Evaluation and validation of new animal and behavioural models for the study of Alzheimer`s disease

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Erklärung:

Hiermit erkläre ich, dass ich diese Arbeit selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

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Abbreviations

Aβ	Beta amyloid
Aβ42	Beta amyloid containing 42 amino acids
ACh	Aceylcholine
AChE	Acelylcholine esterase
AChEi	Acelylcholine esterase inhibitor
AD	Alzheimer´disease
AGM	Agranular medial cortex
ANOVA	Aalysis of variance
Apo E	Apolipoprotein E
APP	Amyloid precursor protein
APP_{SL}	Amyloid precursor protein with “Swdish” and “London” mutation
BFCS	Basal forebrain cholinergic system
BG	Basal ganglia
BM	Barnes Maze (syn. circular platform)
CAA	Cerebral amyloid angiopathy
CF	Cone field
ChAT	Choline acetyl transferase
Ch1-Ch6	Cholinergic sectors of central cholinergic pathways
CNS	Central nervous system
DA	Dopamine
dACA	Dorsal anterior cingulated area
ffx	Fimbria and dorsal fornix bundle
FTDP	Frontotemporal dementia and Parkinson`s disease
GABA	Gamma-amino butyric acid
Glu	Glutamate, glutamatergic
HB	Holeboard
hdb	Horizontal limb nucleus of the diagonal band of Broca
Ibo	Ibotenic acid
i.c.v.	Intracerebroventricular
IL	Infralimbic cortex
i.p.	Intra-peritoneal
ITI	Inter-trial interval

KA	Kainic acid
Lc	Line crossings
LTM	Long-term memory
LTP	Long- term potentiation
mAb	Monoclonal antibody
mBM	Modified Barnes Maze
md	Medio-dorsal
mdTh	Medio-dorsal thalamic nucleus
min	Minute(s)
mPFC	Medial prefrontal cortex
MS	Nucleus of the medial septum
MTL	Medial temporal lobe
mu p75 SAP	Anti-murine-anti-p75 NGFR binding antibody with a linked saporin molecule
mut	Mutant
MWM	Morris Water Maze
NAC	Nucleus accumbens
NBM	Nucleus basalis of Meynert (magnocellularis in rodents)
NFT	Neurofibrillary tangles
NGF	Nerve growth factor
NGFR	Nerve growth factor receptor
NP	Neuritic plaques
NSAIDs	Non-stereoidale anti-inflammatory drugs
NT	Neurotransmitter
PL	Prelimbic cortex
PS1	Presenilin 1
PS2	Presenilin 2
QA	Quinolinic acid
RAM	Radial arm maze (syn. Olton Maze)
s	Second(s)
s.c.	Subcutaneous
SNr	Substantia nigra pars reticulata
SC	Spinal cord
STM	Short- term memory
T-DAT	T-maze delayed alternation task

vdb	Ventral limb nucleus of the diagonal band of Broca
VP	Ventral pallidum
VTA	Ventral tegmental area
wt	Wild-type
Y-DAT	Y-maze delayed alternation task

Zusammenfassung

Die Wahrscheinlichkeit, an der Alzheimer`schen Demenz (AD) zu erkranken steigt mit der zunehmender Lebenserwartung der Menschen und stellt eine enorme Belastung für die Betroffenen, ihre Angehörigen und das Gesundheitssystem dar. Bisherige Tiermodelle zielten lediglich auf Teilaspekte der AD Pathologie ab. Die vorliegende Arbeit kombiniert Gentechnik mit selektiv invasiven Versuchsmethoden mit dem Ziel, ein besseres Tiermodell für die Erforschung von AD zu identifizieren.

Die Eignung der Tiere, Aufgaben in Verhaltensmodellen zu erlernen ist entscheidend und kann grossen Einfluss auf die Messung kognitiver Leistungen nehmen. Daher wurden mehrere Mausstämme in verschiedenen Verhaltensmodellen untersucht. Insbesondere C57BL/6 Mäuse zeigten konstant hohe Leistungen im T-Maze (T-CAT), Holeboard, Barnes Maze (BM) und im Morris Water Maze (MWM). Ungenügende Leistungen im originalen Testaufbau des object recognition task (ORT) konnten durch Modifikationen im Aufbau und Protokoll hochsignifikant verbessert werden. Basierend auf diesen Ergebnissen wurden vier verschiedene C57BL/6 Genotypen mit unterschiedlichem β-Amyloid Protein (Aβ) Niveau in einer Longitudinalstudie in vier Verhaltensmodellen getestet. Danach wurde bei einem Teil der Tiere eine selektive Nucleus Basalis Magnocellularis (NBM) Läsion induziert, gefolgt von erneuter Prüfung in den vier Verhaltensmodellen. Die Studie ergab eine Aβ-abhängige Verschlechterung im BM und MWM (*spatial reference memory*) für die erste und zweite Testung. Die Beeinträchtigung in der zweiten Testung war unabhängig von den gesetzten NBM-Läsionen. Die Lernerfolge im ORT (*working memory*) waren unbeeinträchtigt von Aβ (erste und zweite Testung). In der zweiten Testung zeigten die Tiere jedoch eine NBM-Läsions-abhängige Verschlechterung. Die Lernerfolge im T-CAT (*spatial working memory*) waren unbeeinträchtigt von Aβ in der ersten Testung. Interessanterweise war der Lernerfolg im T-CAT sowohl Aβ- als auch läsionsabhängig in der zweiten Testung. Darüberhinaus konnte die humane Standardtherapie (Donepezil) den Lernerfolg im T-CAT signifikant verbessern.

Die vorliegenden Ergebnisse zeigen, dass die Art (Aβ, Läsion) und Intensität (einfach-, doppelmutant) der Pathologie unterschiedliche Lernaufgaben beinträchtigt. Das komplexe Krankheitsbild der humanen AD wird allerdings am besten von NBM-lesionierten, Aβ-überexprimierenden Tieren wiedergegeben, die im T-CAT getestet wurden. Die hier präsentierten Daten, stellen somit einen entscheidenden Beitrag zum Verständnis der

Zusammenhänge zwischen Pathologie und Lernverhalten dar und führen zu einer Verbesserung von Tier- und Verhaltensmodellen für die Erforschung der AD.

Abstract

The incidence of suffering from Alzheimer`s disease (AD) increases steadily with augmenting life expectancy in humans, generating an enormous burden for patients, their families and the national health system. Models used in previous publications covered only parts of the AD pathology. The work presented here combines transgenic technology and selective invasive methods to identify an improved animal model for the study of AD.

The suitability of animals to acquire task demands of behavioural models is an important issue that may bias measure of cognition. Therefore, several mouse strains were tested in different behavioural models. Especially C57BL/6 mice displayed constantly high performance in the T-Maze (T-CAT), Holeboard, Barnes Maze (BM) and in the Morris Water Maze (MWM). Insufficient performance in the original version of the object recognition task (ORT) was significantly ameliorated with modifications of set-up and test procedure. Against this background, four different C57BL/6 genotypes expressing different ß-amyloid protein (Aß) niveau were tested in a longitudinal study in four behavioural models. Upon first testing, the nucleus basalis magnocellularis (NBM) of 50 % of the animals was selectively lesioned. All animals were again subjected to a second testing procedure. The results show Aβ-dependent impairment in the BM and MWM (spatial reference memory) in the first and second testing. Impairment was independent from NBM lesions. Learning performance in the ORT (working memory) was not influenced by Aβ (first and second testing). However, performance was impaired by NBM lesions in the second testing. In the T-CAT (spatial working memory), learning was unimpaired by Aβ when first tested. Interestingly, performance was reduced with Aβ and NBM lesions in the second testing. Additionally, standard therapy used in human patients (Donepezil) significantly improved learning performance in the T-CAT.

These results indicate that type (Aβ, NBM) and intensity (single- or double mutatnt) of pathology influences learning tasks in a different manner. However, the diverse symptomatology of human AD is predominantly reflected by NBM lesioned and Aß overexpressing animals tested in the T-CAT. In conclusion, data presented here provide important contributions for the understanding of connections between pathology and learning, leading to an improvement of animal- and behavioural models in AD research.

Chapter I: General introduction

1.1. Alzheimer´s disease

Alzheimer´s disease (AD) was first reported by Alois Alzheimer at a congress in Tübingen (Alzheimer, 1907). He described the behaviour of a 51 years-old woman:

A 51 years-old woman displayed initial disease symptoms in forms of strong jealousy towards her husband. Soon, a rapidly increasing mental loss was observed, she got lost in her apartment, she carried objects from one place to another, hid them, sometimes she believed that people were out to kill her and started screaming loudly. […] Her ability to memorise was strongly disturbed. She was able to name objects that were shown to her correctly, but she forgot everything in an instance (Translated from German Original Source).

The incidence of suffering from age-related mental diseases, especially from AD, increases steadily with augmenting life expectancy in humans (Molnar and Dalziel, 1997). Epidemiologic data show, that prevalence of AD doubles every 5 years after the age of 60 increasing from a prevalence of 1 % among 60- to 64 years-old to up to 40 % of those aged 85 years and older (Katzman, 1986; Von Strauss et al., 1999). The majority of AD cases are sporadic and typically relate to elderly persons. About 5 % of AD cases are familial with an autosomal dominant inheritance (Shastry and Giblin, 1999) and potentially carry early-onset character (early-onset: age of 40-65 years; late-onset: < 65years) (Molnar and Dalziel, 1997; Jorm et al., 1987). Genotype investigations in familial AD cases revealed several mutations associated with AD (for review see Cummings et al., 1998; Shastry and Giblin, 1999), which enormously sped up preclinical (for review see Higgins and Jacobsen, 2003; Jaffar et al., 2000; also see section 2, mouse models) and clinical research (Heston et al., 1981; Cook et al., 1981). However, the causative agent of AD is still unknown.

Diagnosis of definite AD requires both, the clinical features of probable AD, and histopathological confirmation by biopsy or autopsy (McKhann et al., 1984; Hyman et al., 1989).

1.1.1. Clinical symptoms of AD

Gradual loss of concentration and attention (Hinterhuber, 1996; Sahakianet al., 1993), spatial disorientation (Henderson et al., 1989) and decline of recent or short- term memory (Jones et

al., 1992; Kensinger et al., 2003; Caplan and Waters, 1999) are described as the initial symptoms relating to the mental decline in AD. Memory can usefully be categorized according to the type of information stored into declarative (explicit) and procedural (implicit) memory (for detailed introduction see chapter II; Schacter, 1987; Squire, 1982; Tulving, 1985. For review see Zola-Morgan and Squire, 1993). Declarative memory is reported to gradually decline in AD patients. In contrast, procedural memory, remains spared in AD patients (Postle et al., 1996; Jelicic et al., 1995), at least in the early course of the illness (Devi and Silver, 2000). The American Psychiatric Association (1994) resumes clinical diagnostic symptoms of AD as progressive deterioration of memory (amnesia), judgement, abstract thinking, intelligence and changes in personality. The malignant course of AD allows a survival rate of usually 7-10 years after the outcome of the first symptoms (Bracco et al., 1994). Patients typically die from bronchitis or pneumonia (Beard et al., 1996).

1.1.2. Histopathologal hallmarks of AD

The histopathological hallmarks of AD include neuritic plaques (NPs), neurofibrillary tangles (NFT) and cholinergic neurodegeneration (for example Braak et al., 1998; Hyman, 1989). Moreover, loss of synapses and neurons, granulovacuolar degeneration and activated microglia, have been detected in brains from AD patients (Cummings et al., 1998). The pathological changes tend to be concentrated in the frontal and temporal lobes of the neocortex and in the hippocampal formation (Carlesimo and Oscar-Berman, 1992; Devi and Silver, 2000; Hyman et al., 1984; Cummings and Cole, 2002).

1.1.2.1. Cholinergic system

1.1.2.1.1. Central cholinergic system

Mesulam and colleges (1983) introduced a widely accepted classification and nomenclature of the central cholinergic pathways. They classified cholinergic cells into six groups or sectors (Figure 1). This classification is based on connectivity patterns of projection fields. The first group (Ch1) is composed of the cholinergic cells of medial septal nucleus (MS), and the cells in the ventral limb nucleus of the diagonal band of Broca (vdb) (Ch2). These two groups provide the major cholinergic projection to the hippocampus. Ch2 further provide cholinergic afferents to hypothalamus, cingulated cortex and olfactory bulb. The horizontal limb nucleus of the diagonal band of Broca (hdb) that mainly projects to the olfactory bulb is classified as Ch3. Cells from the fourth sector (Ch4) most closely refer to the NBM but also cells from the

nucleus of the ansa lenticularis, nucleus of the ansa peduncularis, medulla laminae of the globus pallidus and the substantia nigra are included to the Ch4 section. Nuclei from sections Ch1-Ch4 are functionally designated as the basal forebrain cholinergic system (BFCS). Comparative neuroanatomic studies indicate that the major part of this cholinergic system in primates consists of the NBM (Meynert, 1872; Gorry, 1963). Large (magnocellular) pyramidal cell populations of at least 30µm in diameter characterise cholinergic NBM neurons (Chui et al., 1984; Whitehouse et al., 1982). The NBM provides the main cholinergic projection to neocortical structures (Divac, 1975; Johnston et al., 1979; Vogels et al., 1990; Wenk et al., 1980). Additionally, the NBM regulates indirectly limbic and hippocampal structures via connections to the amygdala (Wenk, 1996). The two remaining groups within the cholinergic system, Ch5 and Ch6, form connections to the thalamus. They are located in the pedunculopontine nucleus (Ch5) and laterodorsal tegmental nucleus (Ch6). Although all sections (Ch1-6) are generally considered to be cholinergic, they contain also other types of cells. For example, only 10-20 % of the cells in the Ch3 nucleus are cholinergic, whereas the proportion can be as high as 80-90 %, as in Ch4. Most of the studies concerning the role of the cholinergic system in learning and memory have concentrated on Ch1/Ch2 and Ch4, because they are supposed to be the most important ones based on their projection areas (hippocampus and neocortex).

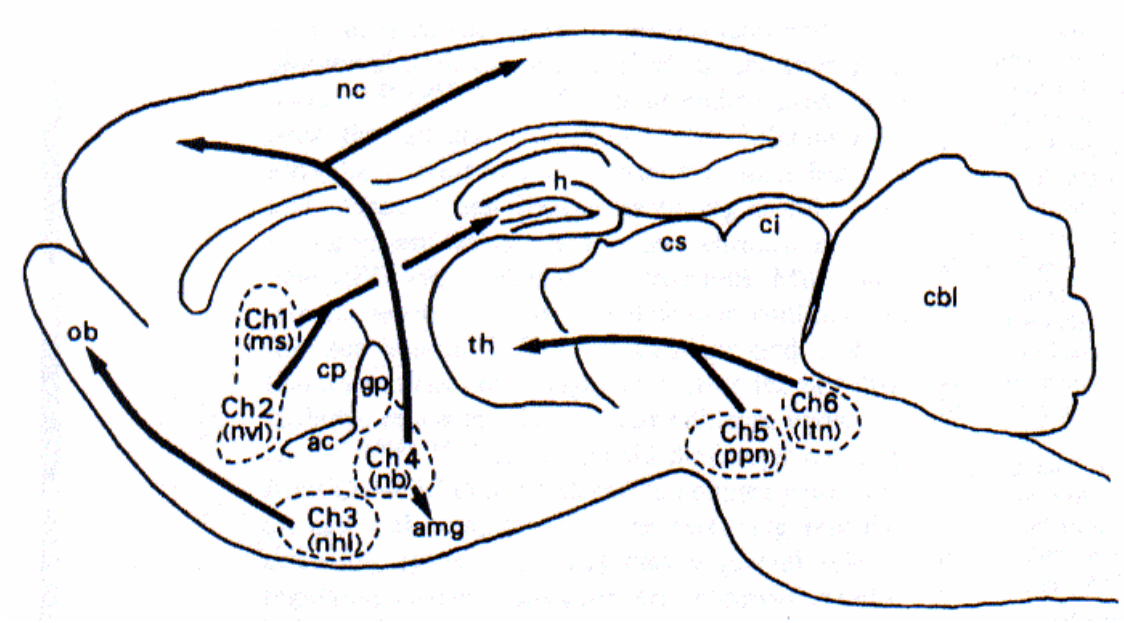


Figure 1: Central cholinergic pathways. The six major cholinergic projection groups (sectors) are depicted (Ch1-Ch6). Therein, projections from Ch1/Ch2 and Ch4 to hippocampal, limbic and neocortical areas are implicated in learning and memory processes.

1.1.2.1.2. Cholinergic neurotransmission

Cholinergic neurons are characterized by the predominace of the neurotransmitter acetylcholine (ACh). Synthesis of ACh is catalyzed by the enzyme choline acetyltransferase (ChAT) from acetyl CoA and choline with the release of coenzyme A. ACh is stored in synaptic vesicles. Action potentials that reach the synapse induce the release of ACh into the synaptic cleft to receptors located in the presynaptic and postsynaptic membranes. Two main classes of cholinergic receptors are known based on the ability to mimic the effect of ACh, nicotinic (nAChR) and muscarinic (mAChR) ACh receptors. nAChRs belong to a superfamily of ligand-gated ion channels (Albuquerque et al., 1997a, 1997b; Dani and Mayer, 1995; Dani, 2001). Binding of nicotin or ACh to presynaptic nicotinic autoreceptors results in enhanced neurotransmitter release, binding to postsynaptic nAChRs mediates fast excitatory neurotransmission. Reduction of nAChRs ranging between 20 % and 50 % were consistently observed at autopsy in a number of neocortical areas and hippocampi of patients with AD (Perry et al., 1995; Nordberg, 1992). The other receptor type, mAChR, accounts for the majority of effects of ACh and is spread over multiple brain areas and the peripheral nervous system (for review see Caulfield, 1993). Currently, five mAChRs are classified: M_1-M_5. All receptors are placed at postsynaptic membranes with the exception of M_2 and M_4 that mostly occur presynaptically as inhibitory autoreceptors. No major or consistent changes in mAChRs were observed in the cerebral cortex in AD patients (Nordberg, 1992), thus therapeutic approaches to enhance the effect of ACh, may act at mAChRs. The enzyme acetylcholine esterase (AChE), which is located in the postsynaptic membrane, regulates the effect duration of ACh at any receptor, by degrading ACh into acetic acid and choline. A reuptake of choline into the cholinergic neuron is granted by a high affine transport mechanism. Inhibition of AChE to enhance the effective concentration of ACh represents the therapeutic standard to ameliorate the cognitive decline in AD patients

1.1.2.1.3. Cholinergic hypothesis of memory dysfunction in AD

Early clinical neuropsycholpharmacological approaches pointed at the involvement of the central cholinergic system in memory processes, and correlations between cholinergic hypofunction and memory decline were detected. Drugs that block central ACh M-receptors were found to disrupt higher congnitive functions and induce transient amnestic states (Longo, 1966). The observation by Drachman and Leavitt (1974) that the muscarinic antagonist scopolamine, administered to healthy young volunteers induces a cognitive state resembling that found in senile dementia proved prophetic when it later became apparent that

the most consistent biochemical alteration in AD was a dramatic reduction of the cholinergic markers AChE (up to 50 %) and ChAT (60-90 %) in cortex, hippocampus and the BFCS (Bartus et al., 1982; Davies and Maloney, 1976; Ezrin-Waters and Resch, 1986; Whitehouse et al., 1982). Profound destruction of cholinergic neurons within the BFCS proved to be central to the AD pathology (Greferath et al., 2000; Whitehouse et al., 1982). Therein, special focus is put on the neuropathology within the NBM (Arendt et al., 1983; Vogels et al., 1990). Extreme neuronal loss and other degenerative alteration within the NBM (Cullen and Halliday, 1998; Ezrin-Waters and Resch, 1986; Rinne et al., 1987; Vogels et al., 1990) relate to the NBM as a useful animal model to mimic important aspects of cholinergic hypofunction in AD (Coyle et al., 1983a,b; Dunnett et al., 1991; Lerer et al., 1985).

1.1.2.2. Histopathology of neuritic plaques and Aβ synthesis

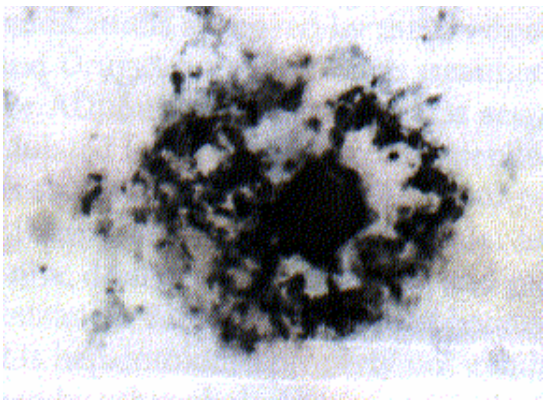


Figure 2: Morphology of a neuritic plaque from the brain of an individual with Alzheimer`s disease (labelled with a mAb for human amyloid peptide using diaminobenzidine combined with hematoxylin counterstain, x2500 magnification) Taken from Cummings et al., 2002.

Several types of amyloid-related neuritic plaques (NPs) are recognized in the brain of AD patients (Terry, 1997). The classical NP is a spherical 50 to 200μm in diameter, consisting of a central amyloid core surrounded by dystrophic neuritis, which are primarily axon terminals (Coyle et al., 1983a). (Figure 2). The neuritis often contain paired helical filaments, normal glial processes, and abnormal organelles. In addition, NPs include tau (τ) protein, α_1-antichymotrypsin, apolipoprotein E (ApoE), and glycosaminoglycans, among other components. Reactive astrocytes and microglia are also found within the plaque and at the plaque periphery (Mandybur and Chuirazzi, 1990).

From molecular view, NPs consist of accumulated amyloid beta (Aß) protein, derived from its amyloid precursor protein (APP) (Cummings et al., 1998). In familial AD cases, mutations around the APP gene and the proteolytic cleavage site have been demonstrated (Hardy, 1997; Mullan et al., 1992a, 1992b). APP is a transmembrane protein with an intracellular domain and an N-terminal in the extracellular region (Figure 3). Dependent on the secretase (α, β, or γ-secretase) the cleaving site within the precursor protein is variable. Cleavage by an α-secreatase results in a soluble, degradable APP fragment with neuroprotective properties (Allinson et al., 2003; Kogel et al., 2003; Masliah, 1997; Masliah et al., 1997; Mattson et al.,

1993), which is the case in healthy persons. β -or γ-secretase cleavage, however, produces insoluble pathogenic Aß peptides, 39-42 amino acid fragments that can accumulate in NP deposits (Irizarry et al., 2001; Liu et al., 2002). In most cases, Aß42 molecules were observed. Moreover, Mucke et al. (2000) found synaptotoxic activity with Aβ. Mutations, on chromosome 21, to the β-secretase have been found in a Swedish family with strong AD inheritance ("Swedisch mutation") (Haass and Selkoe, 1993). Mutations to the γ-secretase were identified in the "London mutation" (Goate et al., 1991). Further critical mutations in familial AD pathology were identified at the presenilin-1 -and 2 (PS1 and PS2) gene (Price et al., 1998; Sherrington et al., 1995). These mutations also increase $Aß_{42}$ brain level. The elevation of $Aß_{42}$ brain level is considered to be an early and critical key step in the pathogenesis of AD (Hardy, 1997).

Characteristically, highest concentrations of deposits are found in the hippocampal formation and cerebral cortex (Irizarry et al., 2001; Liu et al., 2002a, 2002b; Van Hoesen et al., 1991).

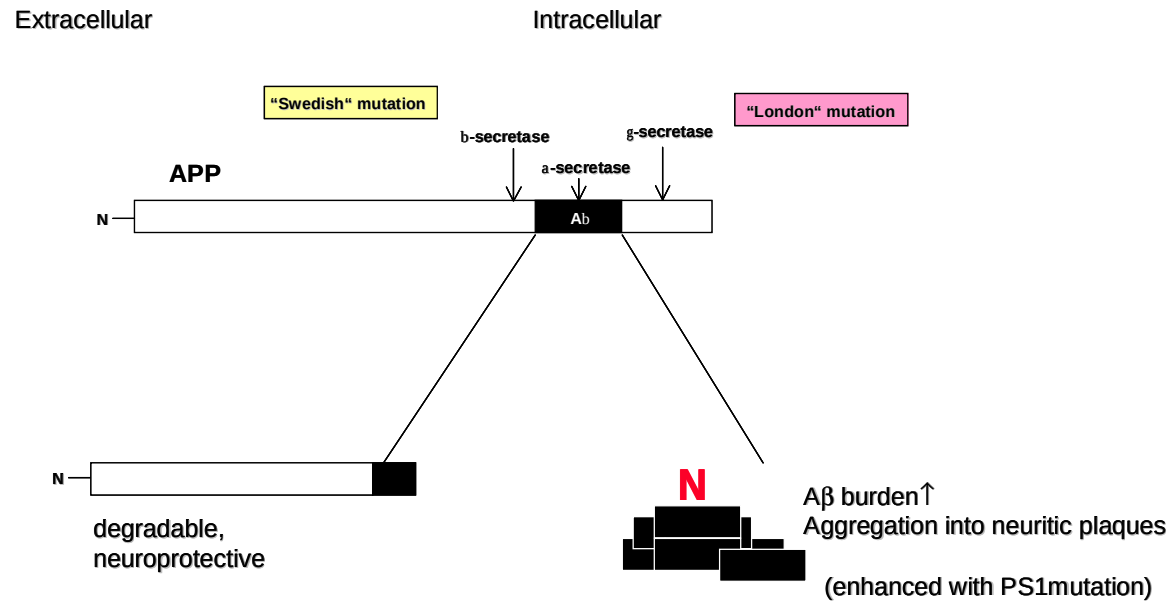


Figure 3: Schematic illustration of the amyloid precursor protein (APP), cleavage site of α, β and γ secretase and the location of the Swedish and London mutation in familial Alzheimer`s disease. APP is a transmembrane protein with an extracellular N-terminal and four transmembrane domains containing the Aß

1.1.2.3. Histopathology of neurofibrillary tangles

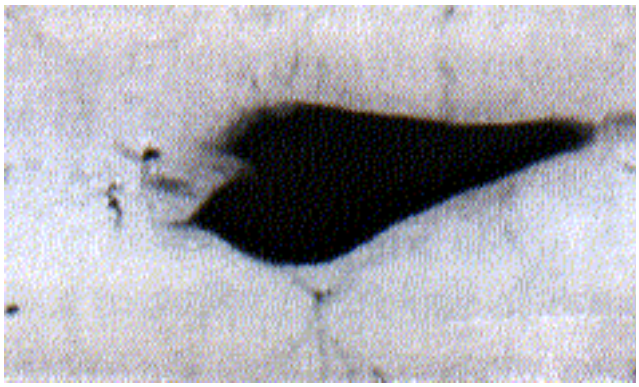


Figure 4: Morphology of a neurofibrillary tangle from the brain of an individual with Alzheimer`s disease (labelled with Gallays silver stain, x2500 magnification)
Taken from Cummings et al., 2002.

Neurofibrillary tangles (NFTs) represent another characteristic histopathological change observed in AD (Figure 4). While NPs represent extracellular changes in the pathogensis of AD, NFTs represent intracellular changes. NFTs consist of paired helical filaments (PHF) that occupy the cell body and may extend into the dendrites but do not occur in the axon. PHF consist of protofilaments arreanged to form a tubule and containing abnormally phosphorylated τ-protein (Selkoe et al., 1982a,b). NFTs were also diagnosed in non-AD CNS diseases, including postencephalic Parkinson´s disease, supranuclear palsy or subacute sclerosing panencephalitis (McGeer et al., 1994; for review see Spillantini and Goedert, 1998). Therefore, NFTs are less specific to AD than NPs, which are almost unique to AD and to so-called normal aging.

1.1.3. Therapeutic approaches

A causal therapy to prevent the onset of AD is the most conspicuous aim, however the causal agent has not been discovered so far. Most current therapeutic approaches relate to drugs effective to AD symptoms. They should improve cognitive impairments, control the behavioural and neurological symptoms, and they should delay progression of the disease. Current therapeutic research focuses at two aims: enhancement of cholinergic function and protection of neurons from pathological changes typical for AD.

To enhance cholinergic function, several therapeutic approaches have been reported and successfully administered to AD patients. Acetylcholinesterase inhibitors (AChEi) improve cognition by enhancing residual cholinergic activity. They block hydrolyses of ACh, which enlarges bioavailability of ACh in the synaptic cleft and the effect at cholinergic receptors. First generation AChEi such as Tacrine with undeserved side effects like hepatotoxicity (Watkins et al., 1998) were replaced by improved second generation AChEi such as donepezil, metrifonate, rivastigmine, eptastiminge or galantamine (Tariot and Schneider,

1996). Other cholinergic approaches point at postsynaptic M_1 -and M_3-receptors, because they seem to be preserved, which provides a suitable target for cholinomimetic agents, acting synergistic with ACh at those receptors (Kemp et al., 2003; Gu et al., 2003). Enhancement of ACh transmission from synaptic vesicles can be mediated by either nicotinic agonists acting at the presynaptic nicotinic receptors (Albuquerque et al., 2001) or by M_2-receptor antagonists that block inhibitory presynaptic M_2-autoreceptors. Analogues to transplantation of foetal tissue in patients with Parkinson`s disease in order to replace degenerated dopaminergic cells, cholinergic foetal neurons have successfully been transplanted in animal models (Dunnett et al., 1982; Low et al., 1982; Nilsson, 1990).

Therapeutic approaches to protect neurons from pathological changes typical for AD are multi-focal. Most promising approaches for treating amyloidosis in AD were presented by Schenk and colleges (1999). In preclinical studies, active vaccination with human Aβ42 was performed in young (6 weeks old) and old (11 months old) transgenic PDAPP mice that develop plaque depositions (see Chapter I, genetic mouse models). Hippocampal Aβ burden was measured at higher ages (Schenk et al., 1999). In both groups, vaccination-dependent decrease of Aβ level was observed. It is assumed that anti-Aβ antibodies prevent Aβ deposition or enhances Aβ clearance. There was also a reduction in neuritic pathology and reactive astrocytosis found in treated animals. Passive vaccination was performed with similar results (Check, 2003). In addition to Aβ burden, vaccination also successfully ameliorated cognitive deficits in transgenic mice with induced amyloidosis pathology (Dodart et al., 2002; Janus et al., 2000; Morgan et al., 2000). Clinical vaccination studies in AD patients were partially successful, but they had to be interrupted due to cases of CNS inflammation (Check, 2003). Inhibition of γ-secretase is another therapeutic approach to treat amyloidosis (Dewachter and van Leuven, 2002; Wong et al., 2004; Conway et al., 2003). It reduces cleavage of APP at the V717F site, thus resulting in higher intracellular levels of C-terminal fragments of APP by α- or β-secretase cleavage in favour of Aβ42. This effect is also expected by increasing or amplifying α-secretase pathways (Dewachter and van Leuven, 2002). Neuroprotective anti-inflammatory agents have successfully been employed to slow the progress and to defer the onset of AD (Irizarry and Hyman, 2001). In particular, α -tocopherol and seleginine effectively retarded AD symptoms (Sano et al., 1997). Non-stereoidal anti-inflammatory drugs (NSAIDs) or steroids also represent drugs with predictable side effects especially for chronic use (Clinard et al., 2001; Eriksen et al., 2003). Monoamine oxidase inhibitors reduce radicals and protect neurons from oxidative stress (Rosler et al., 1998; Yu, 1994). Moreover, excitotoxic cell death by excitatory amino acids has been

demonstrated in AD, thus application of calcium channel blocker prove beneficial against these symptoms (Kane and Robinson, 1999; LeVere and Walker, 1991; Weiss et al., 1993,1994).

In order to attain the targets of therapeutic investigations, cell and animal models are needed on which to test pathogenic hypothesis and demonstrate the potential effectiveness of new drugs. Moreover, evaluation of appropriate behavioural tasks is demanded to elaborate cognitive and sensorimotor phenotypes of animal models.

1.2. Mouse models

Preclinical models in AD have two purposes: to investigate the pathogenetic mechanism, and identify potentially effective drugs, their mechanism of action and toxicity. There is no model available reflecting all pathological symptoms of AD. Therefore, the current models should be considered as animal models of neurodegenerative disorder relating to pathological changes in AD, rather than proper AD models.

1.2.1. Model validity

Different types of validity must be considered to estimate the value of an animal model as a model for the study of human diseases (D`Mello and Steckler, 1996; Sarter et al., 1992a;b; Willner, 1991). The model should have *face validity*, which defines the degree of similarity in cognitive processes or pathology between the animal model and humans. In the present study, this claim should aim at the pathological changes observed in AD. A model has *predictive validity* if it the evidence taken from the model extends the knowledge of pathology in humans or if inferences made from the model reliably translate to AD. *Construct validity* is optimal for an animal model. It closely mimics the disease itself. Validity of the models used in the present study will be estimated by these criteria.

In current literature, use of the term “animal model” is inconstant. It describes both, manipulation or pathology induced to an animal, and learning tasks for measuring behaviour. Therefore terminological distinction will be introduced in the present thesis. The term “animal models” that describes pathological changes (including control animals) will still be referred to “animal models”. “Animal or learning models” describing means to measure behaviour, i.e. cognitive abilities, will be referred to “learning tasks” or “behavioural model”.

1.2.2. Genetic animal models

Genetic causes of AD are heterogeneous and include mutations or variants in several genes including those for APP, PS1 and ApoE (Cruts and Broeckhoven, 1998). Also, mutations leading to NFT pathology have been identified and integrated into mouse modelling approaches (Dammerman et al., 1988; Gotz , 2001; Gotz et al., 2001; Phinney et al., 2003).

1.2.2.1. Amyloidosis (APP and PS)

Important mutations have been found to the chromosomes 21, 14 and 1 in familial AD cases (Lendon et al., 1997; Shastry and Giblin, 1999). Specific mutations in the APP gene on chromosome 21 were identified referring to alternations in the cleavage sites of APP. As outlined before (see 1.2.2.), these mutations generate high Aβ brain level, thus contributing to the amyloidosis, which is an important hallmark of AD. Mutations located on chromosome 14 have been correlated to alterations of PS1, mutations on chromosome 1 were shown in alternations of PS2 (Price et al., 1998; Sherrington et al., 1995). Especially mutations at the PS1 in combination with the APP gene refer to enhanced Aβ burden and deposition in NPs (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996).

On this background, various genetic manipulations were evaluated in order to generate animal (mouse) models of amyloidosis that closely resemble the pathology observed in AD patients (for review see Higgins and Jacobsen, 2003).

The *NSEAPP* mouse, first described by Quon et al. (1991) carried the human APP751 isoform (Quon et al., 1991), with enhanced Aβ burden, but only few mature NPs (Higgins et al., 1994, 1995). Age related cognitive impairments of tasks that require hippocampal integrity were described (Moran et al., 1995).

The *PDAPP* mouse carried human APP gene containing the V717F mutation. Profound enhancement of Aβ production was observed, including NPs, CNS inflammatory markers such as activated astro- and microglia and synapse loss (Games et al., 1995). Cognitive decline was observed before plaque deposits could be detected (Dodart et al., 1999; Chen et al., 2000).

The *Tg2576* mouse, first reported by Hsaio et al. (1996), was composed of the “Swedish” isoform APP695 containing the mutation K670N/M671L. Mature NPs were detected, especially in the hippocampal formation (McGowan et al, 1999). No NFTs were observed. The amount of Aβ deposited in AD brain did not correlate with the degree of clinical symptoms (Gomez-Isla et al., 1996a; Hyman et al., 1993) or the amount of neuronal loss

(Gomez-Isla et al., 1996). Cognitive impairments were observed before plaque depositions were detected. However, age-related cognitive decline was reported in a few studies (Chapman et al., 1999; King et al., 1999; Westerman et al., 2002).

The *APP23* mouse is composed of a hAPP751 construct containing the K670N/M671L mutation. Activated astro- and microglia (Bornemann and Staufenbiel, 2000; Bornemann et al., 2001) and neurodegeneration in the hippocampal region were detected, which correlated with the plaque load (Calhoun et al., 1998). Furthermore, development of cerebral amyloid angiopathy (CAA) has been detected (Winkler et al., 2001). Age-dependent memory impairments in hippocampus dependent tasks were found before NPs formation (Sturchler-Pierrat et al., 1997).

Mice, over-expressing the PS1 gene mutation M146L were crossed with Tg2576 mice to generate a double mutant animal model, the *PSAPP* mouse (Holcomb et al, 1998). The pure PS1 mutant mouse has shown an increase in Aβ 1-42 burden but no plaque deposition (Duff et al., 1996). In combination with Tg2576 mice, double mutant mice show profound increase in plaque pathology in forms of plaque size and NP distribution including gliosis (Holcomb et al, 1998). Moreover, first formation of NP deposits was accelerated from 9-12 months in Tg2576 to 3 months in the PSAPP mice (McGowan et al., 1999; Wengenack et al., 2000). Influence on cognition has been described ambiguously (Holcomb et al., 1999; Arendash et al., 2001). It is assumed that the clearest cognitive deficits emerge at the age of 15-17 months (McGowan et al., 1999; Matsuoka et al., 2001; Jantzen et al., 2002).

APP_{SL}, containing the “Swedish” mutation K670N/M671L, together with the “London” mutation V717F, express enhanced Aβ and show activated microglia. However, no NP deposits were found in these mice (in-house data). Double mutant forms, APP_{SL} x $PS1_{mut}$ develop stronger inflammation than APP_{SL} and develop plaques at the age of 6 months (personal communication). Transgenic controls for the double mutant mice, APP_{SL} x $PS1_{wt}$, show similar pathological phenotype as the $APP_{SL.}$

No NFT formation was detected in the presented animal models of amyloidosis.

1.2.2.2. Tau pathology

Mutations in the tau gene have been discovered as for example in frontotemporal dementia and Parkinson`s disease (FTDP) (Hutton et al., 1998; Spillantini et al., 1998) that directly link abnormalities in tau to neurodegenerative diseases, yet there are no tau mutations described in AD.

The *JNPL5 (tau)* mouse contains the most prevalent tau mutation associated with FTDP, the FTDP-17 mutation P301L (Gotz, 2001; Lewis et al., 2000). The animals develop NFTs but also hypolocomotion and muscular weakness (Lewis et al., 2000).
Andorfer and colleges (2003) generated the *htau* mouse that develops pathology from non-mutant human tau. Age-related accumulation of human NFTs was reported.

1.2.2.3. Model validity: comparison of amyloidosis and tau pathology in human AD cases versus mouse model

1.2.2.3.1. Animal model of amyloidosis

In AD patients, high level of extracellular Aβ amyloid and gradual deposition of NPs surrounded by dystrophic neuritis, astrocytosis and gliosis characterise the pathological processes summarised as amyloidosis in AD (Ghiso and Frangione, 2002). First deposits are found in the hippocampal formation (Mann, 1989; Thal et al., 2000), further extending throughout the temporal and parietal lobes with high NP deposition throughout the complete cortex and subcortical areas at the latest stages of AD (Mann, 1989). All animal models presented meet *face* and *predictive validity* for the high degree of pathogenic Aβ42 burden. All but NSEAPP mutant mice developed profound NP deposits with strong hippocampal implication. In addition, dependent on respective mutation, dystrophic neuritis and inflammatory processes were observed. As a consequence, these models meet *face* and *predictive validity* for both, aberrant $Aß_{42}$ burden and NP deposits. Schenk et al (1999) grounded his milestone investigation towards active vaccination to treating amyloidosis by use of PDAPP mice. The activity and potential toxicity of γ-secretase inhibitors and anti-inflammatory neuroprotective agents is tested on the basis of these animal models (Lanz et al., 2003). In addition, cognitive impairment, which depended on hippocampal integrity, was observed in some of the animal models (Chen et al., 2000; Moran et al., 1995), resembling features of cognitive decline observed in AD patients. Taken together, animal model of amyloidosis possess high *face* and *predictive validity* for several aberrant changes in AD and to a certain degree *construct validity* for the amyloidosis of AD if studied exclusively.

1.2.2.3.2. Animal model of Tau pathology

NFT formation has been observed in mice expressing mutant human tau transgenes (Gotz, 2001), making them good models for inheritant tauopathies with *face* and *predictive validity*, but less valuable to the study of pathogenesis in AD.

1.2.3. Cholinergic neurodegeneration

Extensive neurodegeneration of cholinergic cells in the BFCS, as it is the case in AD, has not been demonstrated in the aforementioned genetic animal models (Irizarry et al., 1997; Takeuchi et al., 2000).

As outlined before (see 1.2.1. cholinergic system), cholinergic hypofunction represents the central neurodegenerative process in AD (Coyle et al., 1983; Davies and Maloney, 1976; Perry, 1986; Rossor et al., 1984; Whitehouse et al, 1982). Profound cholinergic cell loss is related to mnemonic degradation in AD patients (Bartus et ;al., 1985; Davies and Maloney, 1976; Hefti et al., 1984). Histopathological correlation with neuropsychological data relies upon post-mortem assessment of cholinergic degeneration that may be temporally distant from time of cognitive assessment. Moreover, restricted means for pharmacological manipulation or incisions to patients limit investigation of the disease. Thus, ablation of cholinergic neurons as an animal model reflecting important parts of AD pathology is very beneficial for the study of cognition enhancing drug therapy (Berger-Sweeney et al., 2001; Collerton, 1986; Wiley et al., 1991). In addition, synergistic activity or amplification of pathological processes induced by cholinergic neurodegeneration can be investigated in conjunction with transgenic animal models (amyloidosis) and may enlarge knowledge of pathology and behavioural outcome.

Intracerebroventricular (i.c.v.) application of neurotoxins can generate neuronal loss that partially resembles that observed in AD (Collerton, 1986). An optimal animal model of cholinergic neurodegneration should imply high selectivity and profound ablation of cholinergic neurons (Olton and Wenk, 1997).

1.2.3.1. Cholinergic deficit induced by standard neurotoxins

AF64A structurally resembles ACh and irreversibly blocks high affinity choline transport into synaptosomes (Fisher, et al., 1980; Rylett and Colhoun, 1980). However, i.c.v. or direct intraparenchymal application into the BFCS and hippocampus not only destroyed cholinergic, but also non-cholinergic neurons (Dunnett et al., 1991; McGurk et al., 1987; Jarrard et al., 1985).

Excitotoxins such as *kainic (KA), quinolinic (QA) or ibotenic (Ibo) acid* deplete neurons by an overstimmulation of glutamatergic receptors. As a result, excitotoxic calcium currents destroy cell somata in the circumference of the injection area (Schwarcz et al., 1984). Lesions to the BFCS can reduce the number of cholinergic neurons and hippocampal cholinergic innervation

up to 50 % (Waite and Thal, 1996; for more details see review Olton and Wenk, 1997; Heckers et al., 1994). Hyperactivity was subsequently reported with QA and Ibo BFCS lesions (Steckler et al., 1993).

1.2.3.2. Cholinergic deficit induced by immunotoxins

A new approach to selectively lesion cholinergic neurons within the BFCS in mice was introduced by Berger-Sweeney and colleges (2001). They adopted the mechanism of the immunotoxin 192-IgG-saporin (Wiley et al., 1991) specifically developed for rats, and created the anti-murine immunotoxin mu p75 SAP. Mu p57 SAP is a chemical conjugate of a rat monoclonal antibody (mAb) nerve growth factor (NGF) receptor (p75) and the ribosome-inactivating protein saporin. The immunotoxin targets the p75 NGF receptor (NGFR) localised on cholinergic cell bodies in the BFCS and on their nerve cell terminals in neocortex and hippocampus (Springer, 1988; Torres et al., 1994; Wenk et al., 1994). BFCS neurons have been shown to express by far the highest level of p75 NGFR immunoreactivity in the brain of adult rodents (Bothwell, 1991; Kiss et al., 1988; Yan and Johnson, 1989). Following i.c.v. injections, mu p75 SAP is taken up ("internalisation") by cholinergic neurons and transported to the cell body by retrograde transport. The saporin catalytically inactivates ribosomes and irreversibly inhibits protein synthesis resulting in the apoptotic cell death (Holley et al., 1994; Wiley, 1991, scheme depicted in figure 20). The specificity for cholinergic neurons allows selective lesions while other neurons remain intact (Berger-Sweeney et al., 2001; Rossner et al., 2000). Reduction of ChAT activity, as a cholinergic marker, was shown in the hippocampus up to 75 % and in the neocortex up to 42 % after i.c.v. injections of mu p75 SAP (Berger-Sweeney et al., 2001). To our knowledge, no publication refers to local application of mu p75 SAP into discrete brain areas, so far.

1.2.3.3. Model validity: comparison of cholinergic pathology in human AD cases versus mouse model

Ablation of cholinergic cells to mimic the cholinergic hypofunction in AD patients can be achieved to a certain degree with standard neurotoxins, as outlined previous section. *AF64A* was designed to target the cholinergic transport systems. Degeneration of cholinergic cells with AF64A and with excitotoxins was reported for up to 60 % (Altman et al., 1985; Boegman et al., 1985; El-Defrawy et al., 1985). In comparison, approximately 90 % loss of cholinergic neurons was shown in human tissue of AD patients (Whitehouse et al., 1982). Moreover, specificity for cholinergic neurons was not shown with any of these neurotoxins.

All standard neurotoxins presented only partially meet *face* and *predictive validity,* because (1) cholinergic neurodegeneration is less pronounced than in AD cases and (2) non-specific cell damage confounds the animal model of a pure model of cholinergic neurodegeneration.
High selectivity for cholinergic neurons within the BFCS was found with the immunotoxin mu p75 SAP. The degree of cholinergic ablation almost reaches degeneration values of tissues from AD patients. Thus, lesions with mu p75 SAP meet *face* and *predictive validity* as an animal model to study the cholinergic neurodegneration of AD. The model meets *construct validity* if cholinergic neurodegeration and cognitive impairments as a result of the lesion is studied as a model for the cholinergic hypofunction in AD exclusively. The clear removal of cholinergic BFCS neurons constitutes an important animal model and can be used in conjunction with knockout models for the study of cholinergic influence on behaviour, neural plasticity or plasticity of other systems in response to loss, replacement therapies or drug effects and dependence.

1.3. Aim of the thesis

1.3.1. Evaluation and validation of appropriate behavioural models of learning and memory to define mouse phenotype (Chapter II)

Mouse behaviour is a phenotype of considerable interest for comprehensive assessment of neurodegenerative changes in any mouse model. There is no standard implementation of any single test that is deeply entrenched and most tests are done in a manner that is unique in each laboratory (Wahlsten, 2001). There is considerable evidence that parametric differences in the details of many tasks are important for the outcome of genetic experiments (Boehm et al., 2000; Chesler et al., 2002; Gerlai, 2001) and minor changes in a task can sometimes yield large benefits for the value of a test.

Therefore, the study should introduce a broad overview of behavioural models. Neuroanatomical substrates and type of learning and memory should be compared for respective model. Studies comparing motivational aspects, together with task modifications enhancing intrinsic motivation of the animal to acquire the task should optimise testing procedure for most unambiguous and least confounded data read out. In conclusion, this should enlarge comprehension for the selection of behavioural models in AD, and for other investigational applications.

1.3.2. Evaluation and validation of animal models in a longitudinal study (Chapter III)

Central aim of the study is the development of new animal models to mimic symptoms of AD in mice. Therefore, a novel concept utilizing a combination of genetically manipulated mice (C57BL/6 wild-type, APP mutant and APP x PS1 double mutant mice) and selective cholinergic lesions of the NBM should be developed. To achieve selective degeneration, a novel type of immunotoxin (mu p75 SAP) should be investigated to induce discrete ablation of cholinergic neurons in the NBM.

Taken together, the results obtained from the tests should increase our understanding of learning behaviour in mice, and the impact of these mouse models on cognition. The study should provide a useful extension of already established mouse models of AD, thus relating closer to the clinical symptomatology in AD patients. This should support research and development of new therapeutic approaches for the treatment of DAT.

Chapter II: Evaluation and validation of appropriate behavioural models of learning and memory to define mouse phenotype (Methodical chapter)

2.1. Overview from the literature (Part I)

Behavioural models of learning and memory characteristically imply acquisition, consolidation and recall of information that is given to a subject. The majority of models admit testing of these steps separately (Riekkinen et al., 1998). The ability to store and recall information is essential to adopt behaviour to continuously changing environmental conditions. At least two main forms of memory are described, declarative (explicit) and procedural (implicit) memory (Zola-Morgan & Squire, 1993; Schacter, 1987; Squire 1982; Tulving 1985; Markowitsch, 1998; Tulving & Markovitsch, 1998). Evidence suggests that during learning, neuroanatomic structures for both implicit and explicit memory systems are activated simultaneously and that in some learning situations competitive interference exists between these two systems (Fleischman and Gabrieli, 1999; Packard and Knowlton, 2002; Sherry and Schacter, 1987; White and McDonald, 2002).

