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Automated behavioral phenotyping of inbred mouse strains and mouse models of Alzheimer disease

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ABSTRACT

Behavioral characterization of various mouse strains created as models for human diseases such as Alzheimer disease requires robust phenotyping methods. Previous work on inbred mouse strains has shown that some of the widely used behavioral methods yield inconsistent results across laboratories, in spite of standardization efforts. One approach to minimize experimenter induced variability relies on development of automated methods.

The aims of this thesis were to evaluate an automated device - *the IntelliCage* - which enables behavioral testing of group-housed mice. In a multi-center study, inter-laboratory consistency of behavioral measurements in IntelliCage was evaluated [study I]. Three strains of mice: C57BL/6NCrl (B6), DBA/2NCrl (D2) and (C57BL/6 x DBA/2) F1/NCrl (C6D2F1) were tested simultaneously in four laboratories (n=78/lab). No statistically significant interaction effect of *Laboratory* x *Strain* was obtained, indicating that strains were consistently ranked across laboratories. Significant *Laboratory* effects were obtained for several *Activity* and *Learning* variables due to uncontrolled local factors. Phenotypically, the mouse strains were not discriminated during the initial exploratory phase. During the following adaptation phases the B6 mice made more visits to IntelliCage corners than the D2 mice. For unconditioned phases, the visit number for F1 mice was between that of the inbred strains. For conditioned phases F1 mice performed the smallest number of visits. B6 mice discriminated best following place learning and D2 were best at re-learning the task. F1 ranked last on both place learning and reversal measures of learning.

Using the same multi-center study design and the same mouse strains, we evaluated the effect of additional components (add-ons) availability on IntelliCage measures [study II]. In the enriched condition (IntelliMaze) access to additional space was made through the “SocialBox” and “AnimalGate” add-on devices. The unconditioned activity during adaptation dark phases was reduced in the presence of add-ons. During the place conditioning paradigms, the overall number of trials needed to reach the learning criterion, was lower in the presence of add-ons. The strain ranks for activity measures were consistent with the results of study I.

Dissociation in cognitive abilities of B6 and D2 mice has been proposed as a natural model to study hippocampal (dys)function. Behavioral predictive validity of animal models for Alzheimer disease is implied by impairments in hippocampal dependent tasks.

In study III, a double transgenic Amyloid precursor protein model of Alzheiemer disease, (the tg-ArcSwe) was tested longitudinally in the IntelliCage. Lower body weight was found throughout the adult life-span of the tg-APPArcSwe mice. Lower activity counts were seen at 4 month of age, but not at 14 months. A deficit in extinguishing place preference for a previously rewarded corner at 4 month was shown. At 14 months the tg-APPArcSwe mice were impaired in a passive avoidance test in the IntelliCage. During the training phase of the passive avoidance test the behavior (preference for the punished corner) of tg-ArcSwe was found to moderately and inversely correlate with the level of CALB immunoreactivity in the polymorphic layer of the DG.

Finally, the effects of IntelliCage exposure as well as relationships between variables obtained during IntelliCage testing and Elevated Plus Maze, Open field, Rotarod, Morris Water Maze and Fear conditioning were explored [study IV]. We found that only a limited amount of variance in the conventional tests could be accounted for by IntelliCage variables.

In conclusion we have shown that mouse strains can be discriminated using the IntelliCage. Similarly, the behavior of tg-ArcSwe and non-tg mice was dissociated by this metholodogy. Although some degree of correlation was found between the results of conventional studies and IntelliCage variables, only a small part of the variance in conventional studies was explained by variables obtained in the IntelliCage.

To my family

"*Home is not the one tame place in a world of adventure;*
it is the one wild place in a world of rules and set tasks."
G. K. Chesterton

LIST OF PUBLICATIONS

I. Consistent behavioral phenotype differences between inbred mouse strains in the IntelliCage

S. Krackow, E. Vannoni, **A. Codita**, A. H. Mohammed, F. Cirulli, I. Branchi, E. Alleva, A. Reichelt, A. Willuweit, V. Voikar, G. Colacicco, D. P.Wolfer, J. U. F. Buschmann, K. Safi, H.-P. Lipp

Genes Brain Behav. 2010; 9:722-31

II. Effects of spatial and cognitive enrichment on activity pattern and learning performance in three strains of mice in the IntelliMaze

A. Codita, A. H. Mohammed, A. Willuweit, A. Reichelt, E. Alleva, I. Branchi, F. Cirulli, G. Colacicco, V. Voikar, D.P. Wolfer, F.J.U. Buschmann, H-P Lipp, E. Vannoni, S. Krackow

Submitted manuscript

III. Impaired behavior of female tg-ArcSwe APP mice in the IntelliCage: A longitudinal study

A. Codita, A.Gumucio, L. Lannfelt, P. Gellerfors, B. Winblad, A. H. Mohammed, L.N.G. Nilsson

Behav Brain Res. 2010; 215:83-94

IV. Influence of IntelliCage testing on subsequent behavioral measures; Intra-test and inter-test relationships between automated measures of home cage behavior and behavioral measures from conventional tests

A. Codita, M. Mitrovic, A. Horvath, R. Rabl, B. Hutter–Paier, M. Windisch, A. H. Mohammed

Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer disease
APP	Amyloid-β precursor protein
Aβ	Amyloid-β
B6	C57BL/6
CALB	Calbindin-D28k
CNS	Central nervous system
D2	DBA/2
EE	Environmental enrichment
ELISA	Enzyme-linked immunosorbent assay
EPM	Elevated Plus Maze
FC	Fear Conditioning
IC	IntelliCage™
KO	Knock-out
MWM	Morris Water Maze
NFT	Neurofibrilary tangle
NP	Nosepoke
OF	Open Field
Tg	Transgenic
Wt	Wild type

1 INTRODUCTION

Genetic, epigenetic and environmental factors shape the biology and functions of the Central Nervous System (CNS). Behavior is the distilled expression of CNS function while altered behavior accompanies neuropsychiatric and neurodegenerative diseases. In the attempt to understand the mechanisms which lead to human brain disease and find potential cures, research takes advantage of reductionist model systems. Currently, the mouse is the most widely used mammal serving as a complex model organism for human physiology and disease. Given both the obvious and the less well understood differences between these two species, translating paradigms between them is not a trivial task. Furthermore, specific methodological obstacles are encountered when behavior is the phenotype of interest. These theoretical aspects are introduced below.
This thesis presents work performed to validate a novel approach for behavioral testing of mice, as part of a collaborative project, involving four European labs, as well as work to tests the applicability of that approach for phenotyping Alzheimer disease (AD) mouse models.

1.1 THE MOUSE LIFE: A BACKGROUND

According to current biological classifications, laboratory mice and their wild relatives belong to the order *Rodentia* (class *Mammalia*, subphylum *Vertebrata*, phylum *Chordata*, kingdom *Animalia*). The order comprises about 30 different families, in approximately 2000 species having in common a well developed gnawing apparatus. Rodents have rootless, continuously growing incisors (only one for each quadrant), a diastema (gap), followed by pre-molars or molars (in the mouse). The incisors are maintained at a physiological length by chewing. The skeleto-muscular apparatus supporting gnawing (e.g. Masseter muscle, skull bones) has characteristics which vary between rodent families. The order *Rodentia* clusters inter alia families of mice, voles, rats, hamsters, gerbils, beavers, lemmings, squirrels and gophers. Wild mice and rats belong to the *Myomorpha* suborder, *Muridae* family, currently the largest family – over 25%- of all living mammals (Havenaar et al., 2001). Four different subgenera, are categorized in the genus *Mus* (Veyrunes et al., 2006), of the *Murinae* subfamily in the *Muridae* family: Coelomys (Indomalayan areal, shrew mice), Mus sensu stricto (Eurasian, North and South American, Australian and North African areal; field mice and house mice), Nannomys (African pigmy mice) and Pyromys (Indomalayan areal,

spiny mice). The common house mouse belongs to the Mus musculus species (for a detailed description of subspecies and areal see (Tucker, 2007). Phylogenetic studies have shown that laboratory mice have a mixed genetic contribution from different Mus musculus subspecies: M. m. castaneus, M. m. domesticus, M. m. musculus, and a hybrid M. m. molossinus (Mott, 2007, Yang et al., 2007). Unless otherwise specified, the information provided below refers to the Mus musculus laboratory mice - *mus laboratorius*, as they are sometimes colloquially called (Guenet and Bonhomme, 2003).

