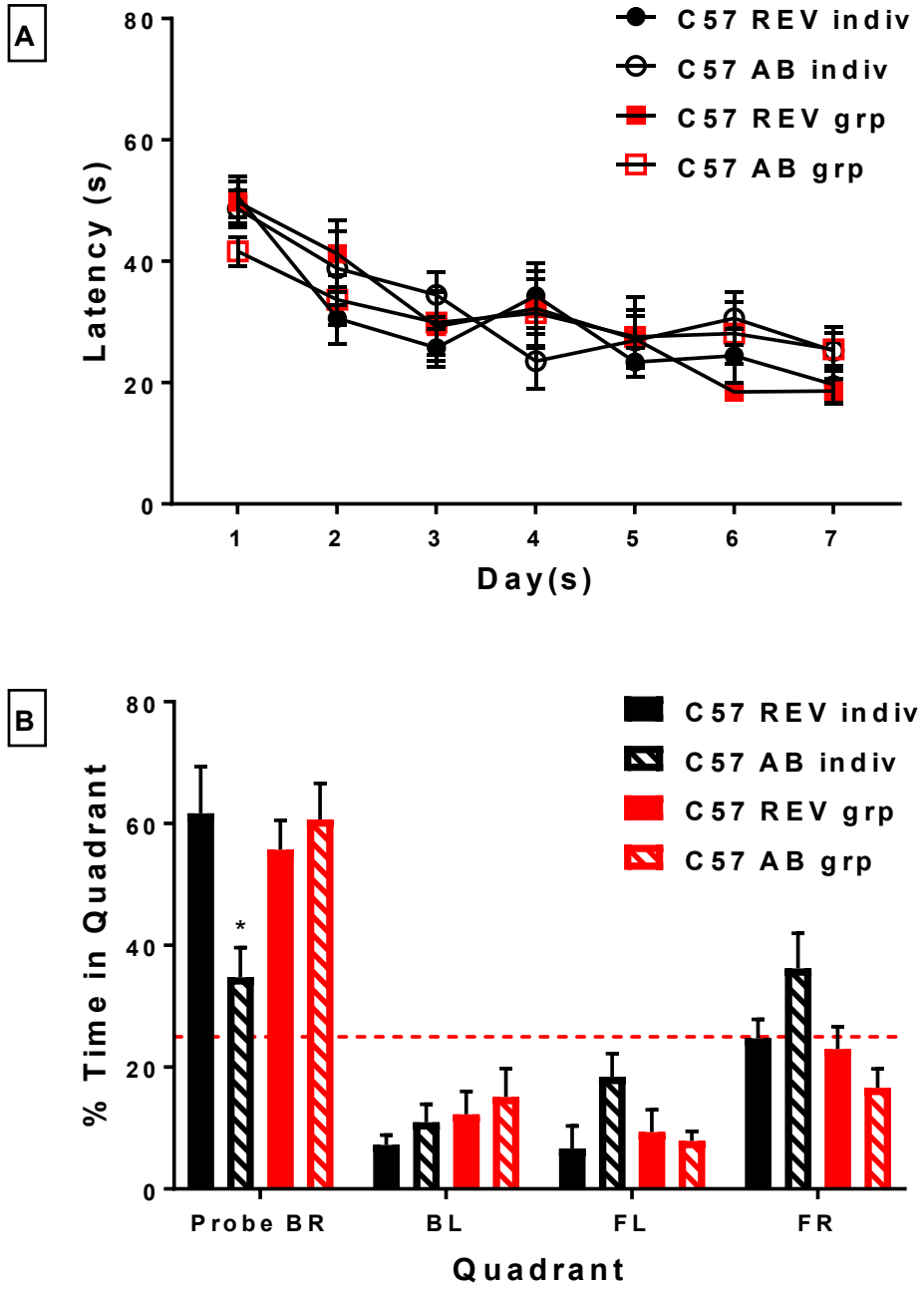

Figure 11. Spatial learning and reference memory in the hard MWM task in three month old C57BL/6J males tested one week post-ICV.