2.1.1. Explicit memory, spatial memory

Explicit or declarative memory affords the capacity for conscious acquisition and recollection about facts (semantic memory) and events (episodic memory). Typical to this form of memory is the spatial working (WM) and reference (RM) memory, or in general, encoding of spatial memory (Kim & Levin 1996; Morris et al., 1982; Seamans & Phillips, 1994; Watanabe et al., 1992).

2.1.1.1. Neuroanatomy

Explicit memory is related to three critical brain regions (due to the important position of the hippocampal formation, this form of memory often relates to the “hippocampal system” as a synonym for all brain regions involved):

1. The medial temporal lobe (MTL) with the hippocampal formation as the central area for this type of memory. The hippocampus admits of the subdivisions subiculum, gyrus

dentatus, perihippocampal region including entorhinal cortex, perirhinal and parahippocampal cortex.

2. The medial diencephalons with mammilary bodies and the medial and anterior thalamus.
3. The BFCS with the NBM, MS and vdb (Tulving and Markovitsch, 1997; Zola-Morgan and Squire, 1993).

Moreover, cortical structures are involved, receiving and storing information from aforementioned brain regions. The complete procession flow is related to the "papez-circuit" (depicted in figure 5; Papez, 1937), starting with sensory inputs from the environment and processed sensory impressions from higher associative cortices to the entorhinal cortex. The tractus perforcance forms an important pathway from the entorhinal cortex to the hippocampus. Projection terminates to the mossy fibres of the gyrus dentatus within the

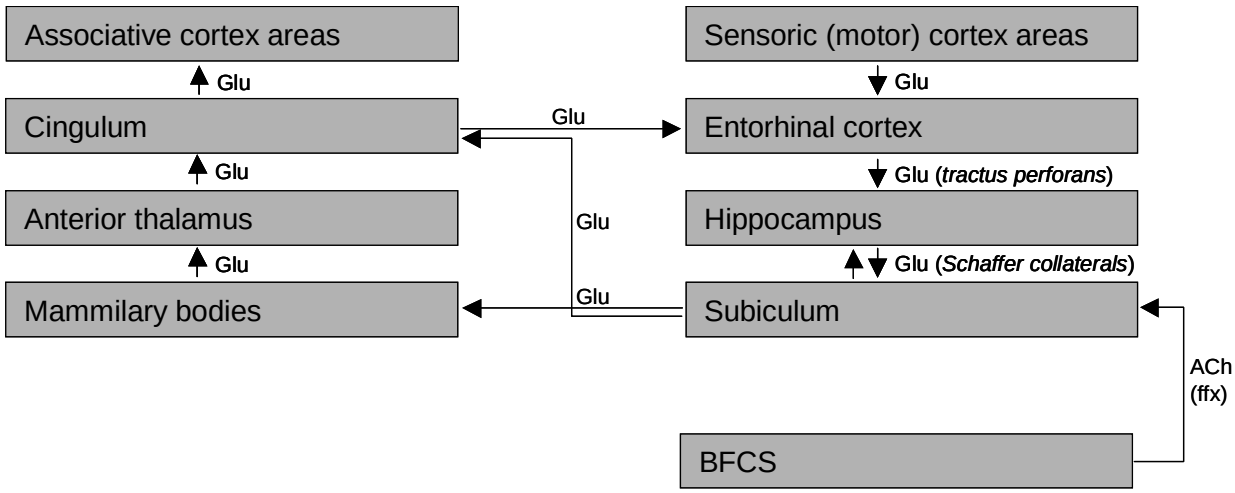


Figure 5: Scheme of projection areas within the **papez-circuit**. Main input structures to the hippocampus, the central area within the cicuit, are the entorhinal cortex, projecting sensori (-motor) information from the environment via tractus perforans, and the subiculum, receiving cholinergic afferents from the BFCS via ffx. Information process follows indicated brain areas including various feedback loops (not depicted). Main neurotransmitter (NT) system implicated constitutes the glutamatergic (Glu) system, however GABA-ergic, dopaminergic and other NTs are involved (not depicted).

hippocampus. Mossy fibres project to the CA4 (in humans) and CA3 pyramidal cells that are interconnected with CA2 and CA1 cells via Schaffer collaterals. The hippocampus receives another important input from the BFCS. The fimbria and the dorsal fornix (ffx) bundle strongly connect the cholinergic MS and vdb and the hippocampus ("septo-hippocampal system"). Afferents from the NBM reach the hippocampus indirectly by projections to the amygdala that is closely connected to the hippocampus. All inputs to the hippocampus are processed in the CA1-CA3 regions and further projected to the subiculum, the central output

structure of the hippocampal formation. Projection leads to diencephalic structures, to the cingulum and finally to higher associative cortices in the frontal lobe of the brain or back to the hippocampus (papez-circuit depicted in figure 5). Associative areas generate further procession and integration of the memory traces to form “memory” or “remembering”, as referred to in ordinary language. Most areas form reciprocal and interconnective projections for procession refinement (not depicted).

2.1.1.2. Molecular basis of memory

Procession in the hippocampus contains modulation of synaptic connections, enhanced transmitter release, up-regulation of glutamategic NMDA-receptors, their sensitivity and duration of the post-synaptic potentials. The resulting amplification of information transmission is related to long-term potentiation (LTP). LTP is strongly anticipated to represent the cellular basis for learning and memory (“acquisition” and “consolidation”) (Bliss & Lomo, 1973; Tulving & Markovitsch, 1997, 1998; Nadel & Moscovitch, 1997; Moser et al., 1998; Sanes & Lichtman, 1999).

2.1.1.3. Pathological changes within the papez-circuit in patients with AD

At the early stages of AD, the pathology is largely restricted to the hippocampus and nearby medial temporal cortical structures (Braak and Braak, 1991). The first histological changes observed in AD relate to the tractus perforance and a disruptive connection between the entorhinal cortex and the hippocampus (Hyman et al., 1984). Destruction of cholinergic neurons in the BFCS affects important projections to the hippocampus and connections to the amygdala. Taken together, critical afferents to the hippocampus degrade in AD. As a consequence, integrative projections to the hippocampus and processing of recent memory traces, especially mapping of spatial information, gradually decline. In addition, NP deposits and NFTs within the entorhinal cortex and hippocampus occur early in the course of the disease (Arnold et al., 1991; Davison, 1987; Hyman et al., 1984).

2.1.1.4. Type of memory

Dependent on frequency and intensity of synaptic activation, short-term memory (STM) gradually transforms to long-term memory (LTM), which is stored in higher cortical regions. STM is also referred to WM, a term applied to the type of memory that is active and relevant only for a short period of time (Honig, 1978). Spatial WM is considered to imply an important behavioural function of the hippocampal system (Jackson et al., 1998; Olton et al., 1979a,b).

It has been demonstrated that there is a significant and neuroanatomically selective involvement of the hippocampal system in WM procedures in which discriminative stimuli were extra-maze cues (see 1.1.5.1.). WM is either forgotten within a short period of time or captured due to reactivation into LTM or RM. This form is a permanent inscription on neuronal circuitry due to learning.

An important behavioural function of the hippocampal system emphasises spatial organised behaviours, especially those using cognitive maps (O`Keefe et al., 1976; O`Keefe, 1979; Bures et al., 1997). The internal representation of the environment in forms of a cognitive map was found for CA1 and CA3 hippocampal cells, thus they are referred to as “place cells” (O`Keefe, 1979; O`Keefe and Dostrovsky, 1971). The place where one of these cells fires is called “place field”. They fire in respect to the animals position and locomotion.

2.1.1.5. Behavioural models to study explicit memory

The degree of hippocampal involvement in learning and memory can depend upon the type of cues represented and the learning procedure. These tasks are sensitive to either hippocampal damage or destruction of afferent structures into the hippocampus (Leherizy et al., 1993; Olton et al., 1978; Perry et al., 1977), whereas lesions to brain areas within the ventral loop has little effect on these tasks (see section 2.1.2.). In the following, typical hippocampus related behavioural tasks are presented.

2.1.1.5.1. Allocentric learning

Orientation in space can be accomplished by using landmarks or “cues” available in the external environment, so called “extra-maze cues”. The allocentric coding system is based on memory for the target coordinates relative to remote extra-maze cues, which leads to the coding of absolute space within a spatial map. Path integration, i.e. a permanent re-update of changing cue relations due to movement, guaranties continuous knowledge about the position (Moghaddam and Bures, 1996). Behavioural models of allocentric learning and memory imply acquisition of given extra-maze cues in relation to a given aim, such as escape or location of a food reward. A simple test for the allocentric nature of a task is shifting or rotating the maze once the task was acquired. Animals that rely on allocentric orientation display impaired recall of the learned task in a new spatial environment. Allocentric learning is opposed to egocentric learning (see section 2.1.2.4.1.), which is also based on orientational aspects. Although both forms of learning require navigation in space, the term “allocentric learning” is often used in synonym sense of “spatial learning” for at least two reasons: first,

allocentric orientation and learning is based on visual information taken from the environment. Learning refers to spatial cues and the representation of the environment as a cognitive spatial map (O`Keefe and Nadel, 1978). Second, allocentric learning requires information processing in the place cells of the hippocampal formation, which is not the case for egocentric learning.

2.1.1.5.2. Working memory (WM)

In a WM procedure, stimulus information is useful for one trial of an experiment, but not for subsequent trials (Honig, 1978). One example is the protocol of a radial arm maze (RAM) task, where four out of eight arms are baited (Olton and Samuelson, 1976). The animal reaches highest search strategy efficacy, if no arm is revisited within the same trial. Previous arm visits have to be kept in mind to avoid unrewarded arm visits, independent of the animals` search pattern. This flexible stimulus-response requirement is characteristic of WM procedures. The animal must remember not only which stimuli have been presented, but also when they were presented.

2.1.1.5.3. Reference memory (RM)

In a RM procedure, information is useful for many trails and usually for the entire experiment (Honig, 1978; Simard and Reekum, 1999). To stay in the example with the RAM, the animal must remember the position of the baited arms, independent of his WM that only holds up information about the latest arm visits. Visits to unbaited arms are considered as RM errors.

2.1.1.5.4. Standard behavioural models of allocentric WM and RM

The present section emphasis the description of the type of memory tested in the respective tasks, rather than illustrating the set-up and procedure of the test, which will be accomplished later in the current chapter (Part II), or by referring to the relevant literature. A summary of the present section is depicted in table 1, p.44.

2.1.1.5.4.1. Radial Arm Maze (RAM)

The experiment, first introduced by Olton and Samuelson (1976), was created to study WM and RM by means of allocentric navigation through a maze bearing several compartments that were appetitively motivated (Olton and Samuelson, 1976; Olton et al., 1979). Assessment of WM and RM was described in previous sections. A series of experiments has demonstrated that the animals identified and remembered each arm on the basis of the extra-maze cues, which defined its location in the test room. The standard allocentric RM task comprises a win-

shift version, where successful performance is achieved if the subject avoids re-entries by shifting to other arms than previously visited. One set demonstrated the unimportance of alternative strategies such as response chains (Olton and Samuelson, 1976; Maki et al., 1979). A series of lesioning experiments accomplished neuroanatomic dissociations for the RAM task. Groups of rats with bilateral lesions of the neocortex (Olton et al., 1978), caudate nucleus, sulcus frontal cortex, medial frontal cortex (Becker et al., 1978) or amygdala were tested in the RAM task. Only cognitive deficits produced by the damage in the hippocampal system were found, while the other lesions spared learning in the RAM.

2.1.1.5.4.2. Morris Water Maze (MWM)

The task was designed to test allocentric RM in rodents (Morris, 1984_J). The animal navigates through the cold water in search of a hidden platform for escape. It must remember one single place of the hidden platform by being subjected to four consecutive trials with different starting positions during one daily session. This procedure is implemented with the rationale to randomly assign the animal to four directions in the maze in order to avoid habit learning and to equalise the task severity for every subject participating the test. During one session, a progressive improvement from trial to trial is observable. However, performance in the first trial on a given task is lower, compared to performance in the last trial on the previous day. (personal observations and discussions). In order to evaluate the amount of learning within one session, a fifth trial can be implemented that is identical with the first starting position. In this case, definition of WM should be enlarged from “within-trial memory” to “within-session memory”. This modification should deliver information about WM procedures within the MWM and thus enlarge utility of the task, which was originally designed to only test RM. Assessment of WM in the MWM is also conductable with another protocol, containing daily sessions of two trials, one presentation trial, one testing trial. The platform has to be moved daily to different locations (Janus, 2004). This procedure requires naïve animals lacking previous experiences with a RM test arrangement.

2.1.1.5.4.3. Barnes Maze (BM)

BM represents a “dry” equivalent of the MWM spatial learning task (Gerlai, 1999; Steckler et al., 1993). A circular platform was designed that requires discrimination of a particular place (Barnes, 1979; Inman-Wood et al., 2000; King et al., 1999; Pompl et al., 1999). In addition to allocentric RM, search pattern analysis also allows assessment about the use of WM (Pompl et al., 1999). The test meets the criterion for a “matching-to-location” arrangement, where

animals have to discriminate between false and correct locations (Mumby et al., 2002). Animals search for an escape motivated by natural exploratory behaviour, curiosity, preference for dark environments or to escape from artificially added bright light, fan or noise from a buzzer.

2.1.1.5.4.4. Holeboard (HB) and Cone field (CF)

Allocentric RM and WM can be assessed in the HB (Douma et al., 1998; Heim and Sontag, 1994; Oades and Isaacson, 1978; De Oritz et al., 2000). The task is appetitively motivated. A classical arrangement is rewarding four holes out of sixteen for rats (Froehlich et al., 1995; Gaspar et al., 1992; Hoyer et al., 1999; Oades and Issacson, 1978), or one out of four for mice (Brosnan-Watters et al., 1996; Brosnan-Watters and Wozniak, 1997; Galey and Jaffard, 1992; Wozniak et al., 1996).

The spatial CF task (van der Staay et al., 1990) has been developed on the basis of the HB task first described by Oades and Isaacson (1978). Holes were replaced by 16 cones with little cups on the tip where food pellets can be placed. The modification was introduced to avoid ambiguity such as indicated nose pokes or accidental hole visits. Visits to the cone are automatically defined as a learning response to the cone (Blokland et al., 1992; Blokland et al., 1998).

2.1.1.5.5. Other behavioural models to test hippocampus related learning and memory

2.1.1.5.5.1. T-Maze continuous alternation task (T-CAT)

Spontaneous alternation in the T-Maze has long been observed for multiple purposes (for review see Dember and Fowler, 1958; Dennis, 1939; Tolman; 1925). Exploratory and curiosity behaviour (Dember and Earl, 1957; Montgomery, 1951) but also memory and perception have been investigated with the model (Gerlai, 1998; Johnson et al., 1977; Morgan and Wood, 1943). Alternation is considered to reflect the animals` ability to explore its environment in most effective manner. T-CAT requires allocentric WM and spatial discrimination. Lesions to the hippocampus (Gerlai, 1998; Kirkby et al., 1967; Means et al., 1971; Roberts et al., 1962), entorhinal cortex (Ramirez and Stein, 1984), septum (Douglas and Raphaelson, 1966; Thomas, 1972), but also to the NBM depletion (Murray and Fibiger, 1986; Salamone et al., 1984), transection of afferent structures such as the ffx, or efferent pathways to the mamillay bodies of the hypothalamus and to the anterior thalamus impair alternation performance (Ameral and Witter, 1995). Performance of the T-CAT is not confined to the

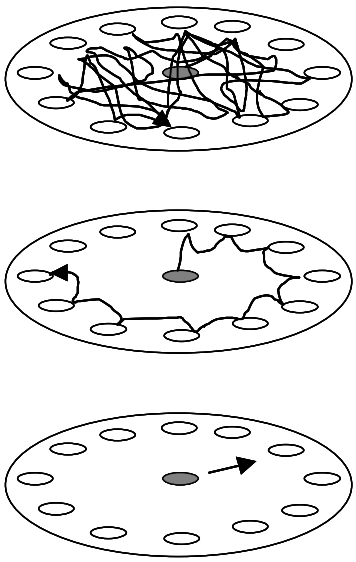
hippocampal formation. The prefrontal cortex and parts of the basal ganglia system are also implicated in spontaneous alternation performance (Divac et al., 1975).

2.1.1.5.5.2. Object recognition task (ORT)

A general classification of recognition memory distinguishes two different processes: memory for spatial context (allocentric memory) and memory for objects (non-spatial memory) (Kolb et al., 1994; Rampon et al., 2000; Steckler et al., 1998 a,b). The first one includes pivotal brain regions in the papez-circuit, the second one includes temporal cortical association areas, rhinal cortex and md thalamic nuclei (Mumby et al., 1996; Myhrer, 1988; Phillips et al., 1988; Steckler et al., 1998b). The task requires WM holding up information about the familiar object within a critical time frame (Ennaceur and Delacour, 1988) and it avoids the learning of a rule (Dodart et al., 1997). Integrity of the cholinergic system is required to perform the task (Dodart et al., 1997). Learning of the ORT task can be categorised to a "matching-to-sample" arrangement, which specifically depends on hippocampal integrity (Bunsey and Eichenbaum, 1996; Dodart et al., 1997). The intensity of memory storage can be tested by variation of the inter-trial interval (ITI) between the first presentation trial T1 and the challenge trial T2 where a familiar object is replaced by a new object. No positive or negative reinforcer is needed to motivate the animal. The task is based on spontaneous explorative behaviour to novelty (Dodart et al., 1997; Ennaceur et al., 1989; Myhrer, 1988).

2.1.1.5.6. General rules for allocentric learning tasks

Typical to allocentric learning is a change in search pattern with progressive knowledge about the task requirements. The search pattern is categorized into three different types, which are assessable from video tracking plots where x and y coordinate points were connected sequentially for individual animals (Barnes, 1979; Fox et al., 1998; Sanchez-Alavez et al., 2000). Animals usually search initially in *random* pattern, they often cross the centre of the maze area and switch from the edge to central parts of the area. With more experience, a *serial* search strategy is observable. They approach from one hole or arm to the adjacent one with no or little centre crossing. In case of the MWM, only one aim is possible, thus in this stage, animals improve their strategy by a more precise restriction to the periphery, where the platform is placed. The third searching category relates to actual *spatial* or allocentric knowledge about the aim. The animal targets the aim with high degree of accuracy and little or no false additional inspections. This sequence indicates that the animal becomes more and more accurate and efficient locating the correct position of the aim.



Random search pattern
Animals search without observable strategy, many centre crossings and switching from the edge to central parts

Serial search pattern
Animals search at the edge from hole to hole with little centre crossings

Spatial search pattern
Direct approach to the goal, no or little false inspections

Figure 6: Development of search pattern in allocentric learning tests; illustration is related to set-up of the original Barnes Maze behavioural task. Grey circle represents the starting position, black lines indicate travelling pathways, holes are located at the periphery of the arena.

Although all tests presented in the allocentric section were proved to relate on spatial orientation, egocentric components can be evaluated using a modified testing protocol (Liu et al., 1994; Moghaddam and Bures, 1996; Pompl et al., 1999; Sutherland and Dyck, 1984). Such a modification was shown for the MWM task. The animals were tested for example in complete darkness and assigned to one single starting position in several consecutive trials. The study has shown, that the animals were able to acquire the task by kinaesthetic information. They learn the position of the platform as a habit, with no conscious effort. However, control groups learned the platform position markedly faster in the presence of allocentric cues. A non-spatial modification can also include cues proximal to the target (Ahlander et al., 1999; Janus, 2004; Morris, 1984). These experiments show that not the maze, but the set-up and procedure of the test determine the nature including the neuronal substrate of memory assessed.

2.1.2. Implicit memory

Implicit memory includes learning of non-conscious skills, habits, priming, some forms of classical conditioning and stresses high emphasis on motor learning (Zola-Morgan and Squire, 1993; Melia et al., 1996).

2.1.2.1. Neuroanatomy

Implicit memory is also arranged in a feed back circuit, the "ventral loop", with two central brain regions and numerous interactive brain areas:

1. medial prefrontal cortex (mPFC) (dorso-lateral cortex in humans) including subregions such as agranular medial cortex (AGM), dorsal anterior cingulated area (dACA), infralimbic cortex (IL) and prelimbic cortex (PL)
2. ventral part of the subcortical basal ganglia (BG) system: nucleus accumbens (NAC), ventral pallidum (VP), substantia nigra pars reticulata (SNr), medio-dorsal thalamic nucleus (mdTh)
3. ventral tegmental area (VTA), amygdala, (spinal cord (SC))

(Brodman, 1909; Alexander et al., 1986; Packard and Knowlton, 2002)

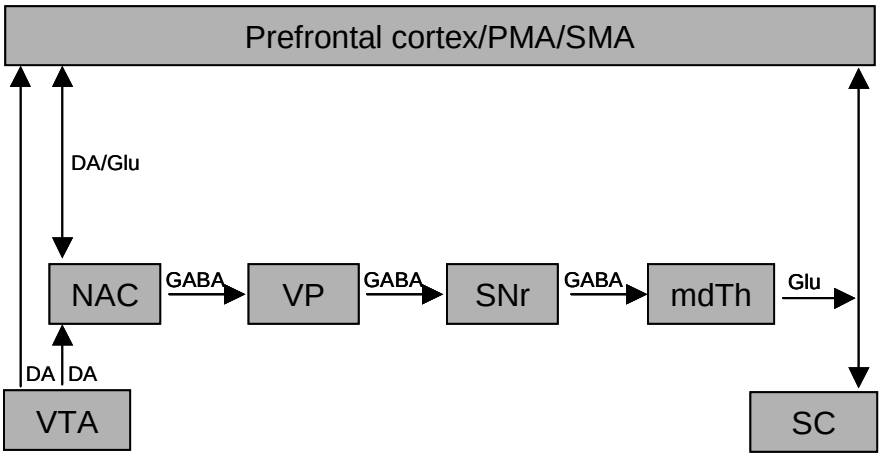


Figure 7: Scheme of projection areas within the **ventral loop**. Pivotal structures are the subcortical nucleus accumbens (NAC), and the prefrontal cortex including accessory areas. VP:ventral pallidum, SNr: substantia nigra pars reticulata, mdTh: medial thalamus, SC: spinal cord, VTA: ventral tegmental area

The BG occupies the main part of the ventral loop. Herein, the NAC represents the central input structure within the BG. It receives afferents from cortical pathway and from the VTA, thus mediating an important linkage between the cortical and the mesencephalic system. Information processing follows the VP, SNr and the mdTh, the central afferent relais for cortical projections (Packard and Knowlton, 2002; Rose and Woosley, 1948$_l$). These projections terminate mainly but not exclusively in the mPFC (Kievit and Kuypers, 1977; Golman-Rakic and Porrino, 1985; Ilinski et al., 1985). In addition to cortical projections, the

BG project to motor neurons in the SC, the executive instance of motor coordination. These inputs contribute to complex behavioural (motor) performance.

2.1.2.2. Type of memory

The mPFC has predominat functions, because it represents the only neocortical area within this circuit. The precentral location within the motor cortex characterises the mPFC for motor processes (Groenewegen et al., 1997). Moreover, the mPFC is influenced by limbic connections to the subregions AGM, dACA; IL and PL and by afferents from the NAC, amygdala and the hypothalamus. These projections and interconnections to association areas and further brain regions allow multiple integrative information processing (Joel et al., 1997). A complex behavioural repertoire refers to this brain area, such as “decision making”, a selection of “good” versus “bad” options, reaction and instant adoption of behaviour to changing situations, rule learning, learning of skills and habits, or planning (Bechara et al., 1994, 1996, 1997). An important basis for these features is the WM of the mPFC that keeps relevant information actual for a critical period of time (Fuster, 1990; Baddeley, 1992; Goldman-Rakic, 1992; Bechara et al., 1998). Destruction or dysfunction within this area is referred to pathophysiology and psychiatric changes such as schizophrenia or perseverative behaviour (Adams et al., 1997; Carter et al., 1996; Goldman-Rakic & Selemon, 1997; Karreman & Moghaddam, 1996; Moghaddam et al., 1997; Verma & Moghaddam, 1996).

Within the BG, a critical focus is put on the NAC and its contribution to implicit learning and memory. The NAC is referred to the “neuronal interface” as it integrates information from motoric and limbic/motivationally characterised brain areas (Annett et al., 1989). Several investigators claim, this area is “putting will into action” (Annette et al., 1989; Kim and Levin, 1996) or changes locomotor activity dependent on motivational level (Pijnenburg & Van Rossum, 1973; Robbins & Everitt, 1982). The NAC is also characteristic for the memory of biological reinforcement in reward related behaviour such as appetitive conditioning or drug addiction (Di-Chiara et al., 1999; Kalivas & Nakamura, 1999; Mark et al., 1999; McBride et al., 1999).

The BG is also critical for stimulus-response (S-R) associations or habit learning. Therein, a reinforcer only modulates the strength of learning, but is not itself represented in the association formed (for review see Packard and Knowlton, 2002; Graybiel 1998; Knowlton et al. 1996; Packard et al., 1989;White, 1997).

2.1.2.3. Pathological changes within the ventral loop in AD

The brain areas within the ventral loop remain relatively unaffected in the early stages of AD (Devi and Silver, 2000; Irrizary et al., 2001), which is clinically reflected in the spared implicit memory of AD patients (Postle et al., 1996; Jelicic et al., 1995). Every day habits are still intact, such as getting dressed, eating and drinking or orientation in familiar surroundings. It has been reported that patients are able to even navigate as a habit in complex well-known paths, but they rather forget having passed the path. In the later course of the disease, NP depositions, NFTs and inflammatory processes were observed in the ventral loop, especially in cortical brain areas (Mann et al., 1988; Hyman et al., 1984). In this stadium, patients even loose their habits and skills of every days life, and need to be placed in residential care or institutionalised for continuous supervision and help of caretakers.

2.1.2.4. Behavioural models to study implicit memory

Learning tasks for the study of implicit memory that require orientation in a given environment to successfully solve the task demands refer to egocentric orientation and memory. However, implicit memory is not confined to orientation but also to other forms of learning processes, such as S-R associations and habit learning. In the following sections, egocentric and other characteristic implicit learning tasks are introduced.

2.1.2.4.1. Egocentric learning

Acquisition of egocentric memory utilises kinaesthetic information from proprioceptive reception together with information from the vestibular system (Klatzky et al., 1990; Presson and Montello, 1994; Wang and Spelke, 2000). A continuous “spatial updating” or “path integration” during ego-motions automatically integrates the new position of a subject, which occurs without conscious effort, similar to a habit (Amorim and Strucchi, 1997; Farrell and Robertson, 1998; Hollins and Kelley, 1988; Jog et al., 1999). Egocentric orientation thus integrates kinaesthetic information flow and proximal visual cues, rather than distal cues as spatial landmarks. In a testing arrangement, displacement of distal cues should have no influence on egocentric orientation. (Riecke, 2003).

2.1.2.4.2. Standard behavioural models of egocentric learninig

2.1.2.4.2.1. T-maze or Y-maze delayed alternation task (T-DAT or Y-DAT)

The DAT uses a number of processes associated with mPFC function such as WM (Goldman-Rakic and Selemon, 1997), egocentric orientation processing (Kesner et al., 1988) and

inhibition of proactive interference and inappropriate motor responses (Mishikin, 1964; Kolb, 1990). The interposed delay between respective trials is strongly associated with the mPFC and implicit memory (Larsen and Divac, 1978; Van Haaren et al., 1985). Animals are appetitively motivated to learn the rule of the task (alternate between left and right arm) and need to keep up information about the previous arm visit during the delay. Some investigators interpose forced runs (forced T-DAT) in order to enhance the mnemonic demand of the rule, i.e. enter the opposite arm after the forced trial and alternate during free-choice trials (Goricanec and Kretschmer, 2004). Destruction or pharmacological manipulation of either the PFC (Bubser and Schmitd, 1990; Hauber and Schmidt, 1989; Zarth et al., 1997) or the striatum, an important part of the BG (Westerink and Mulder, 1981), was shown to impair DAT in the T-Maze. In contrast, lesions to the mPFC were not capable to affect allocentric memory in the RAM (Bubser and Schmidt, 1990).

2.1.2.4.2.2. T-CAT

As indicated in section 2.1.1.5.5.1., T-CAT performance is also assigned to brain regions within the ventral loop (Divac, 1975). Spontaneous alternation rates were reduced in mutant mice with vestibular damage (Douglas et al., 1979). Thus, implication of egocentric orientation in the T-CAT is possible.

2.1.2.4.2.3. Plus-Maze task (PM)

Animals are appetitively motivated to learn the fix place of one baited arm (e.g. west) within the maze and they are trained to approach the maze from the same start box (e.g. south) on each trial (Packard and McGaugh, 1996). After extensive training, the animals are challenged by shifting the start box to the opposite arm (north). Entry to the east arm is considered as “response” learning as the animal displays the same body turn as in previous training trials. Egocentric information serves for orientation. Correct entry of the west arm, however, is considered to refer to “place” learning as the animal corrects the shift using visual cues, i.e. allocentric information. Pharmacological manipulation within the BG impaired response learning in favour of place learning (Hicks, 1964; Ritchie et al., 1950), manipulations to the hippocampal formation had the opposite effect (Packard and Gaugh, 1996).

2.1.2.4.3. Other behavioural models to study implicit memory

2.1.2.4.3.1. S-R associations and habit learning (S-R A)

The win-shift task of the allocentric RAM task can be modified to a win-stay version. Animals obtain food by visiting four illuminated arms twice during a trial, while the other four arms remain unbaited and unlit (Packard et al., 1989). Learning relates to the association between arm and light, whereas the food only serves as reinforcer without a direct association to the stimulus. Lesions to the hippocampal system impair the win-shift task, whereas the win-stay S-R associated task remain unaffected. The opposite effect was observed in striatum lesioned animals.

2.1.2.4.3.2. Active/ passive avoidance task (AA, PA)

A foot-shock is being paired with a particular compartment in the maze. The animal responds to the stimulus (electric foot-shock) with an active or passive avoidance of the punishment, dependent on the task protocol. Performance of this conditioned avoidance behaviour was shown to depend on processing within the BG (Kirkby and Polgar, 1974; Winocur and Mills, 1969).

2.1.3. Comment

Although implicit and explicit memory refer to distinct anatomical substrates, it has been postulated that during learning both memory systems appear to be activated simultaneously (McDonald and White, 1994; Packard and McGaugh, 1996; White and McDonald, 2002). The “racehorse model” states a competitive relationship, with the system that comes up with the most valid and reinforced response being strengthened in its control of behaviour (Packard and Knowlton, 2002). Evidence of this hypothesis came up from studies lesioning important brain areas in one of these systems. Lesions to the hippocampal system enhanced acquisition of the BG related win-stay RAM behaviour (Packard et al., 1989; McDonald and White, 1993). On reverse, damage to the caudate putamen of the BG facilitated acquisition of a spatial Y-maze discrimination task (Mitchell and Hall, 1988). Furthermore, neuroimaging studies demonstrated initial stress on medial temporal lobe structures, which declines with training and shifts towards BG related learning. Dependent on the memory system addressed, this shift occurs early with implicit tasks, but also with excessive explicit task training (Poldrack, 2001).

Table 1: Overview behavioural models for testing rodent learning behaviour. Tests include working and reference memory, allocentric and egocentric memory testing, in combination or separately.

Behavioural models	Trial protocol example	Parameters measurable	Motivation to acquire the task	Type of memory				Brain areas mainly involved	Pivotal Reference
				WM	RM	Allocentric orientation	Egocentric orientation		
Radial Arm Maze (RAM)	Habituation for 3 day with food scattered in the maze Test: 4trials/day; 10consecutive days	n incorrect arm visits to never baited arms	Appetitive reinforcement (food restriction)		X	X		Hippocampal formation (papez-circuit)	Olton and Samuelson, 1976 Olton and Wenk, 1987
		n of consecutive arm visits (baited or unbaited) within one trial		X		X			
		Duration; speed		X	X	X			
		Searching pattern		X	X	X			
Morris Water Maze (MWM)	4trials/day; 5consecutive days swimming time: restricted to e.g.120s	Distance to platform Path lenght Trial duration; speed	Escape from the cold water (potentially threat of life)		X	X		Hippocampal formation (papez-circuit)	Morris (1984) Janus, 2004 Whishaw and Auer, 1989 Wozniak et al., 1996
MWM modification	5trials/day; 5consecutive days 5th same as 1st trial; Moving hidden platform from day to day	Distance to platform Path lenght Trial duration; speed		X	X	X			
	Probe trial: 30s without platform	Per cent of time spent in a quadrant			X	X			
Barnes Maze (BM) "Matching to location"	Habituation 2 days Test: 2 consecutive trials or with an inter-trial interval of 15min Often: training to criterion	Errors Distance Duration, speed Searching pattern n of trials to reach criterion	Natural explorative activity, preference for dark locations, artificially: fan, noise, bright light (No reinforcer necessary)	X	X	X		Hippocampal formation (papez-circuit)	Barnes, 1979 Barnes, 1988 Inmann-Wood Koopmans et al., 2003
Object recognition (ORT) "Matching to sample"(see saghal et al., 1992 "human")	Habituation to the maze for 2 days One presentation trial T1 (1 or 2 identical objects), One challenge trial T2 (1 familiar object from T1, 1 new object) ITI variable to estimate forgetting	Memory parameters: Discrimination indices d1, d2 Exploratory parameters: Exploration time for each object Habituation to the objects	Exploration; Novelty No reinforcer necessary	X		(X)	(X)	Hippocampal formation (papez-circuit); Striatum; rhinal cortex; Nucleus accumbens	Ennaceur and Delacour, 1988 Steckler et al., 1998 Sargolini et al., 2003

Holeboard or Cone field	Habituation 2 days Test: 4 trials/day; 10consec. Days Often: testing to criterion	Errors Distance Duration, speed Searching pattern n of trials to reach criterion	Appetitive reinforcement (food restriction), Exploration	X	X	X		Hippocampal formation (papez-circuit)	Oades and Isaacson, 1978 Brosnan-Watters et al., 1996 Blokland et al., 1998
T-Maze Spontaneous alternation (T-CAT)	15 consecutive free choice trials in one single session	Per cent alternation Time to choice Total time (per trial)	Exploration, curiosity; reactive inhibition; stimulus satitaion no artificial reinforcer necessary	X		(X)	(X)	Hippocampal formation (papez-circuit); Also: mPFC, striatum, raphe system, vestibular system, cerebellum	Gerlai, 1998 Dember and Fowler, 1958 Lalonde, 2002 Hull, 1943 Glanzer, 1953
T-DAT or Y-DAT	20 consecutive free choice trials with 30sec confinement in the start box (delay)	Per cent alternation N of errors (incorrect choices) Time to choice Total time (per trial)	Appetitive reinforcement (food restriction), Exploration	X			X	Ventral loop, striatum in general	Hauber, 1993 Bubser and Schmidt, 1990 Murphy et al., 1996
Forced T-DAT	10 free choice trials, 10 forced trials in random order								Goricanec and Kretschmer, 2004
Active or passive avoidance	Forgetting curve: ITI variable to test time frame of forgetting	Duration until leaving punished compartment to (active avoidance) Duration until punished compartment is entered (passive avoidance)	Conditioned avoidance behaviour	X	X		X	Ventral loop, striatum in general, amygdala	McIntyre and Reichert, 1971; Kurtz and Palfai, 1972;

2.2. Experimental validation of behavioural models for the study of AD (Part II)

2.2.1. Abstract

Motivational means and cognitive abilities were analysed in hippocampus-related learning tasks. 129S6/SvEvTac and C57BL/6J mice, contributing to the parental background of most of the genetically engineered mouse mutants served as subjects. The T-CAT revealed performance at chance level in 129S6/SvEvTac and low motor activity; C57BL/6J displayed alternation above chance level and high motor activity. In the mBM, 129S6/SvEvTac reduced the amount of incorrect hole visits, equally to C57BL/6J, whereas parameters displaying locomotor activity, i.e. distance and duration and speed were significantly were only improved by C57BL/6J. In the HB task 129S6/SvEvTac failed to acquire the task. C57BL/6J successfully reduced errors and distance. Both groups acquired the MWM task at similar level. In conclusion, the study revealed high motivational interference with learning performance in the "dry mazes" for 129S6/SvEvTac, indicated by high duration values, high distance due to thigmotaxis, low speed values and high standard error means for all parameters. In contrast, motivation to perform the Morris Water Maze task was sufficient to show improvement of all parameters with low standard error means in both mouse strains.

The second experiment assessed the issue of whether adopting a test to a given mouse strain or choosing the mouse strain that performs best in a given test. Performance of several mouse strains and groups were comparatively analysed for discriminative abilities and exploration in an "original" and modified version of the ORT. Impact of session duration was included into analysis. The study revealed that prolongation of session duration from 3 to 5 min had no influence on discrimination performance in any strain. Young, old C57BL/6 and APP_{SL} x C57BL/6 displayed low exploration and discriminative values in the original version, which was strongly ameliorated in the modified ORT version. OF1 and NMRI successfully performed the task in both versions, however showing enhanced discrimination and constant exploration performance with modifications. SJL only enhanced explorative activity with modification. They failed to discriminate in any ORT version.

In conclusion, it was shown that (1) C57BL/6 displayed high motivation to perform all tasks if set-up and protocol were adapted to the innate behaviour of the animal, (2) C57BL/6 acquired all tasks, (3) 129S6/SvEvTac was only motivated to perform the MWM, (4) OF1 and NMRI are high performers of the ORT, independent of modifications and (5) SJL failed to perform the ORT, irrespective of modifications.

2.2.2. Introduction

For the study of neurodegenerative processes, the mouse model gains increasing interest due to enormous advantages of genetic engineering techniques (http://jax.org/recources/documents/imr/). To understand the impact of gene-associated alteration of the nervous system, a comprehensive testing of behaviour in mutant but also in wild-type animals is required. Thereby, examination of learning and memory is a crucial focus, in particular for the investigation of AD. Tasks requiring integrative processing in the hippocampal system constitute a main emphasis in the study of AD. Behavioural models that meet this criterion were therefore chosen for the present studies.

Previous studies have demonstrated that learning performance in different mouse strains vary in dependence on the task applied (Crawley et al., 1997a,b; Lathe, 1996). Motor activity underlies almost every mouse behavioural paradigm. As a consequence, the learning task has to be chosen carefully by [1]motivational aspects to mouse behaviour. Low motivated testing set-ups may produce false negatives on behaviour of interests in the transgenic and knockout but also in the wild-type mouse strains. Highly motivated testing arrangement may induce anxiety and stress in the animals, which interferes with cognitive behaviour *per se*. The assessment of the optimal cognitive behavioural model of interest in turns of motivational aspects may thus help avoiding interference with these confounding factors.

In the first experiment of the present study, four hippocampus-related behavioural models were chosen to assess cognitive abilities in mice and their quality of motivational factors used to induce learning performance. Mice were tested in (1) the T-CAT, that is motivated by explorative behaviour, (2) in the modified BM task, that profits from the natural behaviour of rodents to avoid open and bright areas, (3) in the HB task that requires food restriction to motivate the animal in searching for an appetitive stimulus, and (4) in the MWM task that forces the animal to search for a platform to escape from the water. Two critical mouse strains, C57BL/6 and 129SvEv, for the engineering of mutant mouse models will be subjected to the tests. Background genes from parental strains, such as 129SvEv, the embryonic stem cell donator of most of transgenic mice may interact the mutated gene in a manner that could compromise interpretation of the mutant phenotype (Crawley et al., 1997). C57BL/6 is the strain commonly used for breeding and hybridisation with genetically shaped 129SvEv mice, thus contributing to the background of most of the transgenic or knockout mice (Crawley,

[1] In the present study, the term “motivation” refers to the sense in ordinary language, enlarging the restricted definition used by behavioural scientits.

1996; Crawley et al., 1997; Gerlai, 1996; Lathe, 1996). Knowledge about cognitive behaviour in the parental strains will shed light on this important issue of background interference. Learning performance, motivation and the consequence of stress will be comparatively assessed in these mice.

Another important aspect in measuring behaviour is stressed on the testing protocol. To have maximal utility, a good behavioural model should yield valid data for most of the commonly used mouse strains. It was reported that minor changes of the testing protocol can provide large benefits concerning the measured performance (Goldowitz and Koch, 1986; Wahlsten et al., 2003).

The ORT, a one trial paradigm based on spontaneous exploration, which is enhanced on novel objects compared to familiar ones, was chosen to study this hypothesis. No rule learning is necessary to perform the task properly (Ennaceur and Delacour, 1988). The animals show no persisting learning effect, thus the task can be repeated several times without interference with previous testing experiences. Application of drugs for pharmacological investigations can therefore be repeated in the same animal.

We demonstrated that the C57BL/6 mice supplied by Charles River are not suited for evaluating putative cognition enhancing compounds in the ORT, in a given protocol version (Prickaerts et al., 2002; Sik et al., 2003). These mice displayed very low object exploration levels that may bias the commonly used discrimination index toward extreme values and reduce the accuracy of the data obtained. However, we found that minor changes of the task protocol in accordance to innate mouse behaviour, potentially improved performance in C57BL/6 mice.

Aim of this second experiment was to improve utility of the ORT to study learning and memory in C57BL/6, the strain most frequently used for cognitive studies in wild-type and gene-targeted mice. The effect on young versus old C57BL/6 and gene manipulated APP_{SL} x C57BL/6 mice was investigated. Moreover, additional mouse strains, i.e. NMRI, OF1 and SJl/Orl, were investigated to identify mouse strains with high exploration times and good retention performance in the ORT. The impact of trial duration on exploration values was included into the study.

2.2.3. Material and Methods

2.2.3.1. Animals

Experiment 1a-d: 14 week old, at the beginning of testing, male C57BL/6J (n=12) (IFFA CREDO) and 14 week old 129S6/SvEvTac (n=12) (Taconic) mice served as subjects for the T-CAT, BM and MWM task. For the experiments in the HB, animals had to be confined to a restrictive dietary feeding protocol. With regard to the metabolic changes and potential influence on other tasks, a second batch of animals, 15 week old male C57BL/6J (n=7) and 129S6/SvEvTac (n=8), was used for the HB task.

Experiment 2a: As subjects served 8 week old (n=12) and 68 week old (n=12) male mice from the inbred C57BL/6J (IFFA CREDO) and 70 week old male APP_{SL} mutant mice (n=9), back-crossed with C57BL/6J in the 6^{th} generation APPSL x C57BL/6J. Breeding was performed in the BAYER in-house breeding facilities. All animals were housed individually in standard Makrolon® type II cages with sawdust bedding.

Experiment 2b: Male mice from the outbred strains OF1 [Ico:OF1(IOPSCaw)] (n=14) and NMRI [Ico:NMRI(IOPSHan)] (n=13), and male mice from the inbred strain SJL (SJL/OrIIco) (n=13) were supplied by Charles River (Sulzfeld, Germany).

2.2.3.2. Housing conditions

All animals were housed individually in standard Makrolon® cages of type II with sawdust bedding. Room temperature was constant (24±1C°), 60 % humidity and a 12:12 light/dark cycles was maintained, with lights on at 6:00 am. Food (standard chow, Altromine®) and water were delivered ad lib. except for the holoboard procedure. We used a food-rewarded procedure that required dietary restrictions. Therefore, the animals were kept on an "over-night deprivation“ schedule one week before formal testing started. Food was delivered ad lib. in the afternoon for a period of four hours a day, for the rest of the time the animals were restricted from food. The animals received Altromine® standard chow after the trials, cheese pellets (Bio-serv®) served as food reward in the trials. A body weight loss of about 85 % of their free feeding weight was accepted, and kept constant throughout the whole experiment. Experiments were performed in the same room where the animals were housed. A minimum of one week was given to the mice to familiarise to the experimental facility before an experiment started.

2.2.3.3. Experiment 1: Study of motivational factors to acquire hippocampus dependent tasks in C57BL/6 and 129S6/SvEv mouse strains

2.2.3.3.1. Experiment 1a: The T-Maze continuous alternation alternation task

2.2.3.3.1.1. Apparatus

The T-Maze apparatus (depicted in figure 8) was constructed by Sembach (Ratingen, FRG) according to the measures provided by Gerlai (1998). The walls of the maze, made of transparent plexiglas®, were glued to a black Plexiglas square bottom piece. The stem of the T is labelled the start arm, extending to the right and the left goal arm, which were separated by a black retractable guillotine door. A third guillotine door was adjusted at the beginning of the start arm to form a start box. The guillotine doors can be operated by the experimenter through a system of pulley strings.

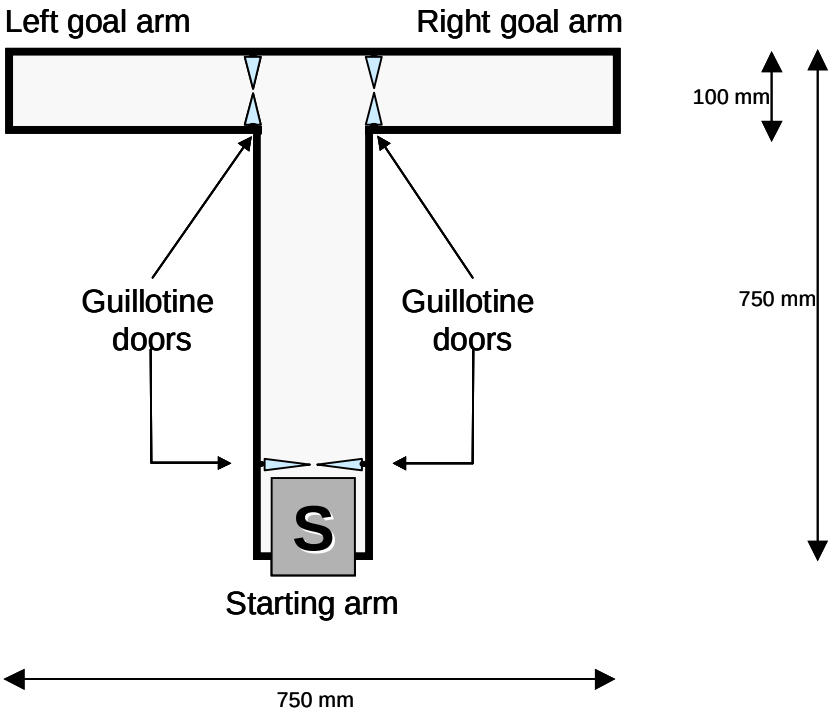


Figure 8: T-Maze: The apparatus consisted of a starting arm, including a starting box (S) and two goal arms, separable by retractable guillotine doors. It was built of transparent plexiglas to allow permanent observation of the mouse without additional video equipment above the maze or standing position of the experimenter, potentially distracting the mouse.

2.2.3.3.1.2. Procedure

The animals were subjected to one single session of 15 consecutive trials, beginning with one forced-choice trial, followed by 14 free-choice trials. No initial habituation procedure was necessary.

Forced-choice trial: in the first run, one of the two goal arms was blocked by lowering the guillotine door of the arm. The forced choice trial started by raising the guillotine door in the start arm and allowing the animal to explore the maze. The time to enter the open arm and the total time to return to the start position was recorded. As soon as the mouse returned to the start box, the guillotine door of the starting arm was lowered, whereas the guillotine doors of both goal arms were raised to initiate the free-choice trial.

Free-choice trial: by raising the guillotine door of the start arm, the mouse was allowed to choose between both goal arms. Once the mouse entered one of the goal arms, entry to the other goal arm was blocked. The time to enter the arm and the total time to return to the start box was recorded. The mouse was confined for 3 seconds in the start box. During the confinement, the door blocking one of the goal arms was lifted. The starting arm door was then lifted and the second trial began. A total of 14 free-choice trials were carried out during one continuous recording session. The session was also terminated as soon as 30 min have elapsed. During the session, the animals were never handled by the experimenter. Animals from the two groups were tested in alternating order in a random selection.

The T-CAT depends on hippocampal integrity (Gerlai, 1998). Successful completion of the task requires intact WM memory that keeps information about the previous trial on-line.