In the wild, the longevity of a mouse depends on many factors, like availability of resources, predation, diseases, and accidents. In the protected laboratory environment the mouse life span reaches 1-2 (3) years (Havenaar et al., 2001). Under extreme genetic (e.g. Growth hormone receptor – knock-out mice (Bartke and Brown-Borg, 2004)) and environmental conditions (mice on caloric restriction diet (Dhahbi et al., 2004)), mice can survive up to 4 years. Adult female mice weigh between 25 to 40 g and males between 20 to 40 g (Havenaar et al., 2001). The estimated average food intake is 5-7 g/day (Ritskes-Hotinga and Chwalibog, 2003) and water intake 15ml/100g/day, for the adult mouse (Havenaar et al., 2001).

Mice are altricial animals and rely on maternal care until weaning, which occurs in the laboratory after approximately 3 weeks (21-28 days). Both in the wild and in the laboratory, nest sharing, cooperative breeding and communal nesting have been described (Sayler and Salmon, 1969, Branchi and Alleva, 2006, Singleton and Krebs, 2007). During this period of neonatal development, the pups communicate with the dam by ultrasonic vocalizations (Branchi et al., 2004, Crawley, 2004, Scattoni et al., 2009). Littermate interactions in the nest contribute to the development of behavior (Branchi et al., 2010), along with other types of social and individual experiences and the maturation of the CNS. Puberty is installed at approximately 5 weeks for female mice and can be accelerated by exposure to male urine (Vandenbergh effect). Sexual maturity (breeding age) occurs at approximately 8 weeks of age. The gestational period lasts on average 19 days (18-21 days). Females are polyestrous (several ovulations per breeding season), and become fertile post-partum (oestrus stage). Therefore pregnancy and lactation can be concomitant in mice. Social housing conditions influence the frequency and regularity of the oestrous cycle of female laboratory mice. Isolated females tend to have regular cycles, while females housed in unisex groups to become anoestrus (Lee-Boot effect). If a male mouse (or its urine) is introduced into a female colony, the oestrous cycle of females becomes synchronized within 72 hours (Whitten

effect). Chemical signals (pheromones) present in mouse urine, have an effect both on reproductive fitness and maturation as well as behavior. Fear and aggression can be directly influenced by pheromones, whereas indirect effects on behavior occur through altered hormone levels (Flanagan et al., 2011).

Following field and laboratory studies, several models of social hierarchy have been proposed for mouse colonies (Singleton and Krebs, 2007). Evidence suggests a strong territorial structure is established in laboratory colonies, characterized by low to medium population densities. Inter con-specific aggression is a common event with some laboratory mice (see below) and may induce site-specific measures. Currently there is no consensus on how individual housing of males or females affects the validity of research results. Field studies support the theory that during the breeding season mice are not highly territorial, but attached to site, and social dominance is established based on body size. The social model can change according to environmental factors, a process known as social plasticity (Singleton and Krebs, 2007).

Mice probably accompanied human communities since the Neolithic, when humans started to gather and store grain crops. The origins of their name suggest mice were not very popular but regarded as a pest (the Sanskrit word for mouse, mūṣaka, is based on the root mūṣ which means to steal or rob; Greek mys; Latin mus). In ancient mythology, mice were a symbol of desire, frailty, and hidden intentions. Along common history, they were a subject for human philosophical thinking, presented in the arts and finally a topic and a means in the scientific endeavor. Mice were domesticated, bred and commercialized as pets for unusual esthetical characteristics ("fancy mice"), first on the Asian Continent and later adopted by the Western culture. One such colony, kept by Miss Abbie Lathrop, was geographically close to the laboratory of William Ernest Castle – the "Pioneer Mammalian Geneticist" at Harvard Bussey Institution for Applied Biology.

Some of the characteristics that make mice interesting for biomedical research are their short inter-generational time, short gestational time and good breeding performance (6-12 pups per litter). Furthermore, mice proved to be robust to genetic manipulations, which were more easily performed on mouse biological material than on material from other mammalian species (O'Sullivan et al., 2006, Smits and Gould, 2009). The mouse contributed to seminal discoveries in research fields such as cancer research, genetics, immunology and the neurosciences.

The use of animals in research is subject to strict regulations. In the European Union (EU) the Directive for the Protection and Use of Vertebrate Animals used for

Experimental and other Scientific Purposes (86/60-9/EEC) set the standards for the use of mice in the laboratory (and other vertebrates). The Directive 86/609/EEC was replaced by the Directive 2010/63/EU on the protection of animals used for scientific purposes, which revised the species which fall under its incidence, developmental stages included, standards for animal welfare, record keeping and killing procedures, among other aspects. In Sweden, additional requirements are in place through the Animal Welfare Act (1988:534) and the Animal Welfare Ordinance (1988:539). Animal research is guided by the 3Rs principles - Replacement, Reduction, Refinement - formulated by Russell and Burch in 1959 (Russell, 1995, Burch, 2009). According to these principles, all effort should be made to: (1) promote the development of alternative models; (2) opt for alternatives if these are available; (3) ensure that animals are used towards defined legitimate goals; (4) by trained personnel; (5) who should reduce to an optimum the number of animals used in experiments; (6) pain and distress must be avoided. Furthermore, the facilities and procedures employed are subject to legitimate inspection and public accountability (de Greeve et al., 2001). The interaction between the research community and *Animal rights* organizations has pushed forward the development of alternative and improved methodology for animal experimentation. Relevant resources on this topic can be accessed online:

- http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm (European Commission resources on Laboratory animals)
- http://ecvam.jrc.ec.europa.eu/ (European Center for the Validation of Alternative Methods)
- http://awic.nal.usda.gov/nal_display/index.php?info_center=3&tax_level=1&tax_subject=185 (Animal welfare information center; USA Department of Agriculture)
- http://altweb.jhsph.edu/ (Johns Hopkinks Bloomberg School of Public Health)
- http://www.isogenic.info/index.html (M. Festing website)

1.2 GENETIC DIVERSITY OF LABORATORY MICE

From a genetic standpoint, mice are “close to humans” (Ji et al., 2002). The International Mouse Genome Sequencing Consortium (IMGSC) published a draft sequence for more than 96% of the mouse genome in 2002, less than 1 year after the draft of the human genome sequence was assembled. High quality, finished sequences have since been published (Brown and Hancock, 2006). Access to detailed information

from IMGSC work and comparisons between the mouse and human genomes are freely available online (Karolchik, 2003, Birney, 2004, Wheeler, 2004). The mouse nuclear DNA is organized in 2n=40 chromosomes and the human 2n=46. The total number of pair bases is about 15% higher for the human genome, whereas the predicted number of protein-coding genes is similar between the two species (Morse, 2007). As of April 2011, a total of 17,840 mouse genes were known to have orthologs in humans. Updated, high quality, high resolution data regarding gene homology between mouse and man can be accessed on the internet at:

- http://www.informatics.jax.org/reports/homologymap/ mouse_human.shtml
- http://www.ensembl.org/Multi/martview
- http:// www.ncbi.nlm.nih.gov/HomoloGene.

Commercial breeding farms provide many of the mice in biomedical research, due to space requirements and high maintenance costs. Depending on the breeding system mouse populations are: “outbred stocks”, “random bred” and “inbred strains”.

1.2.1 Outbred stocks and random bred colonies

Outbred stocks provided an estimated 30% of the mice used in published research in 2005 (Chia et al., 2005). They are bred so that the genetic relatedness between the individuals is kept to a low level, a standard which requires a high number of breeding pairs for each breeding step (Morse, 2007). Although limited by the initial number of breeding animals, the level of genetic variability between individuals in a stock remains higher than in the case of inbred strains (see below). Some researchers consider this genetic variability a better way to mimic variability in human populations. Others argue that a higher number of outbred animals are necessary to detect the same effect, which is against the 3Rs principles. Alternative experimental designs were proposed for experiments where outbred mice are traditionally preferred (Festing, 2010). Outbred mice weigh more, and might offer an advantage when good breeding performance is required.