Average latencies to reach the platform across training days (acquisition phase; **A**) and average percent times spent in each quadrant (probe trial; **B**). REV indiv (n=6), AB indiv (n=7), REV grp (n=7) and AB grp (n=8) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$; indiv: individually housed; grp: group housed. *$p < 0.05$

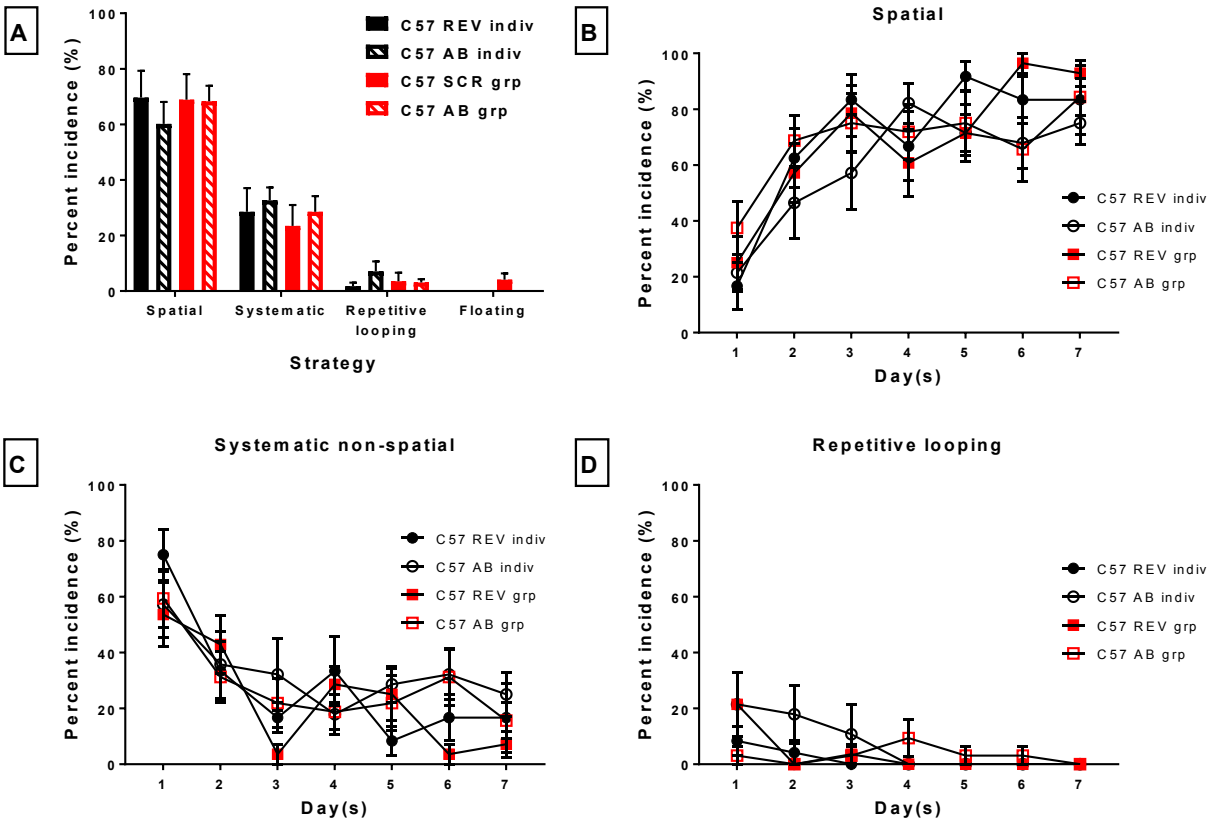

Figure 12. Search strategy incidences in the hard MWM task in three month old C57BL/6J males tested one week post-ICV.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). REV indiv (n=6), AB indiv (n=7), REV grp (n=7) and AB grp (n=8) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$; indiv: individually housed; grp: group housed.