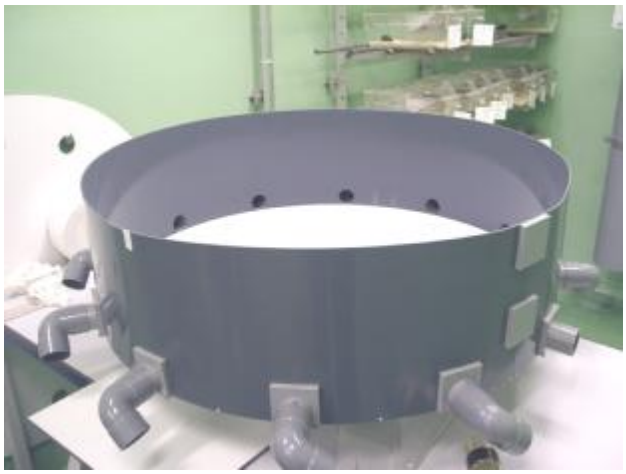
2.2.3.3.1.3. Data analysis

The data of all animals that completed less than 8 free-choice trials during 30 min were excluded from further analysis. The overall *alternation rate* during the 14 free-choice trials was calculated (0 % = no alternation, 100 % = alternation at each trial, 50 % = random alternation), the overall *time to choice* and the *mean session duration* was calculated statistically using the one-way analysis of variance (ANOVA) with the factor “group”. In addition, the Student`s *t*-test was used to evaluated if percent alternation performance was significantly over chance level. It was calculated by percent alternation minus 50%, and significant if the difference score differed from zero.

2.2.3.3.2. Experiment 1b: The modified Barnes Maze task

2.2.3.3.2.1. Apparatus

The mBM apparatus (depicted in figure 9), according to modifications of the original BM (1979) by Koopmans et al. (2003), consisted of a white circular platform, 950 mm in diameter, adjusted to a pillar, which was approximately 1 m in height. A downstream retractable cylinder, 110 mm in diameter, in the centre of the platform served as a starting chamber. The side wall, 250 mm in height, was perforated with 12 equidistantly spaces holes, 50 mm in diameter and 5 mm above the floor. The holes were connected to open, L-shaped plastic tunnels. An escape tunnel, 250 mm in length, was adjusted to only one of the L-shaped tunnels, leading to the home cage of the animal. The position of the escape hole remained in fixed relation to distal environmental cues. A centrally mounted camera provided a picture of the arena on a TV monitor. Performance was manually registered by means of an Observer program, which was designed and programmed by BAYER CNS Research support.



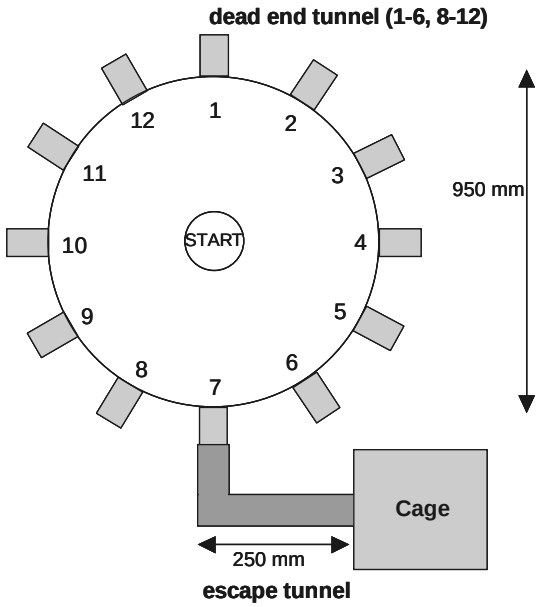


Figure 9: modified Barnes Maze: The apparatus consisted of a central platform, surrounded by a plexiglas wall, which is perforated with 12 holes. An extension was adjustable to each hole to form an escape tunnel to the home cage. A retractable cylinder forms the starting box in the centre of the platform. A video camera above the maze provided pictures of the testing procedure to a monitor.

2.2.3.3.2.2. Procedure

Animals were habituated to the apparatus without the escape tunnel for 5 min per animal on 5 consecutive days.

Each animal was assigned to an individual predetermined escape hole position, which was dissimilar between animals, in order to minimise potential olfactory orientation. Landmarks for orientation were provided by extra maze cues and their relative position to one another.

One daily session comprised two consecutive training trials. The mouse was inserted into the starting chamber facing a different direction in each trial. This procedure should help avoiding habit learning, which may occur if animals start consecutively from the same direction. The trial started by lowering the starting chamber, giving the mouse assess to explore the maze for the escape tunnel for 5 min. Mice that failed to find to descend into the tunnel within the time allotted, were guided gently to the escape hole with a grid during the very first trial. The mouse was allowed to remain in the escape box for 1 min. In the meantime, the platform was wiped clean with fresh tap water. After 1 min in the escape box, the mouse was taken out of the box and inserted into the starting chamber again, to perform the second trial. The second trial was implemented like the first one, except for the mouse being removed from the escape box after 15 seconds and set back into its home cage. The platform was cleaned and the home cage of the next mouse was positioned to the appropriate new hole. Acquisition training (learning of the correct hole position) was accomplished for 19 daily sessions with 2 trials per session. One week post acquisition training, the mice were subjected to a retention test of five daily sessions. Position of the escape holes and procedure were identical to acquisition training. Animals from the two groups were tested in alternating order. The task highly depends on spatial reference and working memory (Barnes, 1979; Inmann-Wood et al., 2001).

2.2.3.3.2.3. Data analysis

The mean *error rate* (amount of incorrect hole visits), *distance* travelled in the arena, *duration* to complete the trial, and *speed* of locomotion were assessed and statistically analysed using the two-way ANOVA for repeated measures over groups and days. A *post-hoc* Student´s *t*-test was additionally used to assess differences between groups particularly. A difference between groups was considered significant if the associated probability (p value) was below 0.05.

2.2.3.3.3. Experiment 1c: The Holeboard task

2.2.3.3.3.1. Apparatus

The HB apparatus (depicted in figure 10) was composed of an open-topped box, consisting of 500 mm high walls made of transparent plastic and a square 700 x 700 mm arena consisting of grey plastic with four holes arranged in respective corners of the maze, 20 mm in diameter and 20 mm in depth. The HB was placed onto a small table, 1 m above the room floor. Numerous spatial cues for orientation were visible. Above the apparatus, a camera and three halogen spot lights were adjusted to a beam arranged in close proximity to the apparatus. Furthermore, placed on a table next to the apparatus, there was a monitor and a video recorder for experiment documentation. Scoring of the hole visits and latency was conducted using an etholog observation program derived from the internet (http://www.geocites.com/ ebottoni/ethohome/html), which was run on a computer, placed on a smaller table in front of the monitor. The experimenter remained constantly on a seat between the HB and the computer, always visible to the animals. Data were scored directly to the observation program. Pellets were put into every exposed hole. The pellets in "unbaited" holes were covered by a grid, thus inaccessible for the animal. This procedure prevented the mouse from discriminating between baited and unbaited holes by orientation on olfactory cues.

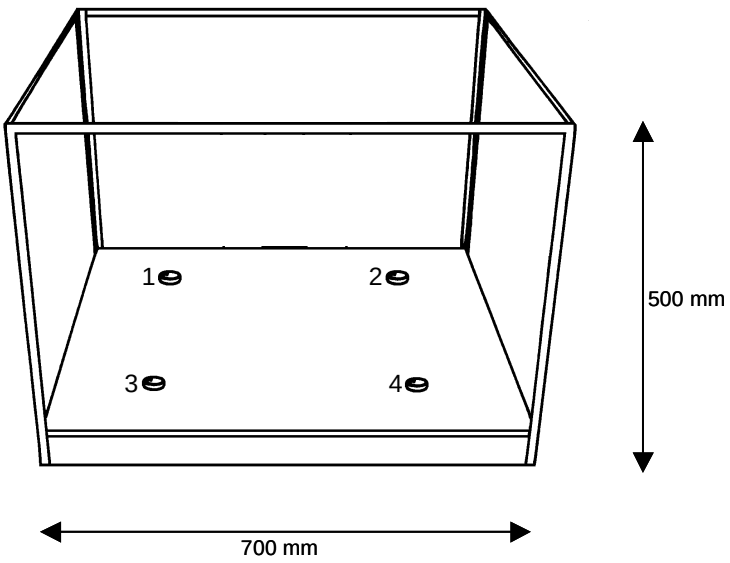


Figure 10: Holeboard: The apparatus consisted of a central square platform with four 20 mm deep holes. Plexiglas walls surround the area. A video camera above the maze provided pictures of the testing procedure to a monitor.

2.2.3.3.3.2. Procedure

The animals were placed singly into the HB apparatus for habituation. Pellets were scattered on the floor and each hole baited with one pellet. Before testing, each mouse was allowed to explore the apparatus for 5 min.

Each animal was tested in finding and learning the position of one baited hole. The mouse was put into a retractable start tube, placed in the centre of the HB. The trial started by raising the tube manually. It was completed as soon as the mouse found the pellet or until 5 min had elapsed, whichever event came first. Between each trial, the droppings were removed and the HB was wiped clean with tip water to minimize odour-based orientation. A daily session comprised three consecutive trials per mouse. Animals from the two groups were tested in alternating order. Acquisition of the test was performed on 16 days. Successful completion of the task requires intact spatial reference and working memory (Oades and Isaacson, 1978; De Oritz et al., 2000).

2.2.3.3.3.3. Data analysis

The mean *error rate* (amount of incorrect hole visits), *distance* travelled in the arena, *duration* to complete the trial, and *speed* of locomotion were assessed and statistically analysed using the two-way ANOVA for repeated measures over groups and days. A *post-hoc* Student´s *t*-test for pairwise comparison was additionally applied. A difference between groups was considered significant if the associated probability (p value) was below 0.05.

2.2.3.3.4. Experiment 1d: The Morris Water Maze task

2.2.3.3.4.1. Apparatus

Spatial navigation was examined using a modified MWM (Morris, 1982, 1984) (depicted in figure 11). The circular pool, 700 mm in diameter x 400 mm in height, was built of grey polyethylene. The pool was filled to a depth of 35 cm with clear tab water at a temperature of 22 ± 1°C. Four points around the circumference of the pool are arbitrarily designated East, South, West, of North, on this basis, the pool area divided into 4 quadrants. During the acquisition phase, a platform, 75 mm in diameter, placed in the western quadrant and arranged to be 10 mm below the water surface, and though invisible to the animal in the water, served as escape. Four equally spaced points around the edge of the pool were used as starting points. Around the pool, several extra maze cues served for spatial orientation. The test was conducted in dim light condition to minimise reflections from the water surface. Performance

of mice was registered automatically using the video tracking system EthoVision® (Noldus Information Technology, Wageningen, The Netherlands). A video camera, mounted in the centre above the pool, provided a picture of the pool on a TV monitor.

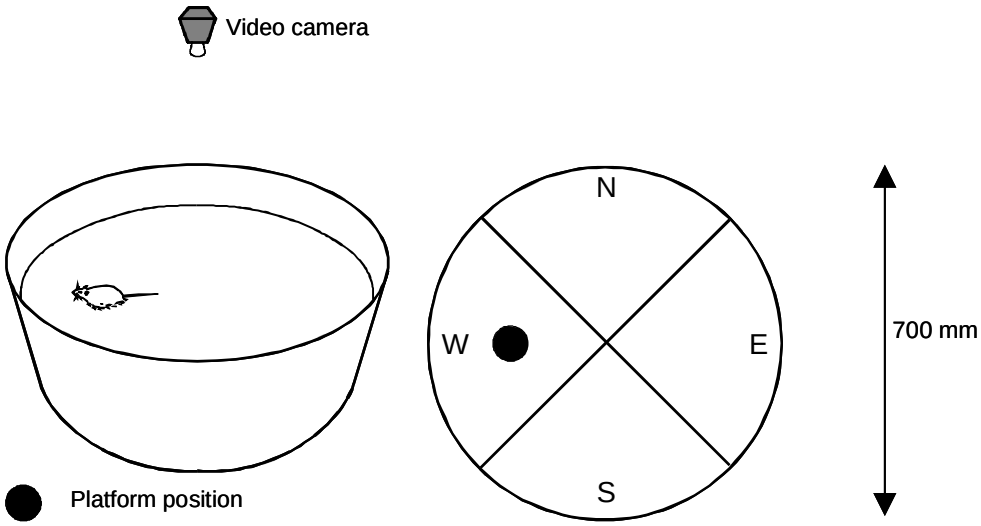


Figure 11: Morris Water Maze: The apparatus consisted of a circular pool, 700 mm in diameter, 400 mm in depth. The arena was virtually divided into four quadrants with a fix platform position in the western quadrant. A video camera above the maze provided pictures of the testing procedure to a monitor (picture van der Staay).

2.2.3.3.4.2. Procedure

Animals were trained on a repeated acquisition schedule to find the submerged escape platform for refuge from the water. One daily session consisted of four consecutive trials with releasing the animal from 4 different start points in a randomly assigned pattern. This pattern changed from session to session in a randomised sequence. A trial was started by putting the mouse in the pool, facing the wall of the pool. The animal was allowed to explore the pool for the escape platform within 60 seconds. The trial was terminated as soon as the animal climbed onto the platform or if 60 seconds elapsed, whichever occurred first. The animal was allowed to remain on the platform for 30 seconds before the start of the consecutive trial. Mice that failed to locate the platform in the allotted time, were placed by hand on the platform for 30 seconds and an escape latency of 60 seconds was recorded for respective trial. This repeated acquisition procedure was performed for five daily sessions. Animals from the two groups were tested in alternating order.

To determine the extent of spatial learning, an additional probe trial was given on the fifth session after the acquisition trials were completed. During the probe trial, the platform was removed and the time spent in the four quadrants was measured for 30 seconds. Here, all animals were released from the eastern quadrant. Successful completion of the task requires intact spatial reference and working memory (Morris, 1984).

2.2.3.3.4.3. Data analysis

Repeated acquisition analysis: Mean platform *escape latency*, mean *distance travelled* in the Water Maze, and mean *swimming speed* were measured and statistically analysed using the two-way ANOVA for repeated measures over days. A Student´s *t*-test was additionally used to assess differences between groups particularly. A difference between groups was considered significant if the associated probability (p value) was below 0.05.

Probe trial: Group differences in the *time spent the quadrants* were assessed by ANOVA. Group effects were analysed with a repeated ANOVA over Quadrant. Group differences were evaluated in more detail by Fisher´s LSD *post hoc* comparison ($p < 0.05$).

2.2.3.3.5. Experiment 2: The object recognition task (ORT)

2.2.3.3.5.1. Apparatus

Original version (depicted in figure 12, A): The observation arena consisted of a circular open field, 480 mm in diameter. The wall (height: 400 mm) was made of transparent Makrolon®. The floor consisted of transparent Makrolon® (Figure 1). The light intensity in the arena of the apparatus was held constant at 700 lux (Sik et al., 2003; adapted for mice from Ennaceur and Delacour, 1988; Prickaerts et al., 2002a).

Modified version (depicted in figure 12, B): The observation arena was a square field, 450 x 450 mm, with slightly outwards inclined walls (height: 500 mm), built entirely out of grey opaque plastic. Dim light intensity of 4 lux was applied. A camera was adjusted above the arena.

In both versions, four different sets of objects, made of aluminium, were used. All objects were available in triplicate. They could not be displaced by the mouse nor could the mouse climb onto or hide in or under the objects. The objects had no natural significance and they were never associated with any kind of reinforcer. Objects were cleaned with fresh tab water and detergents after each trial. Spatial and non-spatial WM is required to successfully perform

the ORT (Gaffan, 1992 Eur J neurosci4; Kolb et al., 1994; Rampon et al., 2000; Steckler et al.,1998 a,b).

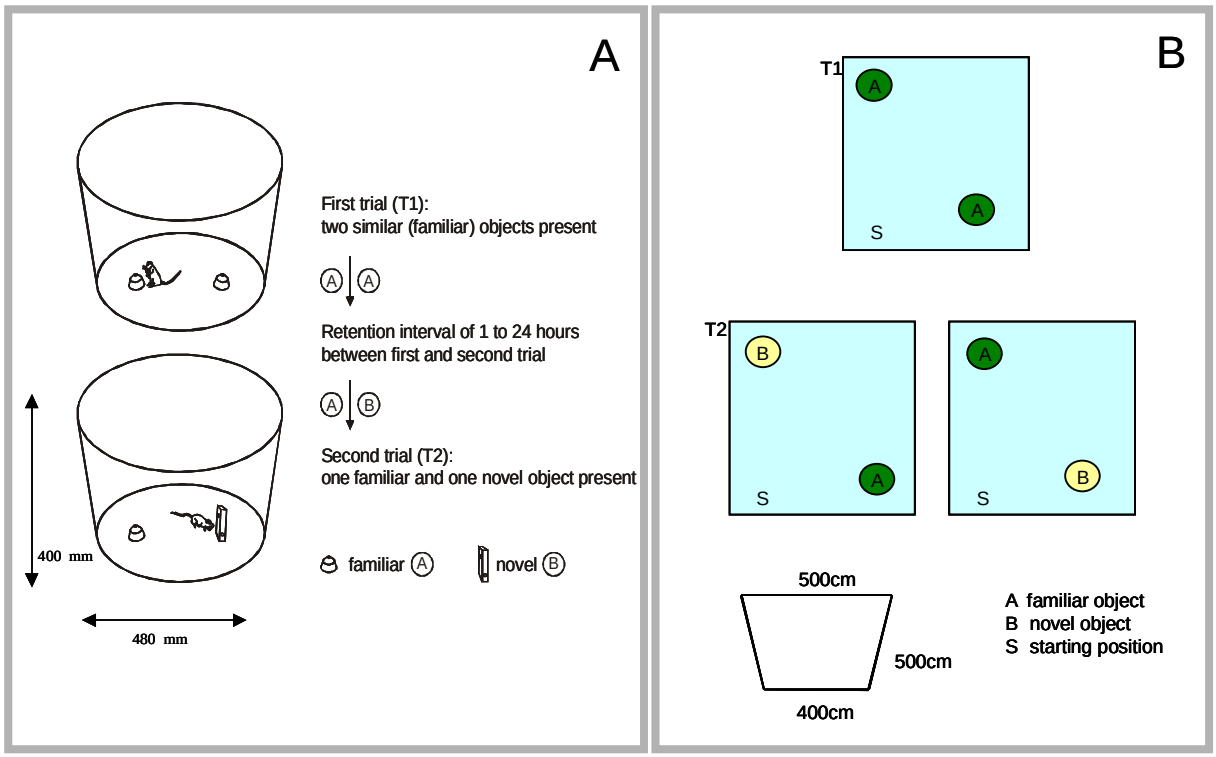


Figure 12: **A: original version of the ORT**: The apparatus consisted of a circular open field, 480 mm in diameter with transparent makrolon walls, 400 mm in height. Light intensity was 700 lux. The experimenter was sitting in front of the maze for direct scoring, always visible to the mouse. (picture van der Staay) **B: modified version of the ORT.** The maze consisted of a square floor, 450 x 450 mm surrounded by a wall, 500 mm in height. The maze was built from grey opaque plastic. Light intensity ranged at 4 lux. A video camera above the maze provided pictures of the testing procedure to a monitor for scoring. The experimenter was never visible to the mouse

2.2.3.3.5.2. Procedure

The ORT was performed according to previously described experiments (*for rats:* Ennaceur & Delacour, 1988; Ennaceur et al., 1989; Ennaceur & Meliani, 1992; Prickaerts et al., 2002a,b; *for mice:* Dodart et al., 1997; Messier, 1997; Sik et al., 2003).

During two consecutive days, the mice were habituated to the apparatus and the testing procedure. They were allowed to explore the empty apparatus twice for 5 min each day (one morning and one afternoon session).

Animals were trained in pairs of two trials that were separated by a retention interval of one hour. During the first trial (T1) the apparatus contained two identical objects, “A1” and “A2” (see Figure 1). These objects were placed in a symmetrical position about 120 mm (with reference to the centre of the object) away from the wall.

A mouse was taken from its home cage and placed into the apparatus, equidistant from the two objects, facing the wall in front of the experimenter. In order to assess the importance of trial duration, the animal was allowed to explore the objects for 3 min and in another session for 5 min, respectively. After T1 the mouse was transferred to its home cage. One hour after T1, the animal was again placed into the apparatus for the second trial (T2). Now, the exploration arena contained two different objects, a copy of the familiar one “A” from T1 and a novel object “B”. Exploration time was again, three or 5 min, in accordance to T1. A retention interval of one hour was chosen, because normally mice show good retention performance after this short interval, whereas complete forgetting occurs after a retention interval of 24 hours (Rosa et al., 2003; Sik et al., 2003). The time spent exploring the two objects during T1 and T2 was manually registered by the experimenter using a personal computer.

Modified version: Exploration was recorded with a camera and displayed on a monitor, thus the experimenter was invisible for the mouse.

2.2.3.3.5.3. Data analysis

Differences between groups were analysed separately for each maze (modified and original ORT) and the effect of session duration (3 min versus 5 min in each ORT version) was assessed. Moreover, performance of each single group was directly compared in both maze versions. Therefore, the mean time (in s) exploring the familiar object A (*a*) and the mean time exploring the novel object B (*b*) during T2 was measured. From these data, exploration index *e2* and two different indices for discrimination performance, *d1* and *d2*, were calculated:

Table 2: Overview of parameters, indices and calculation

Parameters analysed	**Index**	**Calculation**
Exploration time for both objects during T2	e2	e2 = a + b
Discrimination between familiar and novel object during T2	d1	d1 = b - a
Discrimination between familiar and novel object during T2, a relative measure corrected for explorative activity (e2)	d2	d2 = (b – a) / (a + b) = (b – a) / e2

Calculation of a virtual group with a mean of zero and SEM that corresponds with the average SEM of the discrimination parameter show that values of d2 below 0.15 can be considered as a failure to discriminate (Sik et al., 2003), as they do not differ from zero. This value will refer to as the "discrimination level" in the present study.

The values of *e2, d1 and d2* were averaged per group over the three and 5 min testing and analysed statistically by one-way ANOVA for the factors *group*, *maze* (modification versus original protocol) and *session duration*, supplemented with Duncan's post-hoc comparisons.

2.3.4. Results

2.3.4.1. Experiment 1

2.3.4.1.1. Experiment 1a: The T-Maze continuous alternation task

Alternation: The 129S6/SvEvTac mice show 53.6 % alternations, indicating a performance close to chance level (= 50 % alternation). Student`s *t*-test post hoc analysis demonstrated that alternation was not significantly over chance level. The C57BL/6J mice alternated at 60.1 %, which was significantly over chance level ($t_{(12)} = 3.5$, $p = 0.049$). No significant difference between groups ($F_{(1,17)} = 2.82$, $p = 0.111$) was detected (depicted in figure 13, A).

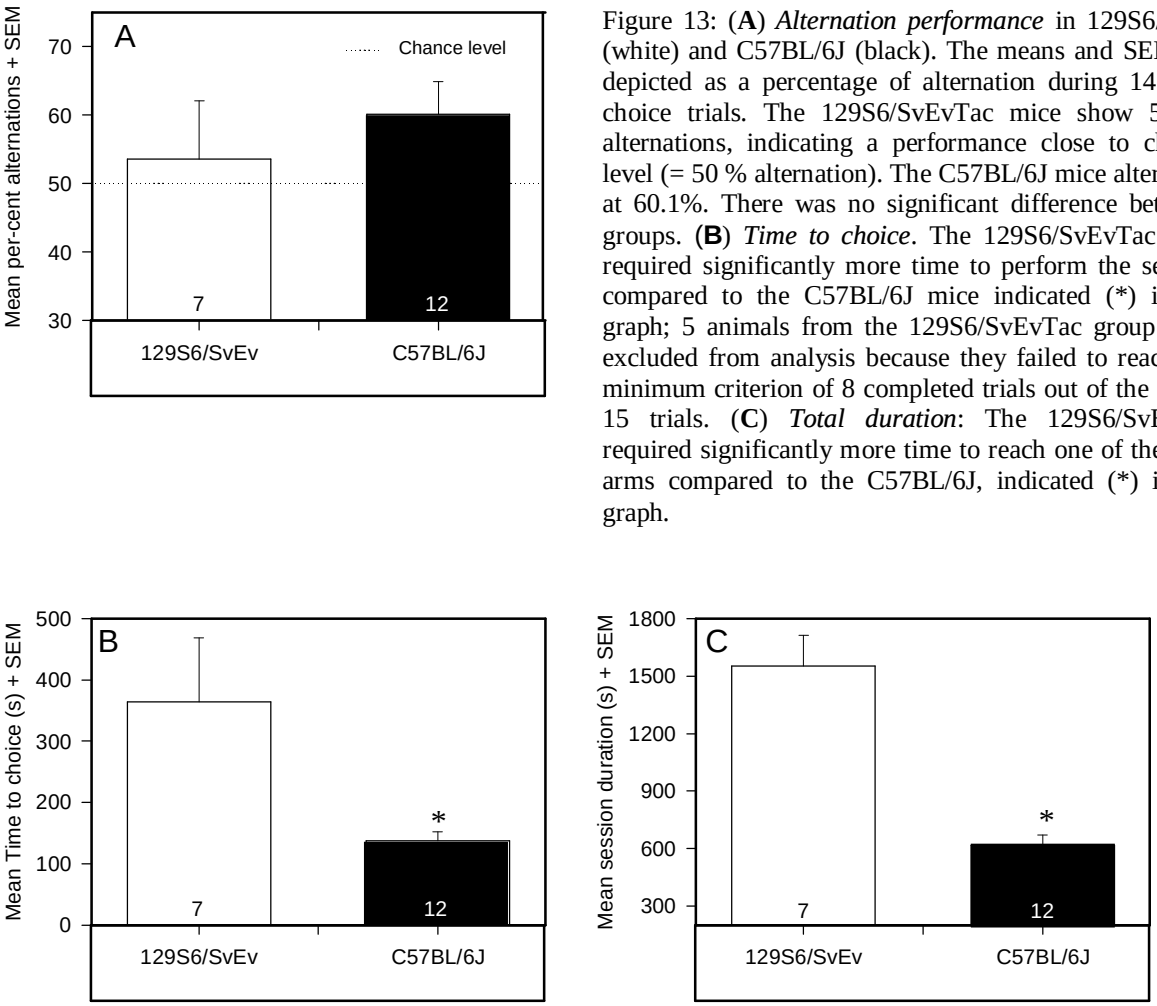


Figure 13: (**A**) *Alternation performance* in 129S6/SvEv (white) and C57BL/6J (black). The means and SEM are depicted as a percentage of alternation during 14 free-choice trials. The 129S6/SvEvTac mice show 53.6% alternations, indicating a performance close to chance level (= 50 % alternation). The C57BL/6J mice alternated at 60.1%. There was no significant difference between groups. (**B**) *Time to choice*. The 129S6/SvEvTac mice required significantly more time to perform the session compared to the C57BL/6J mice indicated (*) in the graph; 5 animals from the 129S6/SvEvTac group were excluded from analysis because they failed to reach the minimum criterion of 8 completed trials out of the set of 15 trials. (**C**) *Total duration*: The 129S6/SvEvTac required significantly more time to reach one of the goal arms compared to the C57BL/6J, indicated (*) in the graph.

Session duration: The 129S6/SvEvTac mice required significantly more time to perform the session compared to the C57BL/6J mice ($F_{(1,17)} = 44.53$, $p < 0.0001$, as indicated (*) in the

graph). Five animals from the 129S6/SvEvTac group (n = 12 subjected to the test) were excluded from analysis because they failed to reach the minimum criterion of eight completed trials out of the set of 15 trials (depicted in figure 13, B).

Time to choice: The 129S6/SvEvTac required significantly more time to reach one of the goal arms compared to the C57BL/6J ($_{(F(1,17)}$ = 30.17, $p < 0.0001$) (depicted in figure 13, C).

2.3.4.1.2. Experiment 1b: The modified Barnes Maze task

Error rate (acquisition): Averaged over sessions, there was no difference between groups (General means: $F_{(1,18)} = 1.26$, $p = 0.274$). The amount of incorrect hole visits decreased significantly over sessions (Sessions: $F_{(1,18)} = 2.75$, $p = 0.0002$) with higher decrease in the C57BL/6J group. The rate of decrease over sessions was similar in both groups (Session by Group: $F_{(1,18)} = 1.53$, $p = 0.077$) (depicted in figure 14, A).

Distance (acquisition): Averaged over sessions, a difference was shown between groups (General means: $F_{(1,18)} = 4.84$, $p = 0.039$). The distance decreased significantly over sessions (Sessions: $F_{(1,18)} = 2.48$, $p = 0.0008$) but different between groups over sessions (Session by Group: $F_{(1,18)} = 1.28$, $p = 0.198$). *Post-hoc* t-test comparison revealed more travelling in 129S6/SvEvTac mice in five sessions (depicted in figure 14, B).

Duration (acquisition): Over all sessions, there was a significant difference between groups (General means: $F_{(1,18)} = 39.47$, $p < 0.0001$). Duration decreased over sessions (Sessions: $F_{(1,18)} = 5.39$, $p < 0.0001$). The decrease over sessions was similar for both groups (Session by Group: $F_{(1,18)} = 0.91$, $p = 0.571$). *Post-hoc* t-test comparison revealed higher duration times in 129S6/SvEvTac mice for all sessions (depicted in figure 14, C).

Speed (acquisition): Averaged over sessions, there was a difference between groups (General means: $F_{(1,18)} = 25.36$, $p < 0.0001$). The speed changed significantly over sessions (Sessions: $F_{(1,18)} = 2.61$, $p = 0.0004$) with C57BL/6J mice increasing the speed continuously, whereas the 129S6/SvEvTac mice slightly increase speed within the first 12 sessions with a decrease and stagnation in the rest of sessions. The trend was similar between groups over sessions (Session by Group: $F_{(1,18)} = 0.82$, $p = 0.678$). *Post-hoc* t-test comparison revealed higher speed in C57BL/6J for almost all sessions (depicted in figure 14, C).

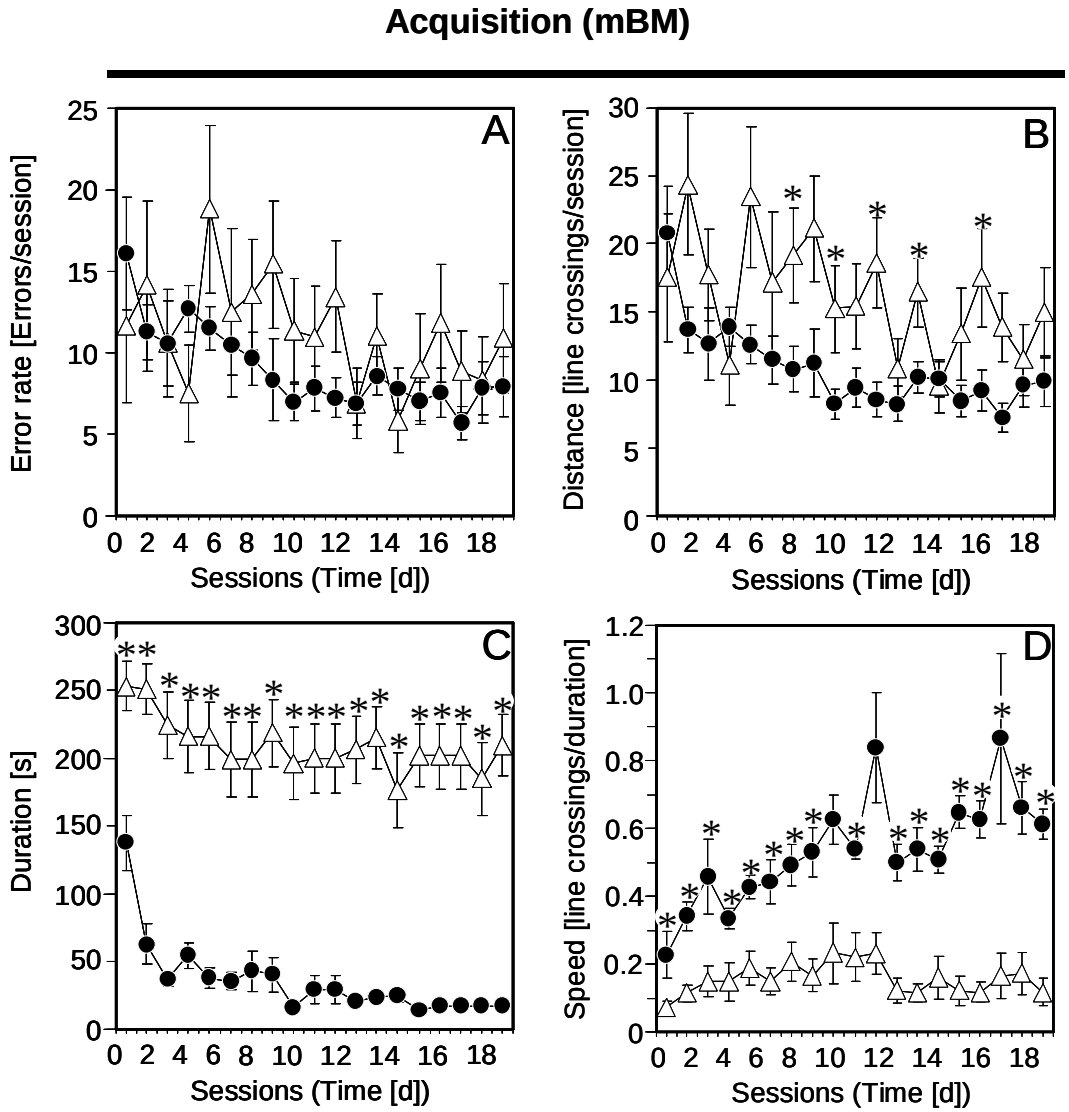


Figure 14: Differences between C57BL/6J (n = 12; black circles) and 129S6/SvEvTac (n = 12; white triangles) in the acquisition session of the modified Barnes Maze task (mBM), with differences between groups (*p < 0.05) (**A**) The *mean error rate* indicates higher error rates in the 129S6/SvEv, however, groups reduced errors similarly. (**B**) The *mean distance* was higher in the 129S6/SvEvTac. (**C**) *Mean trial duration* was clearly higher in the 129S6/SvEvTac. (**D**) *Mean speed* was higher in the C57BL/6J and constantly enhanced in the course of trials.

Error rate (retention): The mean error rate over session revealed no significant difference between groups (General means: $F_{(1,4)} = 3.32$, $p = 0.082$). The animal did not show significant changes in the error rate over sessions (Sessions: $F_{(1,4)} = 1.05$, $p = 0.387$). There was no difference between groups (Session by Group: $F_{(1,4)} = 0.85$, $p = 0.499$) (depicted in figure 15, A).

Distance (retention): Averaged over sessions, there was a significant difference between groups (General means: $F_{(1,4)} = 6.02$, $p = 0.023$) with C57BL/6J requiring less line-crossings

to find the hole. The distance showed no changes over sessions (Sessions: $F_{(1,4)} = 0.87$, $p = 0.485$). Both groups displayed similar distances across sessions (Session by Group: $F_{(1,4)} = 0.44$, $p = 0.078$). *Post-hoc* t-test comparison revealed higher distance in 129S6/SvEvTac on the first session day (depicted in figure 15, B).

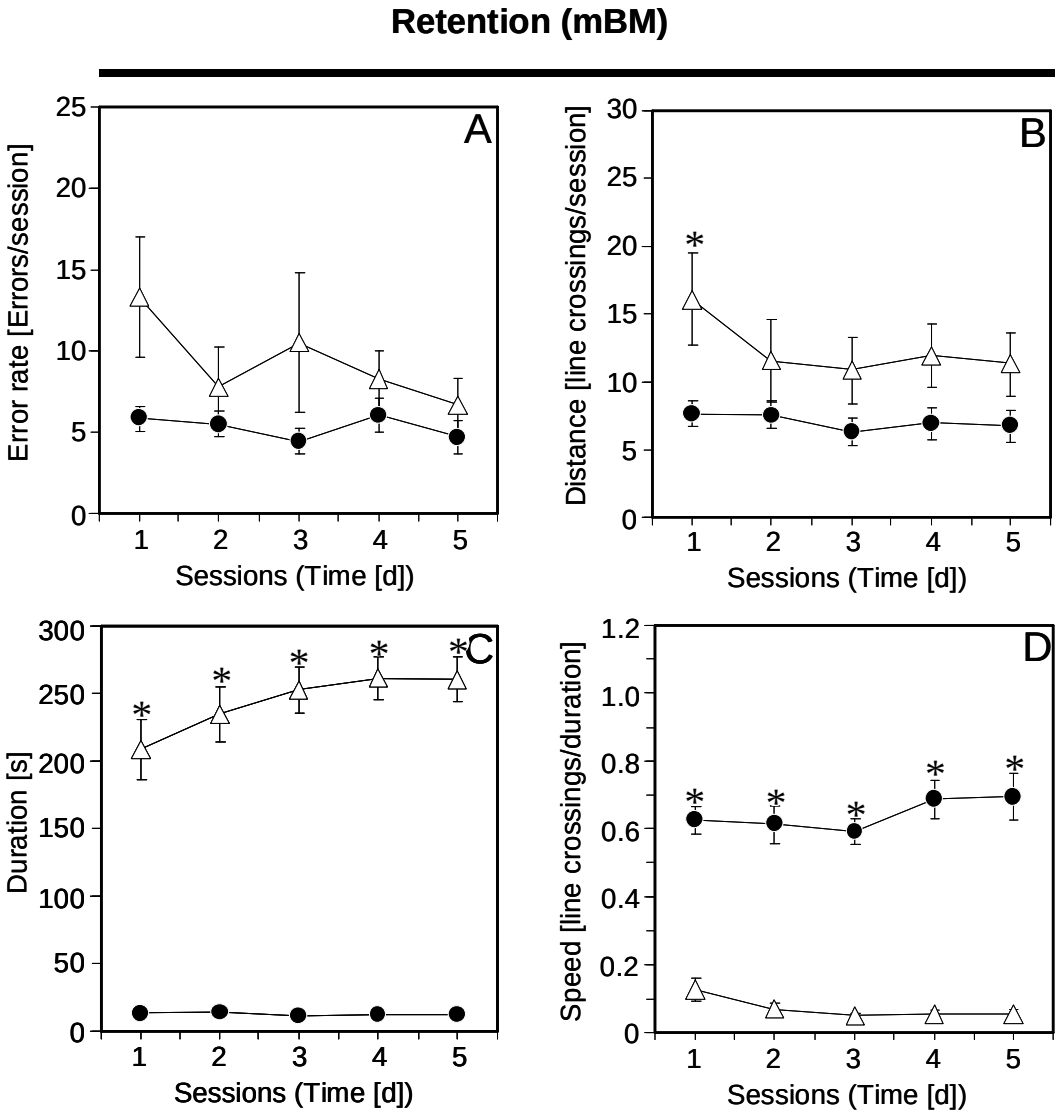


Figure 15: Differences between C57BL/6J (n = 12; black circles) and 129S6/SvEvTac (n = 12; white triangles) in the retention session of the modified Barnes Maze task (mBM), with differences between groups (*p < 0.05). (**A**) The *mean error rate* revealed higher error rates in the 129S6/SvEv, however, both groups continued retention performance at the same level as acquisition performance ended, indicated preserved reference memory. (**B**) *Mean distance* was higher in the 129S6/SvEvTac. (**C**) *Mean trial duration* was clearly higher in the 129S6/SvEvTac. (**D**) *Mean speed* was higher in the C57BL/6J and instantly at the well-trained level of acquisition performance.

Duration (retention): Averaged over the session there was significant difference between groups (General means: $F_{(1,4)} = 171$, $p < 0.0001$). Duration was not changed over sessions

(Sessions: $F_{(1,4)} = 1.88$, $p = 0.121$). The decrease over sessions was dissimilar for the groups (Session by Group: $F_{(1,18)} = 0.91$, $p = 0.571$). *Post-hoc* t-test comparison showed that C57BL/6J performed faster for all session days (depicted in figure 15, C).

Speed (retention): Averaged over sessions, there was a difference between groups (General means: $F_{(1,4)} = 245.22$, $p < 0.0001$). Speed did not change over sessions (Sessions: $F_{(1,4)} = 1.00$, $p = 0.412$), which was similar in both groups over sessions (Session by Group: $F_{(1,4)} = 1.64$, $p = 0.171$). Post-hoc t-test comparison assessed higher speed in C57BL/6J for all session days (depicted in figure 15, D).

2.3.4.1.3. Experiment 1c: The Holeboard task

Error rate: The mean error rate over session was significantly higher in C57BL/6J compared to the 129S6/SvEvTac mouse group (General means: $F_{(1,7)} = 62.14$, $p < 0.0001$). This phenomenon is related to obvious immobility and lack of inspections (see also *duration* and *speed*) of 129S6/SvEvTac rather than to higher cognitive performance. Few inspections resulted in low error scores. The error rate showed to significant changes over sessions (Sessions: $F_{(1,7)} = 3.21$, $p = 0.004$), which was different between groups (Session by Group: $F_{(1,7)} = 2.87$, $p = 0.009$). C57BL/6J decreased the amount of errors constantly over days, whereas 129S6/SvEvTac performed very inconstant with only a slight improvement between day 3 and day 6. *Post-hoc* t-test calculation showed higher error rates in C57BL/6J in all sessions (depicted in figure 16, A).

Distance: Averaged over sessions, there was a significant difference between groups (General means: $F_{(1,7)} = 5.01$, $p = 0.043$) with C57BL/6J travelling less to find the hole compared to 129S6/SvEvTac. Distance was significantly reduced over sessions (Sessions: $F_{(1,7)} = 12.67$, $p < 0.0001$), which was not similar between groups (Session by Group: $F_{(1,7)} = 4.31$, $p = 0.0004$). *Post-hoc* t-test comparison revealed longer paths in C57BL/6J on session one, but longer distances in 129S6/SvEvTac for session four to seven (depicted in figure 16, B).

Duration: The mean time to find the baited hole differed significantly between groups (General means: $F_{(1,7)} = 946$, $p < 0.0001$). C57BL/6J mice required a few seconds to find the baited hole, whereas 129S6/SvEvTac mice searched between 200 and 250 seconds for the food pellet. There was no difference in duration over sessions (Sessions: $F_{(1,7)} = 0.94$, $p = 0.476$), which was similar between groups (Session by Group: $F_{(1,7)} = 1.03$, $p = 0.415$).

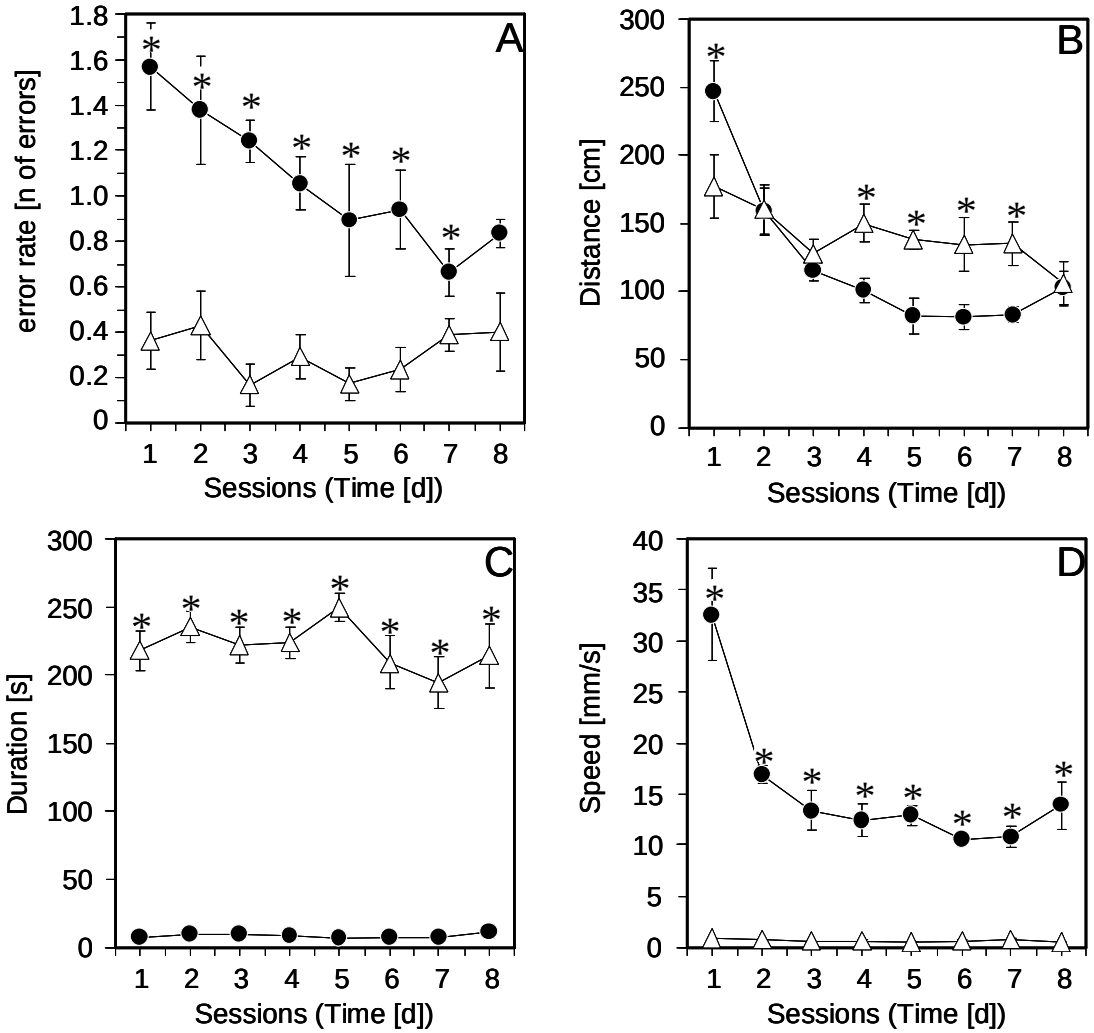


Figure 16: Differences between C57BL/6J (n = 12; black circles) and 129S6/SvEvTac (n = 12; white triangles) in the holeboard, with differences between groups (*p < 0.05). (**A**) The *mean error rate* revealed very low error rates in the 129S6/SvEv, resulting from lacking hole inspections rather than mnemonic dominance. (**B**) *Mean distance* was higher in the 129S6/SvEvTac. (**C**) *Mean trial duration* was clearly higher in the 129S6/SvEvTac. (**D**) *Mean speed* was higher in the C57BL/6J. They displayed goal-directed behaviour, as error rates were diminished over sessions at rather constant speed measures. 129S6/SvEvTac, however failed to acquire the task demands.

Post-hoc t-test comparison revealed longer duration in 129S6/SvEvTac mice in all sessions (depicted in figure 16, C).

Speed: The mean duration to find the baited hole differed significantly between groups (General means: $F_{(1,7)} = 946$, $p < 0.0001$). C57BL/6J mice travelled with an averaged speed between 10 and 15 mm / s. Low speed of about 1 mm / s was assessed for 129S6/SvEvTac mice who even remained in phases of total rigidity during testing. There was no difference in duration over sessions (Sessions: $F_{(1,7)} = 0.94$, $p = 0.476$), which was similar between groups

(Session by Group: $F_{(1,7)} = 1.03$, $p = 0.415$). *Post-hoc* t-test comparison revealed higher speed in all sessions for C57BL/6J mice (depicted in figure 16, D).

2.3.4.1.4. Experiment 1d: The Morris Water Maze task

Platform escape latency: The mean escape latency was similar between groups (General means: $F_{(1,4)} = 0.29$ $p = 0.597$). There was a significant reduction of escape latency over sessions (Sessions: $F_{(1,4)} = 64.57$, $p < 0.0001$), which was dissimilar between groups (Session by Group: $F_{(1,4)} = 3.99$, $p = 0.005$). Initially, 129S6/SvEvTac travelled longer to find the platform, but they reduced escape latency more efficiently than C57BL/6J over sessions (depicted in figure 17, A).

Travelled distance: Averaged over sessions, there was no significant difference between groups (General means: $F_{(1,4)} = 1.14$, $p = 0.297$). Distance was significantly reduced over sessions (Sessions: $F_{(1,4)} = 90.33$, $p < 0.0001$), in a different manner between groups (Session by Group: $F_{(1,4)} = 8.61$, $p < 0.0001$). Post-hoc t-test comparison revealed differences between groups (depicted in figure 17, B).