The “random breeding” system uses a random assignment of the animals to the breeding pairs. With this system, mating between siblings can also occur, which increases the relatedness within a colony more rapidly than in the outbred stocks.

1.2.2 Inbred mouse strains: not all mice are equal

By definition, inbred strains are kept by brother and sister mating (bxs) for more than 20 generations; in some cases breeding between off-spring and the youngest parent is

used. For the 21st generation of bxs progenies, the percentage of initially heterozygous loci fixed in a homozygous state reaches 98.4 % (van Zutphen et al., 2001), and the mice are considered genetically uniform (isogenic). Substrains are created if breeding pairs from an existing inbred strain are maintained for 8-19 generations of bxs mating, and further maintained without intercrosses for a minimum of 12 more generations. Both evidence of genetic differences and transfer to another investigator are considered sufficient for the creation of a new substrain.

Genetic and phenotypic differences between inbred mouse strains in for example development (Wahlsten, 1975), anatomy (Fredens, 1981), physiology (Nguyen et al., 2000), susceptibility to infections (Lloyd et al., 2009a, Lloyd et al., 2009b), pathology (Higuchi et al., 1991) and behavior (Crawley et al., 1997) are notorious. Taking advantage of this knowledge increases the chance to detect phenotypic changes using a lower number of animals (3Rs: reduction) and recognize genetic modifiers (Holmes and Hariri, 2003, Yoshiki and Moriwaki, 2006, Doetschman, 2009) in genetically modified models. The Mouse Phenome Database (http://phenome.jax.org/) offers information about strain characteristics.

Following the Guidelines for Nomenclature of Mouse and Rat Strains, inbred mouse strains are named with short names (capitals) or roman numbers; and substrains by providing the name of the strain they originated from, followed by a slash and the abbreviated name for the laboratory or the researcher who maintains the substrain (http://www.informatics.jax.org/greenbook/chapters/chapter6.shtml).

1.2.2.1 C57BL/6 and DBA/2 inbred strains

Two of the oldest inbred families of strains used in research are the C57BL/6 (“the black mice” - B6) and DBA/2 (“the gray mice” – henceforward D2). Their ancestry can be traced back to the work of Miss Abbie Lathrop.

The B6/J strain was transferred at The Jackson Laboratory (JAX®) where it reached generation F226pF227 (02-JAN-10). Generally, B6/J mice are good breeders and have a relatively long life span. They are the most popular strain in research and B6/J DNA served as material for the Mouse Genome Project. The strain is suitable for genetic, cardiovascular and metabolic research being susceptible to diet-induced obesity, Type 2 diabetes, and atherosclerosis. B6/J manifests age-related hearing loss (onset at 10 months), has a high susceptibility to noise induced hearing loss and microphthalmia.

B6/J mice are preferred in neuroscience research due to "good cognitive abilities" (Crawley et al., 1997), explaining why backcrossing onto a B6/J background is almost common practice. B6/Js are resistant to audiogenic seizures and develop addiction to alcohol and morphine. Due to over-grooming they can experience hair loss. Breeding pairs from the B6/J colony were transferred to National Institutes of Health (NIH; B6/N) and further to Charles River Laboratories (Crl; B6/NCrl). Genetic (Bothe et al., 2004, Mekada et al., 2009, Zurita et al., 2010) and behavioral (Bryant et al., 2008, Matsuo et al., 2010) differences between mice acquired from these separate colonies (substrains) have also been described (Kiselycznyk and Holmes, 2011).

The DBA/2J (Dilute Brown Non-Agouti - D2/J) strain is maintained at JAX® where it reached generation F219pF223 (02-JAN-10). Phenotypically they exhibit many opposing characteristics to the B6/J mice: poor breeders, low-susceptibility to diet-induced atherosclerosis; high frequency hearing loss (onset at weaning); age related ocular degeneration (glaucoma); susceptibility to audiogenic seizures (young mice); intolerance to alcohol and morphine; low preference for sweet tasting substances. Breeding pairs were transferred to Mider, from Mider to NIH, from NIH to Charles River Laboratories (D2/NCrl).

1.2.3 Hybrid strains

F1 hybrids result from a cross between individuals of two different inbred strains. Genetically, they are identical (isogenic) and heterozygous at all loci where the parental strains differ. Phenotypically, they exhibit hybrid vigor (robust health, good breeding performance). The Nomenclature of Mouse and Rat Strains requires that the initials for the maternal strain is mentioned first followed by the name of the paternal strain and F1 particle.

As an alternative to outbred stocks, when genetic variability is desired, mosaic populations can be obtained by systematic F1 crosses between individuals from several different strains.

1.3 EXPERIMENTAL MOUSE MODELS FOR HUMAN DISEASES

Several principles guide academic and drug development research relying on animal models (Chadman et al., 2009, van der Staay et al., 2009, Nestler and Hyman, 2010):

a) Construct validity - refers to similarities between the cause of human disease and the cause of phenotype in the model;

b) Face validity - refers to resemblance between human symptoms and murine counterparts;

c) Predictive validity - implies that a drug effective in the animal model is likely to work in human patients.

Under this theoretical framework, based on translation of human characteristics to animal models, a good predictive validity is sought. Hypotheses formulated from findings of epidemiological and genetic studies in humans can be validated or rejected through this type of studies. However, the approach places species specific and typical characteristics as well as behaviors out of focus. If behavior is the phenotype of interest, a significant part of the behavioral repertoire of laboratory animals might fall outside research interest. From the forward genetics approach, where mice are first screened for peculiar phenotypes and then cellular, molecular and genetic correlates are sought, valuable complementary information is obtained (Feusner et al., 2009, Kurien et al., 2005). From an ethological perspective, species specific and typical behaviors are relevant due to their adaptive significance (Branchi and Ricceri, 2004, Deacon and Rawlins, 2005, Deacon, 2006a, Deacon, 2006b, Deacon, 2006c, Crawley, 2007, Deacon, 2009, Bailey and Crawley, 2009, Line et al., 2011). It has been proposed that an integrative approach would contribute to "…experimental protocol (standardization), … improve the quality of data and the welfare of the experimental animal" (Branchi and Ricceri, 2004).

1.3.1 Genetically engineered mice (GEM)

An ever growing methodological tool box enables ingenious manipulations of the mouse genome. Genetic models of relevance for the work presented in the thesis are introduced below.

1.3.1.1 Transgenic models

Transgenic animals (mice) bear foreign genetic material (DNA) integrated into their genome, in every cell. In order to ensure germ line transmission of foreign DNA (eggs or sperm) the insertion of genetic material is performed during early stages of embryonic development.

In the pronuclear microinjection method (Ittner and Gotz, 2007), after isolation of fertilized eggs, the foreign DNA is injected into the male pronucleus. The DNA integration site is random, and sometimes occurs after several cell division cycles. Often, the foreign fragment integrates as multiple tandem copies. Once germ line

transmission becomes established, following further crosses, transgenic mice are obtained.

Due to their unique property of pluripotency (give rise to all types of tissues), mouse embryonic stem cells (mESC) are also used to produce transgenic models. The foreign material can integrate randomly as above or, through homologous recombination it can be guided to integrate at a specific site, in a single copy. After successful integration of the construct (e.g. evaluated by resistance to neomycin), selected mES are transferred into a host embryo. In the resulting organism - *a chimera* – cells from both embryonic origins will be mixed (often the host embryo is chosen from a strain which has a different fur color than the donor mESC, so that chimeras can be easily spotted). If the engineered mESC contribute to the germ line, some offspring of the following cross will carry the DNA fragment of interest in all of their cells. Through homologous recombination *knock-out* models are also created (Wolfer et al., 2002). In these mice, the gene of interest is silenced through homologous recombination.