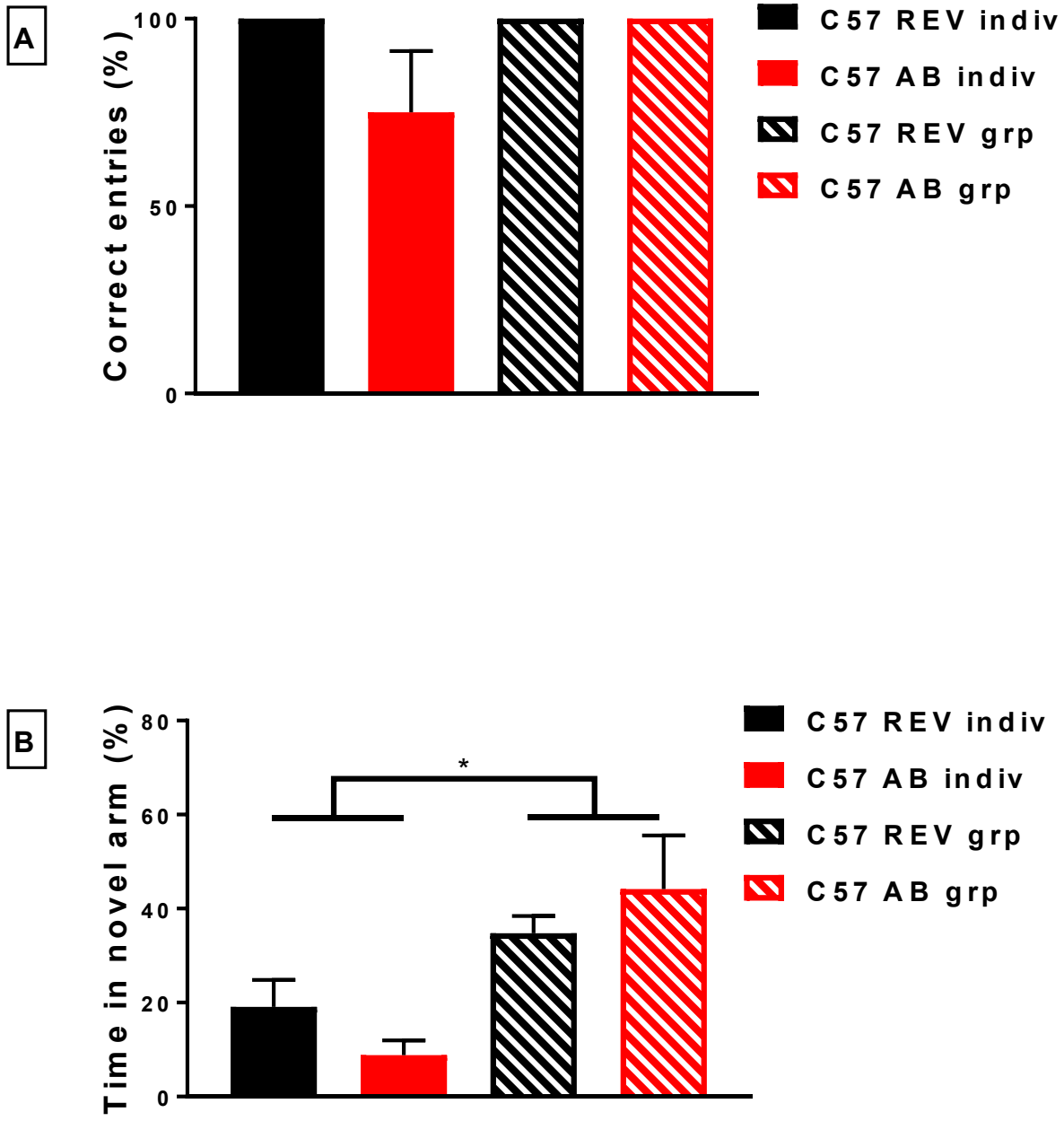

Figure 13. Spatial working memory in the forced alternation in the Y-maze task in three month old C57BL/6J males tested one week post ICV.

Percentage of correct entries into the novel arm (**A**) and percent time spent in the novel arm in the first minute of the trial (**B**). REV indiv (n=8), AB indiv (n=8), REV grp (n=6) and AB grp (n=3) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$; indiv: individually housed; grp: group housed. *$p < 0.05$