Swimming speed: The mean swimming speed was similar between groups (General means: $F_{(1,4)} = 4.17$, $p = 0.053$) with indicated higher speed in the 129S6/SvEvTac. Speed did not change over sessions (Sessions: $F_{(1,4)} = 1.42$, $p = 0.233$), which was dissimilar between groups (Session by Group: $F_{(1,4)} = 1.50$, $p = 0.210$). *Post-hoc* t-test showed that 129S6/SvEvTac swam faster on two session days (depicted in figure 17, C).

Time spent in each quadrant (probe): The averaged time spent in quadrants was different, with the main time spent in the quadrant of the former platform position (Quadrants: $F_{(3,69)} = 17.11$, $p < 0.0001$). The time spent in the training quadrant, however, did not differ between groups (Groups: $F_{(2,23)} = 0.09$, $p = 0.766$) (depicted in figure 17, D).

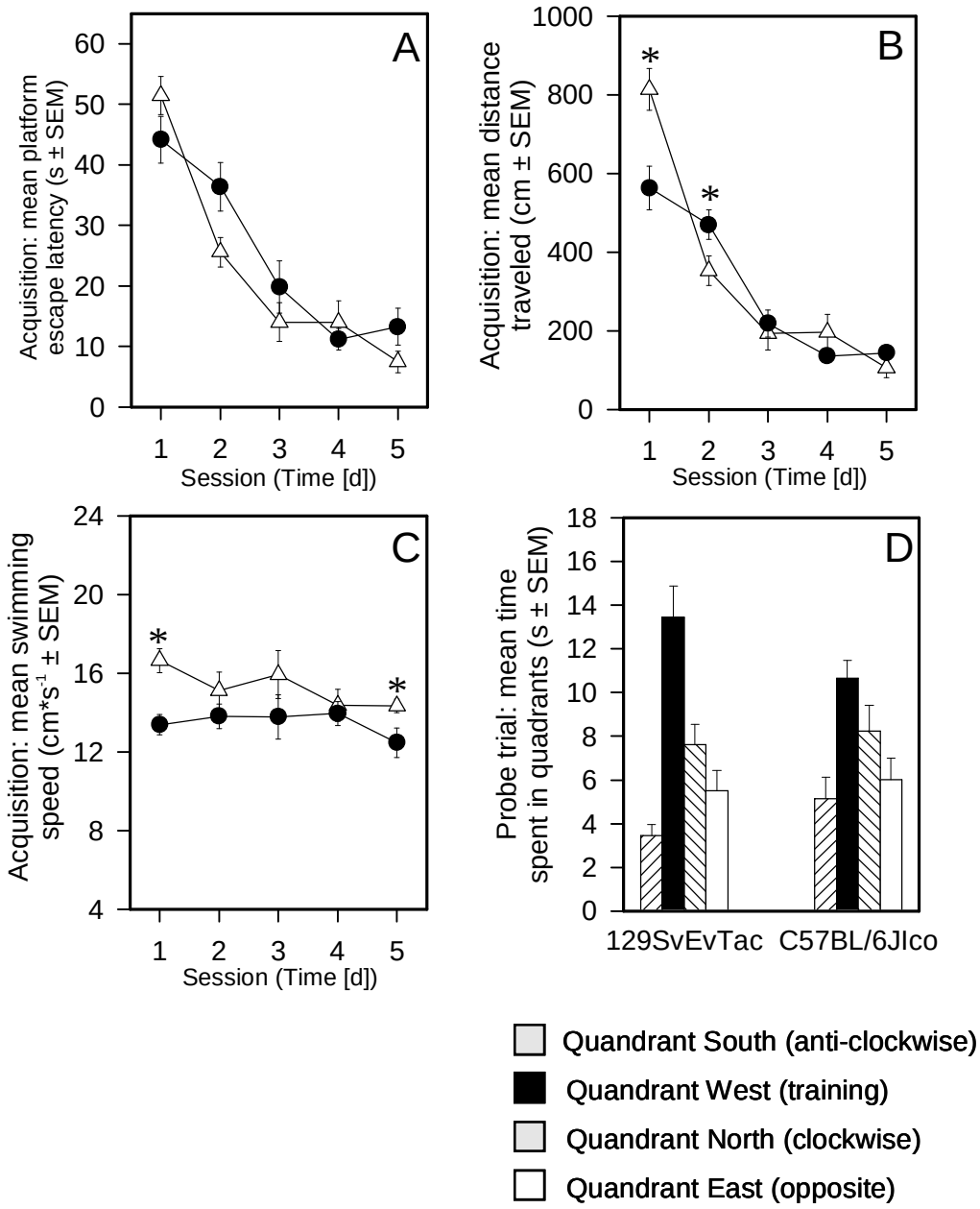


Figure 17: Differences between C57BL/6J (n = 12; black circles) and 129S6/SvEvTac (n = 12; white triangles) in the Morris Water Maze, with differences between groups (*p < 0.05). (**A**) *Mean escape latency* was similar between groups. (**B**) *Mean distance* travelled was initially higher in the 129S6/SvEvTac, however, they reduced paths length stronger than C57BL/6J in the course of sessions. (**C**) *Mean swimming speed* was slightly higher in the 129S6/SvEvTac, but constant for both groups. (**D**) *Probe* trial indicated spatial memory for the platform position in both groups (black bars).

2.3.4.2. Experiment 2

No significant differences between trial duration of 3 min and 5 min (data not shown) for both discrimination indices were detectable. Data analysis will therefore be confined to the tests with 3 min values. Data for 5 min trial duration are not shown.

2.3.4.2.1. Experiment 2a: ORT with young and old C57BL/6 and APP_{SL} mouse groups

Group comparison in the original version: Discrimination indices d1 ($F_{(2,30)} = 0.14$, $p = 0.871$) and d2 ($F_{(2,30)} = 0.02$, $p = 0.982$) were similar in all groups. Shorter exploration times were found in the APP_{SL} mouse group ($F_{(2,30)} = 3.93$, $p = 0.031$) (depicted in figure 18 A-C, left side).

Group comparison in the modified version: APP_{SL} animals displayed a higher discrimination index d1 than the young and old C57BL/6 mice ($F_{(2,30)} = 3.57$, $p = 0.041$). D2 was similar for all groups ($F_{(2,30)} = 0.49$, $p = 0.062$). All animal groups explored the objects in T2 in a similar period of time ($F_{(2,30)} = 1.75$, $p = 0.191$) (depicted in figure 18 A-C, left side).

Maze comparison with young C57BL/6 mice: Young C57BL/6 discriminated higher in the modified ORT version for both indices, d1 ($F_{(1,16)} = 30.05$, $p < 0.0001$) and d2 ($F_{(1,16)} = 7.39$, $p = 0.013$). Modification induced a strong increase of exploration in the mouse group ($F_{(1,16)} = 10.56$, $p = 0.004$) (depicted in figure 18, left side).

Maze comparison with old C57BL/6 mice: Both, d1 ($F_{(1,16)} = 8.85$, $p = 0.007$) and d2 ($F_{(1,16)} = 8.37$, $p = 0.008$) increased with modification. Exploration times were similar for both mazes ($F_{(1,16)} = 3.43$, $p = 0.078$) (depicted in figure 18, left side).

Maze comparison with APPSL mice: All parameters, d1 ($F_{(1,16)} = 12.77$, $p = 0.003$), d2 ($F_{(1,16)} = 5.85$, $p = 0.028$) and e2 ($F_{(1,16)} = 21.42$, $p = 0.0003$) were improved by the modification (depicted in figure 18, left side).

Table 3: Mean exploration times and standard errors of familiar object (a) and novel object (b) in T2 in Experiment 2a

Animal groups	3Min values			
	a	**± SEM**	**b**	**± SEM**
Young C57BL/6, original version	1.52	0.31	2.27	0.42
Young C57BL/6, modified version	2.39	0.53	6.1	0.82
Old C57BL/6, original version	1.78	0.34	2.50	0.39
Old C57BL/6, modified version	1.92	0.39	4.73	0.80
$APP_{SL,}$ original version	0.49	0.24	1.03	0.54
APP_{SL}, modified version	2.04	0.38	8.24	1.60

2.3.4.2.2. Experiment 2b: ORT with OF1, NMRI and SJL mouse groups

Group comparison in the original version: The SJL mouse strain discriminated at lowest level. No differences between groups were found for discrimination index d1 ($F_{(2,37)} = 2.99$, $p = 0.063$) with ANOVA calculation. *Post hoc* analysis, however, revealed differences betweeb SJL and NMRI animals. This phenomenon was also assessed for d2 ($F_{(2,37)} = 2.66$, $p = 0.084$). Exploration was similar in all mouse strains ($F_{(2,37)} = 1.55$, $p = 0.227$) (depicted in figure 18 A-C, right side).

Group comparison in the modified version: The SJL mouse strain discriminated at lowest level, which was similar to the performance in the original version. ANOVA calculation showed no difference between strains for d1 ($F_{(2,37)} = 2.49$, $p = 0.097$), which was due to high SEM values in the SJL group. *Post-hoc* analysis assessed differences between SJL and NMRI mouse strains. Clear differences for d2 was found between SJL and the other groups in the modified version ($F_{(2,37)} = 2.99$, $p = 0.063$). SJL animals failed to discriminate between familiar and new objects. No differences were found for exploration time e2 ($F_{(2,37)} = 2.049$, $p = 0.145$), indicating high activity with low discrimination abilities in SJL animals (depicted in figure 18 A-C, right side).

Maze comparison with OF1 mice: No differences between original and modified ORT version were found with OF1 mice for d1 ($F_{(1,26)} = 1.14$, $p = 0.296$). Differences were found for d2 ($F_{(1,26)} = 9.07$, $p = 0.006$). OF1 mice explored the objects for both versions similarly long ($F_{(1,26)} = 0.01$, $p = 0.919$) (depicted in figure 18, right side).

Maze comparison with NMRI mice: NMRI animals discriminated similar in both versions for d1 ($F_{(1,26)} = 0.62$, $p = 0.439$). Differences between the original and the modified version were found for d2 ($F_{(1,26)} = 6.11$, $p = 0.023$). Exploration times were similar for both ORT versions ($F_{(1,26)} = 0.04$, $p = 0.836$) (depicted in figure 18, right side).

Maze comparison with SJL mice: Animals performed similarly low in both ORT versions for d1 ($F_{(1,26)} = 0.02$, $p = 0.891$) and d2 ($F_{(1,26)} = 0.0$, $p = 0.997$). Exploration was enhanced by the modification ($F_{(1,26)} = 4.43$, $p = 0.046$) (depicted in figure 18, right side).

Table 4: Mean exploration times and standard errors of familiar object (a) and novel object (b) in T2 in Experiment 2b

Animal groups	**3Min values**			
	a	**± SEM**	**b**	**± SEM**
OF1, original version	3.81	0.75	8.16	1.52
OF1 modified version	2.69	0.73	8.94	1.83
NMRI original version	4.57	0.36	11.5	1.59
NMRI modified version	3.47	0.67	12.02	1.79
SJL original version	4.76	0.87	6.64	1.80
SJL modified version	8.01	1.68	10.41	2.09

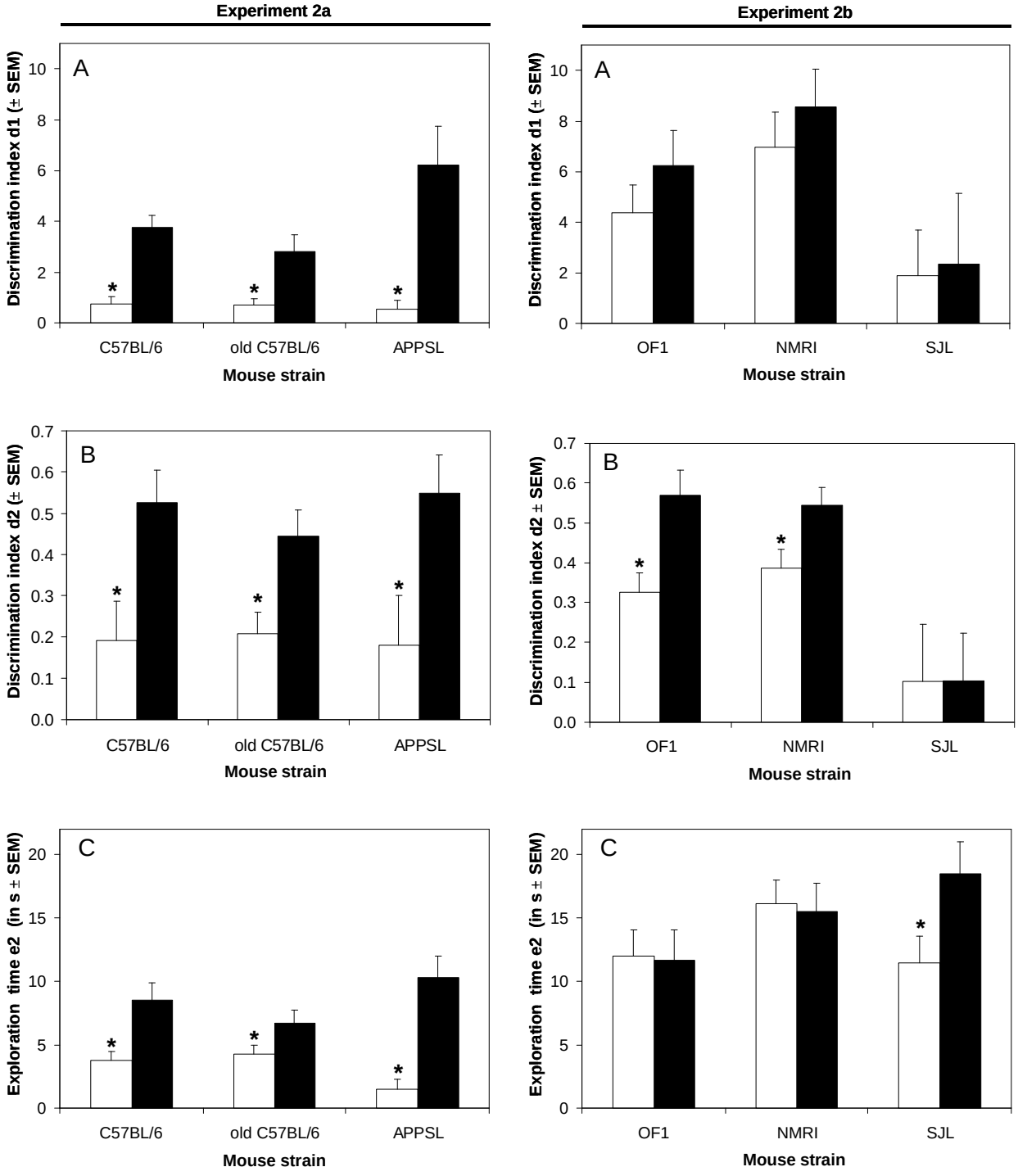


Figure 18: (**A**) *Discrimination index d1*, (**B**) *d2*, and (**C**) *exploration time e2* in Experiment 2a (left side) and 2b (right side) for respective original (white) and modified (black) ORT version are depicted. In Exp.2a, modification improved d1, d2 and e2 measures. In Exp.2b, modification improved d2 in OF1 and NMRI mice. e2 was only enhanced in SJL mice with modification.

2.3.5. Discussion

2.3.5.1. Experiment 1a: The T-Maze continuous alternation task

The analysis of the duration times (mean session duration and time to choice) revealed significant differences between strains. 129S6/SvEvTac needed more than twice the time to perform the task compared to C57Bl/6J. Four 129S6/SvEvTac animals had to be excluded from data analysis due to immobility. Natural motivation to perform a learning task was continuously revealed insufficient in 129S6/SvEvTac (Wahlsten et al., 2003). Analysis of the alternation rate showed that 129S6/SvEvTac performed at chance level, indicating a failure to acquire the task demands in these animals. Another possible interpretation could be the vast time interval between trials that may affect working memory, i.e. the memory of the former arm visit and the interest to visit a new arm. C57Bl/6J showed 60,1% alternation, although data from previous studies (in house studies) reported higher alternation rates in this strain. Duration times, however, were similar to previous studies. Thus, motivation to explore the maze was high enough in C57BL/6J to perform the task and to exceed chance performance for the T-CAT.

Several hypothesis have been postulated concerning the animals motivation to explore the T-Maze and to alternate between two options. One theory was delivered by Montgomery (1951, 1952), which assumes that the curiosity drive is arising by novel stimuli. Alternation occurs as the animal approaches the most novel option. Hull (1949) postulated the "reactive inhibition"(I_R) theory: the previous choice may inhibit the following choice for the same in favour for the opposite goal arm. Another explanation was delivered with Glanzer`s "stimulus satiation" theory ($I_S$) (1953). Opposed to the $I_R$ theory by Hull, based on the ***response*** to a choice for alternation, $I_S$ relates to the ***stimulus***-object association made to an object (goal arm or visual cues in general). The $I_S$ reduces the organism`s tendency to make a response to that object (for more details see Dember and Fowler, 1958). These theories may principally explaine alternation behaviour. However, it still remains to be evaluated why strains such as 129S6/SvEvTac show little motivation to explore the maze.

Correct assessment of animal behaviour is easily confounded by stress and anxiety. Fearful performance in the T-Maze was reported when animals were handled frequently during the testing procedure (Crusio et al., 1990; Dember, 1990), such as picking them up from the goal arm in order to put them back into the starting chamber. This procedure can induce stress to the animal, resulting in freezing or negatively reinforced learning (Gerlai et al., 1994). Therefore, the T-CAT procedure in the present study bears three important advantages: (1) no pretraining or habituation to the apparatus or procedure is necessary, (2) there is no need to

handle the animals during the testing procedure, and (3) one single session is sufficient to elucidate alternation performance in the animals. Stress can effectively be minimised.

2.3.5.2. Experiment 1b: The modified Barnes Maze task

Both strains showed a similar reduction of errors during the acquisition training. The retention test provides information about the memory for the escape hole position acquired and thus, an estimation for the magnitude of forgetting in the animals. Both groups reached the well-trained level of acquisition training within the first and the second day of retention testing, indicating preserved RM in both strains for at least one week. Finally, the interpretation of the error rate may indicate comparable learning success of the correct escape hole for both groups.

In contrast, large differences were revealed for parameters displaying locomotor activity, i.e. distance, duration and speed. Behaviour of the 129S6/SvEvTac was very variable with spending an enormous period of time without activity, and thigmotaxis on the other hand. Thigmotaxis is an instinctive behaviour of rodents searching for an exit along the boarder walls in a given area (Wolfer et al., 1997). This phenomenon often accounts for variability in parameters that measure locomotion and may confound “real learning” analysis. Another possible explanation for the lack of goal directed activity in the 129S6/SvEvTac could be inefficiency in the search strategy. The C57BL/6J, in contrast, decreased distance and duration whilst increasing the speed over training sessions. Taken together, both strains reduced the number of incorrect hole visits and they preserved the information for the correct hole to a similar extend, as it was shown in the retention task. The motivational factors in the modified Barnes Maze emerged sufficient to induce learning performance in C57BL/6J, but the immobility and thigmotaxis observed in the 129S6/SvEvTac shows lack of motivation or pressure to perform the task in these animals. Acquisition of the test is based on the animals innate behaviour of avoiding open, illuminated areas and their preference for a darkened and enclosed shelter (Barnes, 1979).

The BM is claimed to represent the “dry” equivalent of the MWM (Barnes, 1988; Gerlai, 1999; Milani et al., 1998). However, exposure to an open field is not stress-free, but it causes a much smaller increase in stress hormones, such as corticosterone and corticotropin than during MWM sessions (Sternberg et al., 1992). Some investigators found fault with the mild procedure of the BM, considering the disadvantage to vary the motivational pressure of the task in low performing animals. Artificially, bright light, a fan or noise from a buzzer was added to the testing procedure in order to “motivate” these animals (Fox et al., 1998;

Greferath et al., 2000; Inmann-Wood et al., 2000). These aversive stimuli in order to enhance performance also bear stressful components that must be taken into account when interpreting the behaviour measured. Another modulation of the original BM version (Koopmans et al., 2003) concerning the testing procedure was adopted in the present study. During initial pilot studies, we observed hesitation to descend immediately into the escape tunnel, indicating aversive reaction to descend into the escape hole. In theory, motivation to descend into the escape tunnel is driven by exploratory behaviour and by the aversive properties of the open and brightly illuminated area. Thus, the animal is confronted with choosing between an aversive (the open area) and a less-aversive (the shelter) situation. From there, it can be assumed that the animal is confused by a competing situation to choose between two aversive stimuli. Prickaerts and colleagues (Koopmans et al., 2003) have developed a modified version of the BM that comprises an improvement relating to motivational and emotional aspects. Fear of descending vertically into a hole is more presumable than simply passing horizontally into a tunnel. Thus, the position of the holes was turned from vertical to horizontal alignment. Hence, to escape the open area, the animal merely needs to pass directly into the tunnel instead of descending headfirst into it. Demands on motor agility and on motivation are higher in the vertical version than in the horizontal one (personal observation and discussion).
In conclusion, stressful treatments to motivate the animals in performing the BM task can be held to a minor level. It does not involve food deprivation, submission into water, or use of electric shock, instead it is based on natural explorative behaviour and the tendency to avoid open and bright areas. Modifications of the task set-up proved beneficial in reducing potential aversion against the escape hole, thus motivating the animal to immediately descend into the tunnel. The BM is a well-elaborated apparatus for testing spatial cognitive behaviour in rodents, bearing the option to vary the level of stress dependent on the animals` emotionality.

2.3.5.3. Experiment 1c: The Holeboard task

Performance in the HB revealed difference in error rates. 129S6/SvEvTac mice made fewer errors than C57BL/6J. Paradoxically, including 129S6/SvEvTac in the analysis can make the strain look deceptively good as they almost lack inspections. The C57BL/6J significantly reduced incorrect error rates over sessions, whilst the 129S6/SvEvTac failed to learn the correct hole position. In turns of the parameters displaying locomotor activity, i.e. distance, duration and speed, the 129S6/SvEvTac moue strain show almost identical performance as in the modified Barnes Maze. In conclusion, the motivational means in the HB were neither

sufficient to induce learning performance nor to overcome locomotive characteristics in the 129S6/SvEvTac. The food reward was sufficient to motivate C57Bl/6J in the HB task.
Acquisition of the HB task is based on exploratory activity, which is basically driven by the search for food in deprived animals. Deprivation from food is considered as a stress factor (Merali et al., 2003), especially if animals were housed in groups. Dominant animals consume overproportionally more at the expense of weaker cagemates. Single housing, on the other hand, induced additional stress due to absence of cagemates, facing the social character of the animals (Van Loop et al., 2004). Deprivation, but also consumption of food during the test, changes metabolic processes and consolidation of memory traces (Konkle et al., 2003; Davidson, 1993). Together with the failure of 129S6/SvEvTac to acquire the task demands this leads to the conclusion that the HB is less optimal for testing 129Sv mice or mutant mice with a 129Sv background.

2.3.5.4. Experiment 1d: The Morris Water Maze task

Both groups were equally able to learn the MWM escape task, as indicated by reduced escape latency and distance to reach the platform. Swimming speed remained constant for both groups, thus speed did not bias the parameters for learning performance. Measurement of spatial learning requires analysis of spatial selectivity by examining performance on a probe trial, in which the platform is removed and the search pattern of the mouse is evaluated. Herein, both strains spent more time in the quadrant of the former platform position than in the other quadrants. There was no difference between 129S6/SvEvTac and C57BL/6J for the preference of previous platform positions.
With augmenting experience in the maze, animals memorised more precisely the position of the platform, as it is indicated by improved parametric measures. However, most of these parameters, such as escape latency and length of the swimming path typically decrease reverse-proportionally, i.e. the magnitude of improvement is highest within the first two sessions and strongly decreases with each session day. Compared to other multiple-sessions learning tasks, such as the RAM or the BM task, this learning discrepancy between the first two and the remaining sessions is strongest pronounced for the MWM task, giving raise to the theory, that motivation to acquire the task is very high in the MWM task (Hodges, 1996). Contrary, the stress induced by putting the animals into the MWM has to be taken into consideration (Abel et al., 1992). Laboratory animals have no previous experience with swimming, thus first submission to the water may be experienced as potentially life threatening. Moreover, repetitive swim trials can be exerting for the animals, an important

consideration in turns of pharmacological treatment or other manipulation that may interfere swimming performance. Taken together, it can be concluded that high motivation closely relates to stress in the MWM. However, animals can profit from experience they make in the course of sessions (see also discussion in Chapter III), and often show high learning performance potentially unbiased by stress.

2.3.5.5. Experiment 2: The object recognition task with two different versions

Compared to the exploration in experiment 2b, all animals in experiment 2a displayed little exploration in the original ORT version, particularly APP_{SL} mice. The time, animals spent to explore the maze was enhanced in all animals from experiment 2a, and in SJL mice by introducing the modifications. Discrimination performance was very low in all animals from experiment 2a. D1 was below 1.0 and d2 very close to the minimum discrimination level of 0.15. This was only the case in experiment 2b in SJL mice. The modification strongly enhanced d1 and d2 in all animals from experiment 2a and also in OF1 and NMRI mice. SJL mice were the only strain that failed to discriminate in the original version or to improve discrimination in the modified version of the ORT, although exploration times were on a high level for both versions. OF1 and NMRI mice, however, spent more than 12 and 17 s on inspection of the objects in the original version, which is considered as a higher value (Sik et al., 2003). This measure was similar with the modified version. Both strains displayed high recognition memory in the original version for d1 and d2, which was further improved with the introduction of modifications.

It has been reported recently, that strains differences in exploratory behaviour exists (Tang et al, 2002; Voikar et al., 2001). In previous studies, which was confirmed with present data, the C57BL/6 strain display insufficient exploration in the “original” ORT transferring to derogated d1 but also d2 values (Prickaerts et al., 2002; Sik et al., 2003). Low exploration values may result from a lack of motivation or from fearful stress reactions towards the maze. Object inspections occur occasionally with movements or in such a short time frame that manual scoring becomes incorrect. This behaviour can bias discrimination analysis towards extreme or false counts (Sik et al., 2003). We postulate that the higher the exploration time, the better the dissociation between behavioural activity and discrimination performance, and the more precise the measurement of real object retention. In case of experiment 2a, exploration times were less than 5 s in all animals, which negatively influenced discrimination indices. Modifications to the ORT enhanced exploration and revealed clear discrimination performance in all animals. Thus, modified version enables the use of C57BL/6, an important

mouse strain for behavioural studies but also for genetic engineering (Crawley, 1996; Crawley et al., 1997; Gerlai, 1996; Lathe, 1996). It was attested that not only young, but also old C57BL/6 and old transgenic APP_{SL} x C57BL/6 mice with amyloid related mutations successfully performed the task. Experiment 2b revealed that modification enhanced discrimination performance in OF1 and NMRI mice, without taking influence on exploratory activity. These animals are highly discriminating mouse strains, well suited for both testing arrangements. The tests also demonstrated a failure of the SJL mouse strain to acquire the task, independently of the testing arrangement.

Comparison between trial durations of 3 and 5 Min showed no significant difference for discrimination analysis. Although it was shown that memory was longer retained in tests of 10 min versus 6 min duration in male Swiss mice (Dodart et al., 1997), in our experiments, the prolongation of 2 min in the present study had no influence on learning performance.

Taken together, the study identified mouse strains with high discriminative abilities, irrespective of the test set-up and protocol. Furthermore, it was shown that introduction of modifications enlarged applicability of the task for important mouse strains and thus the value for behavioural measurements.

2.3.5.6. General discussion

Learning trials should be designed for optimal reproducibility of learning performance and cognitive abilities. Experiment 1 demonstrated that the motivational means to induce learning performance in the “dry mazes”, i.e. T-Maze, mBM and HB, were effective in the C57BL/6J but not or respectively less in the 129S6/SvEvTac mouse strain. Although 129S6/SvEvTac were successful in reducing the amount of incorrect choices, motor activity was highly inconstant in all dry mazes, thus accounting for potentially false negative results. Remarkably, in the MWM- a task enforcing the animals to motor agility- 129S6/SvEvTac and C57Bl/6J were equally able to acquire the task. However, these results were not constantly attested, as 129Sv mouse strains but also other strains (Royle et al., 1999; Thifault et al. 2002; Yoshida et al., 2001) were also described as poor swimming navigation learners, reflected by thigmotaxis or floating behaviour (Lipp et al., 1995). The 129S6/SvEvTac performed inhomogeneously in the “dry mazes” as it is indicated by high standard errors, whereas performance in the MWM was very similar within the group, confirmed by low standard errors. Thus, the MWM should be given preference in choice of the learning task for testing 129S6/SvEvTac or for mice with similar background.

Behavioural tests of cognition reflect multiple underlying traits such as motivation to induce locomotor and exploratory activity, olfaction and vision, as well as fear and anxiety (Crawley et al., 1997; Dayan and Balleine, 2002). These factors need to be rigorously dissociated from true learning and memory measures. Thus, motivational aspects have to be carefully balanced for highly activating the animals` behaviour, and the induction of stress to avoid confiding variations within mouse (or rat), groups or strains. In low stress environments, general activity probably dominates observed score variance, whereas in stressful test environments, anxiety based factors are likely to be a large component of observed variance in activity. There is no "golden standard" for the degree of pressure that should be set to an animal in order to motivate learning behaviour, facing countless factors taking influence on the animal behaviour *per se.* These factors include breeding and housing conditions, handling, gender, age, strain, transgenic manipulation, even individual differences between littermates can be observed.

To address this problem, standardised housing and handling procedures should found a basis for behavioural testing. Moreover, either high performing strains should be chosen for respective tests (Crawley et al., 1997; Upchurch and Wehner, 1988), or conversely, choice of tests should be adopted to the mouse or rat strain (Wolff et al., 2002; Wahlsten et al., 2003). In addition, adaptation in forms of slight modifications of the testing procedure to respective subjects proved beneficial in many tests of cognitive behaviour (Content et al., 2001; Wahlsten et al., 2003). This high variability of testing arrangements enables research close to the animals` natural behaviour and provides an opportunity to optimise unbiased read-out of information in cognitive tests.

Chaper III: Evaluation and validation of new animal models in a longitudinal study

3.1. Abstract

Single (APP_{SL}, APP_{SL}x x $PS1_{wt}$) and double mutant (APP_{SL}x x $PS1_{mut}$) mice compared to with wild-type controls were tested a study with longitudinal design, constituting a battery of hippocampus related cognitive tasks. Animals were subjected twice to the testing procedure: before and after nucleus basalis magnocellularis (NBM) lesions using a new and selective technique with the immunotoxin mu p75 SAP. Initially executed titration studies revealed a dose-dependent reduction of a cholinergic marker with discrete NBM lesions. Results from behavioural analysis demonstrated a treatment effect in object recognition (ORT) and T-maze continuous alternation task (T-CAT) with NBM lesions, compared to sham operated groups. Genotype effects were found in T-CAT, modified Barnes Maze (mBM) and Morris Water Maze (MWM). Impact of genetic manipulation and surgery were analysed with respect to histopathological changes and implicated brain areas and related to the relevant literature. The findings indicate that selective NBM lesions induced working memory deficits, which is related to neocortical changes. Trangenic manipulations impaired spatial memory, in particular allocentric reference memory, which is particularly related to pathology within the medial temporal lobe, but also to neocortical changes.

3.2. Introduction

In chapter I, a detailed overview of animal models for the study of pathological changes in the course of AD was provided. Investigators profit from genetic analysis of familial inherited AD cases. Studies are based on the “Swedish” and “London” mutation, generating enhanced proteolytic cleavage of APP by ß- or γ- secretase. Genetically engineered mouse models carrying genetic codes for pathological modifications of human APP proved beneficial tools for investigations of Aß and NP load of AD. Double mutant mice carrying additional pathogenic PS1 display higher and earlier Aß load and NPs. It was concluded that most transgenic mouse models of amyloidosis, as an important hallmark of AD, meet *face* and *predictive* model validity.

Cholinergic hypofunction of the BFCS represents the second hallmark of AD. The central cholinergic system is implemented in cognitive processes, and ablation of cholinergic neurons and their pathways was shown to result in mental decline (chapter I, section 1.2.1.). Moreover, it has been postulated that cholinergic degeneration enforces development of NP depositions. Thus, animal models of selective cholinergic neurodegeneration in the BFCS proved valuable tools for the research of synergistic activities in the CNS and of new cognition enhancing drug therapy with high *face* and *predictive* model validity (chapter I, section 2.3).

To our knowledge, the combination of transgenic mouse model of amyloidosis with discrete and selective cholinergic neurodegeneration of the NBM has not been investigated so far. (Berger-Sweeney et al., 2001; Dewachter et al., 2001; Liu et al., 2002; Pepeu, 2001). In the present study, this combination will be investigated as a progressive mouse model. Therefore, four mouse models with different genotypes, APP_{SL}, APP_{SL}x x $PS1_{wt}$, APP_{SL} x $PS1_{mut}$, and C57BL/6 as control animals, will be subjected to the study. These mice generate different niveaus of Aß load or NP depositions. Experiments will be conducted in a longitudinal arrangement containing two identical sets of behavioural models, performed at two different time periods. The learning tests were carefully selected from tests shown in chapter II and have to meet the criterion of high dependence on hippocampal integrity (Wilkerson and Levin, 1999). Prior to the second set of testing, cholinergic lesions by means of the immunotoxin mu p75 SAP will be carried out in 50 % of the animals in each groups. This arrangement allows assessment of the cognitive state in these animals (1) at younger stages in the absence of additional cholinergic neurodegeneration, (2) at older stages, when plaque pathology should be fully pronounced in appropriate mouse groups, (3) and in absence (sham lesion) and presence of cholinergic NBM neurodegeneration in these animals. In addition, (4) impact of gender will be additionally analysed.

To allow monitoring of cholinergic destruction at a given time point (second testing), another group of C57BL/6 was subjected to cholinergtic lesions with mu p75 SAP in a parallel satellite study. Satellite animals were sacrificed after the same incubation period as animals from the longitudinal study, and brains were biochemically analysed for cholinergic markers.

3.3. Material and Methods

3.3.1. General

3.3.1.1. Animals

As subjects served adult C57BL/6J (n = 19) (IFFA CREDO), $APP_{Swedish/London}$ (APP_{SL}) mutant mice (n = 20), double mutant (mut) "APP_{SL} x $PS1_{mut}$"(n = 26), and animals of the wildtype (wt) control group "APP_{SL} x $PS1_{wt}$"(n = 22). All transgenic mice were delivered by the in-house breeder of the Bayer-Health Care AG. Mice of the APP_{SL} mutant group were backcrossed with C57BL/6Jico in 6^{th} generation. PS1mut or PS1wt mutant mice were first backcrossed with C57BL/6, and finally crossed with APP_{SL} mutant mice to APP_{SL} (x C57BL/6Jico) x PS1mut (x C57BL/6Jico) or APP_{SL} (x C57BL/6Jico) x PS1wt (x C57BL/6Jico) (Table 5).

Table 5: Animal groups enlisted providing an overview for the detailed size of groups and the nomenclature used in this study

Animals Genetic nomenclature	**Animals** Nomenclature during experiment	**Age at the beginning of experiments**	**n male animals**	**n female animals**	**total**
C57BL/6Jico	C57BL/6	8 month old	19		19
APP_{SL} x C57BL/6Jico	APP_{SL}	8 month old	9	11	20
APP_{SL} x $PS1_{wt}$ x C57BL/6Jico	APP_{SL} x $PS1_{wt}$	4 month old	13	9	22
APP_{SL} x $PS1_{mut}$ x C57BL/6Jico	APP_{SL} x $PS1_{mut}$	4 month old	15	11	26

3.3.1.2. Study set-up

All animals were subjected to a longitudinal study. Testing procedure was set at two time points including an extensive testing battery (Table 5). C57BL/6 and APP_{SL} were tested at an age of 8 months (M) and at the age of 14M. Previous experiments have shown that APP_{SL} mice do not develop plaques (data not shown) at any age. APP_{SL} x $PS1_{wt}$ and APP_{SL} x $PS1_{mut}$ were tested at the age of 4M and 14M. This schedule allows assessment of cognitive abilities at an early time point (4M) were no plaque formation has been reported and at an age when dense plaque deposits have been described (personal communications K.H. Baumann).

Prior to the second testing set, all animals received NBM or sham lesions and were allowed to recover for ten days. The procedure provides information about the "unimpaired" cognitive abilities as a negative control, about the impact of the lesion and the impact of the mutation. For the transgenic animal groups, the impact of gender will additionally be analysed.

The testing set comprised several behavioural testing procedures. The (1) T-CAT, (2) the ORT, the modified version of the (3) BM and the (4) MWM were used. The tasks are associated with different motivational factors and motor requirements (see chapter II). This, should lead to profound information concerning behaviour, cognition and sensitivity of the tasks used for these different genotypes. This following detailed readout was use

(1) effect of gender in the transgenic/ lesioned mice
(2) impact of genotype (comparison of all groups)
(3) impact of lesions (NBM lesioned versus non lesioned C57BL/6)
(4) impact of NBM lesions in APP_{SL} mutant mice, e.g. amplification of learning deficits
(5) amplification of learning deficits in NBM lesioned double mutant mice, e.g. in mice that develop plaque deposits

Grouping of animals for data analysis- scheme for a detailed read-out of performance in all learning tests, respectively:

Impact of genotype (of transgenic manipulation, i.e. Aβ burden):
The four genotype groups were directly compared:

(i) before lesioning in the first learning battery (B1)
(ii) all sham operated animals, second testing battery (B2sham)
(iii) all lesioned animals, second testing battery (B2les)

Impact of surgery (cholinergic ablation) on each single genotype:

(i) C57BL/6 B1, B2sham and B2les were compared
(ii) APP_{SL} B1, B2sham and B2les were compared
(iii) APP_{SL} x PS1wt B1, B2sham and B2les were compared
(iv) APP_{SL} x PS1mut B1, B2sham and B2les were compared

Treatment (surgery) versus genotype effect
Data will be depicted for each genotype and treatment (sham versus lesioned) combined into conclusive schemes. Representative sessions and parameters will be selected for the schemes. These graphs will help elucidate the impact of each pathology.

Impact of gender

Data was sorted as in *lesion/sham operation analysis.* Groups were analysed separately for B1, B2sham and B2les.

***Working memory** analysis* (restricted to WMW)

Data was sorted by ***strains*** and separated for B1, B2sham and B2les groups. The analysis will elucidate the degree of learning within one session and the degree of forgetting between daily sessions.

Table 6: Experiment schedule of the testing procedures for the longitudinal study. Both experimental groups of animals were tested at two time points, to elucidate the impact of compromised Aß$_{42}$ processing and the plaque formation pathology. Half of all animals received NBM lesions with the immunotoxin mu p75 SAP, the other half was sham operated. The testing battery includes assessment of learning in the T-Maze continuous alternation, the object recognition task, the modified Barnes Maze, and in the Water Maze task. Impact of gender was additionally determined

Animals	***Testing battery (B1)*** **T-Maze Object recognition Modif. Barnes Maze Morris Water Maze**	**Lesions**	***Testing battery (B2)*** **T-Maze Object recognition Modif. Barnes Maze Morris Water Maze**
APP_{SL} C57BL/6Jico	8 month old	Ten days prior to second testing set: Counterbalanced immunotoxic and sham lesions	14 month old
APP_{SL} x $PS1_{wt}$ APP_{sL} x $PS1_{mut}$	4 month old	Ten days prior to second testing set: Counterbalanced immunotoxic and sham lesions	14 month old

3.3.1.3. Housing conditions

All animals were housed individually in standard Makrolon® cages of type II with sawdust bedding. Room temperature was constant (24 ± 1C°), 60% humidity and a 12:12 light/dark cycles was maintained, with lights on at 6:00 am. Food (standard chow, Altromine®) and water were delivered *ad lib.*. The experiments were performed in the same room where animals were housed.

3.3.1.4. Immunotoxic and sham lesions

For the longitudinal and satellite study, the immunotoxine mu p75 SAP (Bioserve®) was used. Mu p75 SAP is a conjugate of rat anti-mouse NGFR (p75) monoclonal antibody (mAb) and the toxin saporin, a ribosome-inactivating protein. The mAb specifically binds to p75 receptors, which are mainly represented by cholinergic neurons in the BFCS and on their nerve cell terminals in neocortex and hippocampus (Springer, 1988; Torres et al., 1994; Wenk et al., 1994). Upon binding of mAb to the NGFR, the receptor-mAb-complex is being internalised and degraded to its respective components. Binding of the released saporin molecule to ribosomes leads to inhibition of protein biosynthsis and subsequently to cell death (Wiley et al., 1991) (Figure 19). In previous studies it has been shown that i.c.v. injections of mu p75 SAP lead to degeneration of the cholinergic neurons up to 70 % (Berger-Sweeney et al., 2001). Injections of the immunotoxin into discrete brain areas have not been reported, yet.

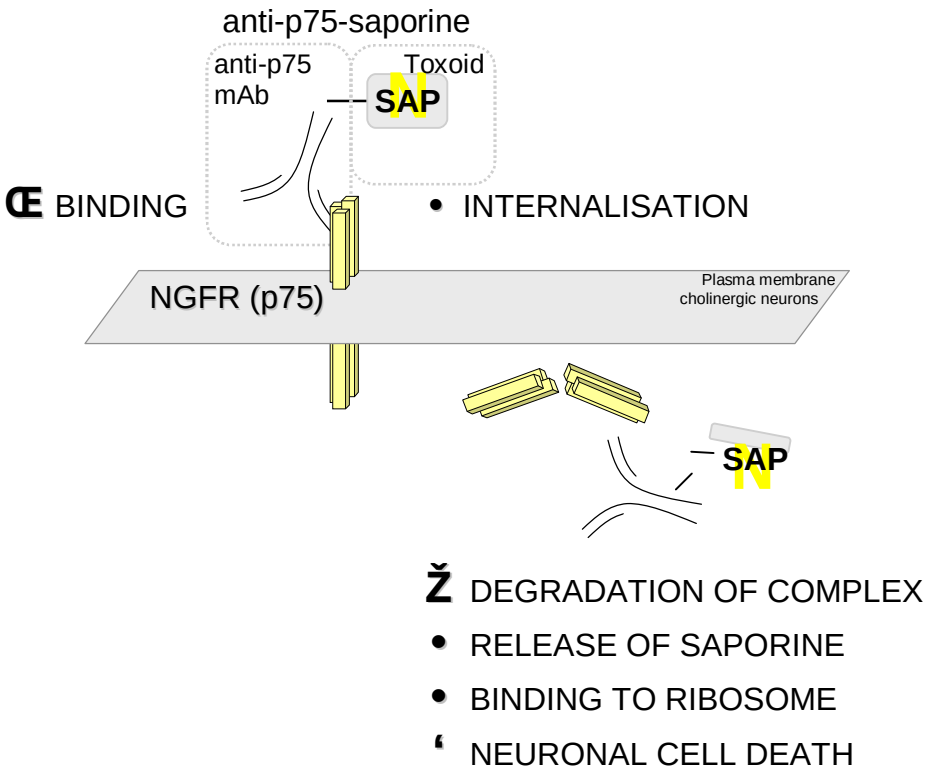


Figure 19: (**1**) mu p75 SAP selectively binds to the NGFR (p75), (**2**) the complex is being internalised and (**3**) degraded in the cell cytosol. (**4**) The toxin saporin is being released and (**5**) binds to the ribosomes of the cell, (**6**) resulting in apoptotic cell death.

Coordinates for the NBM lesions were evaluated according to Franklin & Paxinos mouse brain atlas (Franklin and Paxinos, 1997) by injection of ink or ibotenic acid (Connor et al., 1991) in initial pilot studies (data not shown). On this purpose, other C57BL/6 and

APP_{SL} x $PS1_{wt/mut}$ mice were used to guaranty correct position of the lesion, independent of genetic background.

For the longitudinal study, animals of all genotypes were randomly assigned into equal groups of lesioned and sham operated animals. The mice were deeply anaesthetised with Rompun (2% xylancinhydrochloride, Bayer AG) and Ketavet (100 mg ketaminhydrochloride ml^{-1}, Pharmacia & Upjohn). Solution: 0.8 ml Rompun solution and 1.2 ml Ketavet solution solved in 8 ml NaCl (0.9%). The anaesthetised (6 $ml*kg^{-1}$, i.p.) animal was placed in a Sembach (Ratingen, FRG) stereotaxic apparatus. Mu p75 SAP (c = $1mg*ml^{-1}$) or phosphate buffered saline (PBS) were injected bilaterally at the following coordinates: measured from flat scull, AP ± 0.0 mm, ML ± 2.7 mm, DV – 4.6 mm, 8° angle affixed lateromedially. Infusion was given at a rate of 0.1 µl min^{-1} with 0.1 µl infusion volume per hemisphere by use of a hamilton syringe (0.5 µl volume). 5 min after infusion, the syringe was retracted carefully and the scalp wound was shut with tissue-glue. The animals were allowed to recover for a minimum of ten days before the second battery of experiments started.

3.3.2. The immunotoxin mu p75 SAP – cholinergic deficit induced by discrete injection into the NBM; a new technique to model AD like cholinergic deficit

3.3.2.1. Animals

As subjects served 20 male 19 weeks old C57BL/6 (IFFA CREDO) mice (housing conditions see 3.1.2.) for the satellite study. Moreover, a titration study, 48 male 13 weeks old C57BL/6 (IFFA CREDO), was initially performed to find the optimal concentration of the immunotoxin for discrete injections into the NBM. Animals were initially housed in groups of 10 or 8 in standard Makrolon® cages of type 3 with sawdust bedding. After lesioning, mice were housed individually in Makrolon® cages of type II.

3.3.2.2. Surgery

Immunotoxic lesions were conducted as described in section 3.3.1.4.. A group of 10 animals was bilaterally lesioned with a concentration of c = $1mg*ml^{-1}$, another group of 10 animals was sham lesioned with bilateral injections of PBS. These animals were subjected to the satellite study. Chronically, surgery was conducted after lesioning animals from the longitudinal study.

In addition, previous studies with mu p75 SAP were performed to receive a titration of the immunotoxin in correlation with cortical AChE activity. Therefore, respectively eight animals

were lesioned unilaterally with following concentrations: c = 0.5mg*ml^{-1}, c = 0.75mg*ml^{-1}, c = 1.0mg*ml^{-1}, c = 3.0mg*ml^{-1} and c = 4.0mg*ml^{-1}. Eight animals were bilaterally sham operated with PBS.

3.3.2.3. Biochemical analysis of AChE activity related to protein concentration

All mice were sacrificed by cervical dislocation for biochemical analysis 10 days after surgery. Cortices were removed, left and right hemispheres were transverse sectioned into equal anterior and posterior sections and separately weighed, frozen and stored at –70° until the essay. Biochemical analysis of AChE activity was conducted according to the protocol of Ellmann and colleges (Ellmanns et al., 1961; Ho and Ellman, 1963). In brief, brains were homogenised in 9 volumes of solution D (0.01M Tris HCL pH 7.4, 1M NaCl, 0.01M EDTA, 1 % Triton X-100) per wet weight to liberate the AChE from their membranes. After centrifugation at 14000 rpm for 45 min at 4°C to sediment membranes, supernatants were collected and diluted 1:10 with 0.1M phosphate buffer. Due to a high concentration of AChE activity in the anterior part of the cortices, data analysis is restricted to the frontal brain sections.

Colorimetric determination of protein concentration- the Lowry assay (Lowry et al., 1951)

The Bioserve ABC protein kitt assay was used in a 96-well microtiter plates in order to determine the protein concentration of the supernatant. The analysis uses a standard curve of readings from known amounts of proteins.

Colorimetric determination of AChE activity- the Ellmans assay (Ellman et al., 1961)

Ellmans reagent (2ml DTNB stock solution, 30ml 0.2M phosphate buffer pH 7.4, 28ml A.dest.) was added to respectively 10 µl supernatant samples in 96-well microtiter plates. Following an incubation of 30 min (DTNB reacts with thio-remnant (??) in the homogenate), 20 µl of acetylthiocholine (10mM solution in A.dest) was dispensed to each well. Wells were instantly placed in the ELISA reader. Spectrometric readings at 405nm were taken at regular intervals of 30 s for 10 min.