The foreign DNA fragment introduced (construct) can be engineered so that temporal and spatial control of the expression can be achieved. Tissue-specific promoters (e.g. calcium/calmodulin-dependent kinase II drives the expression of the transgene to the forebrain) or the Cre - recombinase model (Utomo et al., 1999) are used to control the cell population where the transgene is expressed. More recently, systems which enable inducible expression of the gene of interest have been devised (e.g. in the Tet-On/Tet-Off systems doxicycline is used to control the expression of the transgene (Urlinger et al., 2000). The combination and developments of these powerful techniques now allow a better tuned control of transgene expression.

1.3.1.2 Co-isogenic and congenic strains

A congenic inbred strain carries a piece of DNA from another strain or stock. The purpose is to transfer the DNA fragment of interest (donor) onto a homogenous genetic environment (host). Congenic strains carry flanking donor sequences (congenic interval). The initial donor x host cross (N1) is followed by successive backcrosses (Ni) of heterozygous off-springs with individuals from the host strain. After N_{10}; more than 99% of the offspring genome is expected to be similar to that of the recipient strain, with the exception of the transferred region of interest (gene). After N_5 the percentage of host genome becomes higher than 96% (incipient congenic). After the 10^{th} backcross (N_{10}), the gene of interest can be fixed to homozygousity by intercrossing the

heterozygous mice. If this heterozygous x heterozygous breeding takes place sooner, the flanking sequence will also become fixed (Morse, 2007).

1.3.2 Animal models of AD

Homologous models of AD should present relevant pathological characteristics as well as behavioral ones.

1.3.2.1 AD: a brief introduction

Sixty to eighty percent of dementia cases are caused by AD (Fratiglioni and Qiu, 2009), Globally, an estimated 35.6 million people were suffering from Alzheimer disease in 2010 (World Alzheimer Report 2010, Alzheimer's disease International). The number of persons affected will increase in the future, doubling every 20 years, thus turning Alzheimer into a major public health issue (Wimo et al., 2010a, Wimo et al., 2010b).

According to clinical criteria, individuals with AD present memory impairment accompanied by a second cognitive deficit (aphasia, agnosia, apraxia or executive function impairment), which affect the activities of daily living (Diagnostic and Statistical Manual-IV Text Revision, 2000).

A definitive diagnosis requires post-mortem neuropathological examination of brain tissue. Extracellular accumulations of β-amyloid (Aβ plaques), intraneuronal neurofibrillary tangles (NFT), synaptic and neuronal loss are the hallmarks of the disease. The main constituent of Aβ plaques is the β-amyloid peptide (Zhan et al., 1995, Perl, 2010), whereas twisted strands of hyperphosphorylated protein tau form the NFT (Goedert et al., 1988). Both amyloid plaques and NFT can be seen in cognitively normal elderly people (Price et al., 1991, Arriagada et al., 1992b).

As with other neurodegenerative diseases, the etiology of AD is not fully understood. Both genetic and environmental factors are thought to contribute to disease pathogenesis. Sporadic, late-onset AD (> 65 years old) accounts for the majority of AD cases (Fratiglioni et al., 1999). Widely accepted factors associated with a high risk to develop sporadic AD are increased age (the risk doubles every 5 years after 65) and the carrier status for the Apolipoprotein E (*APOE*) ε4 allele. In the general population the most common variant at the *APOE* locus is ε3. The product of the *APOE* gene is a protein involved in cholesterol transport. Apart from major risk factors many other conditions such as brain injury, stroke, mid-life hypertension and obesity increase the risk to develop AD (Kivipelto et al., 2008). Epidemiological studies evidenced protective factors, amongst which a strong association with a reduced risk to develop

AD was found for physical activity and cognitive training (http://www.ahrq.gov/clinic/tp/alzcogtp.htm).

Autosomal dominant (familial, inherited) forms of AD account for less than 1-5% of the total cases. Almost two decades ago mutations in the Amyloid precursor protein (*APP*), Presenilin 1 and 2 (*PSEN* 1 and *PSEN* 2) genes were isolated from AD affected families (Rogaev et al., 1995, Sherrington et al., 1995). The products of these genes are involved in processes linked with the production of β amyloid , the peptide found at the core of amyloid plaques. Thus, inadequate Aβ production or clearance was postulated as the central event in AD pathogenesis according to the *amyloid cascade hypothesis* (Hardy, 2006). Preventing Aβ accumulation is currently a major target for drug development (Grill and Cummings, 2010).

Recently, advances made by studies on cerebrospinal fluid and imaging biomarkers (Hampel et al., 2008, Aisen et al., 2010) have been incorporated into a comprehensive set of criteria aiming to diagnose early stages of the disease (MCI) which will provide a likely target for an effective treatment (Dubois et al., 2007)

To date, no effective cure or preventive treatment is available for AD. The symptomatic treatment available (cholinesterase inhibitors: donepezil, rivastigmine and galantamine; and memantine, an NMDA receptor antagonist) has a time-limited effect on cognition (Geldmacher, 2007). The results of phase III clinical trials of vaccination against Aβ could well provide a definite proof regarding *the amyloid cascade hypothesis* (Mangialasche et al., 2010).

1.3.2.2 Clinical presentation of AD

AD unfolds as a gradual cognitive decline which in time determines impairments in activities of daily living, social dysfunction and finally leads to the deconstruction of self. During the early stage of the disease (mild cognitive impairment - MCI), isolated episodic memory deficits can be ascertained by neuropsychological assessment (Terry et al., 2008, Terry et al., 2011). While age-related cognitive decline is known to occur (Caserta et al., 2009), the magnitude of the impairment is higher for people affected by AD.

The AD clinical presentation is not homogenous (Castellani et al., 2010). Early clinical manifestation of AD can be revealed by neuropsychological test batteries, where test of episodic memory and global cognitive function were found to have good predictive validity (Backman et al., 2005). In advanced stages of AD, psychiatric (e.g. hallucinations, delusions), affective (e.g. depression, anxiety) and behavioral symptoms

(e.g. disinhibition, aberrant locomotor activity) further aggravate the clinical presentation (Ballard et al., 2009).

NFT spread in the brain according to a pattern which follows a recognized anatomic circuitry (Braak and Braak, 1991) and correlates with symptoms of AD (Arriagada et al., 1992a, Jicha and Carr, 2010). Severe neuropathologic changes in the entorhinal cortex, hippocampus, the association cortices and subcortical structures (the nucleus basalis of Meynert) are believed to contribute to the cognitive impairment seen in AD (Gotz et al., 2009).

1.3.2.3 Natural animal models for AD

To date, there is no accepted complete, spontaneous animal model for AD. Nonetheless, amyloid plaques, not accompanied by NFT, were described in guinea pigs, rabbits, cats, dogs, goats, sheep, cows, pigs, polar bears and monkeys (Lemere et al., 2004, Maloney et al., 2004). Comparative studies have revealed high homology for *APP* across species but indicated different regulatory mechanisms for its expression (Maloney et al., 2004).

Recently published reports draw attention to similarities between brain pathology of old degus (Octodon degus) and human AD pathology (Inestrosa et al., 2005, van Groen et al., 2009). Degus are diurnal, highly social and communicative rodents (Wilson, 1982, Fuchs et al., 2010) with an average life-span of 5-7 years, from Chile. They are also one of the 9% bi-parental mammalian species (Helmeke et al., 2009). White matter changes, amyloid (first vascular and then pareanchymal) and tau pathology have been described in brain material from of old wild degus (>3 years). Similar pathology is also found in laboratory bred degus but the onset is delayed (van Groen et al. 2009). Limited information about behavioral age-related changes is available (Popovic et al., 2009).

Elderly dogs experience age related cognitive decline, a syndrome known as the Canine Cognitive Dysfunction Syndrome (CCDS). Behavioral impairments recognized in old dogs (Colle et al., 2000) can be classified as maintenance behaviors and environment-dependent behaviors (such as learned specific behavior, self-control, learned social behavior, and adaptive capabilities). CCDS was documented both for laboratory beagle dogs (Vasilevko and Head, 2009) as well as for elderly pet dogs (Rofina et al., 2006). Amyloid angiopathy was found to correlate with a number of dog maintenance behavioral measures (Colle et al., 2000, Sarasa and Pesini, 2009).