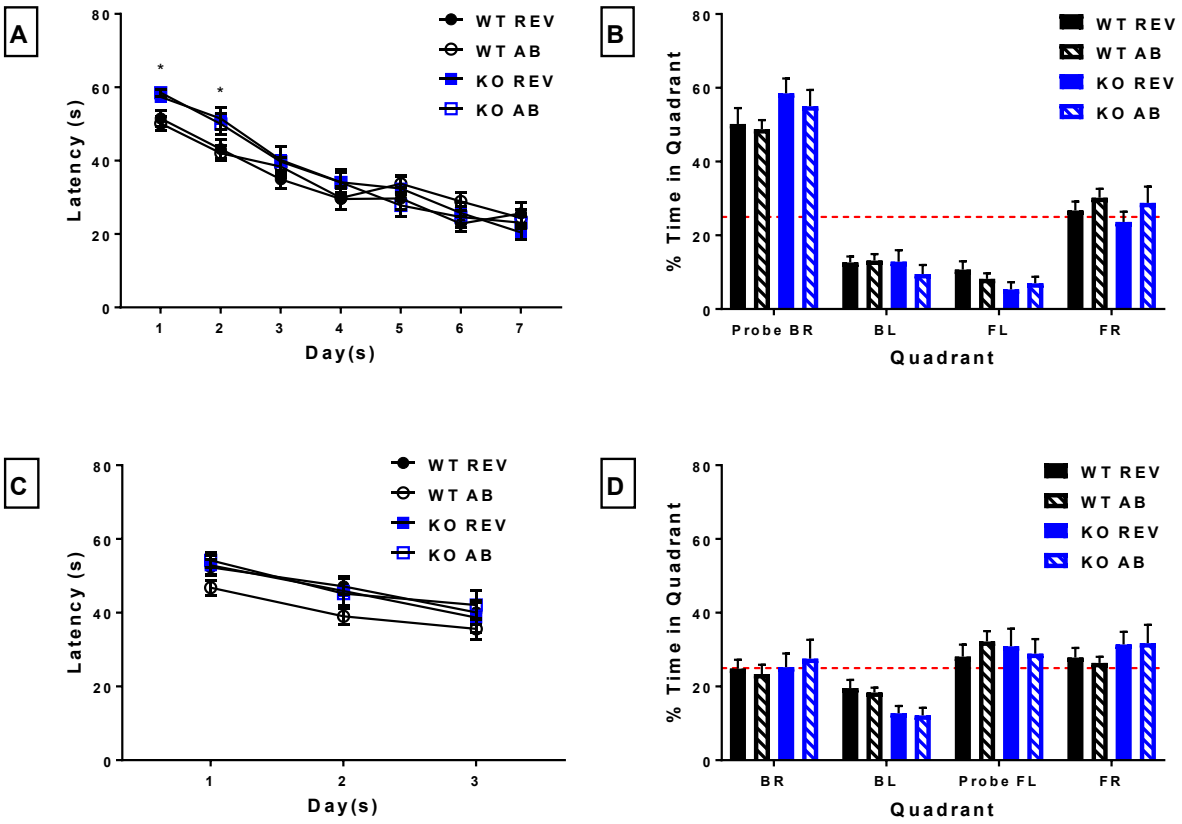

Figure 14. Spatial learning, reference memory, and cognitive flexibility in the hard MWM task in individually housed six month old Sig1R WT and KO males.

Average latencies to reach the platform across training days (acquisition phase, **A**; reversal, **C**) and average percent times spent in each quadrant (probe trial 1, **B**; probe trial 2, **D**). WT REV (n=18), WT AB (n=21), KO REV (n=14) and KO AB (n=15) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$. *$p < 0.05$