3.3.2.4. Data analysis

Protein concentration and AChE activity were correlated and measured as nmol / min*mg protein. Results were expressed as mean and ± SEM percent decrease of AChE activity versus respective unlesioned brain areas and versus sham operated brains. Data was analysed by use of one-way ANOVA, accomplished by Fisher`s LSD test.

3.3.3. Experiment 1: The T-Maze continuous alternation alternation (T-CAT)

3.3.3.1. Apparatus and set-up of the test

The apparatus and set-up of the study have been introduced in chapter II, 2.2.3.3.1.. (Figure 8).

3.3.3.2. Procedure

All animals groups were tested three times in the T-CAT: First, before lesioning, second, after leasioning, and a third time to examine the effect of E2020 (Aricept), an acetylcholinesterase (AChE) inhibitor. E2020 reversibly blocks ACh, thus the bioavailability of acetylcholine is enhanced. It has been shown that AChE inhibitors can improve cognition (Darvesh et al., 2003; Giacobini, 2003). In the present study, we wanted to examine the effect of E2020 ($c = 0.6$ mg*ml^{-1}) on cognition in transgenic mice, with and without cholinergic deficits. Enhanced alternation in lesioned mice in the presence of E2020 would confirm cholinergic character of the lesion.

The animals were subjected to one single session of 15 consecutive trials, beginning with one forced-choice trial, followed by 14 free-choice trials according to the procedure depicted in chapter II, 2.3.3.1..

3.3.3.3. Data analysis

The data of all animals that completed less than 8 free-choice trials during 30 min were excluded from analysis. The overall alternation rate (percent alternation) during the 14 free-choice trials was calculated (0% = no alternation, 100% = alternation at each trial, 50% = random alternation), the overall time to choice and the mean session duration was calculated statistically using the one-way analysis of variance (ANOVA) with the factor *genotype (and gender)* or *treatment (effect of surgery)*, supplemented with Fisher`s least significant difference (LSD) *post hoc* comparisons. A difference between groups was considered significant if the associated probability (p value) was below 0.05. In addition, the Student`s *t*-test was used to evaluate if percent alternation performance was significantly over chance level. It was calculated by percent alternations minus 50%, and significant if the difference score differed from zero.

3.3.4. Experiment 2:The modified object recognition task (ORT)

3.3.4.1. Apparatus and set-up of the test

The apparatus and set-up of the study have been introduced in chapter II, 2.2.3.3.5.. The modified version of the ORT has been adopted for the present test (Figure 12 B).

3.3.4.2. Procedure

The procedure of the test has been introduced in chapter II. However, enlarged tests have been made, which should implicate a short description of the whole procedure for better understanding.

The mice were trained in pairs of two trials per day that were separated by a retention, or ITI. The ITI was varied from day to day beginning with an ITI of 1 min, followed by ITIs of 1h, 3h, 6h and 12h. During the first trial (T1) the apparatus contained two identical objects. These objects were placed in a symmetrical position about 120 mm (with reference to the centre of the object) away from the wall. A mouse was taken from its home cage and placed into the apparatus, equidistant from the two objects, facing the wall in front of the experimenter. The mouse was allowed to explore the objects for 5 min. After T1, the mouse was transferred to its home cage. After respective ITI, the mouse was transferred into the apparatus again for the second trial (T2). During T2, the exploration arena contained two different objects, a copy of the familiar one (from T1) and a novel object. In 5 daily sessions different sets of objects have been presented. The time spent exploring the two objects during T1 and T2 was manually registered by the experimenter using an observer program (designed and programmed by BAYER CNS Research support).

In this ORT, memory for a given object over different retention times is tested. Good retention performance is indicated by short exploration times of the familiar object in T2, because the animal should be habituated to the object. Higher exploration of the familiar object, in relation to exploration of the new object, indicates forgetting. It has been shown, that complete forgetting occurs after an ITI of 24 h. The ORT is created to test WM in mice. We chose a protocol with a daily extended ITI to find out how long mice with the induced pathological changes are able to keep memory of a given object, i.e. to test the impact of ITI duration on forgetting.

3.3.4.3. Data analysis

In accordance to the calculation procedure introduced in chapter II, the values of the discrimination indices d1 and d2 were averaged per mouse over the two sets of testing and

analysed statistically by an analysis of variance (ANOVA) with the factor *genotypes* or *gender*, supplemented with Duncan's *post hoc* comparisons. In addition, strain differences on the discrimination indices were also analysed by ANOVA for each of the two testing series. Repeated measures analysis was implemented to describe the *surgery* effects. A difference between genotypes, surgery groups or gender was considered significant if the associated probability (p value) was below 0.05. Each of the 5 sessions was analysed separately, because all sessions were independent from another.

3.3.5. Experiment 3: The modified Barnes Maze task

3.3.5.1. Apparatus and set-up of the test

The apparatus and set-up have been introduced in chapter II, 2.2.3.3.2. (Figure 9).

3.3.5.2. Procedure

The procedure of the test has been introduced in chapter II, 2.2.3.3.2.

Acquisition training (learning of the correct hole position) was accomplished for 10 daily sessions with two trials per session. In first five sessions, an ITI of 1 min was implemented. The following five sessions were carried out with an ITI of 15 min. The position of the escape hole (for each mouse, respectively) remained identical for both training blocks.

3.3.5.3. Data analysis

The mean *error rate* (amount of incorrect hole visits), *distance* travelled in the arena, *duration* to complete the trial, and *speed* of locomotion were assessed and statistically analysed using the two-way ANOVA for repeated measures over *genotypes*, *surgery groups* or *gender* for sessions as repeated measure factor. A *post hoc* Student´s *t*-test was additionally used to assess differences between groups particularly. A difference between groups was considered significant if the associated probability (p value) was below 0.05.

3.3.6. Experiment 4: The Morris Water Maze (MWM) task

3.3.6.1. Apparatus and set-up of the test

The apparatus and set-up have been introduced in chapter II, 2.2.3.3.4. (Figure 11).

3.3.6.2. Procedure

The procedure has been introduced in chapter II, 2.2.3.3.4.. In the present study, the procedure has been slightly varied to enhance information read-out from of test.

Animals were trained on a repeated acquisition schedule to find the submerged escape platform for refuge from the water. One daily session consisted of 5 consecutive trials with releasing the animal from 4 different start points in a randomly assigned pattern that changed from session to session in a randomised sequence. In the fifth trial, the mouse was released from the same starting point as in the first trial. A trial was started by putting the mouse in the pool, facing the wall of the pool. The animal was allowed to explore the pool for the escape platform within 120 seconds. The trial was terminated as soon as the animal climbed onto the platform or when 120 seconds elapsed, whichever occurred first. The animal remained on the platform for 30 seconds before the start of the consecutive trial. Mice, which failed to locate the platform in the allotted time, were manually placed the platform for 30 seconds and an escape latency of 120 seconds was recorded for that trial. This repeated acquisition procedure was performed for 5 daily sessions.

3.3.6.3. Data analysis

Repeated acquisition analysis: Mean platform *escape latency*, mean *distance travelled* in the Morris Water Maze, mean *swimming speed* and mean *distance to platform* were measured and statistically analysed using the two-way ANOVA for repeated measures over sessions. A Student´s *t*-test was additionally used to assess differences between *genotypes*, *surgery groups* or *gender* particularly.

Working memory analysis: A two-way ANOVA for repeated measures was used to evaluate working memory within a session. The first (trial 1) and the last (trial 5) trial, from respective mouse group, were compared as groups over sessions. *Post-hoc* Fisher`s LSD tests revealed differences between trials for respective session. A difference between groups was considered significant if the associated probability (p value) was below 0.05.

3.4. Results

3.4.1. Results of biochemical analysis

3.4.1.1. Titration study

Three major findings were assessed in the titration study. First, no differences were found between untreated hemispheres in all treatment groups ($F_{(4,28)} = 0.23$, $p = 0.918$) and in comparison to sham operated animals ($F_{(5,75)} = 0.63$, $p = 0.675$). Second, clear treatment effects were observed in all lesioned hemispheres if compared to appropriate unlesioned hemispheres and to the pooled sham group (indicated by asterisks in figure 20, and expressed as percent decrease in table 7). Third, lesion effects were dose-dependent. Clear differences were found between the treatment group c = 1.0mg*ml^{-1} and c = 0.5mg*ml^{-1}, c = 0.75mg*ml^{-1}, and c = 3.0mg*ml^{-1}. Moreover, reduction of AChE activity was significantly higher in the three highest concentrations c = 1.0, 3.0 and 4.0 mg*ml^{-1} if respectively compared to the two groups at lowest concentrations. No differences were found between concentration c = 1.0mg*ml^{-1}, c = 3.0mg*ml^{-1}, and c = 4.0mg*ml^{-1}. However, side-effects by the immunotoxin increased with dosage (aphagia, adipsia; animals had to be fed artificially and supplied with s.c. injections of saline with dosages higher than c = 1.0 mg*ml^{-1}).

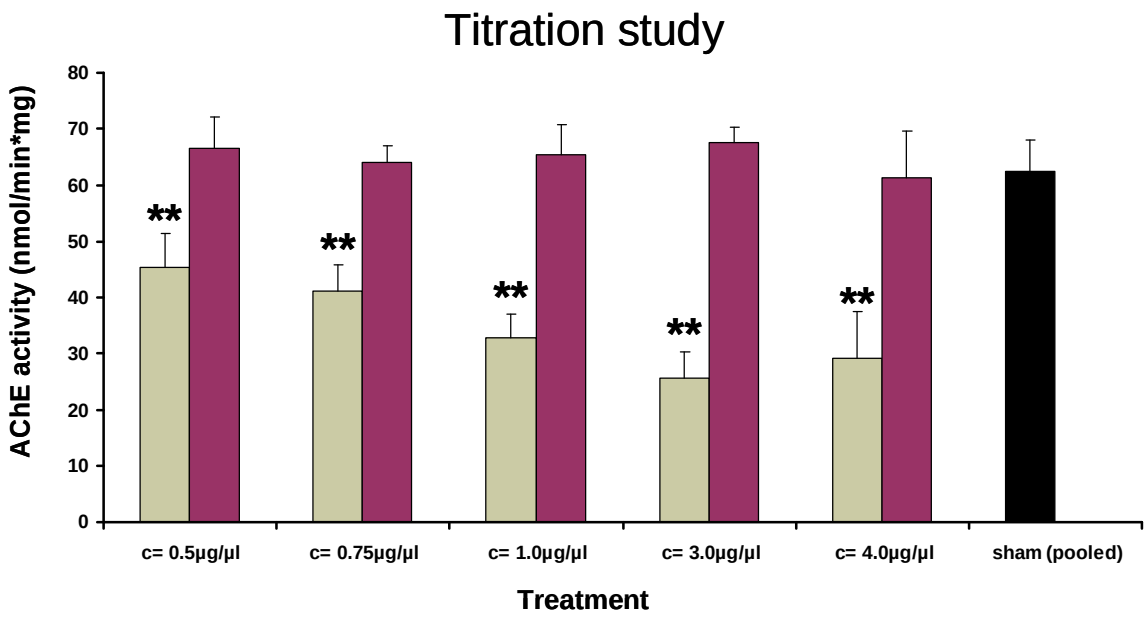


Figure 20: Effects of discrete unilateral NBM lesions (striped bars) with different concentrations of the immunotoxin mu p75 SAP upon. *AChE activity* related to appropriate protein level measure. Lesioned hemispheres are compared to appropriate unlesioned (red bars) hemispheres and bilaterally lesioned sham (black bars) data. Data is represented as mean + SEM. High significance between lesioned and respective unlesioned hemispheres is indicated by ** for $p < 0.01$

Table 7: Percent decrease of cortical AChE activity after NBM lesions with different immunotoxin doses

Concentration of immunotoxin	***Percent decrease of AChE activity* compared to unlesioned side**		***Percent decrease of AChE activity* compared to pooled sham group**	
	Means	**SEM**	**Means**	**SEM**
c = 0.5mg*ml^{-1}	32.10 %	6.47	27.50 %	10.89
c = 0.75mg*ml^{-1}	35.61 %	7.22	33.98 %	9.82
c = 1.0mg*ml^{-1}	49.92 %	6.87	47.55 %	4.68
c = 3.0mg*ml^{-1}	61.93 %	8.94	58.81 %	6.49
c = 4.0mg*ml^{-1}	52.46 %	15.68	53.37 %	12.79

Analysis of respective hippocampi revealed no differences between unlesioned sides ($F_{(4,28)} = 0.307$, $p = 0.851$), unlesioned and sham operated sides ($F_{(5,75)} = 1.18$, $p = 0.338$) or between lesioned hemispheres ($F_{(4,28)} = 0.388$, $p = 0.815$). Data was therefore pooled for all lesioned and unlesioned groups. Hippocampal AChE activity was similar to pooled unlesioned sides ($F_{(1,39)} = 0.414$, $p = 0.524$) and to sham operated sides ($F_{(1,39)} = 0.22$, $p = 0.642$), indicating a sparing effect by the immunotoxin.

Table 8: Percent decrease of hippocampal AChE activity after NBM lesions with different immunotoxin doses (data was pooled).

Hippocampal tissue (pooled data)	***AChE activity (nmol/min*mg)***	
	Means	**± SEM**
unlesioned sides	65.77	1.85
sham operated sides	67.55	2.03
lesioned sides	68.56	3.14

3.4.1.2. Satellite study

Data analysis confirmed similar AChE activity in sham operated animals for respective right and left hemispheres ($F_{(1,9)} = 0.935$, $p = 0.359$). Similar activity was also observed in respective right and left hemispheres of lesioned animals ($F_{(1,9)} = 0.693$, $p = 0.427$). Therefore, AChE activity measure of lesioned animals was pooled and compared to pooled sham data. Clear differences between NBM lesioned and sham treated animals were observed ($F_{(1,19)} = 81.60$, $p < 0.0001$). Activity in c = 1.0mg*ml^{-1} treated animals was reduced to 57.57 % if compared to the sham operated hemispheres.

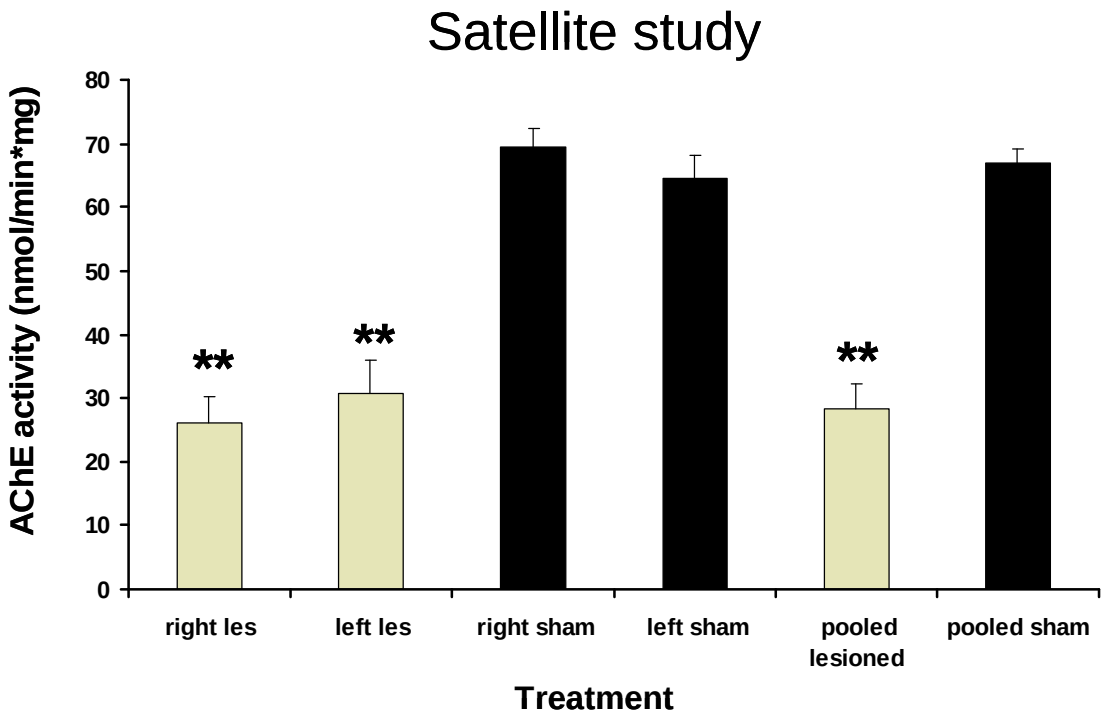


Figure 21: Effects of discrete bilateral NBM lesions with immunotoxin mu p75 SAP at c = 1.0µg/µl upon *AChE activity* related to appropriate protein level measure. Activity in right and left hemispheres are depicted separately and pooled. Data are represented as mean + SEM. High significance between lesioned (striped bars) and sham lesioned (black bars) hemispheres is indicated by ** for $p < 0.01$

3.4.2. Results of longitudinal study

General: following animals died after surgery or during the testing procedure:

C57BL/6: 6 male

APP_{SL}: 3 male, 5 female

APP_{SL} x $PS1_{wt}$: (all animals survived)

APP_{SL} x $PS1_{mut}$: 3 male, 4 female

No gender effect were found in any experiment (data not shown)

3.4.2.1. Experiment 1: The T-Maze continuous alternation task (T-CAT)

3.4.2.1.1. Genotype analysis

The results of the study are depicted in figures 22/23

3.4.2.1.1.1. All genotypes in B1 (all animals from the first testing battery)

Animals from all genotypes displayed mean percent alternation significantly over chance level. There was no difference between groups ($F_{(3,82)} = 0.59$, $p = 0.624$). Mean time to reach the goal arm was similar in all genotypes ($F_{(3,82)} = 0.84$, $p = 0.477$). Time to complete a session was also similar in all genotypes ($F_{(3,82)} = 1.45$, $p = 0.234$), but the control animals C57BL/6 tended to show fastest performance for both "locomotive"- parameters. (depicted in figure 22 A and 23 B1,a and B1,b). At the present time point, previous studies (data not shown) could not detect any NP deposits in the mutant genotypes.

3.4.2.1.1.2. All genotypes in B2sham (all sham animals from second testing battery)

One way ANOVA shows differences in alternation performance between groups ($F_{(3,35)} = 9.78$, $p < 0.0001$) with significantly more alternation in C57BL/6 animals compared to the other three genotypes, as calculated with the Fisher`s LSD test. There was also significant difference between the APP_{SL} and the APP_{SL} x $PS1_{mut}$ animals. All animals alternated significantly above chance level. The time to choose a goal arm was similar in all genotypes ($F_{(3,35)} = 2.58$, $p = 0.069$), but a difference was found between the APP_{SL} x $PS1_{mut}$ and APP_{SL} x $PS1_{wt}$ animals by means of the Fisher`s LSD test. No genotype differences were found for the time to complete the session ($F_{(3,35)} = 0.53$, $p = 0.662$) (depicted in figure 22 B and 23 B2sham,a and B2sham,b). At the present time point, previous studies (data not shown) have shown NP deposits in the APP_{SL} x $PS1_{mut.}$

3.4.2.1.1.3. All genotypes in B2les

The alternation was affected by the genotype ($F_{(3,22)} = 3.90$, $p = 0.022$) with the APP_{SL} animals alternating significantly less then the other genotypes. Alternation was low in all groups and no group alternated above chance level, which indicates, that the lesion effect was more potent to disturb learning performance than the genotype did. The animals showed no difference in time to enter a goal arm ($F_{(3,22)} = 0.41$, $p = 0.748$) or in time to complete the session ($F_{(3,22)} = 0.17$, $p = 0.918$) (depicted in figure 22 C and figure 23 B2les,a and B2les,b). At the present time poin, plaque deposits are expected in the APP_{SL} x $PS1_{mut}$ genotype.

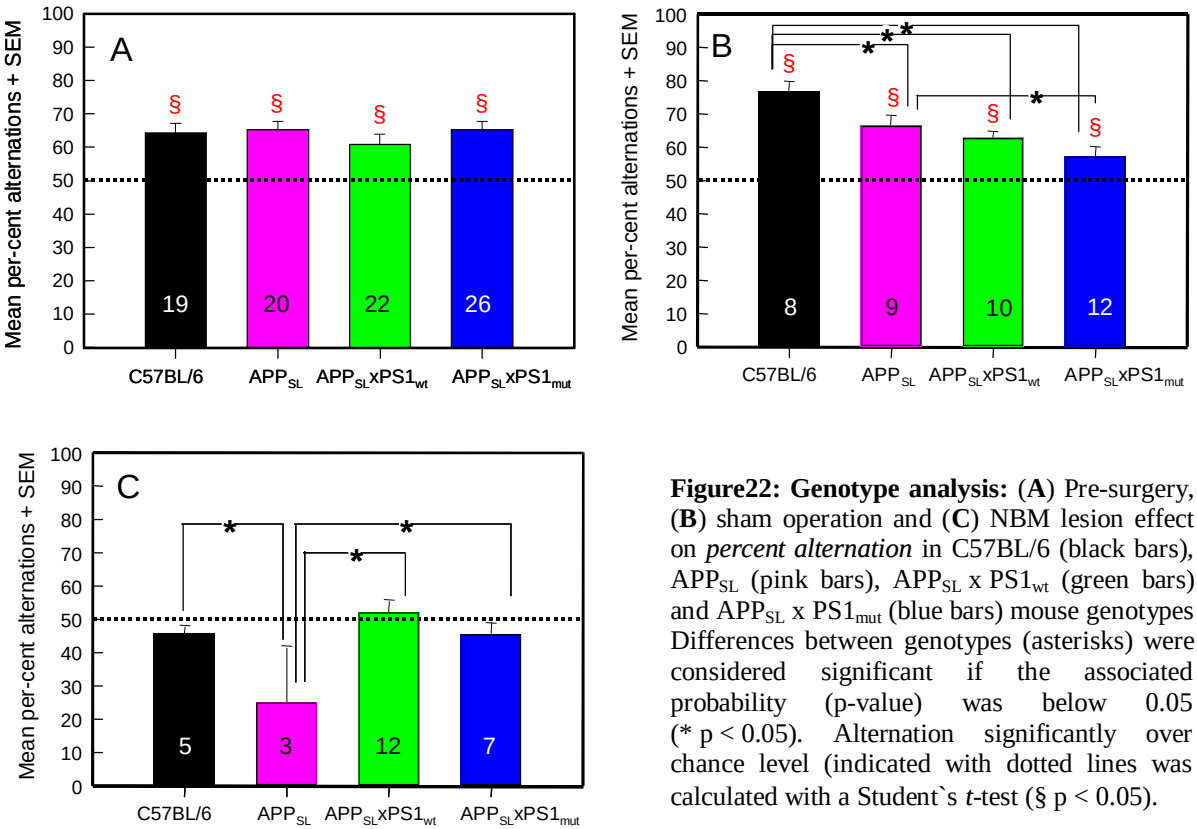


Figure22: Genotype analysis: (**A**) Pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *percent alternation* in C57BL/6 (black bars), APP_{SL} (pink bars), APP_{SL} x $PS1_{wt}$ (green bars) and APP_{SL} x $PS1_{mut}$ (blue bars) mouse genotypes Differences between genotypes (asterisks) were considered significant if the associated probability (p-value) was below 0.05 (* $p < 0.05$). Alternation significantly over chance level (indicated with dotted lines was calculated with a Student`s *t*-test (§ $p < 0.05$).

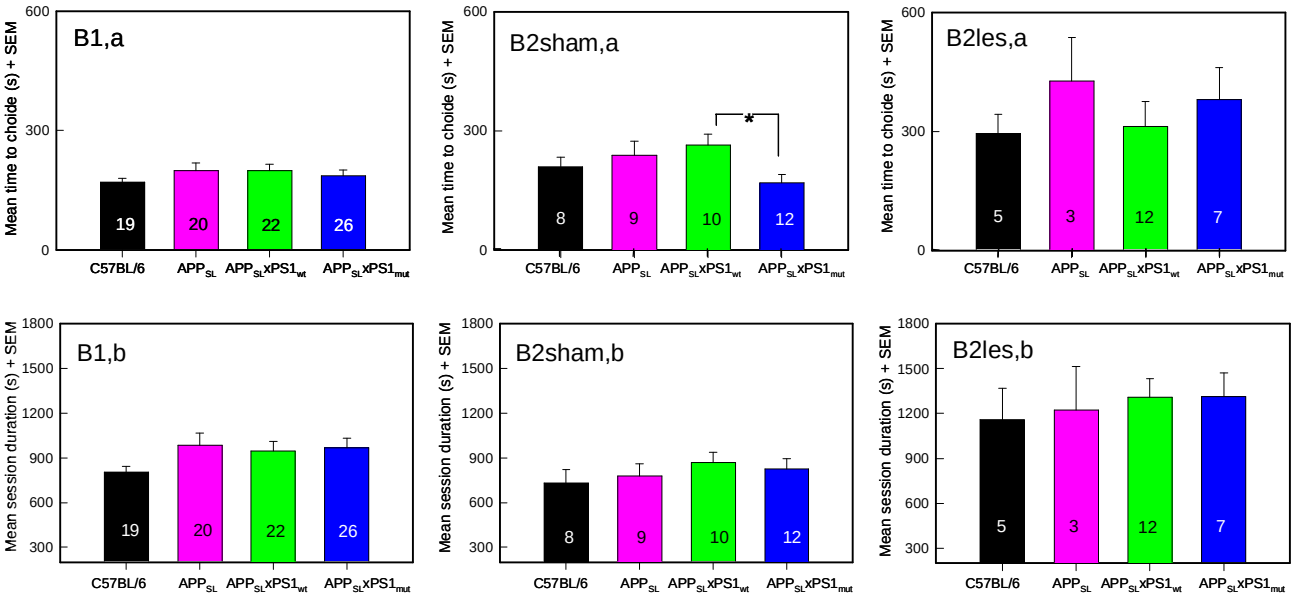


Figure 23: Locomotive parameters (genotype analysis): *Time to choice* (**a**) and *session duration* (**b**) in pre-surgery (**B1**), sham operated (**B2sham**) and NBM lesioned (**B2les**) C67BL/6 (black bars), APP_{SL} (pink bars), APP_{SL} x $PS1_{wt}$ (green bars) and APP_{SL} x $PS1_{mut}$ (blue bars) mouse genotypes. (* $p < 0.05$). Parameters only show a delayed initiation of inspections in sham operated APP_{SL} x $PS1_{mut}$ mice. Remaining parameters were similar, indicating a lack of locomotive interactions with alternation.

3.4.2.1.2. Surgery analysis

Alternation results are depicted in figure 24

3.4.2.1.2.1. C57BL/6 for B1, B2sham and B2les

There was no difference in alternation within the group of untreated animals that were later subjected to the sham or lesioned group ($F_{(1,12)} = 0.03$, $p = 0.871$). Alternation was dissimilar between treatment groups with B2les animals alternating worse than B2sham and B1 and B2sham alternating higher than B1 animals ($F_{(2,30)} = 12.64$, $p < 0.0001$). Mean time to choice ($F_{(2,30)} = 5.53$, $p = 0.007$) and mean session duration ($F_{(2,30)} = 13.9$, $p < 0.0001$) were also different with B2les animals needing most time in both parameters (alternation data is depicted in figure 24 A).

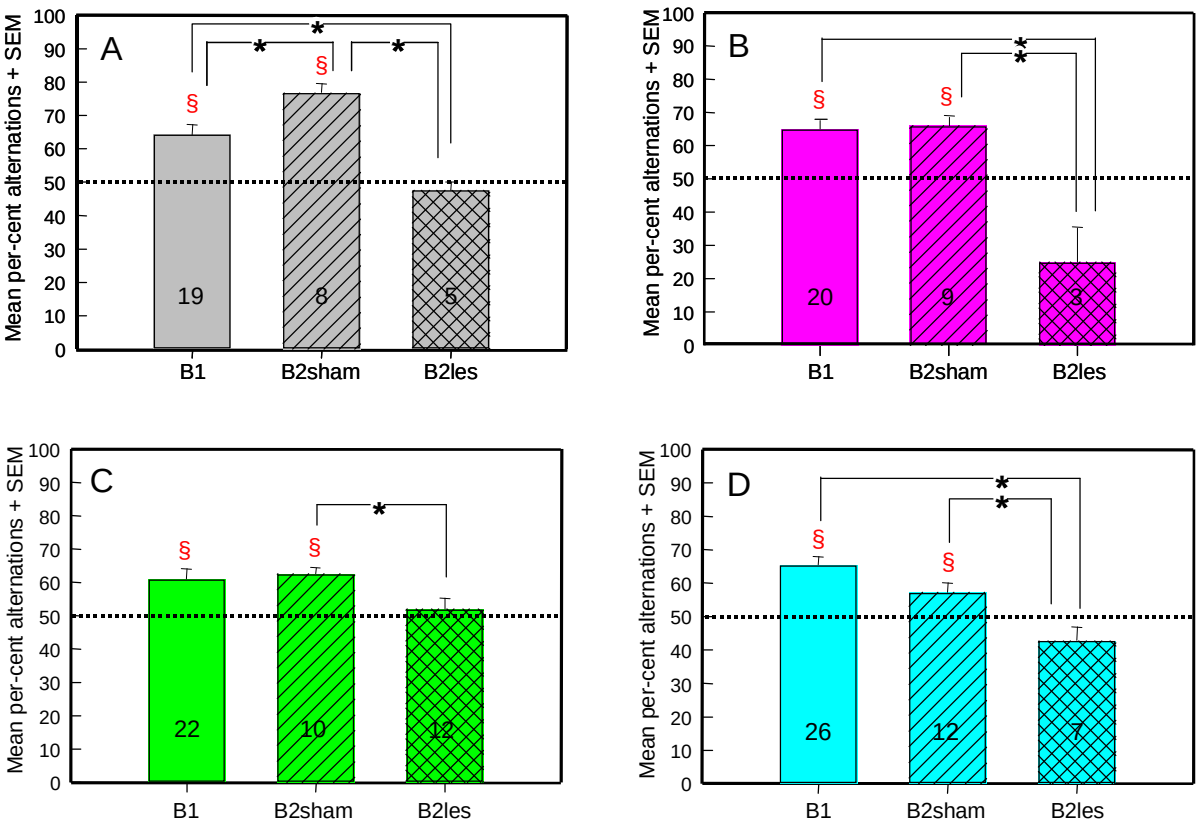


Figure 24: **Surgery analysis**: Impact of surgery on *mean percent alternation* is depicted for (**A**), C57BL/6, (**B**) $APP_{SL,}$ (**C**) APP_{SL} x $PS1_{wt}$ (**D**) and APP_{SL} x $PS1_{mut}$ mouse genotype (* $p < 0.05$). Alternation significantly over chance level (indicated with dotted lines) was calculated by Student`s *t*-test (§ $p < 0.05$). All genotypes were significantly impaired with NBM lesions.

3.4.2.1.2.2. APP_{SL} for B1, B2sham and B2les

There was no difference in alternation in the untreated (B1) groups between the animals that were later subjected to the B2sham and B2les group ($F_{(1,10)} = 0.06$, $p = 0.808$). B2les animals alternated less than the unlesioned groups ($F_{(2,29)} = 14.53$, $p < 0.0001$). Time to enter an arm was highest in the B2les group ($F_{(2,29)} = 4.86$, $p = 0.015$). Total session duration was similar between groups ($F_{(2,29)} = 2.09$, $p = 0.142$) (alternation data is depicted in figure 24 B).

3.4.2.1.2.3. APP_{SL} x $PS1_{wt}$ for B1, B2sham and B2les

Before surgery, there was no difference in alternation in the untreated (B1) groups ($F_{(1,10)} = 0.61$, $p = 0.696$). Alternation was similar between groups ($F_{(2,39)} = 2.74$, $p = 0.077$), however *post-hoc* comparison assessed worse alternation in B2les compared to B2sham

animals. No difference between groups was found for the time to enter an arm ($F_{(2,39)} = 3.17$, $p = 0.053$). B2les animals needed most time to complete the session ($F_{(2,39)} = 6.17$, $p = 0.005$) (alternation data is depicted in figure 24 C).

3.4.2.1.2.4. APP_{SL} x $PS1_{mut}$ for B1, B2sham and B2les

Alternation was similar in the untreated (B1) animal groups that were later subjected to surgery treatment ($F_{(1,17)} = 3.07$, $p = 0.098$). B2les animals alternated at lowest level compared to unlesioned B1 and B2sham animals ($F_{(2,43)} = 11.84$, $p < 0.0001$). Time to choice ($F_{(2,43)} = 14.75$, $p < 0.0001$) and total session duration ($F_{(2,43)} = 6.96$, $p = 0.002$) was highest in the B2les group (alternation data is depicted in figure 24 D).

3.4.2.1.3. Effect of E2020

Alternation results are depicted in figure 25.

3.4.2.1.3.1. Effect of E2020 on C57BL/6 sham and lesioned animals

A treatment effect between sham and NBM lesioned animals could be detected in the absence of E2020 ($F_{(1,11)} = 73.62$, $p < 0.0001$). This effect was reversed completely in the presence of E2020 ($F_{(1,11)} = 0.89$, $p = 0.366$). The time to choice was similar without E2020 ($F_{(1,11)} = 3.24$, $p = 0.099$) and in the presence of E2020 ($F_{(1,11)} = 0.08$, $p = 0.789$). However, total time to complete one trial was similar between sham and lesioned animals without E2020 ($F_{(1,11)} = 4.84$, $p = 0.0501$) and different between E2020 treated animals ($F_{(1,11)} = 4.91$, $p = 0.0488$). These findings indicate that E2020 was potent to increase alternation in NBM lesioned animals, but it had no obvious effect on locomotion (alternation data depicted in figure 25 A)

3.4.2.1.3.2. Effect of E2020 on APP_{SL} sham and lesioned animals

In the absence of E2020, there was a treatment effect between the sham and lesioned animals ($F_{(1,9)} = 34.89$, $p < 0.0001$). E2020 reversed the treatment effect in the lesioned group ($F_{(1,9)} = 0.16$, $p = 0.697$). There was an effect for time to choice between sham and NBM lesioned animals in the absence of E2020 ($F_{(1,9)} = 10.14$, $p = 0.011$) but not in the presence of E2020 ($F_{(1,9)} = 0.106$, $p = 0.764$). There were no differences between groups in the presence ($F_{(1,9)} = 0.39$, $p = 0.546$) or in the absence of E2020 ($F_{(1,9)} = 0.14$, $p = 0.721$) (alternation data depicted in figure 25 B)

3.4.2.1.3.3. Effect of E2020 on APP_{SL} x $PS1_{wt}$ sham and lesioned animals

Alternation was higher in the sham group compared to the NBM lesioned group in the absence of E2020 ($F_{(1,19)} = 8.11$, $p = 0.010$). This difference was reversed in the presence of E2020 ($F_{(1,19)} = 0.04$, $p = 0.848$). Time to choice was similar between sham and NBM lesioned animals in the absence ($F_{(1,19)} = 0.48$, $p = 0.497$) and in the presence of E2020 ($F_{(1,19)} = 3.42$, $p = 0.080$). Total time was different between sham and lesioned animals without E2020 ($F_{(1,19)} = 8.67$, $p = 0.008$) but similar in the presence of E2020 ($F_{(1,19)} = 0.03$, $p = 0.858$) (alternation data depicted in figure 25 C).

3.4.2.1.3.4. Effect of E2020 on APP_{SL} x $PS1_{mut}$ sham and lesioned animals

Alternation was higher in the sham operated animal group compared to the NBM lesioned animal group in the absence of E2020 ($F_{(1,14)} = 8.51$, $p = 0.032$). E2020 reversed the difference between groups, i.e. the effect of NBM lesions ($F_{(1,14)} = 0,00$, $p = 0.953$). Time to choice was different between sham and lesioned animals in absence ($F_{(1,14)} = 11.03$, $p = 0.004$) or in the presence of E2020 ($F_{(1,14)} = 6.31$, $p = 0.023$). Total time to complete a trial was different between sham and lesioned animals in absence of E2020 ($F_{(1,14)} = 9,36$, $p = 0.007$) but similar in the presence of E2020 ($F_{(1,14)} = 0.02$, $p = 0.892$) (alternation data depicted in figure 25 D).

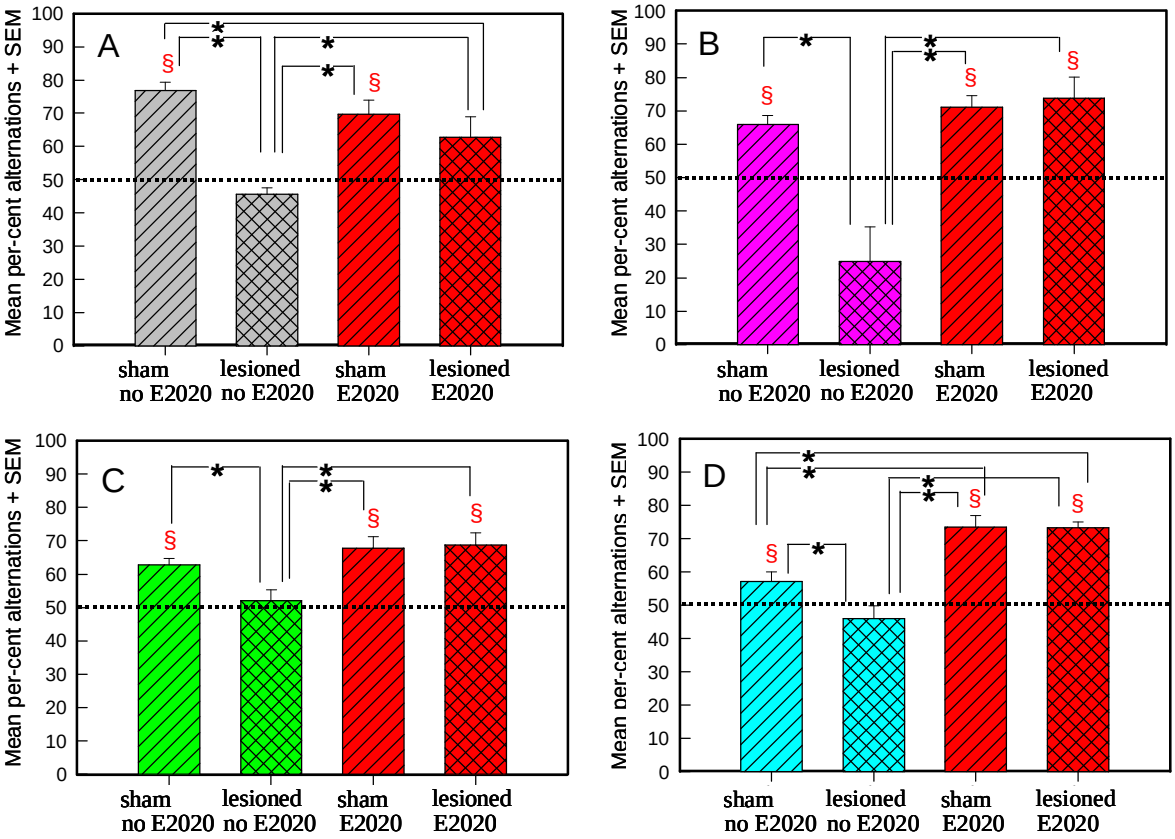


Figure 25: Effect of E2020: *Percent alternation* in sham and lesioned (**A**) C57BL/6, (**B**) $APP_{SL,}$ (**C**) APP_{SL} x $PS1_{wt}$, and (**D**) APP_{SL} x $PS1_{mut}$ mouse genotypes in the absence (original colour) and presence (red bars) of E2020. Differences between groups (asterisks) were considered significant if the associated probability (p-value) was below 0.05 (* $p < 0.05$). Alternation significantly over chance level (indicated with dotted lines) was calculated with a Student`s *t*-test (§ $p < 0.05$. Alternation was enhanced to sham level in the lesioned animal groups in the presence of E2020.

3.4.2.2. Experiment 2: The object recognition task (ORT)

3.4.2.2.1. Genotype analysis

The results of the study are depicted in figure 26.

3.4.2.2.1.1. All genotypes in B1

For the ITI of 1 min, discrimination index d1 indicated differences between groups ($F_{(3,83)} = 3.23$, $p = 0.027$). The C57BL/6 control mice displayed highest discrimination compared to the three mutant genotypes. Analysis of the ITI sessions of 1h, 3h, 6h and 12h have not shown any significant difference between groups (figure 26 A, left side).

Discrimination index d2 was similar between groups for the ANOVA calculation ($F_{(3,83)} = 1.52$, $p = 0.216$) of the 1min ITI session. *Post hoc* Fisher`s LSD analysis revealed a significantly higher discrimination in the C57BL/6 compared to the APP_{SL} x $PS1_{mut}$ genotype. In the ITI session of 1h differences were found ($F_{(3,83)} = 3.77$, $p = 0.014$) with a higher performance in the C57BL/6 compared to the APP_{SL} x $PS1_{mut.}$ During the ITI of 3h, 6h and 12h, animals of all genotypes performed at similar levels (figure 26 A, right side).

3.4.2.2.1.2. All genotypes in B2sham

No difference of d1 could be detected between groups for any of the ITI sessions. This may be due to the facts that the animals within a group performed at variable levels, which resulted in high standard error means (figure 26 B, left side).

Analysis of the discrimination index d2 revealed no differences between groups for ANOVA calculation. For the ITI of 6h ($F_{(3,35)} = 1.53$, $p = 0.224$), the Fisher`s LSD test has shown differences between APP_{SL} and APP_{SL} x $PS1_{mut}$ animals (figure 26 B, right side).

3.4.2.2.1.3. All genotypes in B2les

No difference could be detected for index d1 or d2 by the ANOVA analysis. Again, the variation within one group was very high. For the ITI of 12h, the Fisher`s LSD test has shown a difference between C57BL/6 and APP_{SL} x $PS1_{mut}$ animals for d1 (figure 26 C).

3.4.2.2.2. Surgery analysis

3.4.2.2.2.1. C57BL/6 for B1, B2sham and B2les

Repeated measure analysis has shown that there were no differences in the untreated (B1) animals group before they were separated into sham and NBM lesioned groups ($F_{(1,11)} = 0.09$, $p = 0.768$). There has been a within subject effect for replication, i.e. subjecting the animal for

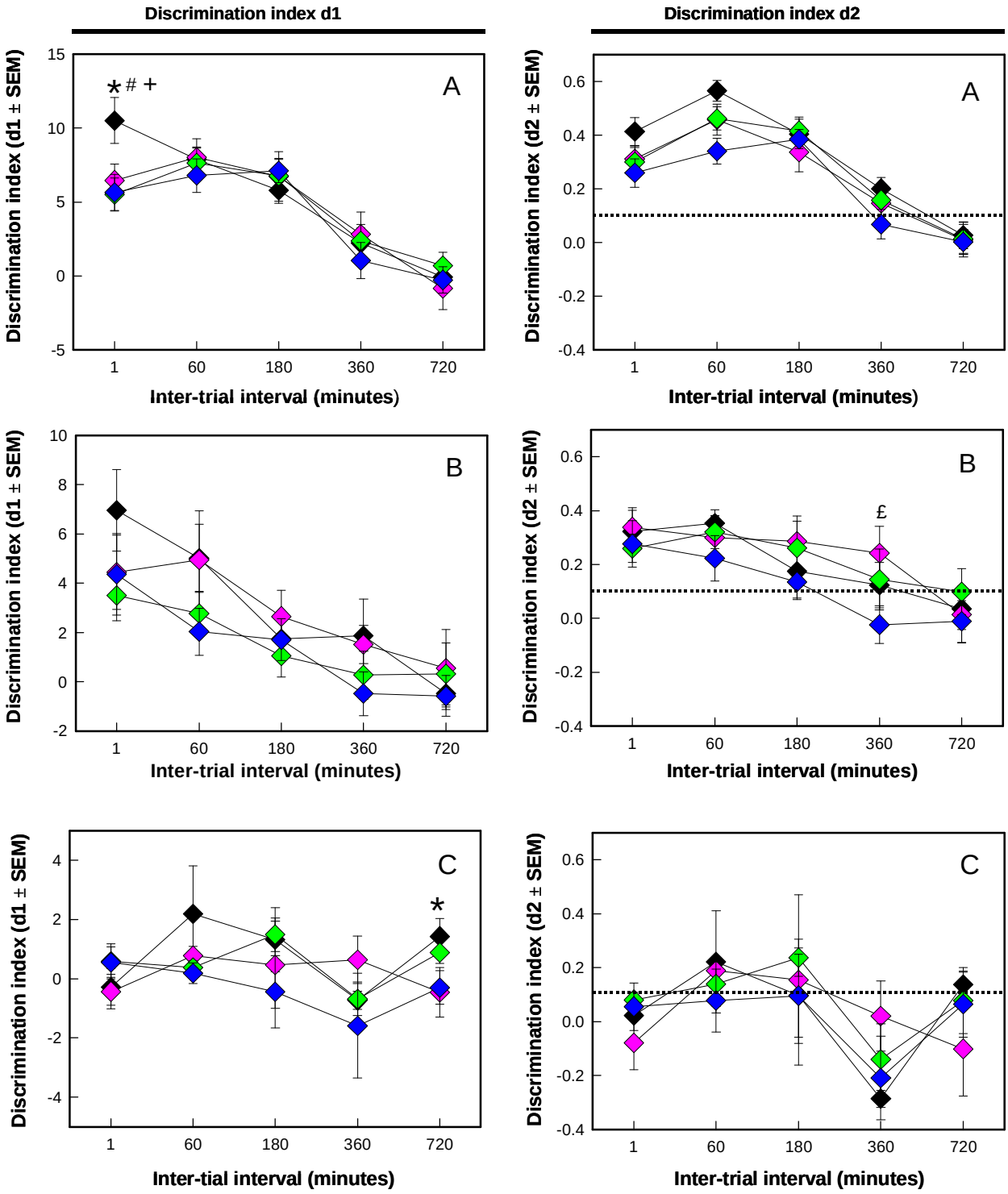


Figure 26: Genotype analysis: (**A**) Pre-surgery, (**B**) sham operation, and (**C**) NBM lesion effect on *discrimination performance d1* (left side) and *d2* (right side) in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$): * = C57BL/6 vs APP_{SL} x $PS1_{mut}$, # = C57BL/6 vs APP_{SL} x PS1wt, + = C57BL/6 vs APP_{SL}, £ = APP_{SL} vs APP_{SL} x $PS1_{mut}$. Discrimination was considered successful if d2 was over discrimination level (indicated with dotted lines). Strong lesion effects were observable, whereas only a slight genotype effect (A, left upper panel) in the C57BL/6 group with an ITI of 1 min was revealed.