With increasing age, amyloid pathology accumulates in the brains of various non-human primate species: lemurs, squirrel monkeys, marmosets, tamarins, cynomolgus monkeys, vervets, rhesus monkeys, orangutans and chimpanzees (Rosen et al., 2011). In view of the close similarity between the human and the primate immune response, vervets are used for safety tests required for AD clinical trials (Lemere et al., 2004, Lemere et al., 2006).

1.3.2.4 Genetically engineered mouse models for AD

A plethora of genetic rodent models (both mice and rats) are currently used for AD research (reviewed in (Gotz and Ittner, 2008, Gotz et al., 2010, Philipson et al., 2010)). Updated resources on AD models can also be accessed online (http://www.alzforum.org/res/com/tra/default.asp and http://research.jax.org/repository/alzheimers.html).

The "first generation" of successful genetic AD mouse models expresses human mutated genes, found in familial AD cases (Games et al., 1995, Hsiao et al., 1995, Hsiao, 1998, Moechars et al., 1998). Following advances in understanding molecular events of AD pathogenesis, new models have since been created. Besides the single transgenic strains (*APP, PSEN*, Tau, *APOE*), the list now includes multiple combinatorial models (2-5xAPP mice (Rockenstein et al., 2001, Lord et al., 2006) ; *APP-PS* (Minkeviciene et al., 2004, Savonenko et al., 2005, Carro et al., 2006)), *APP*-Tau (Ribe et al., 2005, Perez et al., 2005), 3xTg expressing all three mutant genes (Oddo et al., 2003), reviewed in (Codita et al., 2006). APP knock-out mice offer the opportunity to address questions about the physiological role of APP and related proteins (Ring et al., 2007, Bergmans et al., 2010). Currently the field is moving towards humanized immune response (Colton et al., 2006, Zota et al., 2009), humanized APP and tau (Reaume et al., 1996, Andorfer et al., 2003), inducible, targeted expression of mutant APP and tau (Ando et al., 2010, Harris et al., 2010, Sydow and Mandelkow, 2010); with/without alterations of murine *APOE* (Fagan et al., 2002, Pendse et al., 2009).

Single or combinatorial *APP* transgenic mouse models are commonly used in drug development studies. Stable strains are now widely available and findings have been replicated by different laboratories.

1.4 MEASURING BEHAVIOR IN MOUSE MODELS

Elegant experimental paradigms for behavioral research on mice have been developed for many years (Crawley J.N., *What's wrong with my mouse*, Wiley-Lis, 1999). Some were initially introduced by experimental psychologists who tested rats in behavioral studies (Sahgal, 1993). While dry maze protocols developed for rats can be directly applied for mice, several water maze paradigms are not directly translatable, and require various adaptations (Whishaw and Tomie, 1996, Frick et al., 2000). The test repertoire is greatly enriched by input from the ethological perspective on behavioral studies, which addresses species specific and typical behaviors.

Generally, the paradigms used in behavioral research on mice seek to address targeted behavioral domains and imply transitory exposure to a novel environment or stimulus. Behavioral experiments also imply an interaction between the experimenter and the mice. This is a critical aspect known to impact the results of behavioral studies due to its stress inducing potential (Deacon, 2006d).

1.4.1 Behavioral tests: brief overview

1.4.1.1 General health and development

A good "general health" status of the mice is mandatory for obtaining valid results from (behavioral) experiments. Common signs of illness include: abdominal distension, abnormal behavior ("repetitive, unvarying, with no obvious goal or function"- stereotypic behaviors and lethargy) abnormal postures, dehydration, limb abnormalities, malocclusion, ocular, nasal genital discharge, poorly groomed hair coat and uterine/ penile or rectal prolapse. If these signs are present, the animals should be eliminated from further experimental procedures.

The measurement of body weight over time offers a rough means to monitor the wellbeing of the mice. It has been shown that chronic stress and infections determine a reduction of body weight or decreased rate of growth (Havenaar et al., 2001).

Similarly to humans, mouse development is characterized by the achievement of developmental milestones (Heyser, 2004). Developmental traits, such as emotionality at weaning can be a harbinger for certain adulthood behaviors (Marques et al., 2008).

1.4.1.2 Emotionality

Emotions might be defined as "states that evoke a pattern of cognitive, physiological and behavioral reactions to events" which are triggered by an internal or external

stimulus (Passer et al., 2009). Their expression requires appraisal, can be accompanied by physiological responses (e.g. change in heart rate, vasodilatation/vasoconstriction) and includes "behavior tendencies" (Passer et al., 2009). Genetically, emotionality is a complex trait, probably accounted for by multiple small effect genetic factors, gene-gene interactions and gene-environment interactions (Willis-Owen and Flint, 2007).

Comparative biology teaches that emotions promote the "fulfilment of basic needs: mating, affiliation, defense and the avoidance of predator" (Panksepp 1998, cited by (de Sousa). Research on emotion embraced two views. The *discrete emotional state* approach recognizes a number of basic emotional systems, anchored in the subcortical anatomy of the mammalian brain (limbic system): "maternal CARE, FEAR, LUST, separation distress PANIC/GRIEF, physical PLAY, RAGE and SEEKING" (Panksepp, 2010). On the other hand, the *multidimensional approach* seeks to integrate *core affective* characteristics of emotions, namely their valence (positive vs negative) and arousal value (Mendl et al., 2010). Subjective human emotions occupy different positions in this space, for example fear is characterized by high arousal and negative valence. In animals, the biological need to enhance fitness, expressed as reward seeking behavior (e.g. food, water, sex), and to reduce the occurrence/frequency of fitness-threatening events expressed as punishment avoidance behavior (e.g. predator encounter avoidance) are thought to determine emotional valence (Mendl et al., 2010). Recently, an integrative approach of these conflicting views has been proposed (Mendl et al., 2010). The new model accepts the valence/arousal characteristics of *basic emotions* as a "common currency" which enables behavior prioritization.

Emotionality is associated with the activity of the hypothalamo-pituitary-adrenal (stress) axis. Frequently, the interpretation of behavioral response is supported by determinations of stress hormones (Cirulli et al., 1994, Richter et al., 2008, Cirulli et al., 2010). Subcortico-cortical loops between the thalamus, amygdala and the prefrontal cortex are critically involved in emotional responses (LeDoux, 2000).

Tests of emotionality for laboratory mice generally imply that under a given circumstance a specific emotional state is triggered. Due to their increased prevalence and their societal impact anxiety, depression and fear are negative emotional states/moods/disorders for which many behavioral test paradigms were developed in the laboratory.

Anxiety is a natural state of tension or apprehension in response to a threat. In pathological anxiety disorders this state is prolonged and interferes with normal

activities (Passer M. et al, op cit. pp787). Artificial situations where a conflict between the natural tendency to explore (in order to acquire information about the environment and access to resources) competes with a tendency to remain protected in a "safe" area are employed when anxiety is modeled in mice. Behavioral tests commonly used in anxiety research include: the elevated plus maze (EPM), elevated O-maze, light/dark box and social interaction test (Pellow et al., 1985, Belzung and Le Pape, 1994, Rodgers et al., 2002, Walf and Frye, 2007). The EPM test builds on natural tendencies to avoid exposed, lit spaces as well as thigmotactic behavior of rodents. During 5 minutes, the mouse is left to explore a cross shaped maze, placed at a certain height above the floor. Validation with anxiolytic compounds and ethological measures has been performed (Walf and Frye, 2007).

Manifestations of depression in humans can vary. The list of symptoms includes appetite changes (loss or increase), anhedonia (loss of interest in pleasurable activities), difficulties to focus attention, to make decisions and to remember; fatigue; feelings of persistent hopelessness, inappropriate guilt, sadness, worthlessness; insomnia; irritability; psychosomatic pain (e.g. headaches, abdominal cramps which do not respond well to symptomatic treatment), suicidal thoughts and tendencies. Animal paradigms aimed at inducing comparatively similar biological and behavioral changes include restraint stress, uncontrollable electric shocks, forced swimming, forced running, and unpredictable exposure to different kinds of stressors (Willner et al., 1992, Willner, 2005, Castagne et al., 2011). Animal behavioral tests based on inescapable situations have good predictive validity in depression drug development research (Castagne et al., 2011). Another approach looks at hedonic valuation of stimuli (Schweizer et al., 2009, Branchi et al., 2010). Finally, social stress is modeled by isolation, over-crowding (Modigh, 1974), social instability (Branchi et al., 2010) and social defeat situations (Kudryavtseva et al., 1991).