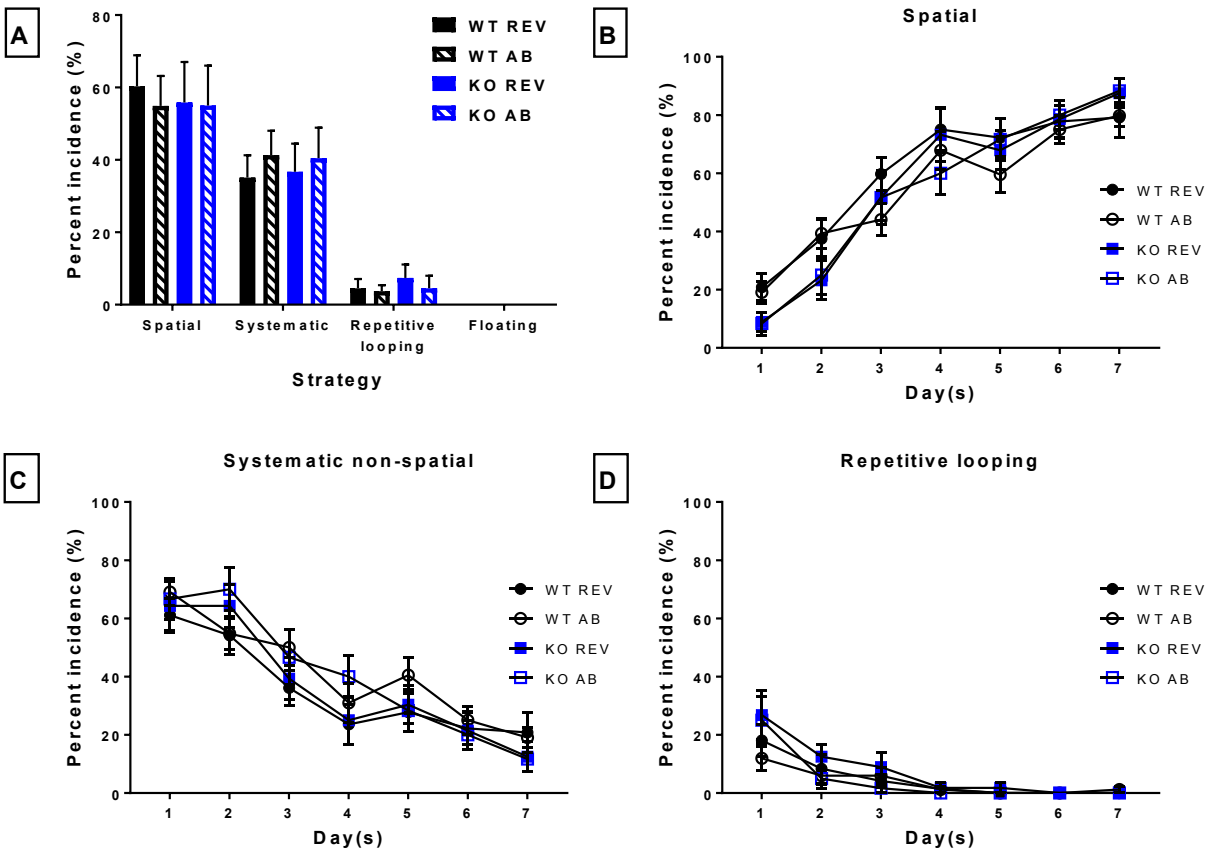

Figure 15. Search strategy incidences in the hard MWM task in individually housed six month old Sig1R WT and KO males.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). WT REV (n=18), WT AB (n=21), KO REV (n=14) and KO AB (n=15) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$. *$p < 0.05$