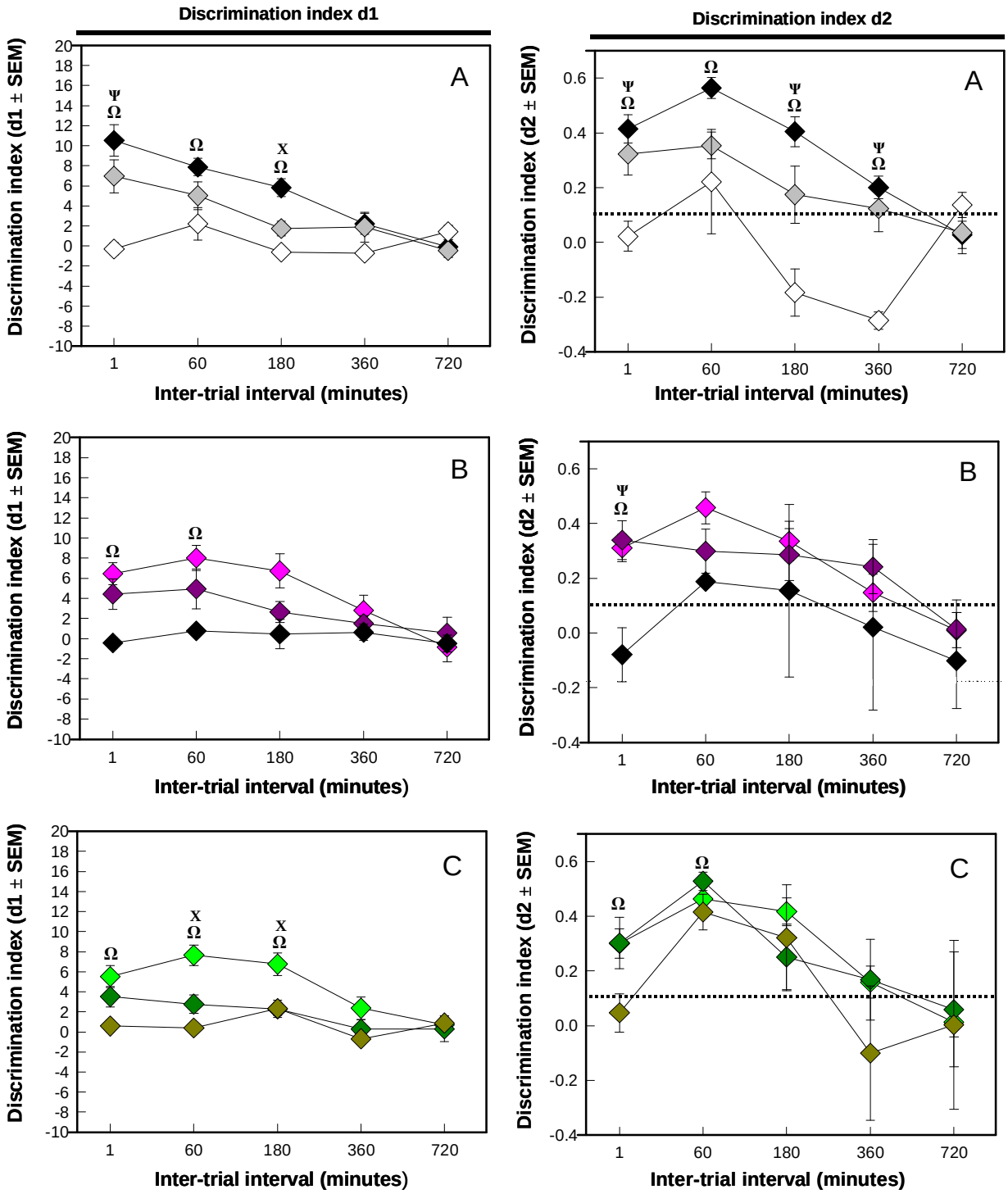


Figure 27/1: Surgery analysis: Discrimination performance for *d1* (left row) and *d2* (right row) in: (**A**) C57BL/6 with pre-surgery (black), B2sham (grey) and B2les (white) groups. (**B**) APP_{SL} with pre-surgery (pink), B2sham (violet) and B2les (black) groups. (**C**) APP_{SL} x $PS1_{wt}$ with pre-surgery (light green), B2sham (green) and B2les (olive) groups. Discrimination performance was analysed for surgery groups, differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les. Discrimination was considered successful if d2 was over discrimination level (indicated with dotted lines). Lesion effects are observable in for d1 and d2.

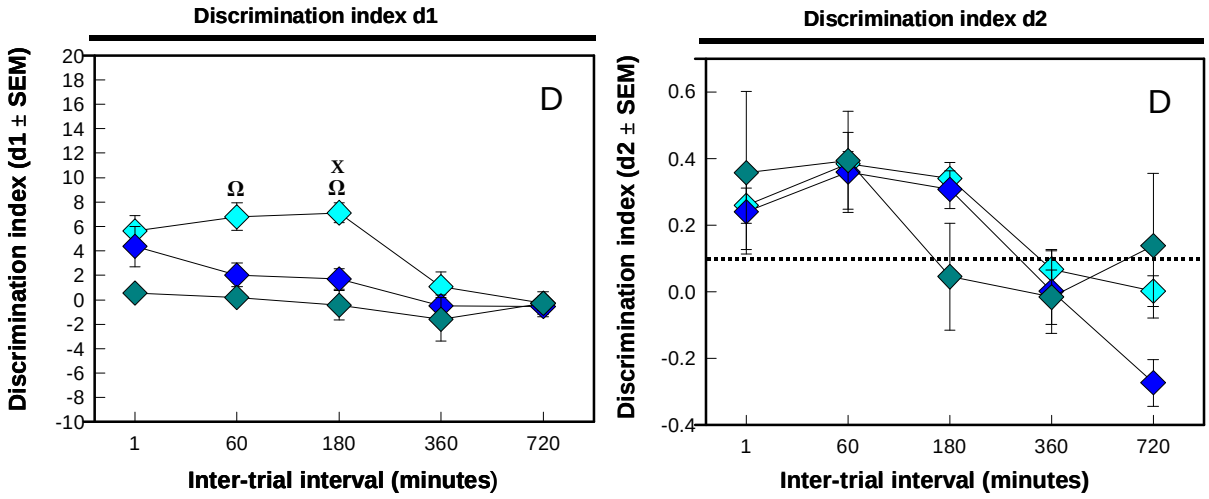


Figure 27/2: Surgery analysis: (**D**) APP_{SL} x $PS1_{mut}$ with pre-surgery (light blue), B2sham (blue) and B2les (turquoise) are depicted for *discrimination index d1* (left pannel) and *d2* (right panel). Discrimination performance was analysed for surgery groups, differences between surgery groups ($p < 0.05$): X = B1 vs B2sham, Ω = B1 vs B2les. Discrimination was considered successful if d2 was over discrimination level (indicated with dotted lines). Lesion effects are observable in for d1.

the first versus the second time to the testing procedure, between the untreated and the operated animals (Replication: $F_{(1,11)} = 4.85$, $p = 0.049$). This trend was similar for all treatment groups (Replication by Treatment $F_{(1,11)} = 1.05$, $p = 0.327$). There were different discrimination effects found for different ITIs (Interval $F_{(4,44)} = 13.09$, $p < 0.0001$) and this trend was not similar between treatment groups (Interval by Treatment $F_{(4,44)} = 0.52$, $p = 0.705$). *Post hoc* analysis has shown, that the NBM lesioned group performed at lowest level for ITI of 1min, 1h and 3h. For discrimination index d2, there was also no difference in the untreated animal group before they were divided into sham and lesioned groups ($F_{(1,11)} = 0.00$, $p = 0.982$). There was also an effect of replication (Replication: $F_{(1,11)} = 14.91$, $p = 0.003$), which was similar for all treatment groups (Replication by Treatment $F_{(1,11)} = 2.18$, $p = 0.167$). Discrimination was different when ITI changed (Interval $F_{(4,44)} = 11.03$, $p < 0.0001$) and this was observable in all treatment groups (Interval by Treatment $F_{(4,44)} = 1.04$, $p = 0.399$). *Post hoc* analysis has shown, that discrimination was lowest in the NBM lesioned group (depicted in figure 27 A, left and right side).

3.4.2.2.2.2. APP_{SL} for B1, B2sham and B2les

No differences could be detected within the untreated animals (B1), if they were divided into later sham or NBM lesioned groups ($F_{(1,10)} = 0.89$, $p = 0.369$). There was a treatment effect,

i.e. effect of replication (Replication: $F_{(1,10)} = 14.21$, $p = 0.004$), and the effect was similar in treatment groups (Replication by Treatment $F_{(1,10)} = 0.29$, $p = 0.602$). Discrimination was different for changing ITI (Interval: $F_{(4,44)} = 4.30$, $p = 0.006$). This observation was made in all treatment groups (Interval by Treatment: $F_{(4,44)} = 1.15$, $p = 0.347$). No differences were found within untreated animals (B1), if they were divided into later sham of NBM lesioned groups for discrimination index d2 ($F_{(1,10)} = 1.89$, $p = 0.199$). There was an effect of replication (Replication: $F_{(1,10)} = 5.22$, $p = 0.045$), which was similar for treatment groups (Replication by Treatment $F_{(1,10)} = 1.20$, $p = 0.298$). Discrimination changed when ITI was enlarged (Interval: $F_{(4,44)} = 4.37$, $p = 0.005$), and this effect was pronounced in all treatment groups (Interval by Treatment: $F_{(4,44)} = 0.60$, $p = 0.666$) (depicted in figure 27 B, left and right side).

3.4.2.2.2.3. APP_{SL} x $PS1_{wt}$ for B1, B2sham and B2les

Upon segregation into sham or NBM lesioned groups, repeated measures analysis has demonstrated that there were no differences within the untreated animals (B1) ($F_{(1,20)} = 2.44$, $p = 0.134$). There was an effect of treatment (Replication: $F_{(1,20)} = 40.08$, $p < 0.0001$) and this effect was dissimilar for treatment groups (Replication by Treatment $F_{(1,20)} = 6.90$, $p = 0.016$). Changes of ITI have an effect on discrimination (Interval: $F_{(4,40)} = 10.18$, $p < 0.001$), which is similar for treatment groups (Interval by Treatment: $F_{(4,40)} = 0.81$, $p = 0.508$). No differences were found within untreated animals (B1), if they were divided into later sham of NBM lesioned groups for discrimination index d2 ($F_{(1,20)} = 0.76$, $p = 0.356$) There was an effect of treatment (Replication: $F_{(1,20)} = 32.78$, $p < 0.0001$). This effect was dissimilar for treatment groups (Replication by Treatment $F_{(1,20)} = 6.90$, $p = 0.016$).Changes of ITI have an effect on discrimination (Interval: $F_{(4,40)} = 14.45$, $p < 0.001$), which is similar for treatment groups (Interval by Treatment: $F_{(4,40)} = 0.42$, $p = 0.648$) (depicted in figure 27 C, left and right side).

3.4.2.2.2.4. APP_{SL} x $PS1_{mut}$ for B1, B2sham and B2les

Upon segregation into sham or NBM lesioned groups, repeated measures analysis has demonstrated that there were no differences within the untreated animals (B1) ($F_{(1,17)} = 0.72$, $p = 0.408$). There was an effect of replication (Replication: $F_{(1,17)} = 28.77$, $p < 0.0001$) and this was similar for treatment groups (Replication by Treatment $F_{(1,17)} = 0.35$, $p = 0.561$). There was a change in discriminative abilities, if ITI was changed (Interval: $F_{(4,68)} = 9.21$, $p < 0.001$), and this was similar for treatment groups (Interval by Treatment: $F_{(4,68)} = 0.26$, $p = 0.896$). There was an effect of treatment (Replication: $F_{(1,17)} = 13.10$, $p = 0.002$). This

effect was similar for treatment groups (Replication by Treatment $F_{(1,17)} = 0.01$, $p = 0.923$). Changes of ITI have an effect on discrimination (Interval: $F_{(4,68)} = 9.36$, $p < 0.001$), which is similar for treatment groups (Interval by Treatment: $F_{(4,68)} = 0.74$, $p = 0.558$) (depicted in figure 27 D, left and right side).

3.4.2.3. Experiment 3: The modified Barnes Maze (mBM)

3.4.2.3.1. Genotype analysis

The results of the complete study are depicted in figures 28-31.

3.4.2.3.1.1. All genotypes in B1

Distance: Averaged over the first session block (day 1-day 5, ITI of 1 min), the distance travelled was similar in all genotypes (General mean: $F_{(3,83)} = 2.04$, $p = 0.114$), but the animals from the APP_{SL} x $PS1_{mut}$ group displayed the most line crossings before finding the escape hole. *Post hoc* analysis showed that the APP_{SL} x $PS1_{mut}$ mice differed significantly from the APP_{SL} group in session 2 and from the control group C57BL/6 in session 5. There was reduction of line crossings across sessions (first block) for all genotypes (Session: $F_{(4,332)} = 109.59$, $p < 0.0001$), and the reduction was similar for all animal groups (Session by Group interaction: $F_{(12,332)} = 0.95$, $p = 0.484$).

Averaged over the second block (day6-day10, ITI of 15 min), performance of the four genotype groups was similar (General mean: $F_{(3,83)} = 1.2$, $p = 0.314$). There was a difference

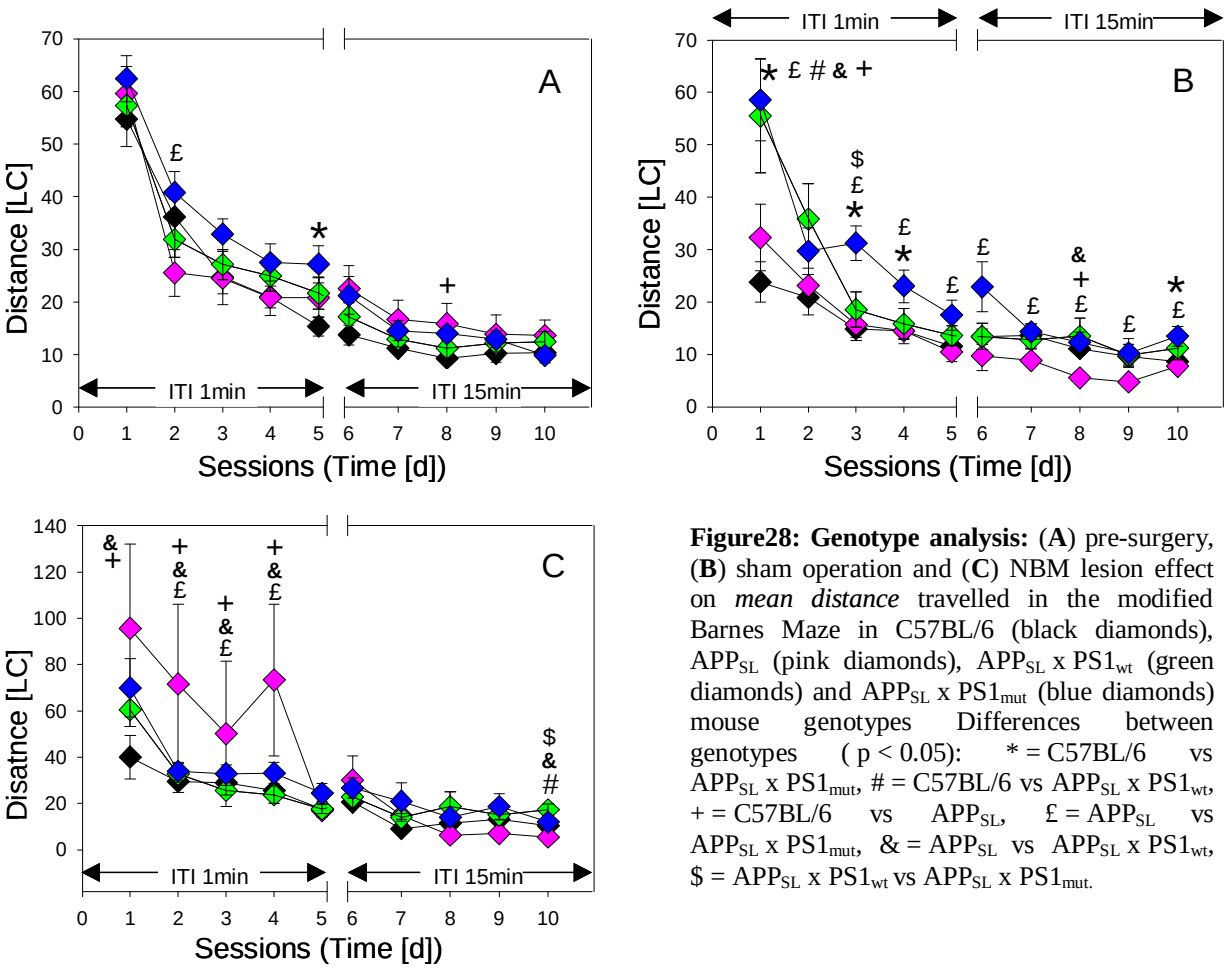


Figure28: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean distance* travelled in the modified Barnes Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$): * = C57BL/6 vs APP_{SL} x $PS1_{mut}$, # = C57BL/6 vs APP_{SL} x $PS1_{wt}$, + = C57BL/6 vs APP_{SL}, £ = APP_{SL} vs APP_{SL} x $PS1_{mut}$, & = APP_{SL} vs APP_{SL} x $PS1_{wt}$, $ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$.

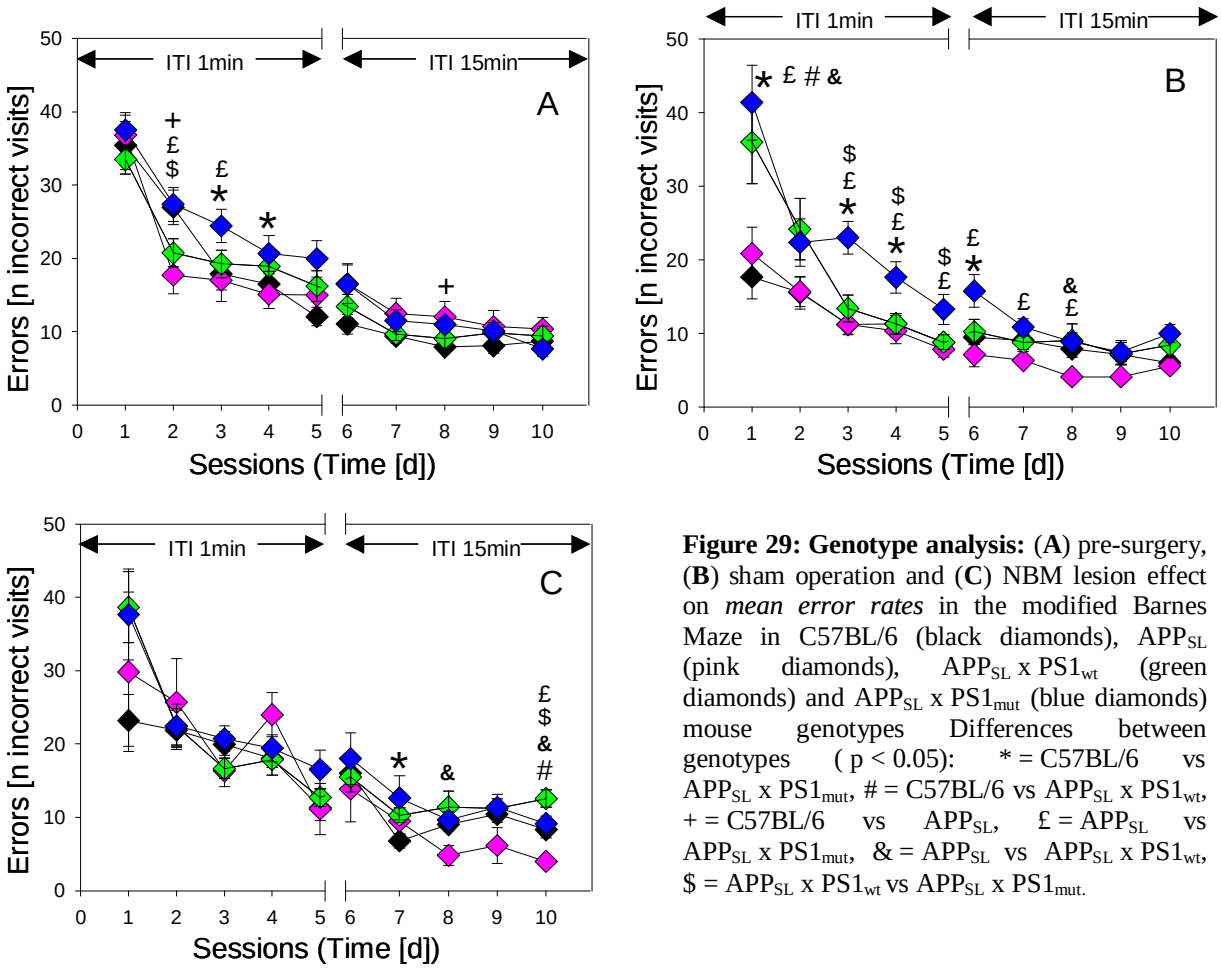


Figure 29: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean error rates* in the modified Barnes Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$): * = C57BL/6 vs APP_{SL} x $PS1_{mut}$, # = C57BL/6 vs APP_{SL} x $PS1_{wt}$, + = C57BL/6 vs APP_{SL}, £ = APP_{SL} vs APP_{SL} x $PS1_{mut}$, & = APP_{SL} vs APP_{SL} x $PS1_{wt}$, $ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$.

between the C57L/6 and the APP_{SL} group in the 8th session, as indicated by the *post hoc* analysis. All genotypes were able to reduce the distance travelled in the course of the second session block (Session: $F_{(4,332)} = 13.82$, $p < 0.0001$) in a similar degree (Session by Group interaction: $F_{(12,332)} = 0.91$, $p = 0.509$) (depicted in figure 28 A).

Errors: The average error rate over the first session block showed a tendency to different learning behaviour between genotypes, but it did not reach significance (General mean: $F_{(3,83)} = 2.54$, $p = 0.062$). Animals from the APP_{SL} x $PS1_{mut}$ genotype constantly performed at highest error rate and post hoc analysis demonstrated, on the second session day, a significant difference between the APP_{SL} x $PS1_{mut}$ and the APP_{SL} x $PS1_{wt}$ and the APP_{SL} animal group, respectively. There was also a difference between the C57BL/6 and the APP_{SL} animal group. On the third session day, again, APP_{SL} x $PS1_{mut}$ animals made significantly more errors than C57BL/6 and APP_{SL} genotypes. There was also a difference between the APP_{SL} x $PS1_{mut}$ animals and the C57BL/6 on the 5th session day. Animals from all groups were able to reduce the amount of errors over the first block of sessions (Session: $F_{(4,332)} = 76.34$, $p < 0.0001$). The

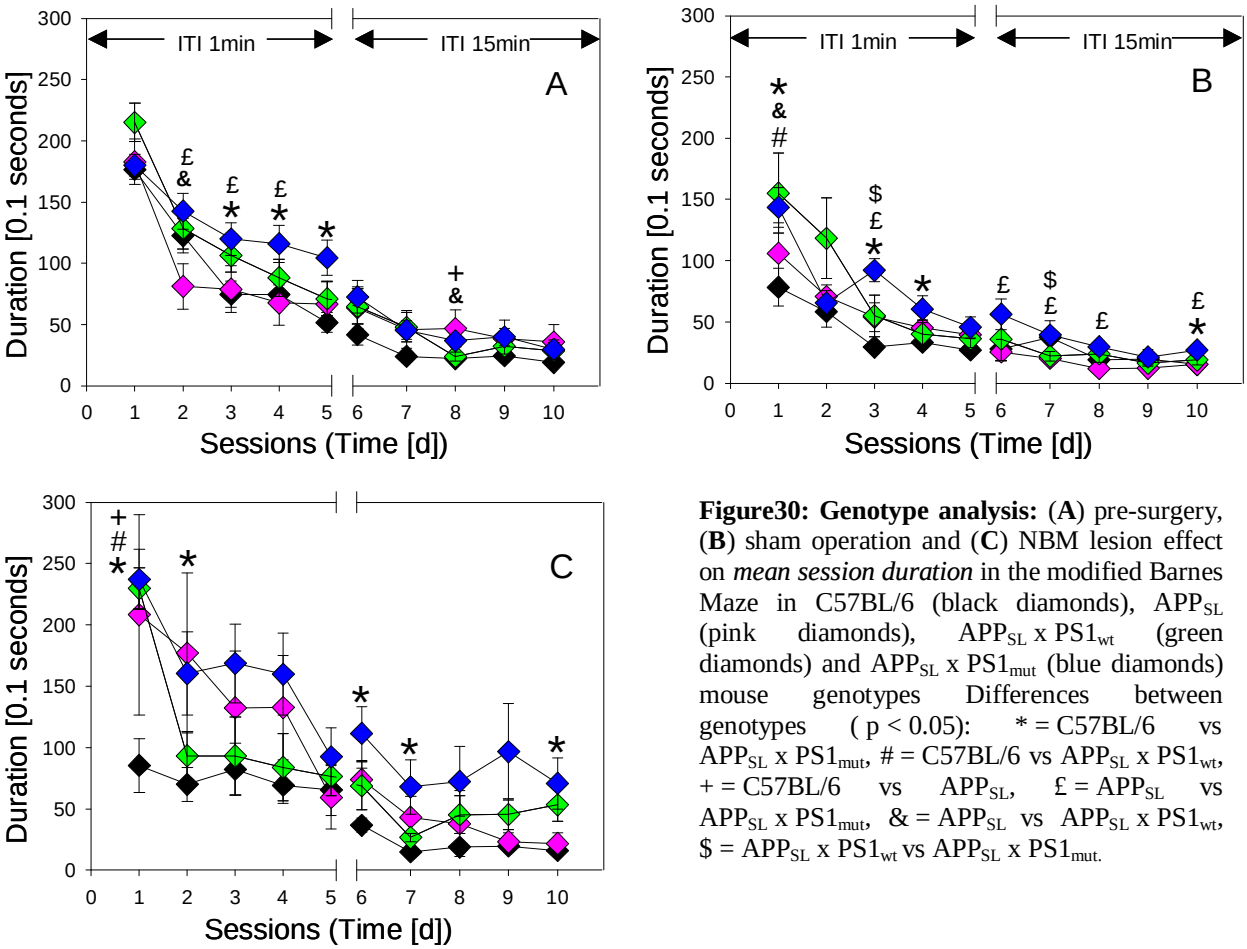


Figure30: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean session duration* in the modified Barnes Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$): * = C57BL/6 vs APP_{SL} x $PS1_{mut}$, # = C57BL/6 vs APP_{SL} x $PS1_{wt}$, + = C57BL/6 vs APP_{SL}, £ = APP_{SL} vs APP_{SL} x $PS1_{mut}$, & = APP_{SL} vs APP_{SL} x $PS1_{wt}$, $ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$.

reduction of errors over days was similar in all genotypes (Session by Group interaction: $F_{(12,332)} = 1.96$, $p = 0.076$).

For the second session block, the mean error rate calculated over sessions showed no difference between genotypes (General mean: $F_{(3,83)} = 1.2$, $p = 0.314$). *Post hoc* analysis revealed a difference between C57BL/6 and APP_{SL} genotypes on the 8th day. The animals were able to reduce the amount of errors during the session block (Session: $F_{(4,332)} = 15.38$, $p < 0.0001$), which was similar between groups (Session by Group: $F_{(12,332)} = 1.06$, $p = 0.389$) (depicted in figure 29 A).

Duration: The mean session duration of the first session block was similar between genotypes (General mean: $F_{(3,83)} = 2.29$, $p = 0.084$). *Post hoc* analysis, anyhow, revealed several differences between groups from session 2 until session 5 (for details see Figure 26) The time to complete a session was reduced by all animal groups in the course of the session block (Session: $F_{(4,332)} = 85.2$, $p < 0.0001$), and there was a difference in reduction between groups

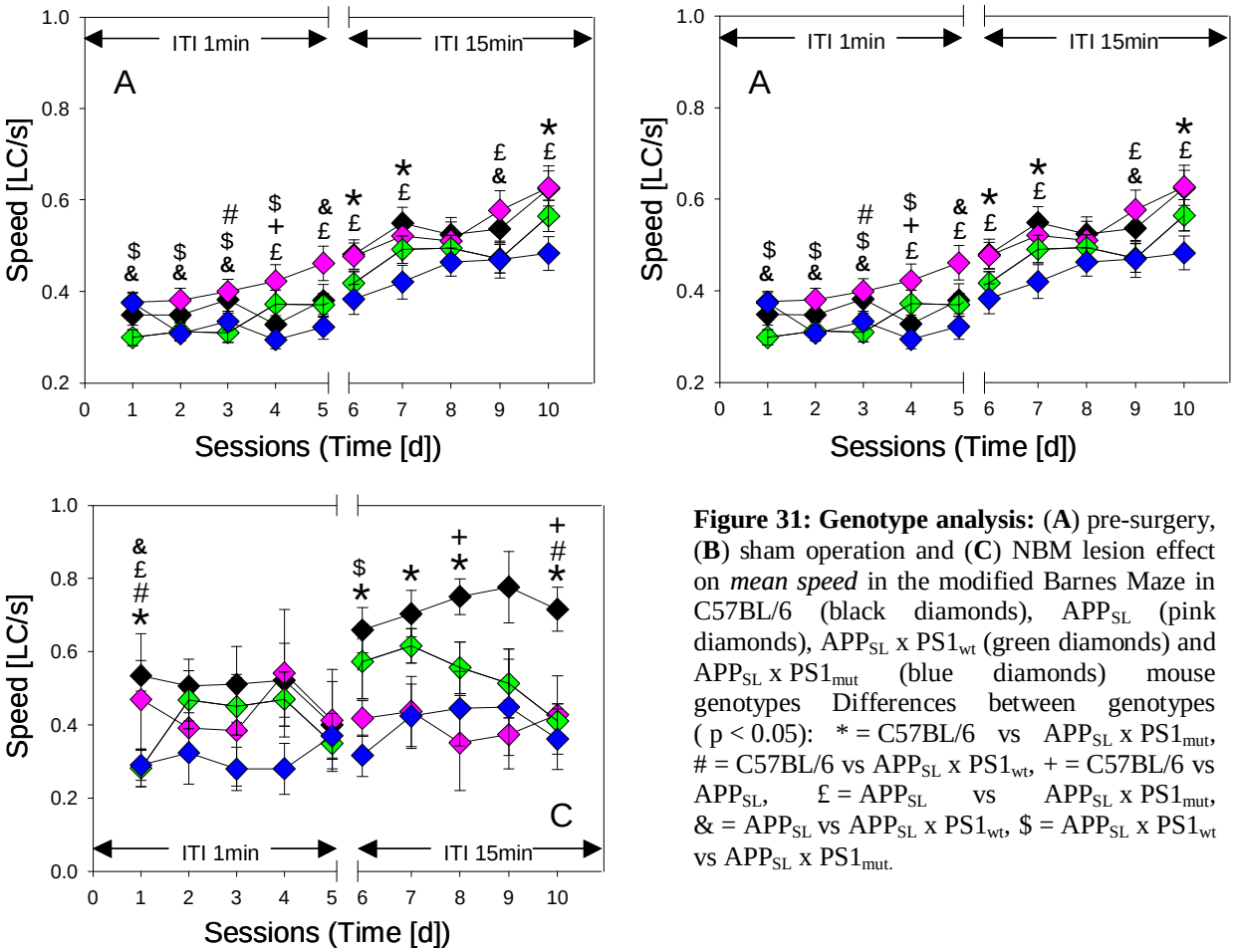


Figure 31: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean speed* in the modified Barnes Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$): * = C57BL/6 vs APP_{SL} x $PS1_{mut}$, # = C57BL/6 vs APP_{SL} x $PS1_{wt}$, + = C57BL/6 vs APP_{SL}, £ = APP_{SL} vs APP_{SL} x $PS1_{mut}$, & = APP_{SL} vs APP_{SL} x $PS1_{wt}$, $ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut.}$

(Session by Group interaction: $F_{(12,332)} = 2.35$, $p = 0.010$), with the C57BL/6 genotype showing constantly shortest duration times.

Averaged over the second session block, the time to complete a session was similar between genotypes (General mean: $F_{(3,83)} = 0.94$, $p = 0.424$). *Post hoc* calculation showed a longer session duration in APP_{SL} genotypes compared to C57BL/6 and APP_{SL} x $PS1_{wt.}$ Session duration decrease in the course of sessions (Session: $F_{(4,332)} = 20.79$, $p < 0.0001$), and the reduction was similar between groups (Session by Group interaction: $F_{(12,332)} = 0.94$, $p = 0.480$) (depicted in figure 30 A).

Speed: Averaged over the first session block, significant differences between genotypes were found (General mean: $F_{(3,83)} = 3.58$, $p = 0.017$) with the APP_{SL} tending to be the fasted group. *Post hoc* analysis showed several dissimilarities between groups (for details see Figure 27). Speed was increase over sessions (Session: $F_{(4,332)} = 3.69$, $p = 0.009$), but not in the same manner between groups (Session by Group: $F_{(12,332)} = 3.16$, $p = 0.001$).

For the second block, the averaged speed over sessions tended to be different between groups (General mean: $F_{(3,83)} = 2.64$, $p = 0.055$). Animals from the APP_{SL} and C57BL/6 tended to be the fastest, APP_{SL} x $PS1_{mut}$ animals were constantly the slowest group. Speed was increase by all groups over sessions (Session: $F_{(4,332)} = 22.13$, $p < 0.0001$) in a similar manner between groups (Session by Group interaction: $F_{(12,332)} = 1.15$, $p = 0.312$) (depicted in figure 31 A).

3.4.2.3.1.2. All genotypes in B2sham

Distance: The mean distance travelled in the first session block was different between groups (General mean: $F_{(3,35)} = 7.02$, $p = 0.001$) with the tendency in the APP_{SL} x $PS1_{mut}$ animal group to travel most before finding the escape hole. Many differences were detected in by the *post hoc* calculation (for details see Figure 24). Distance was significantly reduced over sessions (Session: $F_{(4,140)} = 45.46$, $p < 0.001$), but in a different degree between groups (Session by Group interaction: $F_{(12,140)} = 4.24$, $p = 0.001$).

On average, the distance in the second session block was different between groups (General mean: $F_{(3,35)} = 4.16$, $p = 0.013$) and again, the APP_{SL} x $PS1_{mut}$ animals tended travel most before finding the escape hole. *Post hoc* calculation demonstrated most differences between APP_{SL} x $PS1_{mut}$ animals and the other groups (for details see Figure 24). In the course of the second session block, the distance was also reduced (Session: $F_{(4,140)} = 8.01$, $p < 0.001$), which was similar in all groups (Session by Group interaction: $F_{(12,140)} = 1.15$, $p = 0.340$) (depicted in figure 28 B)

Errors: The mean amount of errors over the first block of sessions differed between animals (General mean: $F_{(3,35)} = 9.42$, $p = 0.0001$). The animals from the APP_{SL} x $PS1_{mut}$ group performed at highest error rates, which was also confirmed by post hoc comparisons (for details see Figure 25). The amount of errors was reduced over sessions (Session: $F_{(4,140)} = 45.62$, $p < 0.001$), but in dissimilar manners between groups (Session by Group interaction: $F_{(12,140)} = 4.24$, $p = 0.001$).

For the second session block, the mean error rate was different between groups (General mean: $F_{(3,35)} = 6.26$, $p = 0.002$) and the animals from the APP_{SL} x $PS1_{mut}$ group also show highest error rates in the second block, whereas the amount of errors was lowest in the APP_{SL} genotype (for details see Figure 25). The error rate decreased over sessions (Session: $F_{(4,140)} = 10.19$, $p < 0.001$) and it was similar for all groups (Session by Group interaction: $F_{(12,140)} = 1.2$, $p = 0.293$) (depicted in figure 29 B).

Duration: The mean session duration of the first session block was different between groups (General mean: $F_{(3,35)} = 3.22$, $p = 0.034$). Thereupon, shortest session duration was registered

for the control group C57BL/6. *Post hoc* analysis revealed several differences between APP_{SL} x $PS1_{mut}$ and the rest of the groups (for details see Figure 26). In the course of sessions, all groups reduced the time to find the escape (Session: $F_{(4,140)} = 42.39$, $p < 0.001$), but the reduction was dissimilar for groups (Session by Group interaction: $F_{(12,140)} = 3.1$, $p = 0.002$).
For the second session block, the averaged duration time was different between groups (General mean: $F_{(3,35)} = 4.23$, $p = 0.012$). The animals from the APP_{SL} x $PS1_{mut}$ group constantly needed most time to descend. The duration time was reduced in the course of sessions by all animal groups (Session: $F_{(4,140)} = 8.13$, $p < 0.001$) in a similar way (Session by Group interaction: $F_{(12,140)} = 1.09$, $p = 0.378$) (depicted in figure 30 B).

Speed: On average, all groups explored the mBM with a similar speed (General mean: $F_{(3,35)} = 1.63= 0.201$). Post hoc calculation revealed many differences (for details see Figure 27). The speed did not change across sessions (Session: $F_{(4,140)} = 1.68$, $p = 0.164$) and the groups performed in dissimilar ways (Session by Group interaction: $F_{(12,140)} = 1.94$, $p = 0.040$).
Mean speed during the second session block was similar between genotypes (General mean: $F_{(3,35)} = 0.98= 0.413$). There were differences between groups on several session days, as it was shown by the post hoc analysis (see Figure 27). There was no change in speed across sessions (Session: $F_{(4,140)} = 1.18$, $p = 0.324$), which was similar in all groups (Session by Group interaction: $F_{(12,140)} = 1.32$, $p = 0.215$) (depicted in figure 31 B).

3.4.2.3.1.3. All genotypes in B2les

Distance: Averaged over the first block of sessions, the distance travelled did not vary between groups (General mean: $F_{(3,18)} = 2.13= 0.132.$). *Post hoc* analysis indicated strong variation in the APP_{SL} group, which is caused by the small amount of animals (for details see Figure 24). There was a reduction of line crossings in the course of sessions (Session: $F_{(4,72)} = 17.52$, $p < 0.001$), which was similar for all groups (Session by Group interaction: $F_{(12,72)} = 1.45$, $p = 0.240$).
The mean distance measured over the second session block was similar between all genotypes (General mean: $F_{(3,18)} = 1.7= 0.204$). The distance was reduced by the animals over sessions (Session: $F_{(4,72)} = 6.55$, $p = 0.0004$), but there was no difference between groups (Session by Group interaction: $F_{(12,72)} = 0.88$, $p = 0.552$) (depicted in figure 28 C).

Errors: Averaged over the first block of sessions, the animals from all genotypes performed at similar error rate level (General mean: $F_{(3,18)} = 1.66= 0.212$). The amount of errors was

reduced over sessions (Session: $F_{(4,72)} = 15.49$, $p < 0.0001$) and the reduction was similar for all groups (Session by Group interaction: $F_{(12,72)} = 1.15$, $p = 0.354$).

On average, the error rate was different between groups in the second session block (General mean: $F_{(3,18)} = 3.78= 0.029$). The amount of errors was decreased over sessions (Session: $F_{(4,72)} = 8.22$, $p < 0.0001$) similarly in all genotypes (Session by Group interaction: $F_{(12,72)} = 0.7$, $p = 0.728$) (depicted in figure 29 B).

Duration: The mean session duration of the fist session block was similar between groups (General mean: $F_{(3,18)} = 2.59= 0.084$). However, *post hoc* analysis has clearly shown a lowest duration in the C57BL/6 and the highest duration in the APP_{SL} x $PS1_{mut}$ group (for details see Figure 26). Duration was reduced over sessions (Session: $F_{(4,72)} = 17.43$ $p < 0.0001$). The reduction was not similar between groups (Session by Group interaction: $F_{(12,72)} = 2.54$, $p = 0.013$).

In the second block of sessions, the time to find the escape hole tended to be different between groups (General mean: $F_{(3,18)} = 3.05= 0.055$). *Post hoc* analysis especially showed differences between APP_{SL} x $PS1_{mut}$, the group with highest session duration, and the C57BL/6, which was on lowest level (for details see graph). The duration times were reduced over sessions (Session: $F_{(4,72)} = 3.25$ $p = 0.026$), which was similar in all groups (Session by Group interaction: $F_{(12,72)} = 0.46$, $p = 0.904$) (depicted in figure 30 C).

Speed: On average, all animals performed with a similar speed (General mean: $F_{(3,18)} = 1.53= 0.241$), but C57BL/6 performed almost constantly at highest speed, whereas animals from the APP_{SL} x $PS1_{mut}$ group explored the mBM at lowest speed, which was also confirmed by the post hoc analysis (for details see Figure 27). The speed did not change over sessions (Session: $F_{(4,72)} = 0.87$ $p = 0.484$), and this was similar for all groups (Session by Group interaction: $F_{(12,72)} = 1.39$, $p = 0.188$).

For the second session block, the averaged speed differed between groups (General mean: $F_{(3,18)} = 4.60$, $p = 0.015$). This was especially the case for the fastest group, the C57BL/6. The speed remained constant across sessions (Session: $F_{(4,72)} = 0.67$, $p = 0.613$), which was similar for all groups (Session by Group interaction: $F_{(12,72)} = 0.79$, $p = 0.655$) (depicted in figure 31 C).

3.4.2.3.2. Surgery analysis

The results of the complete study are depicted in figures 32-35.

3.4.2.3.2.1. C57BL/6 for B1, B2sham and B2les

Distance: Averaged over the first session block, the sham operated animals (B2sham) made fewest line crossings (General mean: $F_{(2,29)} = 12.47$, $p < 0.0001$). The number of line crossing decreased in the course of sessions (Session: $F_{(4,116)} = 13.72$, $p < 0.0001$), which was dissimilar between groups (Session by Group interaction: $F_{(8,116)} = 2.52$, $p = 0.037$).
In the second session block, there was no more difference between groups (General mean: $F_{(2,29)} = 0.60$, $p = 0.553$). There was a session effect (Session: $F_{(4,116)} = 6.48$, $p < 0.0001$), but it was dissimilar for groups (Session by Group interaction: $F_{(8,116)} = 1.69$, $p = 0.111$) (depicted in figure 32 A).

Errors: On average, operated animals visited less incorrect holes compared to the other groups in the first session block (General mean: $F_{(2,29)} = 15.85$, $p < 0.0001$). Over sessions, all animals reduced errors (Session: $F_{(4,116)} = 11.82$, $p < 0.0001$), but in a dissimilar way (Session

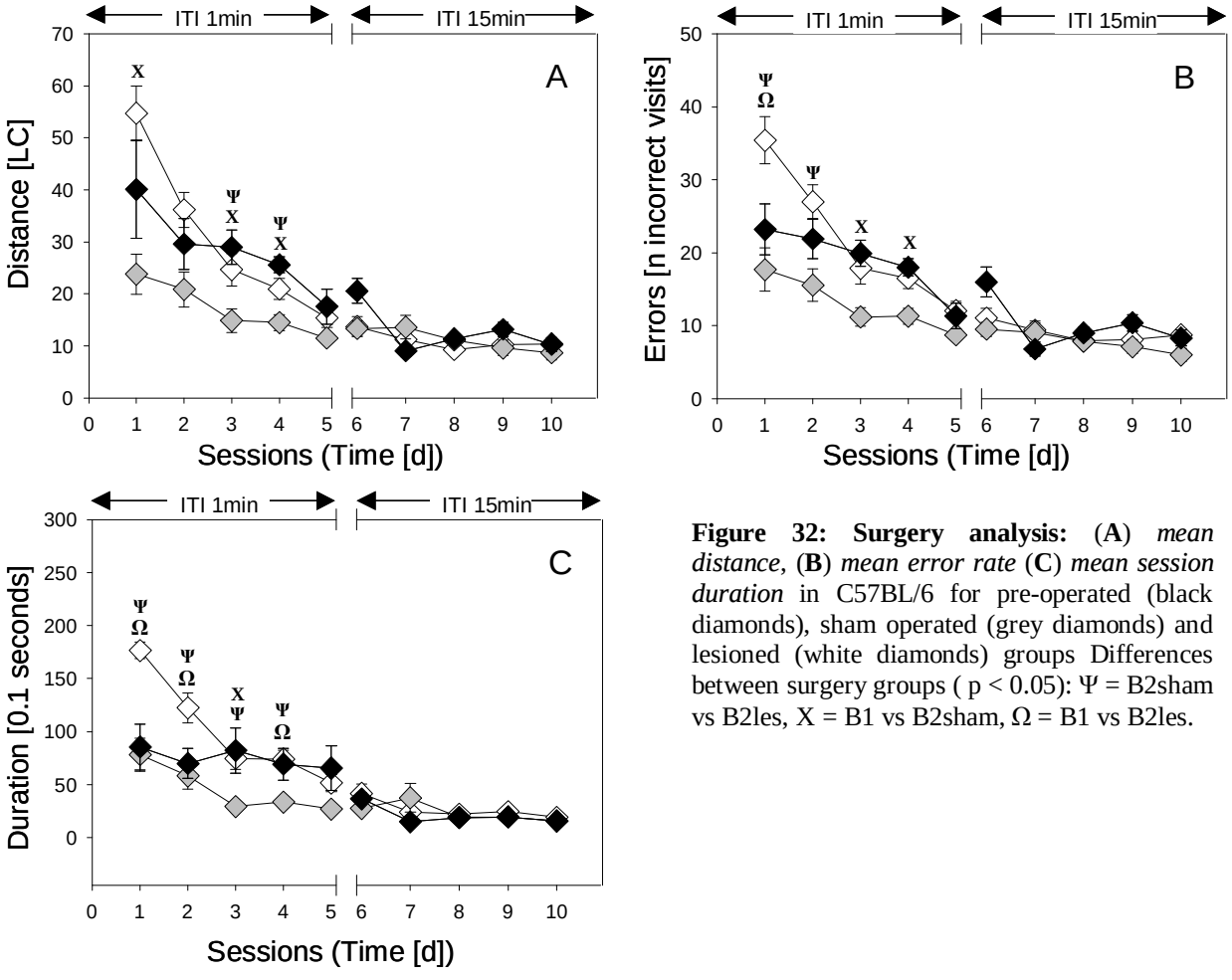


Figure 32: Surgery analysis: (**A**) *mean distance*, (**B**) *mean error rate* (**C**) *mean session duration* in C57BL/6 for pre-operated (black diamonds), sham operated (grey diamonds) and lesioned (white diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

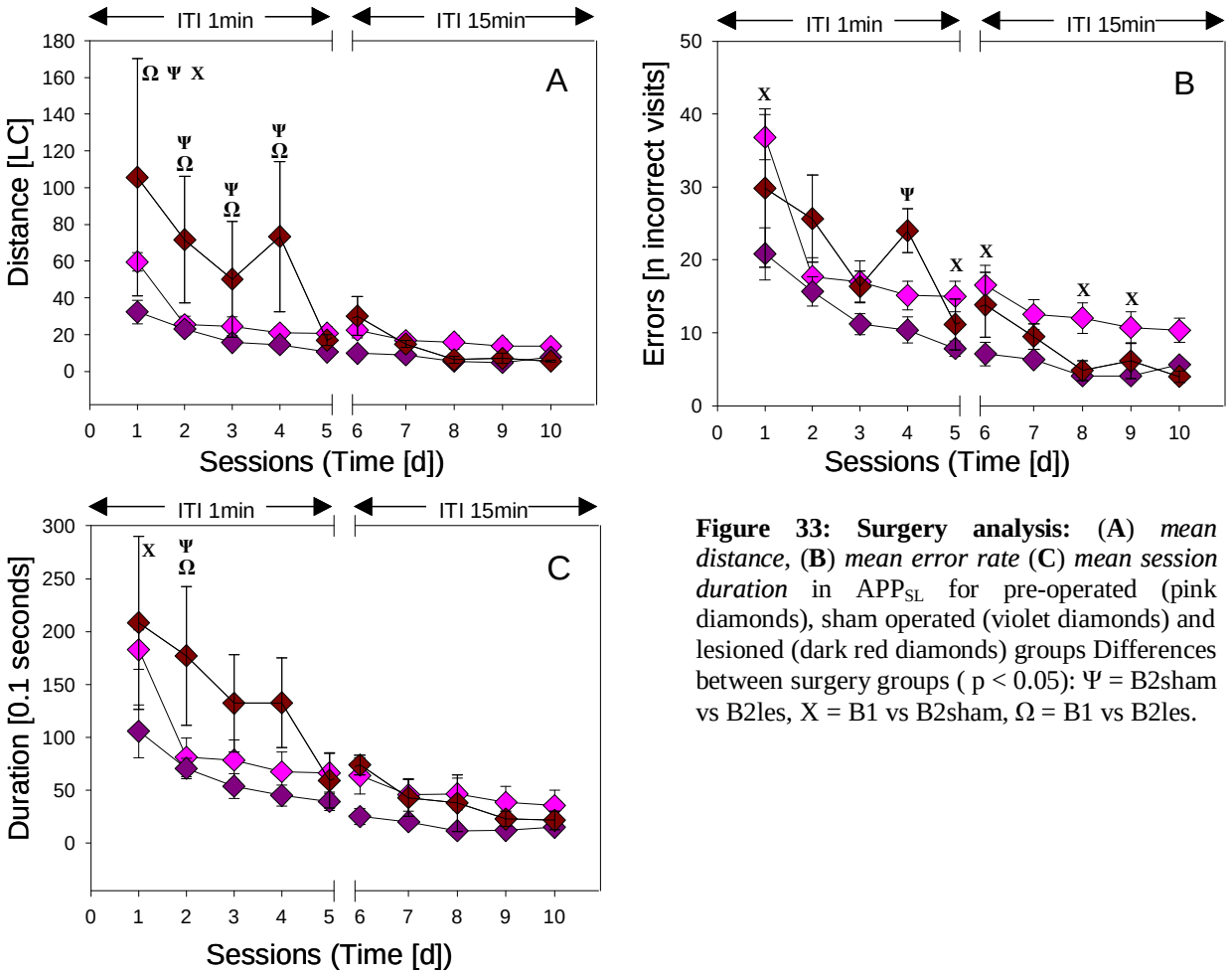


Figure 33: Surgery analysis: (**A**) *mean distance*, (**B**) *mean error rate* (**C**) *mean session duration* in APP_{SL} for pre-operated (pink diamonds), sham operated (violet diamonds) and lesioned (dark red diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

by Group interaction: $F_{(8,116)} = 2.04$, $p = 0.077$).