1.4.1.3 Learning and memory

Information is acquired (learned), stored, retrieved and influences behavior (memory). During the past century, human and animal learning and memory processes have been amply characterized and a taxonomy emerged (Squire, 1992, Tulving and Markowitsch, 1998, Baddeley, 2010).

Learning and memory types considered relevant for the work presented in the thesis will be briefly introduced, following an existing classification (Reznikova, 2007).

Although its mechanisms are not fully elucidated, habituation is considered the simplest form of learning. It may be defined as a reduction in response following repeated presentation of a stimulus. Sensitization is "a paired" process defined by an increase in response if a stimulus is presented after another, salient stimulus. If stimuli are perceived as different, dishabituation occurs, expressed by a response of similar magnitude with the original response to the first stimulus.
A common approach for the study of habituation in the laboratory is the Open Field (OF) test. During this test, the animal (mouse) is exposed to a novel arena and the response (walking in the arena) is monitored over a period of time. The expected response is a decrease in movement over time. Taking into account the spatial aspects of movement in a novel OF arena (central vs periphery), indices of anxiety-like behavior can be extracted (Denenberg, 1969, Lamberty and Gower, 1993).

Associative learning theories see learning as a consequence of paired occurrence of events: stimulus - stimulus and stimulus - consequences (Wasserman and Miller, 1997). Classical conditioning (Pavlovian conditioning) maintains that the emergence of new behaviors occurs through associations between stimuli. The *unconditional stimulus* (US) triggers an automatic, reflex response (*unconditional response* - UCR), "wired" in the CNS. The observation of the same response, following the presentation of a substitute stimulus (*conditioned stimulus* - CS), which had been consistently presented paired with the *unconditional stimulus*, is indicative of learning. US stimuli can be positive (e.g. food) or negative (e.g. pain) and the CS is initially neutral (NS). The main factors influencing learning in classical conditioning paradigms are the intensity of US, the order and timing of US - CS stimuli.
Fear conditioning paradigms currently used in the laboratory for mouse testing offer the possibility to dissociate between fear for the CS ("hippocampal - independent") and fear for the spatial context ("hippocampal - dependent") where training had occurred (Fanselow, 2000, Fanselow, 2010).

Operant conditioning applies to situations where the response is not "innate". Developed by B.F. Skinner the paradigm postulates that a behavior's (response) chance to occur depends on its consequences (reinforcement). Behavioral tests for mice use the lever-press and nose-poke as a response which is manipulated by diverse reinforcement schedules (Haluk and Wickman, 2010).

Processes of learning and memory are closely related. Acquired information is thought to shape behavior through the workings of memory. Short - term memory (STM) holds a limited amount of minimally processed bits of information available for a short while (minutes). At a biological level STM is supported by transient changes at the synapse.
Working memory allows successful management of information during a particular task, "*online memory*". In humans working memory is regarded as a system where "central executive" functions manage information maintained by the "articulatory loop" and visuospatial "sketchpad" (Baddeley, 2010).
To assess working memory in mice, several test apparatuses are employed, such as the radial arm maze, T-maze and Y maze (Gresack and Frick, 2003). While some protocols require food deprivation, others, like spontaneous alternation in the Y and T-maze, are based on species typical behaviors (Deacon and Rawlins, 2006).
Long - term memory (LTM) stores for a long period of time (days-months-years) processed information. Transfer of a memory from STM to LTM (consolidation) requires protein synthesis. During the consolidation process, the information can be altered by subsequent experience.

Human memory can be further classified on the information processed. Declarative memory holds information accessible through recollection and verbal retrieval. Episodic declarative memories have an autobiographic content (egocentric episodes), whereas semantic declarative memories refer to factual knowledge. Implicit memory uses strategies which do not require conscious recollection. In this category procedural learning refers to the acquisition of skills. In the laboratory, acquisition of motor skills is often tested using the Rotarod test. Whereas during the first exposure balance is assessed, over time, as mice become better at running on a rotating rod, a learning curve emerges (Pallier et al., 2009).

Numerous studies have shown that human episodic memory relies on the function of medial temporal lobe structures (Milner, 2005). Direct translation of human episodic memory to animal models is hindered by the definition of episodic memory which links it to the concept of self (Griffiths et al., 1999). However, the memory for *what, when and where* is important for many species (Clayton and Dickinson, 1998). For mice, the Object recognition test is used to assess the ability to recognize *what, when and where* features of stimuli (DeVito and Eichenbaum, 2010). Generally the test is performed in two phases. Higher exploration of the novel object is interpreted as a sign of memory in

this test, as mice prefer to explore novel objects (Bevins and Besheer, 2006). Hippocampal lesions were shown to impair recognition of all these 3 aspects, in line with the hypothesis that the hippocampus integrates the *what, where and when* features of experiences (DeVito and Eichenbaum, 2010).

Space navigation refers to the ability to form and access representations of the outer space. The hippocampus is critically involved in forming the cognitive map (Burgess et al., 2002). In the laboratory spatial navigation abilities of the mice are tested in the Morris water maze task. According to the testing protocol, a mouse can use either spatial cues (allocentric) or nonspatial strategies (egocentric) to orient search to find a platform hidden under the water level (Vorhees and Williams, 2006).

1.4.2 Behavioral phenotyping

Characterization covering a wide array of behavioral domains is referred to as *behavioral phenotyping*. An initial test battery was described by Irwin in the 1960's. Later on, a comprehensive test battery, for the assessment of motor, sensory and cognitive functions – SHIRPA - was validated in adult mice (Rogers et al., 1997).

Major development of means to alter genetic material in mice and the establishment of large mutagenesis programs using N-ethyl-N-nitrosourea (ENU – an alkylating agent which determines the apparition of point mutations) created the need for effective, high-throughput phenotyping screens (Nolan et al., 2000, Brown et al., 2009).

For CNS research, the screening pipelines are partially devoted to characterization of behavior. Due to cost and space limitations, these screens generally incorporate standardized tests enabling rapid behavioral characterization. The behavioral tests selected by different initiatives seek to cover as much as possible of the behavioral repertoire and focus on aspects considered to be highly relevant. For example, the European Mouse Phenotyping Resource of Standardised Screens (EMPRESS) included in EUMODIC pipeline 2 (9-11 weeks old mice), the OF test, modified SHIRPA protocol (Rogers et al., 1997), grip strength, Rotarod, Acoustic startle (PPI- a model for schizophrenia) and hotplate test (a model for pain) (Brown et al., 2005, Mandillo et al., 2008, Gates et al., 2011). The same standardized tests have been selected for the EMPReSSslim primary screen. At the Japan Mouse Clinic "fundamental" aspects of behavior are captured in the "Fundamental" and "In depth" screen (Pipeline1) by testing the mice in the OF and Rotarod test. The second pipeline (8-17 weeks old mice) is entirely devoted to behavioral testing (includes: light/dark transition test, open field, Rotarod, Passive avoidance, Tail Suspension, Hot plate, Tail flick). Following an

intitial “hit”, subsequent tests might be performed (Japan clinic includes Prepulse Inhibition, Object recognition and Fear conditioning test) to provide a fine grained analysis of the deficits. In a seminal study Crabbe et al. 1999 showed that in spite of standardization efforts, different inbred mouse strains were ranked inconsistently between laboratories. Since then efforts for better standardization of protocols, automatization, systematic environmental variation and implementation of ethological approaches were undertaken.

1.4.3 Automated behavioral testing

Valid hypotheses, detailed ethograms, trained observers and systematic observation are the gold standard for behavioral experiments. Apparatuses which automatically record an important number of behavioral measures during classical behavioral tests are commonly employed. In addition, recently developed technologies make possible long term behavioral data collection (de Visser et al., 2005). Technically, home cage behavior can be detected by non-visual sensors (Ganea et al., 2007), video tracking techniques (de Visser et al., 2006) and computer-vision systems for behavior recognition (Jhuang et al., 2010). These approaches permit monitoring of singly housed mice.