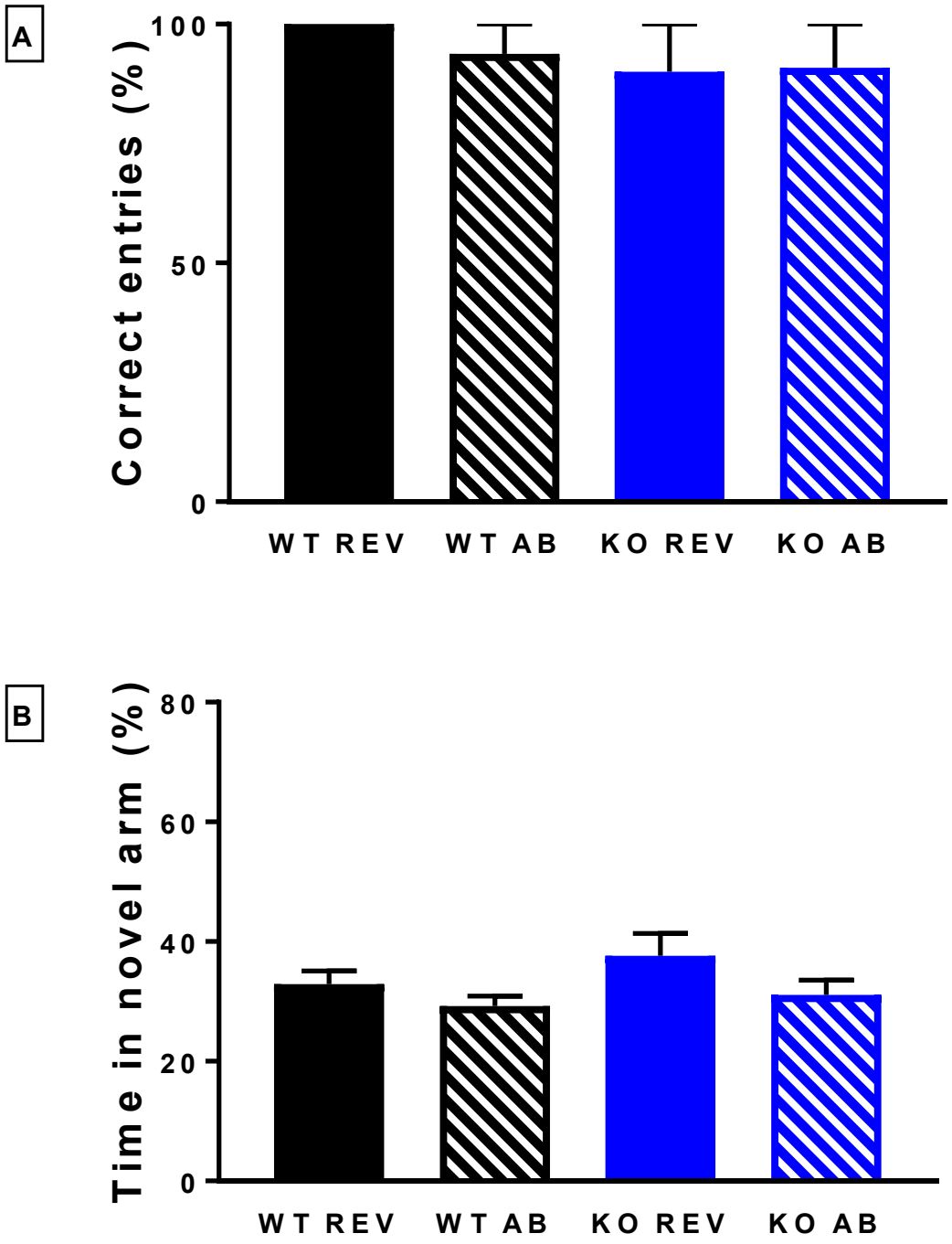

Figure 16. Spatial working memory in the forced alternation in the Y-maze task in individually housed six month old Sig1R WT and KO males.

Percentage of correct entries into the novel arm (**A**) and percent time spent in the novel arm in the first minute of the trial (**B**). WT REV (n=15), WT AB (n=16), KO REV (n=10) and KO AB (n=11) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$.

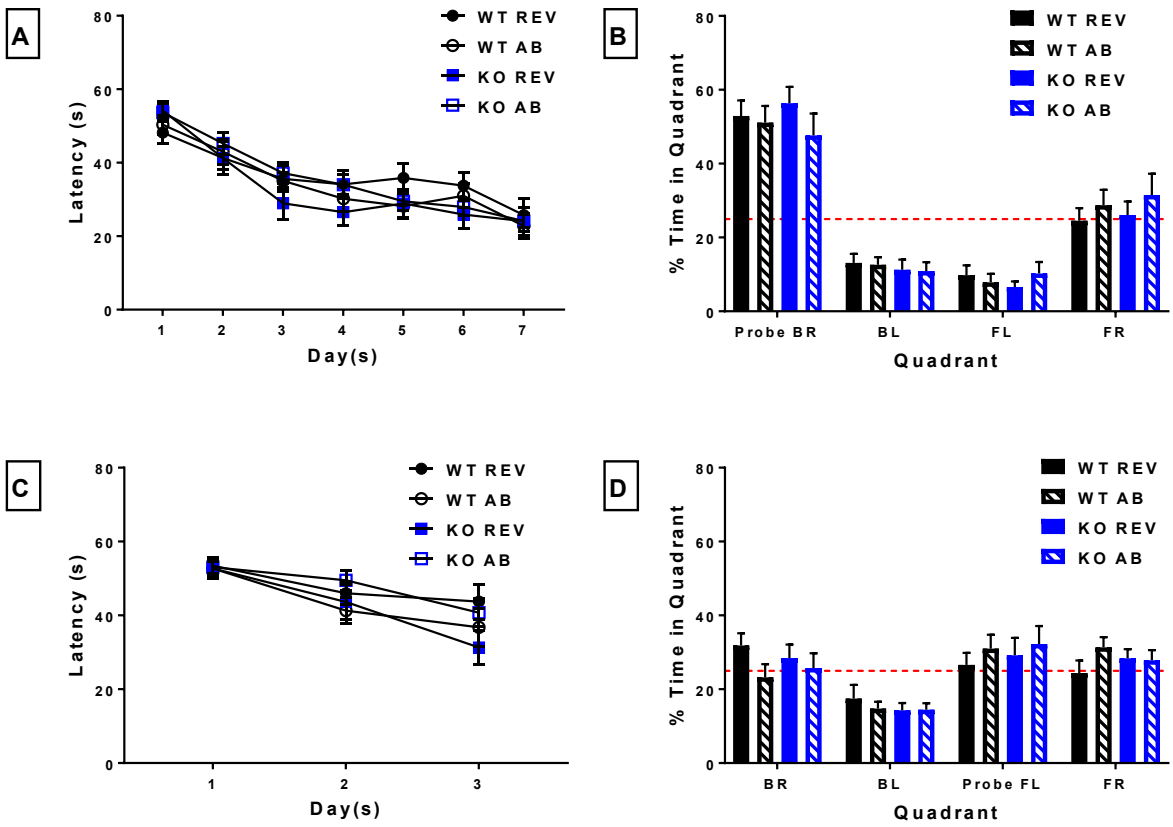

Figure 17. Spatial learning, reference memory, and cognitive flexibility in the hard MWM task in individually housed six month old Sig1R WT and KO females.