In the second session block, animals from all genotypes performed at similar level (General mean: $F_{(2,29)} = 1.16$, $p = 0.327$). Again, number of errors was reduced over sessions (Session: $F_{(4,116)} = 7.17$, $p < 0.0001$), but in a different manner for groups (Session by Group interaction: $F_{(8,116)} = 1.79$, $p = 0.061$) (depicted in figure 32 B).

Duration: In the first session block, there was a difference between genotypes (General mean: $F_{(2,29)} = 12.90$, $p < 0.0001$). The animals reduced time to escape over sessions (Session: $F_{(4,116)} = 13.49$, $p < 0.0001$) and it was dissimilar for groups (Session by Group interaction: $F_{(8,116)} = 4.32$, $p < 0.0001$).

In the second session block, there was no more difference between groups in for session duration (General mean: $F_{(2,29)} = 0.30$, $p = 0.746$). In the course of session, time to find the escape was reduced in all animals (Session: $F_{(4,116)} = 4.32$, $p = 0.006$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.17$, $p = 0.327$) (depicted in figure 32 C).

Speed: On average, untreated (B1) animals travelled fasted through the maze (General mean: $F_{(2,29)} = 6.38$, $p = 0.005$) in the first session block. The speed did not change in the course of sessions (Session: $F_{(4,116)} = 1.58$, $p = 0.185$), with different speed levels between groups (Session by Group interaction: $F_{(8,116)} = 2.56$, $p = 0.014$).

In the second session block, the untreated (B1) animals group performed fasted again (General mean: $F_{(2,29)} = 4.95$, $p = 0.014$). Speed increase over sessions (Session: $F_{(4,116)} = 3.00$, $p = 0.026$), in a similar way between groups (Session by Group interaction: $F_{(8,116)} = 0.94$, $p = 0.485$) (graph not shown).

3.4.2.3.2.2. APP_{SL} for B1, B2sham and B2les

Distance: Averaged over the first session block, there was a surgery effect in the lesioned group (General mean: $F_{(2,29)} = 5.25$, $p = 0.011$), the sham operated group made fewest line crossings. All animals reduced the number of line crossings over sessions (Session: $F_{(4,116)} = 24.47$, $p < 0.0001$), which was different for groups (Session by Group interaction: $F_{(8,116)} = 4.19$, $p = 0.003$).

In the second session block, there was no difference between groups, on average (General mean: $F_{(2,29)} = 1.65$, $p = 0.209$). Animals reduced line crossings over sessions (Session: $F_{(4,116)} = 11.38$, $p < 0.0001$) which tended to be different between groups (Session by Group interaction: $F_{(8,116)} = 2.28$, $p = 0.057$) (depicted in figure 33 A).

Errors: On average, there was a tendency but no difference between groups in the first session block (General mean: $F_{(2,29)} = 3.02$, $p = 0.064$). Animals reduced the amount of incorrect hole visits in the course of sessions (Session: $F_{(4,116)} = 14.03$, $p < 0.0001$), and the reduction was no similar between groups (Session by Group interaction: $F_{(8,116)} = 2.27$, $p = 0.067$).

In the second block of sessions, animals tended to show different error scores (General mean: $F_{(2,29)} = 3.21$, $p = 0.055$). In the course of sessions, there was a reduction in errors (Session: $F_{(4,116)} = 5.86$, $p = 0.002$), which was different between groups (Session by Group interaction: $F_{(8,116)} = 0.86$, $p = 0.522$) (depicted in figure 33 B).

Duration: In the first session block, there was no difference between groups, on average (General mean: $F_{(2,29)} = 1.64$, $p = 0.211$). Animals significantly reduced session duration in the course of time (Session: $F_{(4,116)} = 22.66$, $p < 0.0001$), and the reduction was not similar for groups (Session by Group interaction: $F_{(8,116)} = 2.75$, $p = 0.017$).

In the second session block, there was no difference between groups (General mean: $F_{(2,29)} = 0.88$, $p = 0.426$). There was a reduction of time to complete a session over sessions

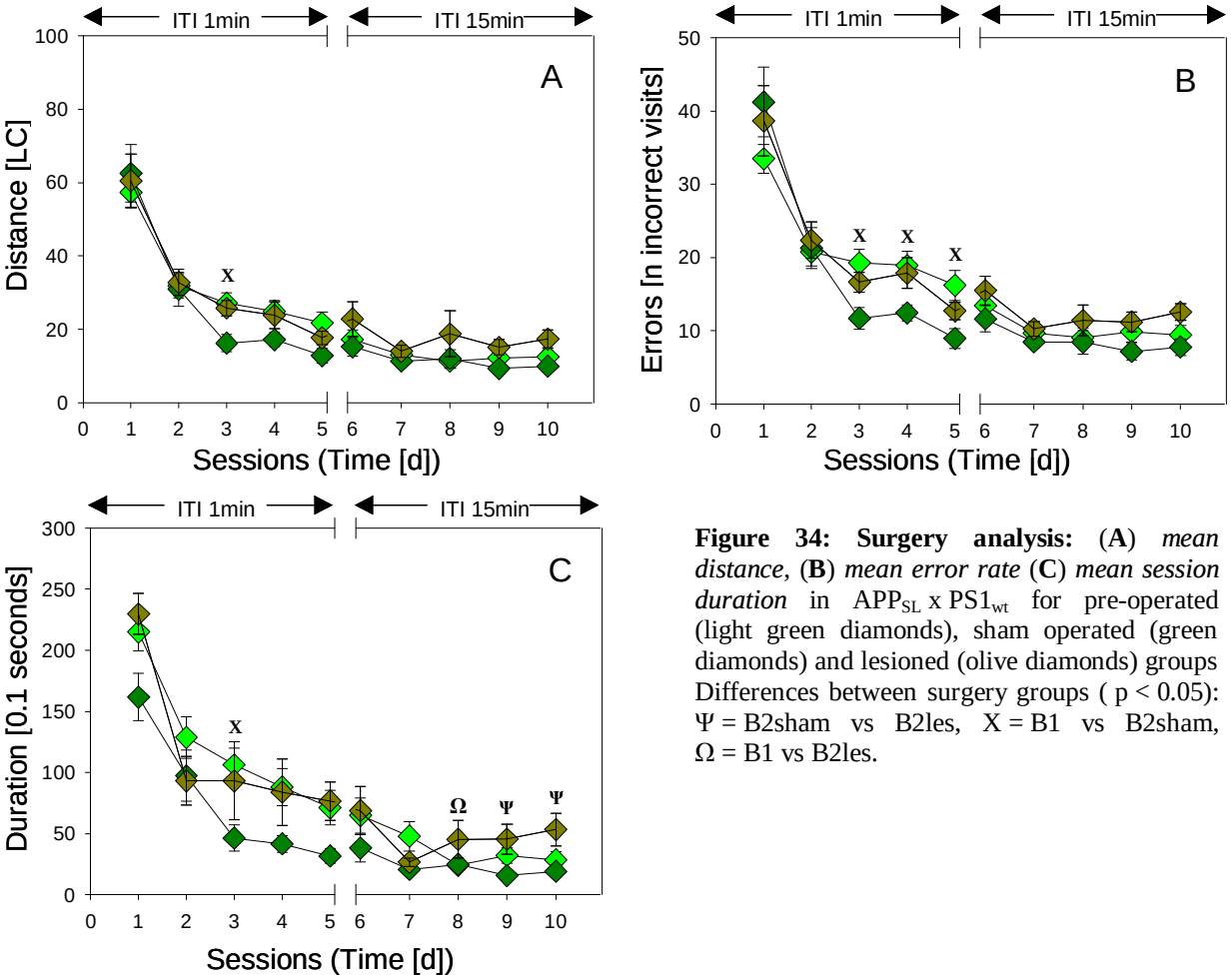


Figure 34: Surgery analysis: (**A**) *mean distance*, (**B**) *mean error rate* (**C**) *mean session duration* in APP_{SL} x $PS1_{wt}$ for pre-operated (light green diamonds), sham operated (green diamonds) and lesioned (olive diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

(Session: $F_{(4,116)} = 10.11$, $p < 0.0001$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.33$, $p = 0.264$) (depicted in figure 33 C).

Speed: On average there was no mean difference between groups in the first session block (General mean: $F_{(2,29)} = 0.19$, $p = 0.831$). Animals tended to increase speed over sessions (Session: $F_{(4,116)} = 2.41$, $p = 0.070$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.22$, $p = 0.303$).

In the second session duration, animals travelled with same speed, on average (General mean: $F_{(2,29)} = 1.36$, $p = 0.274$). There was no change of speed over sessions (Session: $F_{(4,116)} = 0.63$, $p = 0.646$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.35$, $p = 0.226$) (graph not shown).

3.4.2.3.2.3. APP_{SL} x $PS1_{wt}$ for B1, B2sham and B2les

Distance: Averaged over the first session block, there was no difference between groups (General mean: $F_{(2,36)} = 0.86$, $p = 0.431$). Animals reduced line crossings in the course of

sessions (Session: $F_{(4,144)} = 64.6$, $p < 0.0001$), and the reduction was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.09$, $p = 0.370$).

In the second session block, the mean distance was similar between groups (General mean: $F_{(2,36)} = 2.60$, $p = 0.881$). Animals reduced distance in the course of sessions (Session: $F_{(4,144)} = 3.39$, $p = 0.022$), and the reduction was similar in groups (Session by Group interaction: $F_{(8,116)} = 0.37$, $p = 0.913$) (depicted in figure 34 A).

Errors: On average, there was no difference between groups in the first session block (General mean: $F_{(2,36)} = 0.82$, $p = 0.448$). Animals reduced error scores over sessions (Session: $F_{(4,144)} = 55.02$, $p < 0.0001$), but in a dissimilar way for groups (Session by Group interaction: $F_{(8,116)} = 2.81$, $p = 0.013$).

In the second session block, there was no difference between groups, on average (General mean: $F_{(2,36)} = 2.07$, $p = 0.140$). There was a reduction of errors on the course of sessions (Session: $F_{(4,144)} = 4.44$, $p = 0.004$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 0.22$, $p = 0.986$) (depicted in figure 34 B).

Duration: In the first block of sessions, there was a tendency for differences between groups, with the sham operated group escaping at fastest (General mean: $F_{(2,36)} = 3.22$, $p = 0.052$). All animals reduced time to find the escape over sessions (Session: $F_{(4,144)} = 47.07$, $p < 0.0001$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 0.79$, $p = 0.582$).

In the second session block, there was no difference between groups for the mean session duration (General mean: $F_{(2,36)} = 1.94$, $p = 0.158$). In the course of sessions, there was a reduction on session duration (Session: $F_{(4,144)} = 4.26$, $p = 0.007$), which was similar for groups (Session by Group interaction: $F_{(8,116)} = 1.26$, $p = 0.283$) (depicted in figure 34 C).

Speed: On average, untreated (B1) animals travelled with lowest speed (General mean: $F_{(2,36)} = 7.01$, $p = 0.003$). Speed increase over sessions (Session: $F_{(4,144)} = 3.22$, $p = 0.015$), but in a dissimilar way for groups (Session by Group interaction: $F_{(8,116)} = 2.65$, $p = 0.010$).

In the second session block, the mean speed was similar between groups (General mean: $F_{(2,36)} = 1.93$, $p = 0.160$). Over sessions, there was no change in speed (Session: $F_{(4,144)} = 1.00$, $p = 0.407$), which was different in groups (Session by Group interaction: $F_{(8,116)} = 3.13$, $p = 0.003$) (graph not shown).

3.4.2.3.2.4. APP_{SL} x $PS1_{mut}$ for B1, B2sham and B2les

Distance: In the first session block, there was no difference between groups, on average (General mean: $F_{(2,42)} = 1.14$, $p = 0.330$). In the course of sessions, there was a reduction of

line crossings (Session: $F_{(4,168)} = 39.94$, $p < 0.0001$), but similar for groups (Session by Group interaction: $F_{(8,116)} = 0.67$, $p = 0.683$).

There was no difference between groups for the second session block (General mean: $F_{(2,42)} = 0.77$, $p = 0.472$). All animals reduces line crossings in the course of sessions (Session: $F_{(4,168)} = 39.94$, $p < 0.0001$), an the reduction was similar in groups (Session by Group interaction: $F_{(8,116)} = 0.72$, $p = 0.626$) (depicted in figure 35 A).

Errors: On average, there was no difference between groups in the first session block (General mean: $F_{(2,42)} = 0.54$, $p = 0.587$). Error rate was reduced over sessions (Session: $F_{(4,168)} = 32.51$, $p < 0.0001$), in a similar way between groups (Session by Group interaction: $F_{(8,116)} = 0.93$, $p = 0.485$).

There was no difference between groups in the second block of sessions (General mean: $F_{(2,42)} = 0.20$, $p = 0.816$). Error scores were reduced over sessions (Session: $F_{(4,168)} = 11.92$, $p < 0.0001$), in a similar way for groups (Session by Group interaction: $F_{(8,116)} = 0.65$, $p = 0.666$) (depicted in figure 35 B).

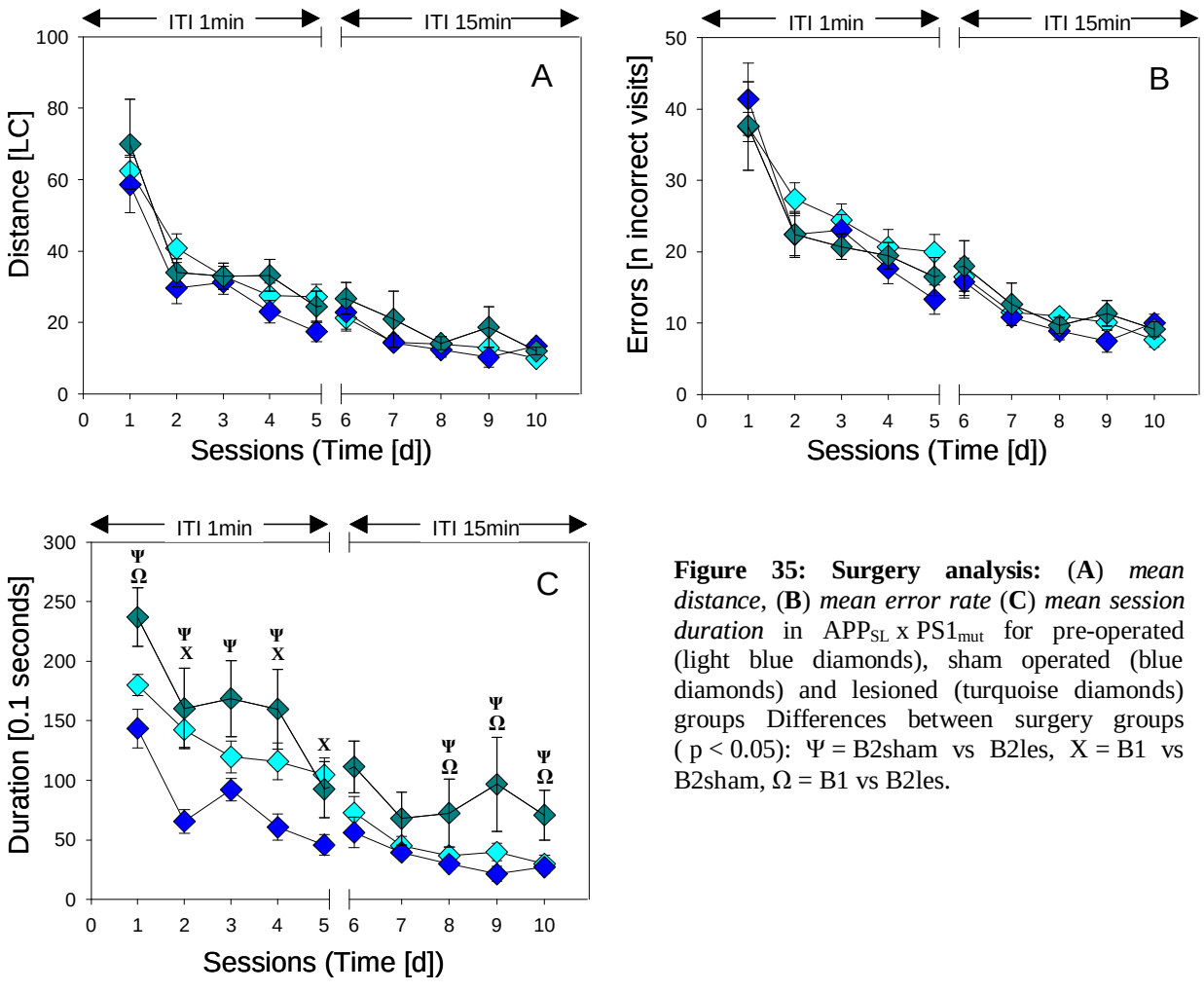


Figure 35: Surgery analysis: (**A**) *mean distance*, (**B**) *mean error rate* (**C**) *mean session duration* in APP_{SL} x $PS1_{mut}$ for pre-operated (light blue diamonds), sham operated (blue diamonds) and lesioned (turquoise diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

Duration: In the first session block, the sham operated animals escaped at fastest, the lesioned animals needed longest time to find the escape hole, on average (General mean: $F_{(2,42)} = 7.36$, $p = 0.002$). Session duration was reduced over sessions (Session: $F_{(4,168)} = 22.63$, $p < 0.0001$), in a similar way for groups (Session by Group interaction: $F_{(8,116)} = 1.51$, $p = 0.159$).
In the second session block, the mean session duration was lowest in the sham operated and highest in the lesioned group (General mean: $F_{(2,42)} = 4.30$, $p = 0.020$). Duration to escape into the hole was reduced over sessions (Session: $F_{(4,168)} = 8.10$, $p < 0.0001$), an the reduction was similar for all groups (Session by Group interaction: $F_{(8,116)} = 0.82$, $p = 0.546$) (depicted in figure 35 C).

Speed: On average, the sham operate group travelled faster than the other groups (General mean: $F_{(2,42)} = 9.69$, $p = 0.0003$). There was no change of speed over sessions (Session: $F_{(4,168)} = 1.64$, $p = 0.165$), and the speed progressed different for groups (Session by Group interaction: $F_{(8,116)} = 2.24$, $p = 0.027$).
In the second session block, the mean speed was similar between groups (General mean: $F_{(2,42)} = 2.17$, $p = 0.127$). Speed increased over sessions (Session: $F_{(4,168)} = 3.97$, $p = 0.004$), in a different manner for groups (Session by Group interaction: $F_{(8,116)} = 2.16$, $p = 0.033$) (graph not shown).

3.4.2.4. Experiment 4: The Morris Water Maze (MWM)

3.4.2.4.1. Genotype analysis

The results of the study are depicted in figures 36-39.

3.4.2.4.1.1. All genotypes in B1

Escape latency: Averaged over all acquisition sessions, animals from the APP_{SL} genotypes had longer escape latencies than the other genotypes (General mean: $F_{(3,83)} = 4.69 = 0.005$). There was a reduction of escape latency across sessions (Session: $F_{(4,332)} = 207.62 p < 0.0001$). The rate of learning was different for the groups (Session by Group interaction: $F_{(12,332)} = 3.1$, $p = 0.002$). (depicted in figure 36 A).

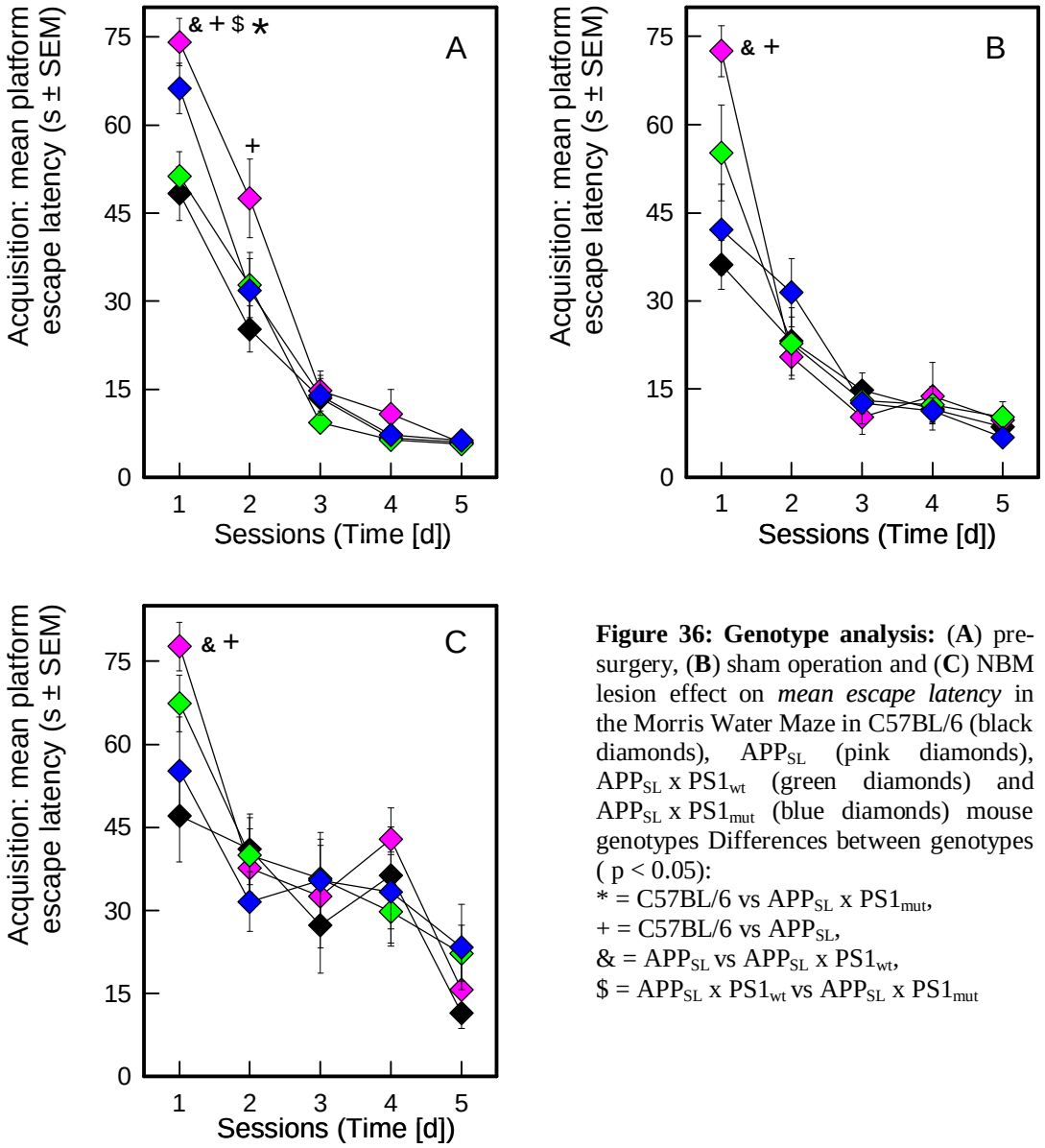


Figure 36: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean escape latency* in the Morris Water Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$):
* = C57BL/6 vs APP_{SL} x $PS1_{mut}$,
+ = C57BL/6 vs APP_{SL},
& = APP_{SL} vs APP_{SL} x $PS1_{wt}$,
$ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$

Distance travelled: The animals from the APP_{SL} genotypes swum, on average, longer paths

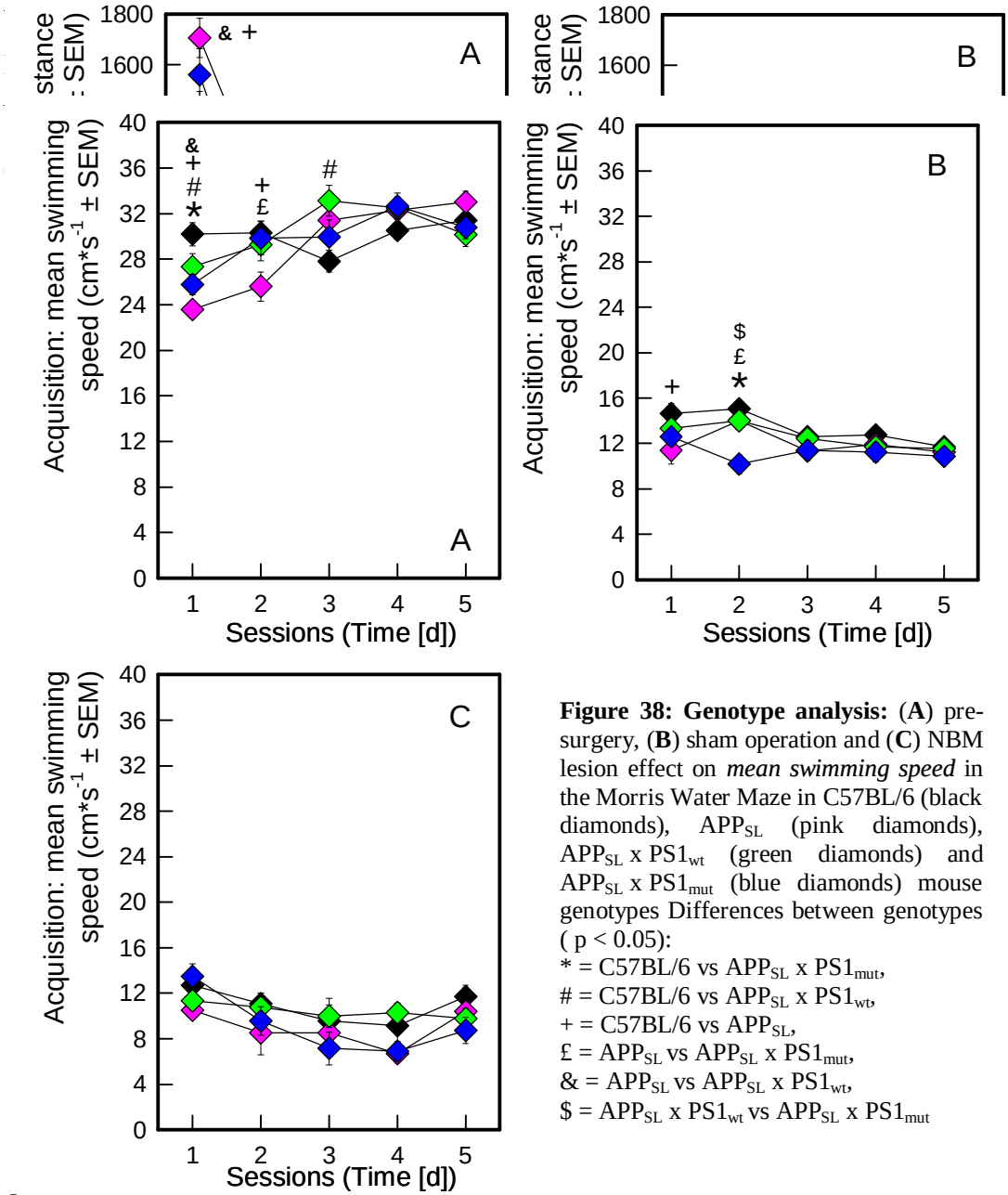


Figure 38: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean swimming speed* in the Morris Water Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$):
* = C57BL/6 vs APP_{SL} x $PS1_{mut}$,
# = C57BL/6 vs APP_{SL} x $PS1_{wt}$,
+ = C57BL/6 vs APP_{SL},
£ = APP_{SL} vs APP_{SL} x $PS1_{mut}$,
& = APP_{SL} vs APP_{SL} x $PS1_{wt}$,
$ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$

S

g

F

interaction: $F_{(12,332)} = 4.56$, $p < 0.0001$) (depicted in figure 38 A).

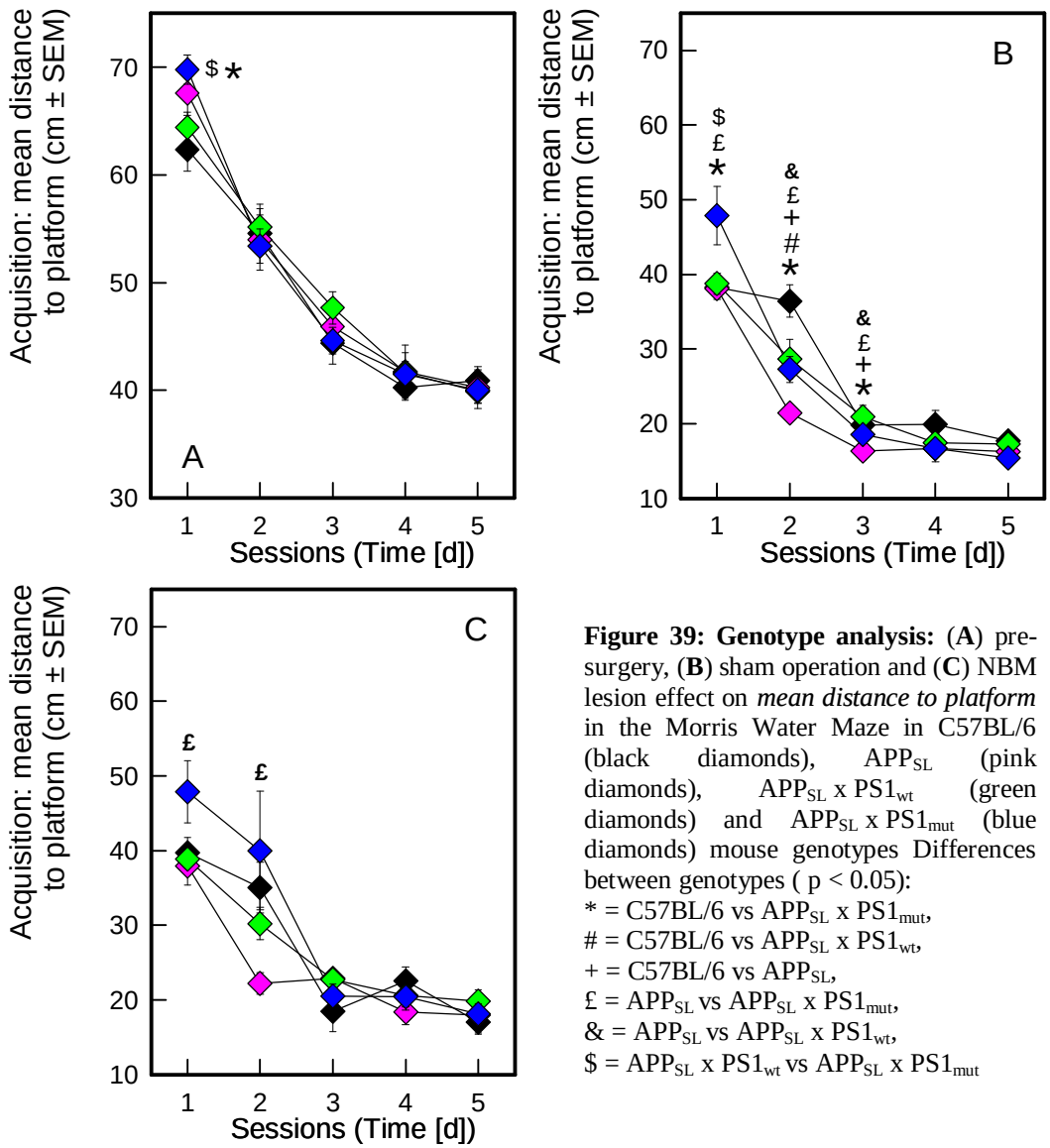


Figure 39: Genotype analysis: (**A**) pre-surgery, (**B**) sham operation and (**C**) NBM lesion effect on *mean distance to platform* in the Morris Water Maze in C57BL/6 (black diamonds), APP_{SL} (pink diamonds), APP_{SL} x $PS1_{wt}$ (green diamonds) and APP_{SL} x $PS1_{mut}$ (blue diamonds) mouse genotypes Differences between genotypes ($p < 0.05$):
* = C57BL/6 vs APP_{SL} x $PS1_{mut}$,
# = C57BL/6 vs APP_{SL} x $PS1_{wt}$,
+ = C57BL/6 vs APP_{SL},
£ = APP_{SL} vs APP_{SL} x $PS1_{mut}$,
& = APP_{SL} vs APP_{SL} x $PS1_{wt}$,
$ = APP_{SL} x $PS1_{wt}$ vs APP_{SL} x $PS1_{mut}$

Distance to platform: The mean proximity to the platform was similar for all genotypes (General mean: $F_{(3,83)} = 0.35 = 0.791$). In the course of training, all genotypes searched in closer proximity to the escape platform (Session: $F_{(4,332)} = 201.39$, $p < 0.0001$) in a similar manner (Session by Group interaction: $F_{(12,92} = 1.39$, $p = 0.170$) (depicted in figure 39).

3.4.2.4.1.2. All genotypes in B2sham

Escape latency: The time to find the escape platform was, on average, similar between groups (General mean: $F_{(3,35)} = 1.17 = 0.334$). Animals from all groups decrease escape latency across

sessions (Session: $F_{(4,140)} = 69.31$, $p < 0.0001$), but the learning curve was different for groups (Session by Group interaction: $F_{(12,140} = 3.44$, $p = 0.002$) (depicted in figure 36 B).

Distance travelled: All animal groups swam, on average, a similar distance to find the platform (General mean: $F_{(3,35)} = 1.59= 0.209$). The distance was reduced in the course of training (Session: $F_{(4,140)} = 77.15$, $p < 0.0001$), to a similar extent (Session by Group interaction: $F_{(12,140} = 1.66$, $p = 0.123$) (depicted in figure 37 B).

Swimming speed: The mean swimming speed tended to be different between groups (General mean: $F_{(3,35)} = 2.75= 0.058$). The C57BL/6 genotype swum at highest speed, the APP_{SL} x $PS1_{mut}$ swum at slowest speed, which was also confirmed by post hoc analysis (for details see Figure 40). Swimming speed decreased over sessions (Session: $F_{(4,140)} = 7.29$, $p < 0.0001$), but it was different for groups (Session by Group interaction: $F_{(12,140} = 2.53$, $p = 0.008$) (depicted in figure 38 B).

Distance to platform: The mean distance to platform was different for genotypes (General mean: $F_{(3,35)} = 3.38= 0.029$). All animals were able to reduce distance to platform, i.e. to search in a closer proximity to the escape platform (Session: $F_{(4,140)} = 166.37$, $p < 0.0001$). The searching pattern was different for groups (Session by Group interaction: $F_{(12,140} = 4.89$, $p < 0.0001$) (depicted in figure 39 B).

3.4.2.4.1.3. All genotypes in B2les

Escape latency: The mean escape latency was similar between genotypes (General mean: $F_{(3,23)} = 0.31= 0.819$). All groups reduced the time to find the escape platform significantly across sessions (Session: $F_{(4,92)} = 21.29$, $p < 0.0001$) to a similar extent (Session by Group interaction: $F_{(12,92} = 1.66$, $p = 0.088$) (depicted in figure 36 C).

Distance travelled: On average, animals from all genotypes swum similar distances to find the escape platform (General mean: $F_{(3,23)} = 0.34= 0.796$). The distance was reduces across sessions in all genotypes (Session: $F_{(4,92)} = 48.8$, $p < 0.0001$) to a similar extent in all groups (Session by Group interaction: $F_{(12,92} = 1.56$, $p = 0.140$) (depicted in figure 37 C).

Swimming speed: The mean swimming speed was similar between groups (General mean: $F_{(3,23)} = 0.89= 0.463$). The swimming speed changed over sessions (Session: $F_{(4,92)} = 11.2$, $p < 0.0001$), but dissimilar for the groups (Session by Group interaction: $F_{(12,92} = 2.27$, $p = 0.016$) (depicted in figure 38 C).

Distance to platform: All groups searched for the platform, on average, in a similar proximity (General mean: $F_{(3,23)} = 1.23= 0.323$). In the course of training, the animals searched in more and more closer proximity to the platform, which indicates successful spatial learning (Session: $F_{(4,92)} = 49.22$, $p < 0.0001$) and this was similar in all groups (Session by Group interaction: $F_{(12,92} = 2.07$, $p = 0.084$) (depicted in figure 39 C).

3.4.2.4.2. Surgery analysis

The results of the complete study are depicted in figures 40-43.

3.4.2.4.2.1. C57BL/6 for B1, B2sham and B2les

Escape latency: On average, lesioned animals spent most time on swimming before escaping onto the platform (General mean: $F_{(2,29)} = 5.61 = 0.009$). Animals reduced escaped latency over sessions (Session: $F_{(4,116)} = 27.25$, $p < 0.0001$), and the reduction was different between groups (Session by Group interaction: $F_{(8,116} = 3.22$, $p = 0.003$) (depicted in figure 40 A).

Distance travelled: On average, untreated animals (B1) swum the longest distance before escaping onto the platform (General mean: $F_{(2,29)} = 13.98$, $p < 0.0001$). All animals groups reduced length of swimming path in the course of training (Session: $F_{(4,116)} = 30.80$, $p < 0.0001$), in a dissimilar way (Session by Group interaction: $F_{(8,116} = 8.53$, $p < 0.0001$)

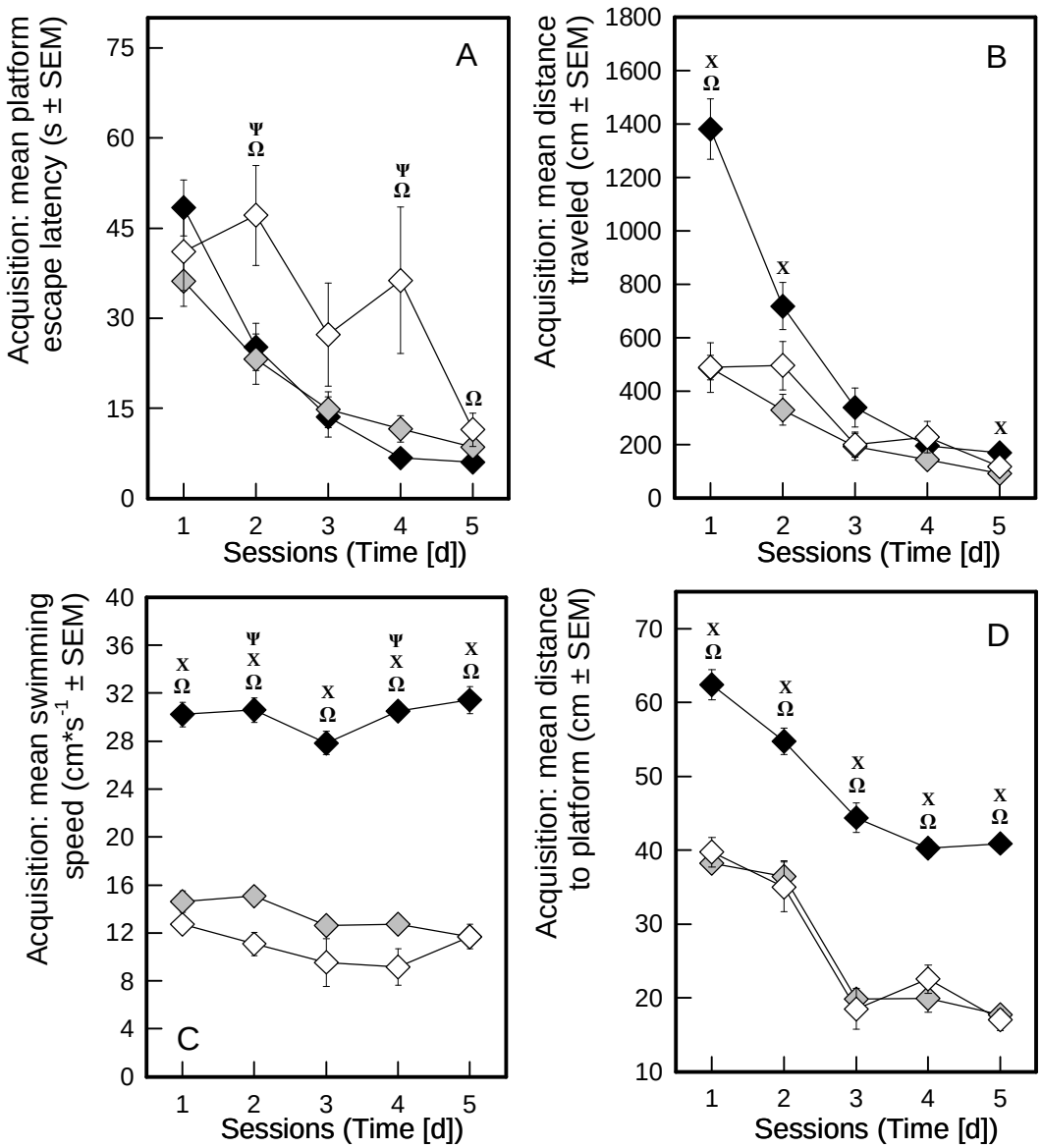


Figure 40: Surgery analysis: (**A**) *mean escape latency*, (**B**) *mean distance travelled* (**C**) *mean swimming speed* and (**D**) *mean distance to platform* in C57BL/6 for pre-operated (black diamonds), sham operated (grey diamonds) and lesioned (white diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

(depicted in figure 40 B).

Swimming speed: On average, the untreated (B1) animal group showed highest swimming speed (General mean: $F_{(2,29)} = 267.86$, $p < 0.0001$). Swimming speed did not change in the course of sessions (Session: $F_{(4,116)} = 2.28$, $p = 0.085$), and it was similar between groups (Session by Group interaction: $F_{(8,116} = 1.23$, $p = 0.301$) (depicted in figure 40 C).

Distance to platform: The untreated (B1) groups searched in widest radius to find the platform (General mean: $F_{(2,29)} = 130.61$, $p < 0.0001$). All animals reduced distance to platform in the course of sessions (Session: $F_{(4,116)} = 71.35$, $p < 0.0001$), which was similar for groups (Session by Group interaction: $F_{(8,116} = 1.08$, $p = 0.385$) (depicted in figure 40 D).

3.4.2.4.2.2. APP_{SL} for B1, B2sham and B2les

Escape latency: The mean escape latency was similar between groups (General mean: $F_{(2,29)} = 2.82$, $p = 0.076$). Latency was reduced over sessions (Session: $F_{(4,116)} = 52.20$, $p < 0.0001$), but the groups reduced latency in a dissimilar way (Session by Group interaction: $F_{(8,116} = 3.24$, $p = 0.006$) (depicted in figure 41 A).

Distance travelled: On average, untreated (B1) animals spent most time on swimming before they finally escaped onto the platform (General mean: $F_{(2,29)} = 17.44$, $p < 0.0001$). Distance was reduced over sessions (Session: $F_{(4,116)} = 31.32$, $p < 0.0001$). The rate of learning, however, was differently affected by treatment (Session by Group interaction: $F_{(8,116} = 6.53$, $p < 0.0001$) (depicted in figure 41 B).

Swimming speed: The mean swimming speed was affected by treatment (General mean: $F_{(2,29)} = 255.87$, $p < 0.0001$). Untreated sham animals swum at highest speed. Swimming speed was changed in the course of training (Session: $F_{(4,116)} = 1672$, $p < 0.0001$), but differently for groups (Session by Group interaction: $F_{(8,116} = 6.55$, $p < 0.0001$) (depicted in figure 41 C).

Distance to platform: The untreated (B1) animal group searched in the widest distance to platform (General mean: $F_{(2,29)} = 86.04$, $p < 0.0001$). All animals reduced distance to platform in the course of sessions (Session: $F_{(4,116)} = 33.33$, $p < 0.0001$), in a similar way (Session by Group interaction: $F_{(8,116} = 1.15$, $p = 0.334$) (depicted in figure 41 C).

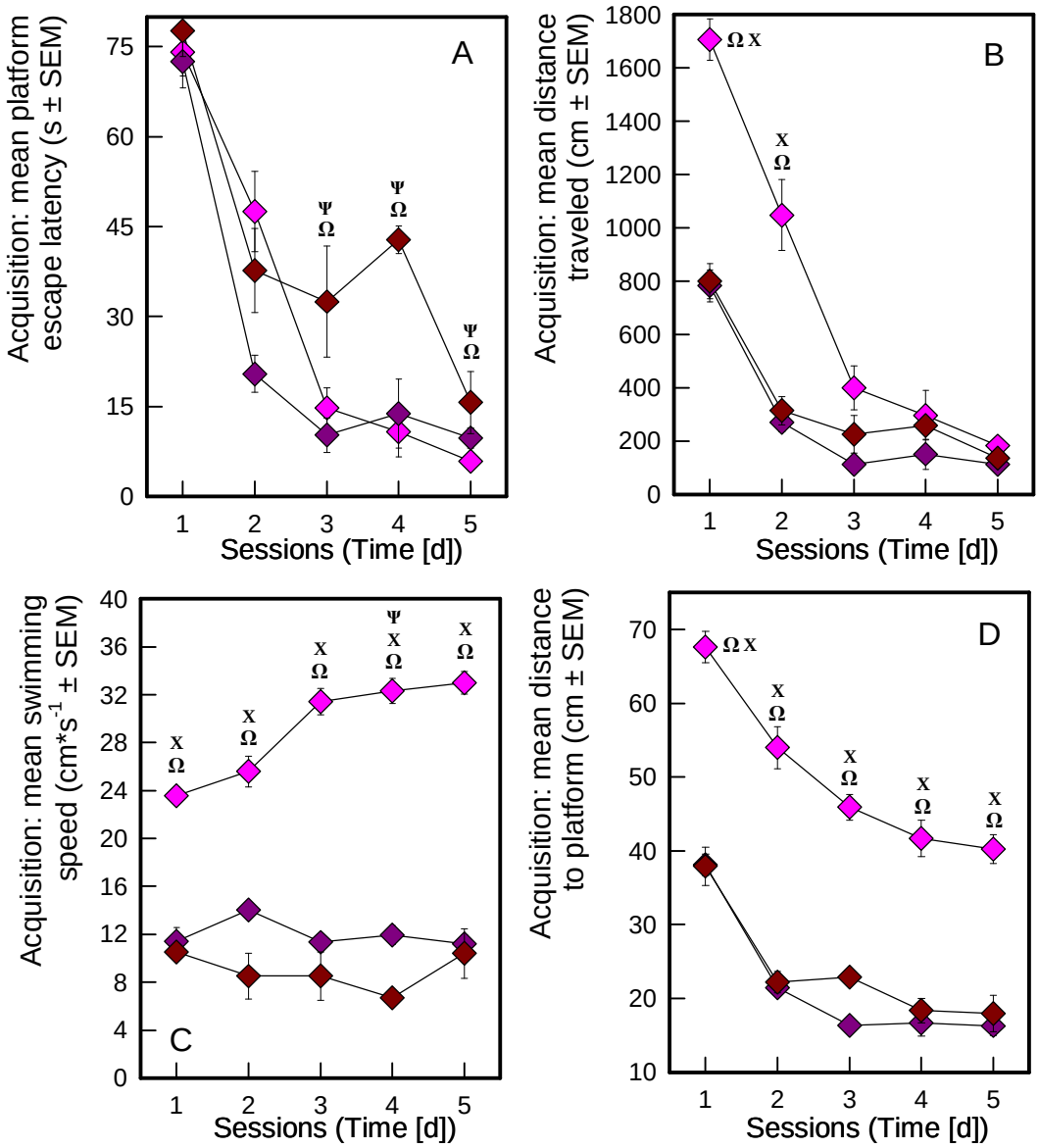


Figure 41: Surgery analysis: (**A**) *mean escape latency*, (**B**) *mean distance travelled* (**C**) *mean swimming speed* and (**D**) *mean distance to platform* in APP_{SL} for pre-operated (pink diamonds), sham operated (violet diamonds) and lesioned (dark red diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

3.4.2.4.2.3. APP_{SL} x $PS1_{wt}$ for B1, B2sham and B2les

Escape latency: Averaged over all acquisition sessions, there was a treatment effect (General mean: $F_{(2,41)} = 10.86$, $p = 0.0002$). Lesioned animals spent most time on searching for the escape platform. Averaged over sessions, there was a reduction of escape latency (Session: $F_{(4,164)} = 63.53$, $p < 0.0001$), which was similar for the treatment groups (Session by Group interaction: $F_{(8,164} = 1.01$, $p = 0.420$) (depicted in figure 42 A).