The IntelliCage system is the only behavioral device for long term testing of group housed mice. The animals can be discriminated by Radio-frequency identification technology, if they enter one of the four conditioning chambers placed in the corners of the IntelliCage. Thus, individual conditioning protocols can be assigned. To date, protocols for aversive (Voikar et al., 2010), appetitive learning (Krackow et al., 2010), and behavioral flexibility (Mechan et al., 2009) have been validated (Table 1).

1.4.4 Behavioral phenotype of B6 and D2 inbred mice

In the OF, B6 are generally “more active” than D2 mice, whereas D2 tend to be more “anxious” (reviewed by Crawley and Paylor, 1997, Brown 1 dataset Mouse Phenome). Home cage “activity” (horizontal photo-beam interruptions) in B6 was also found to be higher than the activity of D2 mice (Tang et al 2002).

D2 mice outperform the B6 mice when the test situation has an important procedural learning component (reviewed by Ammassari-Teule and Castellano, 2004). In hippocampal dependent learning tests, (MWM and contextual FC), the B6 mice use hippocampal strategies whereas D2 mice do not (Logue et al., 1997, Ammassari-Teule et al., 2000). Behavioral dissociation in tests of learning and memory between the B6

and D2 mice, has been proposed as a natural model for hippocampal dysfunction (Logue et al., 1997).

Behavioral findings in B6 and D2 mice are supported by anatomical differences in the brain. Morphologically the number of pyramidal cells in the dorsal hippocampus and the infrapyramidal mossy fiber projection system are smaller in the D2 mice (Logue et al., 1997). Hippocampal protein kainase C (PKC) was shown to be reduced in the poor learners D2 mice (Wehner et al., 1990). Hippocampal expression of long term potentiation (LTP) was also shown to differ between the strains (Menard et al., 2004).

1.4.5 The phenotype of APP mouse models of AD

Increased mortality has been reported for different APP strains and epileptic seizures are cited as a cause of death in these strains (Ziyatdinova et al., 2011). Decreased body weight and disruption in sleep - wakefulness patterns are known to occur (Huitron-Resendiz et al., 2002). Hyperactivity and high frequency of stereotypic behaviors (e.g. circling) as well as aggressive behavior have been documented in many APP-tg mice (Pugh et al., 2007, Ambree et al., 2006). Impairments in species typical behaviors (e.g. burrowing and nesting) were found to have a different age of onset (Deacon et al., 2008). In anxiety tests, depending on the background, APP mice can be distinguished from non-transgenic littermates (Lalonde et al., 2003, Lalonde et al., 2004, Lalonde et al., 2005). In learning and memory tests, deficits have been described in habituation (Deacon et al., 2009), in working memory as assessed in the radial arm maze (Morgan et al., 2000), T or Y maze tests (Holcomb et al., 1999, Deacon et al., 2009, Cotel et al., 2010). Spatial memory impairments in the MWM, are commonly described (Mohajeri and Wolfer, 2009). In some strains the Barnes Maze is used to address the same memory system (Ronnback et al., 2011, Reiserer et al., 2007). Robust impairments were shown in contextual fear conditioning tests (Kilgore et al., 2010) and the Object recognition test (Hillen et al., 2010).
Many of the APP strains are backcrossed to inbred strains with retinal degeneration; hence the sensory function likely interferes with the results of behavioral tests (Rustay et al., 2010).

Ideally, changes in pathology, above a threshold level, should be mirrored by worsening of the behavioral impairment (Arendash et al., 2001).

1.4.5.1 Biological correlates of behavior in APP mouse models of AD

Characteristic features of the human disease such as the progressive accumulation of amyloid, cerebral amyloid angiopathy, tau hyperphosphorylation, synaptic dysfunction, microgliosis, astrocytosis and neurotransmitter alterations have been recapitulated in APP models. Similar to the human condition, it was observed that behavioral impairments in APP mice can be detected prior to Aβ plaque deposition (Dineley et al., 2002). Hence, soluble Aβ species (e.g. dimers, monomers, protofibrils) became the likely candidates to cause behavioral changes. Soluble extracellular Aβ assemblies (Lesne et al., 2006, Selkoe, 2008) and intraneuronal Aβ accumulations have been related to cognitive dysfunction in mice (Billings et al., 2005, Gimenez-Llort et al., 2007). However, others have found different Aβ assembly forms throughout the lifespan of an APP mouse model, thus questioning the idea of a single synaptotoxic Aβ species (Shankar et al., 2009).

It has been argued that other proteins are more reliable surrogate markers of induced neuronal dysfunction. Several biomarkers with modified expression in the APP mice have been described: aberrant expression of neuropeptide Y, reductions in the Fos-/Arc positive granule cells and depletion of calcium dependent protein calbindin (Palop et al., 2011). CALB is a protein with specialized calcium binding domains (EF hands), similar to parvalbumin and calretinin. In the rat hippocampus, CALB was immunohistologicaly localized to the dentate granule cells, some pyramidal neurons in CA1 and CA2, interneurons in the molecular and granule cell layers, basket cells and in the hilus (Sloviter, 1989). In AD mice decreased CALB levels in the granular layer were shown to correlate with behavior (Palop et al., 2003).

1.5 EXPERIENCE, BRAIN AND BEHAVIOR

Following the observation that rats raised as pets were better than laboratory rats at solving mazes, Donald Hebb formulated the hypothesis that early stimulating experiences lead to long-term brain changes which support increased problem solving abilities (Diamond, 1988).

Environmental enrichment is defined as a combination of complex inanimate and social conditions promoting enhanced sensory, cognitive and motor stimulation (Rosenzweig and Bennett, 1996, Olsson and Dahlborn, 2002, Mohammed et al., 2002). Several types of experiences, like exposure to novel stimuli (e.g. toys), cognitive stimulation, social interactions and physical activity have repeatedly been shown to promote brain plastic changes (e.g. increased cortical thickness, hippocampal dendritic branching and spine

density, number of synapses, capillary diameter, neurotrophin levels) and improve learning abilities of rodents at different ages (Diamond et al., 1976, Pham et al., 1999, Ickes et al., 2000, Diamond, 2001, Mohammed et al., 2002, Darmopil et al., 2009, Zhu et al., 2009).

At a molecular level, the mechanisms which support plasticity have only recently started to be elucidated (van Praag et al., 2000, McOmish and Hannan, 2007, Hu et al., 2010, Kempermann et al., 2010). These mechanisms include activation of immediate early genes as well as CREB phosphorylation, which thus link enrichment to the molecular mechanisms of learning (Williams et al., 2001, Toscano et al., 2006, Green et al., 2010).

In AD mouse models, conflicting results have been reported following EE (Richter et al., 2008). Increased Aβ plaque formation (Jankowsky et al., 2005, Jankowsky et al., 2003), reduction of Aβ levels (Lazarov et al., 2005), or no effect on Aβ load (Cotel et al., 2010) following different EE protocols have all been reported. Cognitive function was improved (Costa et al., 2007) or showed no changes (Cotel et al., 2010) and was differently affected by cognitive activity and wheel running (Wolf et al., 2006, Cracchiolo et al., 2007). Both long term and short enrichment exposures have the ability to determine lasting changes of the brain (Ferchmin and Eterovic, 1986). Short term events such as short term enrichment (Paylor et al., 1992) or behavioral testing are also known to affect subsequent behavioral measures (McIlwain et al., 2001, Voikar et al., 2004).

2 AIMS

The overall aim of this thesis was to validate the use of - and develop applications for - a behavioral phenotyping method for group housed laboratory mice, the IntelliCage.

The specific aims of the studies were:

1. Test inter - laboratory *replicability* of behavioral data obtained in different laboratories using the IntelliCage technology [study I, II].

2. Examine the influence of additional devices (add-ons: SocialBox and AnimalGate) on behavior in the IntelliCage [study II].

3. Behaviorally characterize three mouse strains: B6/NCrl and D2/NCrl (inbred) and B6D2F1/NCrl (hybrid) behavior (adaptation, place preference learning and reversal learning) using standardized protocols in the IntelliCage/IntelliMaze [study I, II].