Average latencies to reach the platform across training days (acquisition phase, **A**; reversal, **C**) and average percent times spent in each quadrant (probe trial 1, **B**; probe trial 2, **D**). WT REV (n=10), WT AB (n=12), KO REV (n=11) and KO AB (n=15) mice. The dotted red line at 25% represents the chance of being in one of four quadrants. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$.

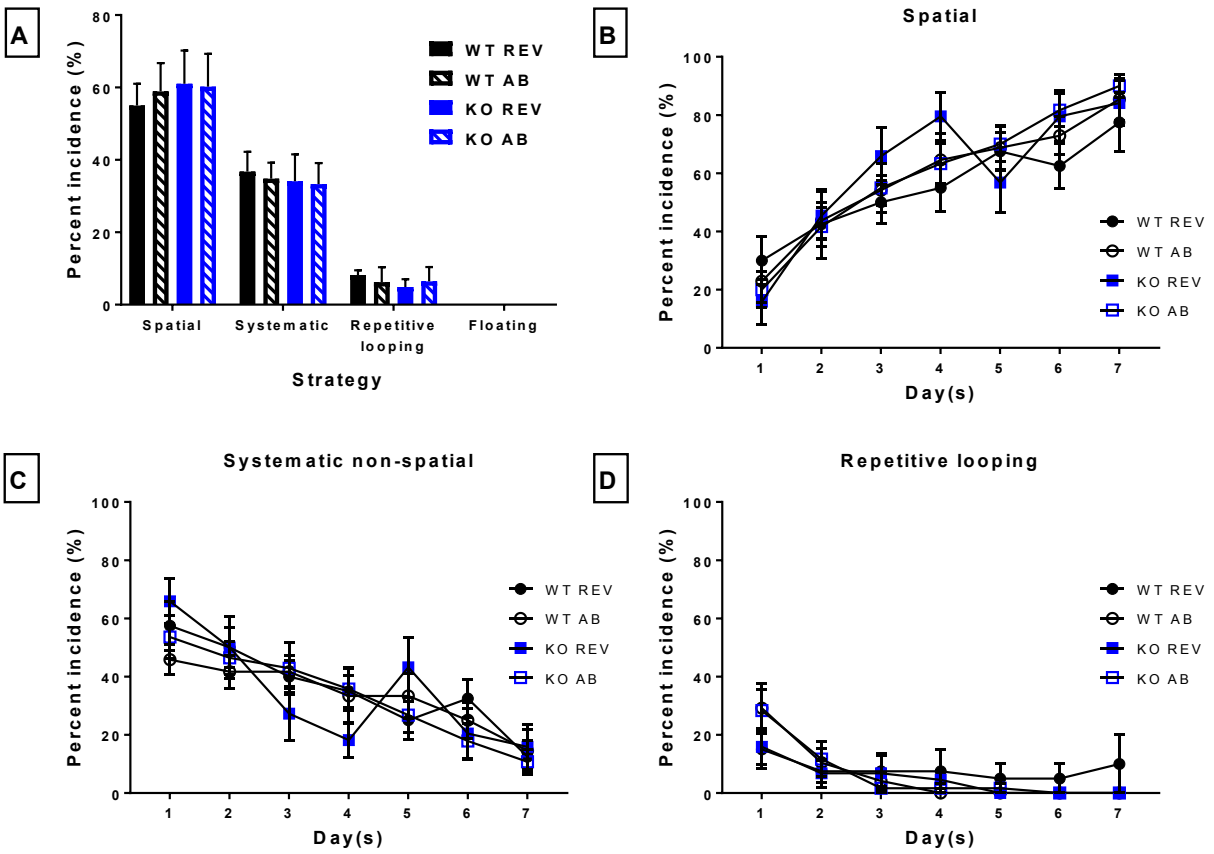

Figure 18. Search strategy incidences in the hard MWM task in individually housed six month old Sig1R WT and KO females.

Average percent incidences of navigational search strategies over all training days (**A**). Percent incidences of spatial, systematic non-spatial, and repetitive looping strategies across training days (**B-D**, respectively). WT REV (n=10), WT AB (n=12), KO REV (n=11) and KO AB (n=15) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35-25}$; AB: $A\beta_{25-35}$.