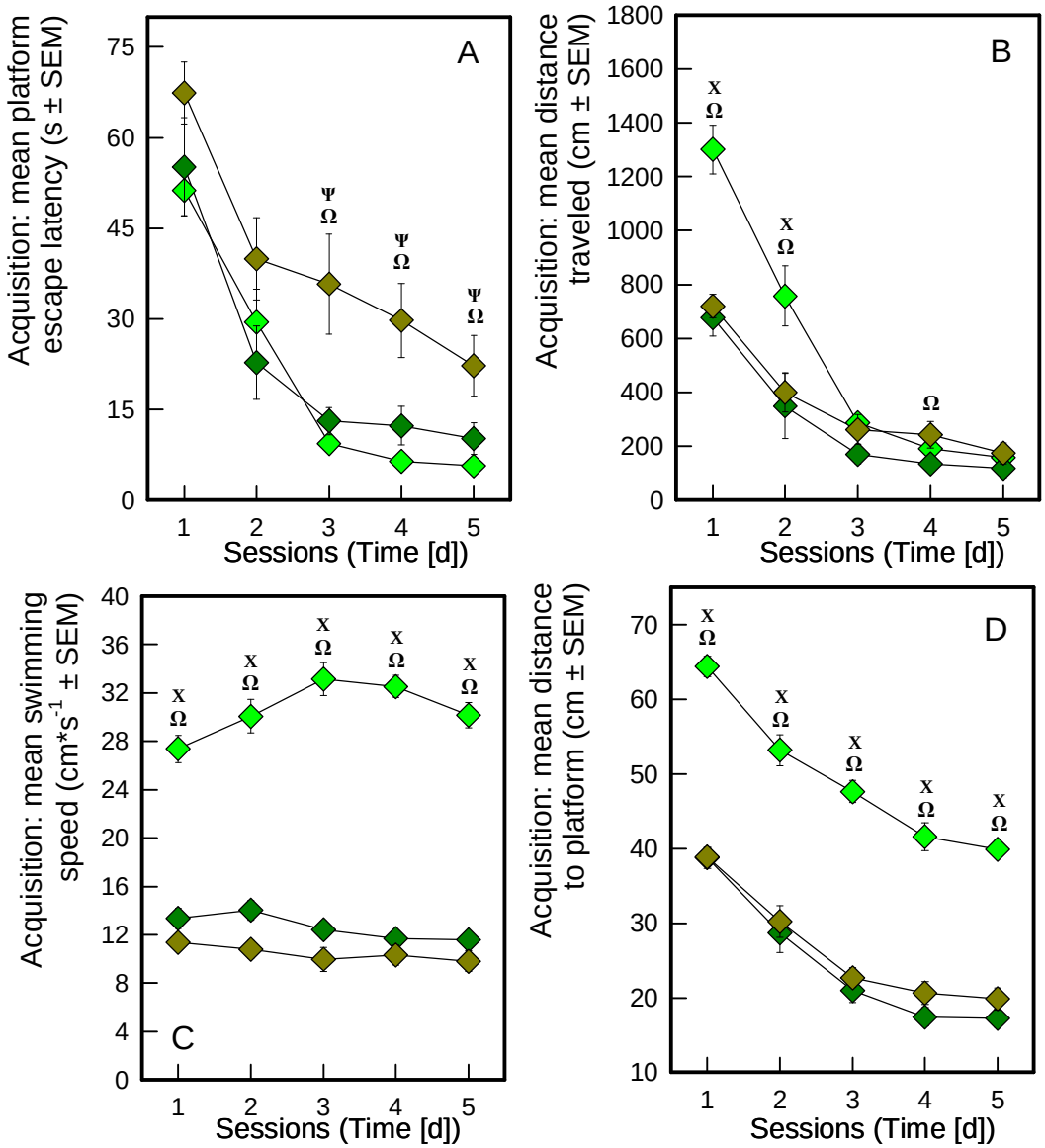


Figure 42: Surgery analysis: (**A**) *mean escape latency*, (**B**) *mean distance travelled* (**C**) *mean swimming speed* and (**D**) *mean distance to platform* in APP_{SL} x $PS1_{wt}$ for pre-operated (light green diamonds), sham operated (green diamonds) and lesioned (olive diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

Distance travelled: On average, untreated (B1) animals swum longest distance before escaping onto the platform (General mean: $F_{(2,41)} = 11.64$, $p < 0.0001$). Swimming path was reduced over sessions (Session: $F_{(4,164)} = 67.11$, $p < 0.0001$), which was differently affected by the treatment (Session by Group interaction: $F_{(8,164} = 6.90$, $p < 0.0001$) (depicted in figure 42 B).

Swimming speed: The mean swimming speed was dissimilar for groups (General mean: $F_{(2,41)} = 225.15$, $p < 0.0001$). Speed remained constant over sessions (Session: $F_{(4,164)} = 1.10$,

p = 0.355), but in a dissimilar way for groups (Session by Group interaction: $F_{(8,164} = 3.67$, p = 0.001). The untreated (B1) animals performed at highest speed (depicted in figure 42 C).

Distance to platform: Mean distance to platform was highest in the untreated (B1) animal group (General mean: $F_{(2,41)} = 193.95$, $p < 0.0001$). The searching radius around the platform decreased over sessions (Session: $F_{(4,164)} = 91.98$, $p < 0.0001$), in a similar way for the treatment groups (Session by Group interaction: $F_{(8,164} = 0.72$, p = 0.658) (depicted in figure 42 D).

3.4.2.4.2.4. APP_{SL} x $PS1_{mut}$ for B1, B2sham and B2les

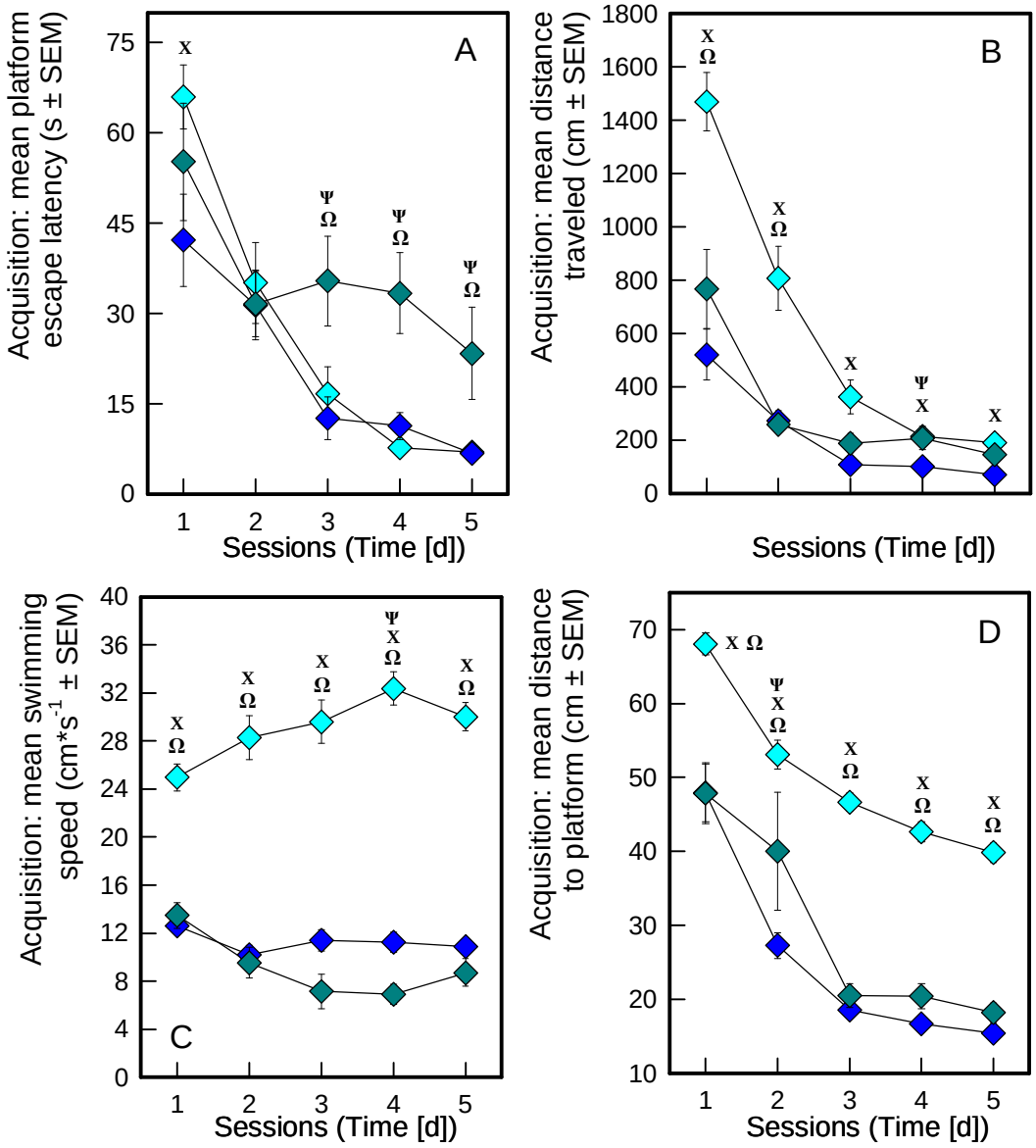


Figure 43: Surgery analysis: (**A**) *mean escape latency*, (**B**) *mean distance travelled* (**C**) *mean swimming speed* and (**D**) *mean distance to platform* in APP_{SL} x $PS1_{mut}$ for pre-operated (light blue diamonds), sham operated (blue diamonds) and lesioned (turquoise diamonds) groups Differences between surgery groups ($p < 0.05$): Ψ = B2sham vs B2les, X = B1 vs B2sham, Ω = B1 vs B2les.

Escape latency: Averaged over all sessions, there was a treatment effect in the lesioned group (General mean: $F_{(2,42)} = 4.33$, $p = 0.020$). Lesioned animals displayed highest durations. Animals reduced escape latency over sessions (Session: $F_{(4,168)} = 39.03$, $p < 0.0001$), which was differently affected in groups (Session by Group interaction: $F_{(8,168} = 4.11$, $p = 0.0006$) (depicted in figure 43 A).

Distance travelled: On average, untreated (B1) animals spent most time to find the escape platform (General mean: $F_{(2,42)} = 20.85$, $p < 0.0001$). Swimming distance decreased over sessions (Session: $F_{(4,168)} = 56.20$, $p < 0.0001$), in a dissimilar way for treatment groups (Session by Group interaction: $F_{(8,168} = 11.48$, $p < 0.0001$) (depicted in figure 43 B).

Swimming speed: The mean swimming speed highest in the untreated (B1) group (General mean: $F_{(2,42)} = 149.26$, $p < 0.0001$). Swimming speed did not change in the course of sessions (Session: $F_{(4,168)} = 0.37$, $p = 0.814$), but it was different for treatment groups (Session by Group interaction: $F_{(8,168} = 5.65$, $p < 0.0001$) (depicted in figure 43 C).

Distance to platform: Mean distance to platform was highest in the untreated (B1) animal group. (General mean: $F_{(2,42)} = 163.06$, $p < 0.0001$). Distance to platform was reduced over sessions (Session: $F_{(4,168)} = 117.15$, $p < 0.0001$), and to a similar way in treatment groups (Session by Group interaction: $F_{(8,168} = 1.38$, $p = 0.226$) (depicted in figure 43 D).

3.4.2.4.3. Working memory analysis

(Figures not shown).

3.4.2.4.3.1. Working memory analysis in C57BL/6 animals in B1

Escape latency: On average, animals reduced the time to find the platform similarly in trial 1 and trial 5 (General mean: $F_{(1,36)} = 0.006$, $p = 0.936$). Escape latency decrease over sessions (Session: $F_{(4,144)} = 25.67$, $p < 0.0001$).

Distance travelled: Averaged over sessions, there was no difference between trials (General mean: $F_{(1,36)} = 3.73$, $p = 0.061$). Distance travelled was reduced over sessions (Session: $F_{(4,144)} = 23.57$, $p < 0.0001$). *Post-hoc* analysis showed that there was a difference on the first and the second session.

Swimming speed: The mean speed was different between trial 1 and trial 5 (General mean: $F_{(1,36)} = 33.22$, $p < 0.001$). Swimming speed was enhanced in the course of sessions (Session:

$F_{(4,144)} = 3.06$, $p = 0.019$). Differences were found in session 1, 2 and 4 by *post-hoc* comparison.

3.4.2.4.3.2. Working memory analysis in C57BL/6 animals in B2sham

Escape latency: On average, animals swum longer in trial 1 compared to trial 5 (General mean: $F_{(1,14)} = 11.26$, $p = 0.005$). Escape latency was strongly reduced over sessions (Session: $F_{(4,56)} = 10.86$, $p < 0.001$). Differences between trials were found in the first and the second session.

Distance travelled: The mean distance swum was different between trials (General mean: $F_{(1,14)} = 21.11$, $p = 0.0004$), with longer distances in the first trial, respectively. Swimming path was reduced over sessions for in both trials (Session: $F_{(4,56)} = 10.76$, $p < 0.001$). *Post-hoc* analysis assessed differences between trials in the first and second session.

Swimming speed: On average, animals displayed higher swimming speed in the first trials (General mean: $F_{(1,14)} = 8.74$, $p = 0.01$). Swimming speed remained constant over sessions (Session: $F_{(4,56)} = 2.50$, $p = 0.053$), with a tendency to speed reduction in the first trials, respectively. Differences between trials were found with *post-hoc* analysis in the first two sessions.

3.4.2.4.3.3. Working memory analysis in C57BL/6 animals in B2les

Escape latency: Averaged over sessions, there was no differences between trials (General mean: $F_{(1,8)} = 3.21$, $p = 0.111$), indicating low recall of the information from previous trials. The time to escape onto the platform was reduced over sessions (Session: $F_{(4,32)} = 3.29$, $p = 0.023$).

Distance travelled: The mean distance swum was similar for both trials (General mean: $F_{(1,8)} = 3.11$, $p = 0.116$). Length of the swimming path was reduced over sessions (Session: $F_{(4,32)} = 10.22$, $p < 0.001$).

Swimming speed: On average, animals displayed higher swimming speed in the first trials (General mean: $F_{(1,8)} = 5.39$, $p = 0.049$). Swimming speed changed in the course of sessions (Session: $F_{(4,32)} = 5.89$, $p = 0.001$). *Post-hoc* comparison showed differences for session 1.

3.4.2.4.3.4. Working memory analysis in APP_{SL} animals in B1

Escape latency: The mean escape latency was higher in the first trails (General mean: $F_{(1,38)} = 6.57$, $p = 0.014$). Animals decreased escape latency in the course of sessions (Session:

$F_{(4,152)} = 71.82$, $p < 0.0001$). Higher duration was assessed for the first trial in the first session by *post-hoc* analysis.

Distance travelled: Animals travelled much longer in the first trials of the test (General mean: $F_{(1,38)} = 77.68$, $p < 0.0001$). Swimming path was reduced over sessions (Session: $F_{(4,152)} = 91.16$, $p < 0.0001$). *Post-hoc* analysis revealed longer distances in the first trial for the first and the second session of the test.

Swimming speed: The mean swimming speed was higher in the first trials (General mean: $F_{(1,38)} = 79.64$, $p < 0.0001$). Swimming speed changed over sessions (Sessions: $F_{(4,152)} = 9.33$, $p < 0.0001$). Differences between the first and the fifth trial were found in session 1,2,4 and 5 by *post-hoc* analysis.

3.4.2.4.3.5. Working memory analysis in APP_{SL} animals in B2sham

Escape latency: On average, there was no difference between trial 1 and trial 5, respectively (General mean: $F_{(1,16)} = 2.30$, $p = 0.149$). Duration of the task was reduced over sessions in both trials (Session: $F_{(4,64)} = 25.86$, $p < 0.0001$). *Post-hoc* analysis revealed higher duration in trial 1 for the first session day.

Distance travelled: Animals travelled longer in the first trails (General mean: $F_{(1,16)} = 29.89$, $p < 0.0001$). Distance was reduced over sessions in both trials, respectively (Session: $F_{(4,64)} = 36.33$, $p < 0.0001$). Animals swum the longest path in the first trial on the first session day.

Swimming speed: The mean swimming speed was higher in the first trials (General mean: $F_{(1,16)} = 19.14$, $p = 0.0005$). The speed changed in the course of sessions (Session: $F_{(4,64)} = 3.88$, $p = 0.007$). *Post-hoc* analysis has shown that speed was higher in the first trials on session 1,2, 3 and 4.

3.4.2.4.3.6. Working memory analysis in APP_{SL} animals in B2les

Escape latency: The mean escape latency was higher in the first trials, compared to the fifth trials (General mean: $F_{(1,4)} = 25.40$, $p = 0.0073$). Trial duration decreased over sessions (Session: $F_{(4,16)} = 3.22$, $p = 0.041$). Animals travelled longer in the first trial than in the fifth trial of session 2.

Distance travelled: The mean distance travelled was higher in the first trials (General mean: $F_{(1,4)} = 42.16$, $p = 0.003$). Animals reduced the distance travelled in the course of sessions

(Session: $F_{(4,16)} = 16.0$, $p < 0.0001$). Differences were found in the first and the second session between trial 1 and trial 5 by *post-hoc* analysis.

Swimming speed: The mean swimming speed was similar between trials (General mean: $F_{(1,4)} = 2.12$, $p = 0.219$). Swimming speed was inconstant in the course of sessions, with a tendency for reduction (Session: $F_{(4,16)} = 3.01$, $p = 0.0497$). Differences between trials were found for the first session.

3.4.2.4.3.7. Working memory analysis in APP_{SL} x PS1wt animals in B1

Escape latency: Duration was higher in the first trials, compared to the fifth trials (General mean: $F_{(1,42)} = 14.54$, $p = 0.0004$). Animals reduced trial duration in the course of sessions (Session: $F_{(4,168)} = 48.86$, $p < 0.0001$). Differences between trial 1 and trial 5 were found for the first two sessions.

Distance travelled: Animals travelled a longer distance to until the platform was found in the first trials (General mean: $F_{(1,42)} = 37.99$, $p < 0.0001$). The distance was reduced over sessions (Session: $F_{(4,168)} = 47.45$, $p < 0.0001$). *Post-hoc* comparison revealed differences between trials in session 1 and 2.

Swimming speed: The mean swimming speed was higher in the first compared to the fifth trial (General mean: $F_{(1,42)} = 12.36$, $p = 0.001$). Animals varied the swimming speed over sessions (Session: $F_{(4,168)} = 3.13$, $p = 0.016$). *Post-hoc* assessment showed a difference between trials for the first session.

3.4.2.4.3.8. Working memory analysis in APP_{SL} x PS1wt animals in B2sham

Escape latency: The mean duration was higher in the first trials (General mean: $F_{(1,18)} = 5.24$, $p = 0.034$). Escape latency was reduced in both trials over sessions (Session: $F_{(4,72)} = 19.44$, $p < 0.0001$). Animals travelled longer in the first trial on session 1, as it was indicated by *Post-hoc* comparison.

Distance travelled: Animals constantly travelled longer distances in the first compared to the fifth trial (General mean: $F_{(1,18)} = 17.33$, $p = 0.006$). They reduced the distance in the course of sessions (Session: $F_{(4,72)} = 3.13$, $p < 0.0001$). *Post-hoc* comparison showed longer distance in the first trial for the first session.

Swimming speed: Animals swum faster in the first trial than in the fifth one (General mean: $F_{(1,18)} = 14.70$, $p = 0.012$). Swimming speed was slightly reduced over sessions (Sessions:

$F_{(4,72)} = 2.74$, $p = 0.035$). *Post-hoc* analysis found differences between trials in session 1,2,3 and 5.

3.4.2.4.3.9. Working memory analysis in APP_{SL} x PS1wt animals in B2les

Escape latency: Averaged over sessions, the duration of trial 1 and 5 were similar, i.e. no improvement occurred over trials (General mean: $F_{(1,22)} = 0.90$, $p = 0.353$). Escape latency was reduced in the course of sessions (Session: $F_{(4,88)} = 13.84$, $p < 0.0001$).

Distance travelled: The mean distance travelled was higher in the first trials (General mean: $F_{(1,22)} = 10.08$, $p = 0.004$). Over sessions, the distance was reduced in both trials (Session: $F_{(4,88)} = 22.52$, $p < 0.0001$). Differences between trials were found in session 1 by *post-hoc* analysis.

Swimming speed: Swimming speed was constantly higher in the first trials (General mean: $F_{(1,22)} = 8.23$, $p = 0.009$). Speed changed in the course of sessions (Session: $F_{(4,88)} = 5.62$, $p = 0.004$). Differences between trials were found in the first and the last session by *post-hoc* comparison.

3.4.2.4.3.10. Working memory analysis in APP_{SL} x PS1mut animals in B1

Escape latency: On average, escape latency was similar in the first and fifth trial (General mean: $F_{(1,50)} = 2.72$, $p = 0.106$). Trial duration was reduced over sessions (Session: $F_{(4,200)} = 71.53$, $p < 0.0001$).

Distance travelled: The mean distance travelled was constantly higher in the first trials of the test (General mean: $F_{(1,50)} = 27.05$, $p < 0.0001$). The distance was diminished over sessions (Session: $F_{(4,200)} = 86.62$, $p < 0.0001$). *Post-hoc* comparison showed differences for the first and the second session day.

Swimming speed: On average, animals swum constantly faster in the first trials, respectively (General mean: $F_{(1,50)} = 19.57$, $p < 0.0001$). Speed changed in the course of sessions (Session: $F_{(4,200)} = 7.02$, $p < 0.0001$). *Post-hoc* analysis revealed differences between trials in session 1,2,4 and 5.

3.4.2.4.3.11. Working memory analysis in APP_{SL} x PS1mut animals in B2sham

Escape latency: The mean time to escape from the water was similar in trial 1 and trial 5, respectively (General mean: $F_{(1,22)} = 3.27$, $p = 0.084$). It was reduced in the course of sessions (Session: $F_{(4,88)} = 12.01$, $p < 0.0001$). *Post-hoc* comparison revealed a difference between trials for the first session.

Distance travelled: On average, a longer swimming path in the respective first trial was assessed (General mean: $F_{(1,22)} = 8.42$, $p = 0.008$). Distance was reduced over sessions (Session: $F_{(4,88)} = 14.35$, $p < 0.0001$). Differences between trials were found in the first session.

Swimming speed: Swimming speed was constantly higher in the first trials of the test (General mean: $F_{(1,22)} = 6.77$, $p = 0.016$). The speed remained similar in the course of sessions (Session: $F_{(4,88)} = 14.35$, $p < 0.0001$).

3.4.2.4.3.12. Working memory analysis in APP_{SL} x PS1mut animals in B2les

Escape latency: The mean time to escape was constantly higher in the first trials (General mean: $F_{(1,12)} = 5.44$, $p = 0.038$). Animals reduced trial durations in the course of sessions (Session: $F_{(4,48)} = 3.49$, $p = 0.014$). *Post-hoc* analysis has shown differences between trials for the first and second session.

Distance travelled: The mean distance travelled was longer in the first trials (General mean: $F_{(1,12)} = 18.77$, $p = 0.001$). Distance was diminished over sessions (Session: $F_{(4,48)} = 24.50$, $p < 0.0001$). Longer swimming path was found in the first trial on session 1 by *post-hoc* comparison.

Swimming speed: The mean swimming time was similar between trials (General mean: $F_{(1,12)} = 4.44$, $p = 0.057$). In the course of session, swimming speed was reduced (Session: $F_{(4,48)} = 24.24$, $p < 0.0001$).

3.5. Discussion

3.5.1. Experiment 1: The T-Maze continous alternation task

The genotype analysis has shown that animals from all genotypes were able to acquire the task demands and alternated significantly over chance level before surgery. Sham operation did not influence the cognitive ability to alternate, i.e. all animals switched between goal arms significantly over chance level. However, *post hoc* analysis showed a higher alternation in the C57BL/6 control group compared to the other genotypes. There was also a difference between the APP_{SL} group and the APP_{SL} x $PS1_{mut}$ group, indicating a worse performance in the double mutant group and thus, a possible influence of the enhanced Aβ procession. All animals from the NBM lesioned groups alternated close to chance level. The parameters "mean time to choice" and "mean session duration" provide information about sensorimotor abilities and motivation to move. Vast time intervals between trials may affect WM, i.e. the memory of the former arm visit and the interest for a new arm. These parameters can bias alternation performance and thus, have to be considered for the complete analysis. In the present study, the time to choice was similar for all genotypes. There was only one difference between the sham operated APP_{SL} x $PS1_{wt}$ and APP_{SL} x $PS1_{mut}$ group, indicating a hesitation in the APP_{SL} x $PS1_{mut}$ group to initiate exploration of the maze. Mean session duration, however, was similar in all groups. In conclusion, the alternation differences seen in the sham and NBM lesioned genotypes were not influenced by the locomotive behaviour of the animals.

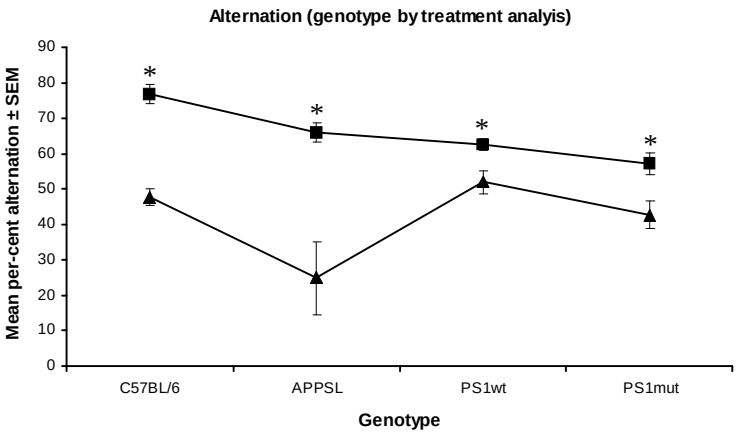


Figure 44: Direct comparison of genotype and treatment effect between sham operated (squares) and lesioned (triangle) animals in the T-CAT. C57BL/6 animals alternated higher than all transgenic mouse groups. There was also a difference between APP_{SL} and APP_{SL} x $PS1_{mut}$ animals. However, a treatment effect (sham vs les.) was observable in all genotypes (* $p < 0.05$).

The analysis of the *surgery effect* constantly showed a significant difference between the NBM lesioned and the sham operated groups, for any genotype. For the groups C57BL/6, APP_{SL} and APP_{SL} x $PS1_{mut,}$ differences were also found between the NBM lesioned and the untreated (B1) group. These results clearly indicate a treatment effect. The cholinergic deficit in the NBM lesioned groups might detract WM, which is necessary for

alternation. However, "time to choice" was also affected by the lesion for C57BL/6, APP_{SL} and APP_{SL} x $PS1_{mut,}$ mice. Only APP_{SL} x $PS1_{wt}$ animals showed no significant difference between surgery groups. All genotypes showed differences for the "mean session duration" between NBM lesioned groups and sham operated and untreated groups. These findings show an influence of NBM lesions on locomotor behaviour. It still has to be evaluated, whether the lesion was extended to further brain areas implicated in locomotion or motivation, or whether the mu p75 SAP induced NBM lesion itself can affect locomotive behaviour.

Taken together, these results show a strong influence of cholinergic ablation and together with an effect of Aβ processing as it is depicted in figure 44. These findings are in line with findings of confirmed Salomone and colleges (1984), showing attenuated performance in the T-maze with NBM lesioned mice. Similar effects were also found for double transgenic mice (Liu et al., 2002; Poulivali et al., 2002). However, Liu and colleges (2002) showed that ffx lesions failed to affect acquisition of the task.

Treatment with E2020 (Figure 25) had an enhancing effect on alternation in all NBM lesioned animals when compared to the NBM lesioned group without E2020 treatment. The AChE inhibitor enhances bioavailability of ACh, thus it may compensate the cholinergic deficit induced by the NBM lesion. The effect on locomotion is diverse, i.e. the effect on alternation may not have been influenced by locomotion.

3.5.2. Experiment 2: The object recognition task

Genotype analysis revealed significantly higher discrimination (d1) with an ITI of 1 min for untreated C57BL/6 control mice compared to the other untreated genotypes. In the sham operated genotype comparison, an effect was found for d2 between the APP_{SL} and the APP_{SL} x $PS1_{mut}$ group with an ITI of 6h that could rather be neglected. Taken together, no differences were found between genotypes. In contrast, NBM lesions had a strong influence on

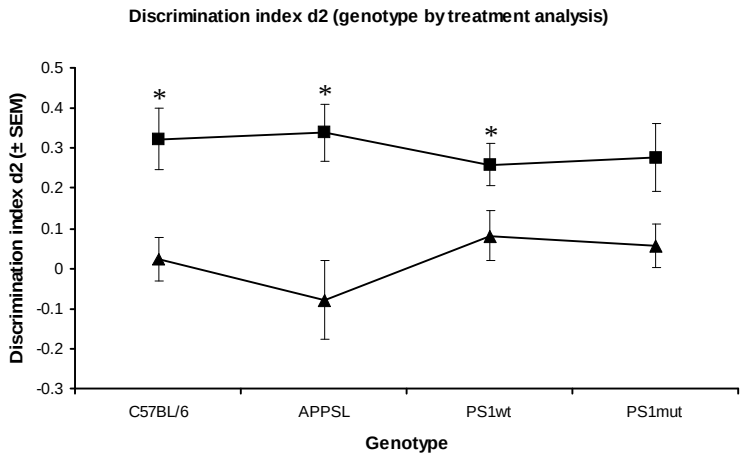


Figure 45: Direct comparison of genotype and treatment effect between sham operated (squares) and lesioned (triangle) animals in the ORT for an ITI of 1h. No differences between genotypes were observed, whereas NBM lesions attenuated discrimination in almost all genotypes (* $p < 0.05$).

discrimination. Discrimination index d2 was lower for all lesioned animal with an averaged discrimination level of 0.1 - 0.15 for the first three ITI, compared to the sham operated animals with an index level about 0.3. NBM lesioned animals had difficulties to hit the task demands.

The *effect of surgery* relates to a decreased discrimination in NBM lesioned animals. This effect was more pronounced for discrimination index d1 than for d2. There is a discussion about choosing d1 in favour of d2 if animals show low explorative activity (Sik et al., 2003). All lesioned animals displayed reduced exploration (data not shown), thus d1 could be a suiting indicator for this application.

In conclusion, analysis of d1 and d2 demonstrated the lowest cognitive performance in most lesioned groups. Genotype by treatment analysis (Figure 45) reveals high impact by NBM lesions, whereas APP pathology had little influence on discrimination.

3.5.3. Experiment 3: The modified Barnes Maze task

Observations revealed that the parameters “errors rate” and “distance” were interdependent. Most mice chose either spatial or serial learning strategies. Even if mice navigated in close proximity to the escape, serial inspection strategy was adapted to efficiently search for the goal, i.e. they explored the maze by means of walking from hole to hole. The number of line crossings in relation to errors (n incorrect hole inspections) is therefore correlated. In general, performance of all mice differed more between genotypes in the first block with an ITI of 1 min compared to the second block with an ITI of 15 min. There are two hypotheses to explain this phenomenon: first, animals were naïve to the test in the first block and the groups differed in the degree of learning. Once the animals acquired the task demands, learning performance reached a more stable state with less errors and variance between animals within a group. Second, ITI of 1 min was observed to be quite stressful to the animals because the instant re-subjection of the mouse into the starting box right after escaping may alter the impression of shelter. Mice tended to extend exploration in the second trial after ITIs of 1 min (data not shown).

Genotype analysis showed that the APP_{SL} x $PS1_{mut}$ group performed, on average, at lowest level for all parameters. This was the case in unlesioned (B1) and sham (B2sham) animals. The performance was also at lowest level in the NBM lesioned group (B2les) for the APP_{SL} x $PS1_{mut}$ group but also in the APP_{SL} group. The NBM lesioned APP_{SL} group consisted

of three animals, which may have taken influence on results. The C57BL/6 control group, however, ranged at highest learning performance level.

The *effect of surgery*: The mBM task requires learning of one aim within several sessions, i.e. sessions are not independent from another as it is the case in T-CAT and ORT. Improvement of learning success depends on experience and knowledge about the demands of the maze. All mice were subjected to the mBM task consisting of 10 daily sessions, for two different time points (B1 and B2). Re-subjection to the mBM, as it was the case in B2sham and B2les animals, revealed that mice remembered the procedure. This was especially supported by lower error rates, distance and duration times in all B2sham groups compared to the naïve groups in B1. The effect was less pronounced in C57BL/6 B2les animals, if compared to B1, which argues for a mild lesion effect in the NBM in this animal group. Transgenic B2les animals, however, performed similar to the B2sham group, indicating a lack of impairment due to NBM lesion. Exploration speed can provide information about the locomotor behaviour of the animals, which may affect measurement of cognitive behaviour. In the present study, no clear result for the parameter *speed* was found.

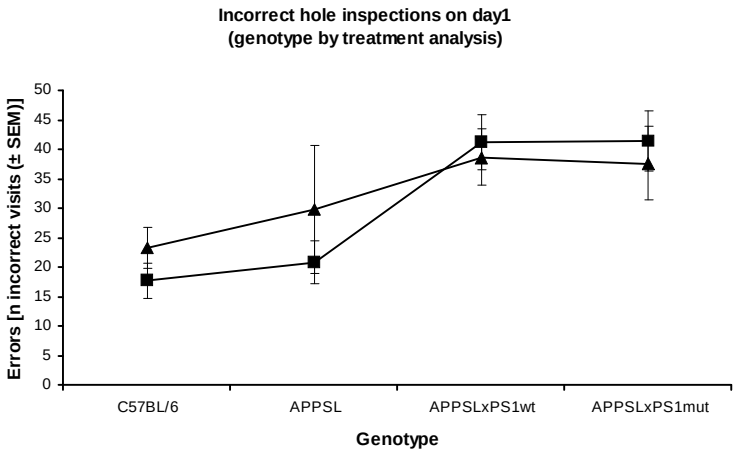


Figure 46: Direct comparison of genotype and treatment effect between sham operated (squares) and lesioned (triangle) animals in the mBM with an ITI of 1min. No differences between sham and lesioned animals were found. However, a strong impairment in the transgenic mouse groups was shown (* $p < 0.05$).

In conclusion, we found that APP_{SL} x $PS1_{mut}$ and APP_{SL} x $PS1_{wt}$ performed worse than the other genotypes, which was the case in untreated, sham and NBM lesioned animals. The deficit induced by increased Aβ processing in the double mutant mice appears to be sufficient for identification of cognitive differences between genotypes in the mBM task. Interestingly, some studies (King et al., 1999; Pompl et al., 1999) reported impairment of session duration, but no increase of errors in APP_{SW} animals. Huitron-Resendiz and colleges (2002) additionally revealed error enhancement in PDAPP mice. In contrast, NBM lesions failed to induce deficits in transgenic mice, as it is indicated in the genotype by treatment analysis

(Figure 46). Similar findings were reported by Steckler and colleges (1993), showing that NBM lesions induced by Ibo and QA had no impact on BM performance.

3.5.4. Experiment 4: The Morris Water Maze task

Genotype analysis showed that the untreated (B1) APP_{SL} group displayed highest escape latencies and distance travelled. This was also the case in B2sham or B2les animals. C57BL/6 animals constantly needed lower escape latencies at all treatments.

Similar to the mBM task, the MWM task also requires experience, i.e. knowledge about the existence and the location of an escape, which is acquired in daily sessions.

The *effect of surgery* analysis assessed for all genotypes that the untreated animals (B1) swum the highest distance, at highest speed and searched in the widest radius for the platform. These findings indicate that animals from both, the B2sham and the B2les groups recalled former experience made in the MWM task from testing in B1, providing evidence that RM was preserved in both groups. The B2les groups performed more similar to respective B2sham animals than to B1. Interestingly, only escape latency was highest in some sessions for B2les animals, which rather resulted from difficulties to climb onto the platform than from mnemonic impairments, facing that the MWM demands high motoric effort. This view is supported by the fact that all remaining parameters were intact in the lesioned group. Treatment versus genotype analysis clearly showed that the MWM task revealed a genotype rather than treatment effect (see figure 47).

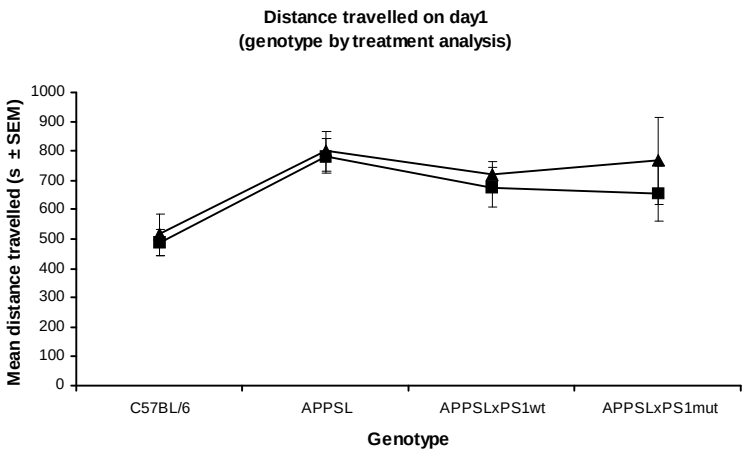


Figure 47: Direct comparison of genotype and treatment effect between sham operated (squares) and lesioned (triangle) animals in the MWM on the first session day. No differences between sham and lesioned animals were found. However, differences between C57BL/6 control and the transgenic mouse groups were identified.

Working memory analysis revealed most differences for the parameters *latency* and *distance* between trial 1 and 5 for the first and the second session, respectively. This effect was mainly observed in B1, where animals first experienced the MWM task. These findings indicate a strong learning process with first experiences in the maze and corroborate that learning of the

MWM task is highly motivated by the potentially life threatening situation of being inserted into the water, as it has been outlined in chapter II.

WM analysis, however, revealed sessions that lack improvements between trial 1 and trial 5, which may be interpreted in two ways: First, consolidation of the information presented within a session probably occurred beyond the time frame of one session. Animals were not able to integrate the acquired information to significantly improve performance within the session, indicating low development or use of WM. This phenomenon would have been expected for mice with high Aß burden, NBM lesions or in mice with combined pathology. Comparison of B2 animals has shown that all genotypes with sham operation performed similar. However, there was a lack of significant improvement in the C57BL/6 group in lesioned animals for both parameters, indicating that NMB lesions but not Aß pathology might influence WM in the MWM. The second interpretation considers that highest learning rate was registered for the first and the second session. The magnitude of improvement and thus the delta between trial 1 and trial 5 decreased over sessions. Improvement of learning performance consequently depended on the extent of learning during the first session, but not on WM *per se*.

Interestingly, all animals in B1 swam almost at constant speed or they enhanced speed over sessions for respective trial 1 or trial 5. Moreover, speed accelerated in respective trial 5 in B1, whereas speed was reduced for trial 5 in B2sham and B2les animal groups. Stress or panic reaction may account for high swimming speed, as animals swum double as fast in B1, where animals first contacted water and the MWM task, compared to the speed in B2 (sham and lesioned), where animals profited from former experiences. In conclusion to this hypothesis, stress was constantly high during the first testing in B1, which was reduced in the second testing in B2 (sham and lesioned).

3.5.5. General discussion

The present study revealed several important findings. A lack of gender differences was confirmed for each learning test applied. This finding may be of importance considering the time and costs consuming procedure of breeding to generate transgenic animals.

A novel immunotoxin was applied into discrete brain areas to selectively lesion cholinergic NBM neurons, representing a new and refined lesioning technique. We found dose-dependent decrease of AChE in cortical tissues of lesioned animals, whereas hippocampal AChE activity remained unaffected. Lesions in the longitudinal study were conducted with a depletion effect

of 50 % and more. Although AChE activity reduction ranging from 40-71 % with Ibo (Altman et al., 1985) or 44-58 % with QA (Boegman et al., 1985; El-Defrawy et al., 1985) into the NBM were reported with similar results, histological analysis revealed gliosis and magnocellular loss in ventral globus pallidus, stria medularis and lateral and preoptical areas of hypothalamus with excitotoxins (Wenk et al., 1984). These collateral damages are strongly reduced in lesions with mu p75 SAP (Berger-Sweeney, 2001). Immunotoxic lesions are virtually selective for cholinergic neurons, however, restriction to the NBM is not granted facing close proximity of other NGFR carrying cholinergic neurons in the BFCS, such as the MS (Ch1) and vdb (Ch2) (Book et al., 1996). Slight damage to cholinergic striatal interneurons (Heckers et al., 1994), or mild AChE activity decrease in hippocampal cells (Berger-Sweeney, 1994) was reported in NBM lesioned animals. Intraventricular administration of mu p75 SAP taxing all cholinergic cells carrying NGFR(p75), including Ch1/Ch2 and non-cholinergic cerebellar Purkinje-cells (Waite and Thal, 1996), resulted in extended lesions and spatial impairments (Berger-Sweeney et al., 2001). These lesions were shown to disturb both, cued and spatial versions of the MWM, and memory decline was correlated with decreased hippocampal ChAT activity (Berger-Sweeney et al., 2001). Interestingly, projections (Ch5 and Ch6) to the thalamus and (from Ch4) to the amygdala are spared following infusion of the toxin, because these cholinergic neurons lack NGFR (Holley et al., 1994). So far, specific NBM lesions by means of an immunotoxin were restricted to rats, or to global NGFR containing neurons in the mouse, the present study enlarges investigational opportunities to study selectively cholinergic neurodegeneration in the mouse model. Advantages of the present lesion technique are further confirmed by the cholinergic specificity and the restriction to NBM cells as it was shown with lack of hippocampal AChE decline and intact spatial memory in mBM or MWM tasks.

Behavioural analysis of the present study revealed memory deficits in lesioned mice for the T-CAT and the ORT, which was preserved in transgenic mice performing the ORT. These tasks proved to be sensitive to NBM lesion effects. In contrast, transgenic mice were impaired in the mBM and MWM, while NBM lesioned animals performed equal to respective sham lesioned groups. These tasks were genotype dependent. Performance of the T-CAT, however, seemed to be affected by both, Aβ pathology and NBM lesions. To interpret these findings, respective brain areas and pathways have to be analysed in correlation with the pathologies induced.

As it was outlined in chapter II, T-CAT and ORT require integration of the hippocampal system but also include processes in other brain regions. In the present investigation of these

tests, pathological effects are stressed on the NBM cholinergic hypofunction and may be related to a deafferation of the cortex resulting in a disconnection of frontal, temporal and parietal cortices (Berger-Sweeney, 1994; Smith, 1988).

Behavioural investigations in NBM lesioned animals revealed impaired WM in a T-DAT (Wenk et al., 1996), T-CAT (Beninger et al., 1986; Murray and Fibiger, 1986), WM adaptation of a spatial navigation task (Moran et al., 1992), in the RAM (Beninger et al., 1986; Wozniak et al., 1989) with excitotoxins. WM was also attenuated in a delayed-matching-to-sample (Baxter et al., 1996) or –position (Robinson et al., 1996) and WM version of the MWM (Waite et al., 1995) with an immunotoxin (but see Berger-Sweeney et al., 1994; Murray and Fibiger, 1985). Effects on spatial RM is ambiguously reported, ranging from no effects in the RAM (Wrenn et al., 1999), MWW (Waite and Thal, 1995) with excitotoxins, to pronounced deficits in the RAM (Murray and Fibiger, 1985), MWM (Nieto-Escamez et al., 2002; Waite et al., 1994) with excitotoxins and MWM deficits with an immunotoxin (Waite and Thal, 1996). These contradictory findings may result from the use of rather unspecific neurotoxins, causing additional non-cholinergic damage. Waite and Thal (1996) found stronger impairments in a spatial MWM version with excitotoxins compared to 192 IgG-saporin lesions, in dosages where cholinergic markers were similarly reduced. Other investigators found a lack of effects in the MWM following immunotoxin lesions to either Ch1/Ch2 or Ch4 cells, however, i.c.v. infusions successfully impaired this test. It was suggested that both neocortical and hippocampal cholinergic levels needed to be substantially reduced to show spatial learning impairments (Baxter et al., 1996; Leanza et al., 1995).

In turn, genotype effects were observed in the spatial RM tasks, mBM and MWM, and in the spatial T-CAT. Deficits in spatial navigation as a consequence of Aβ pathology has been reported by various investigators. Impaired spatial WM (Janus, 2004) and RM (Chen et al., 2000; Janus, 2004; Koistinaho et al., 2001; Moran et al., 1995; Nalbantoglu et al., 1997; Sommer et al., 2000), in particular also spontaneous alternation deficits (Moran et al., 1995) were found in transgenic mice processing human APP. On reverse, preserved memory was shown in a cued version of the MWM task (Janus, 2004). Spatial navigation was also detracted in double mutant mice, carrying APP and PS1 pathology (Arendash et al., 2001; Gordon et al., 2001; Gureviciene et al., 2004; Holcomb et al., 1999; Liu et al., 2003). Liu and colleges found spatial navigation deficits in both, ffx-lesioned and double transgenic mice (Liu et al., 2002). Our results enlarge these findings, as we additionally observed effects between sham operated single mutant APP_{SL} and double mutant APP_{SL} x $PS1_{mut}$ mice on the T-CAT and mBM, i.e. between animals bearing Aβ pathology and animals developing higher

Aβ level and NP deposits. In this context, Holcomb and colleges (1998) observed strong enhancement of $A\beta_{42}$ level (41 %) in double transgenic compared to single transgenic mice. Our finding underscores the effect of the gradual neurodegenerative process by NPs on cognition in AD. It also shows that the tests chosen and adopted for the study were sensitive enough to reveal these differences.

Histopathological investigations related spatial navigation impairments to NP load in important areas of the MTL. Deposits were registered in singly transgenic mice older than 10M in the neocortex, cingulate region with strongest expression in the hippocampus (Higgins and Jacobsen, 2003; Irizzary et al., 1997; Van Dam et al., 2003), especially in the dentate gyrus (Redwine et al., 2003), the termination zone of the perforant path, and in the entorhinal and piriform cortices (McGowan et al., 1999). Deposits are associated with dystrophic neurites and gliosis. Double transgenic mice carry similar pattern of deposits, however, they occur earlier (6M) and they are more distinct (Higgins and Jacobsen, 2003; Holcomb et al., 1998). Liu et al. (2002) assessed highest Aβ burden in double transgenics in the subiculum, the termination zone of BFCS afferents via ffx, followed by dentate gyrus and entorhinal cortex.

3.5.6. Conclusion

The study has shown WM impairment in the ORT, T-CAT as a consequence of selective and restricted lesions to the NBM resulting in deafferation of neocortical projections. This is in particular confirmed by the recent literature. Moreover, deficits in the ORT only show a lesion effect, which was independent of genotype, i.e. a lack of Aβ or NP effects. Thus, intact ORT performance in transgenic mice may relate to a lower dependence on hippocampal function or to stronger compensatory effects in brain regions that are unaffected by Aβ pathology. T-CAT deficits with NBM lesions may result from impaired WM processes in neocortical areas, such as the mPFC. We anticipate that hippocampal function is spared with the lesion, as no AChE activity decrease was assessable in this area and allocentric navigation tasks requiring intact hippocampal processing remained unaffected with the lesion. This finding indicates first, restricted lesion of the NBM sparing Ch1/Ch2 cells within the BFCS and second, an effect due to neurodegeneration in the NBM neocortical pathways.

We assume that deficits in T-CAT, mBM and MWM relate to impaired hippocampal function facing the common spatial demand of the tasks and their vulnerability to hippocampal dysfunction (see chapter II). Brain areas within the MTL structure are particularly affected by

Aβ pathology. These findings give raise to the hypothesis, that first, genotype effects in the present study relate to histopathological Aβ effects in the hippocampal system and second, gradual memory decline relates to increasing Aβ level.

The present study is based on a within-subject design, comparing preoperative and postoperative performance, diminishes between-subject variability, allows each animal to be used as his own control, and minimises the influence of extraneous variables on choice accuracy. Emphasis is placed on large deficits present at the complete rehabilitation of the animal, rather than on transitory changes that may appear early in postoperative phase. In addition, results presented in the previous chapter show how motivational factors and the careful choice of testing set-up and protocol, but also animal strain, can influence the value of a study.

Taken together, the results obtained from the present study increase our understanding of learning behaviour in mice, and the impact of new mouse models that mimic AD like pathology on cognition. Nonetheless, a great deal of research remains to be done before the complex puzzle of memory loss in AD will be solved.

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