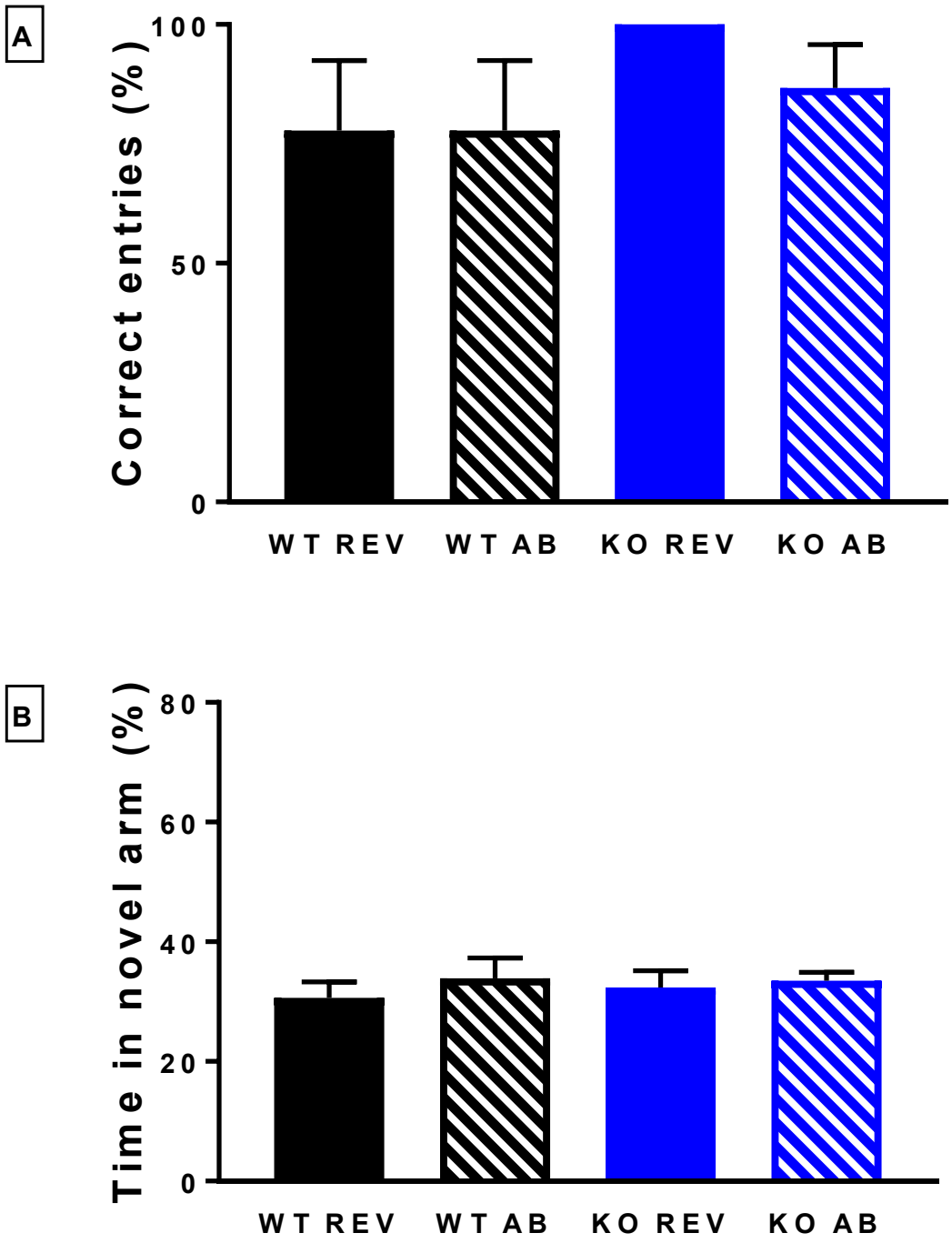

Figure 19. Spatial working memory in the forced alternation in the Y-maze task in individually housed six month old Sig1R WT and KO females.

Percentage of correct entries into the novel arm (**A**) and percent time spent in the novel arm in the first minute of the trial (**B**). WT REV (n=9), WT AB (n=9), KO REV (n=13) and KO AB (n=15) mice. Data expressed as the mean ± SEM. REV: $A\beta_{35\text{-}25}$; AB: $A\beta_{25\text{-}35}$.

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