4. Apply the IntelliCage methodology for behavioral phenotyping of the APP-ArcSwe, a mouse model of AD [study III].

5. Analyze the effects of prior exposure to the IntelliCage on behavior in subsequent classical tests: Open field, Elevated Plus Maze, Rotarod, Morris water maze, Fear Conditioning [study IV].

6. Explore the relationships between behavioral measures in IntelliCage and measures obtained in a battery of classical behavioral tests [study IV].

3 MATERIALS AND METHODS

Experimental procedures for Studies I and II were performed simultaneously in 4 laboratories: Evotec (Evotec Neurosciences, Hamburg, Germany); ISS (Section of Behavioural Neuroscience, Department of Cell Biology and Neuroscience, Instituto Superiore di Sanita Rome, Italy) NKAR (Karolinska Institutet, Alzheimer Disease Research Center, Stockholm, Sweden); UZH (Institute of Anatomy, University of Zurich, Switzerland).

3.1 ANIMALS

[study I, II] For each study, each of four participating laboratories (Evotec, ISS, NKAR and UZH) received 78 female mice from a commercial breeding farm (Charles River, Germany). Thus, in each lab, 26 female mice from each of three strains: B6/NCrl and D2/NCrl (inbred) and B6D2F1/NCrl (hybrid) were used. The mice were approximately 8 weeks old at arrival. They were initially kept in same-strain, group housing conditions (4 mice/group) until the start of behavioral experiments.

[study III] The experimental groups consisted of 13 tg-ArcSwe and 11 non-transgenic littermates (non-tg) from the APP-ArcSwe colony maintained at Uppsala University facilities. Transgenic founder mice were obtained as described previously (Lord et al., 2006) and the colony was maintained by backcrosses to B6/JSca mice (Scanbur, BK Sollentuna, Sweden). The mice tested during the study were offspring from matings following at least six backcrosses (incipient congenics > N_6). We used female mice that were 4 months old at the beginning of behavioral studies.
The tg-ArcSwe mice express human *APP* with both the Swedish (KM670/671NL) and Arctic (E693G) mutations, driven by the murine Thy-1 promoter. The total amount of APP (human and murine) expressed in the brain is three times higher than the level of endogenous APP.
The mice were tested twice: at 4 and at 14 months.

[study IV] Thirty two (32) young adult (3 months old) female mice were used during the study. The first group (n=16) consisted of non transgenic off-springs from the strain B6D2F1.B6D2F1-*APP*^{751tmSD}/JSW (henceforward hAPP$_{SL}$) mice. The colony was maintained at JSW research facility (Graz) on a mixed B6D2F1 background (N10-N12). The second group (n=16) consisted of transgenic offspring from the B6D2F1;

B6D2F1-*p75*^{NTR}/JSW line. These transgenic mice over-express the human p75 neurotrophin receptor (henceforward the p75 mice, (Schwach G. et al., 2006)). The p75 mice were maintained at JSW research facility (Vienna) on a mixed B6D2F1/OlaHsd background (N2-N3).

For each study, prior to the start of behavioral experiments, the mice were group housed according to local standard conditions. For studies performed at Karolinska Institutet facilities [study I, II], the standard conditions were group housing and provision of a Mouse House, Tecniplast™, Italy; for the study performed at Uppsala University facilities [study III] in the standard condition the animals were provided with a cardboard shelter and nesting material; and for study IV (performed at JSW facilities Graz) the standard condition was barren housing.

The dark phase of the light/dark cycle was of 12 h for all the studies. Only for study I the dark cycle was homogenously reversed across laboratories. Humidity varied between 40–70% and temperatures between 20–24 ºC. Food (standard diet) and water were available *ad libitum* in the standard condition. Access to water during IntelliCage behavioral testing was obtained according to the experimental protocol. The body weight of experimental animals was monitored during all studies.

All experimental procedures received ethical approval from local authorities and were performed according to EU and national legislation for animal welfare.

3.2 BEHAVIORAL TESTING IN INTELLICAGE AND INTELLIMAZE

3.2.1 Apparatus and procedures

3.2.1.1 IntelliCage

The IntelliCages consists of a polycarbonate, rectangular cage (20.5 cm high × 58 × 40 cm at top, 55 × 37.5 cm at bottom) containing a conditioning chamber in each corner. The mouse presence in the conditioning chambers is detected by a heat sensor in conjunction with antenna identification of RFID transponders. Following mouse identification, the event is recorded: a - *visit*. On each side of the corner, bottles of water can be accessed through a round hole, gated by a movable door. Pokes at these door openings are registered by a light-beam sensor. Each light beam interruption is recorded as a – *nosepoke event.* Finally, a third event – *lick*, is detected when a change

in voltage of a condenser attached in conjunction to the water bottle support reaches a threshold value.

3.2.1.2 IntelliMaze: Social Box and Animal Gate

An IntelliCage connected to an external device is referred to as IntelliMaze. In our studies, the IntelliCage was either connected to an AnimalGate or to a SocialBox.

The AnimalGate is a tubular passage (30, 3 cm length), equipped with three sliding doors, controlled by the IntelliCage Plus software (NewBehavior AG, Zürich, Switzerland, version 2.6). In the compartment facing the IntelliCage, an antenna enables individual identification of the mice. By sequential door opening, the AnimalGate allows for selective passage of individual mice. During our experiments [study II] the AnimalGates were connected to the short side of the IntelliCage and conjoined with a standard MII cage.

A SocialBox consists of a polycarbonate type II (MII) cage (26.7 x 20.7 x 14.0 cm) connected via a polycarbonate tube (38.5 cm in length, 4 cm diameter), to the short side of an IntelliCage. On the connecting tube, two antennae register in- and outward passages of individuals. In the respective experiments [study II], two SocialBoxes were connected to the short opposite sides of the same IntelliCage.

***3.2.1.3** Procedures*

Prior to the start of behavioral testing, the mice were briefly anaesthetized (we systematically used Isoflurane [studies I-IV]) and a transponder (DataMars, 1 mm diameter and 11 mm length) was injected subcutaneously, into the interscapular region. Transponder retention was checked with a transponder reader before placing the mice into the cages.

In order to ensure proper functionality of all sensors and mechanic components, a calibration procedure was performed according to the IntelliCage manual, before the start of each experiment.

During the studies, the water intake of the mice was monitored daily.

3.2.2 IntelliCage and AnimalGate Protocols

3.2.2.1 Intellicage protocols

The following protocols were validated either during the multi-center studies (study I & study II) or independently, using inbred mice or lesioned mice (Voikar et al., 2010):

- Free Adaptation (studies I-IV): all doors in the IntelliCage are open
  - data analyses from various IntelliCage experiments revealed that mice are highly active immediately following introduction to the cages. Hence we distinguished an initial exploratory phase (study I - 30 min); *Free exploration* (studies III-IV – 1 day).
- Nosepoke Adaptation (study I-IV): nosepoke needed for access to water.
- Drinking Session Adaptation (study I, study III): nosepoke is needed for access to water which is restricted to 2 h / night. For study III, an initial drinking adaptation step was performed when the doors were continuously open during the drinking session.
- Place Learning (studies I-IV): Water available in one corner per animal, one nosepoke needed for access.
- Reversal (Place) Learning (study I-III): Water access shifted to another corner (the diagonal opposite) in the cage. A nosepoke is required for access to water.
- Passive avoidance learning and probe trial memory test [study III]:
  - the baseline preference to IntelliCage corners was first established by monitoring corner preferences of mice for 24 h.
  - day 1 – Training: for 24 h one randomly assigned corner delivered an air-puff (1.5 bars) when the mouse performed a nosepoke, and until the mouse left the corner.
  - day 2 – Delay: the mice were group housed maintaining the experimental groups, in standard cages (24 h).
  - day 3 – Probe trial: the mice were placed back in the IntelliCage. A nosepoke was required for access to any corner.
- Right side learning: only nosepokes to the right side of a corner caused door opening for access to water. After three nosepokes at the left side, LEDs (green, red, blue) were turned on in the respective side of the corner. The LEDs were switched off at the end of the visit.
- Side learning reversal: rewarded corner was switched from right to left.

The following protocols have not been validated by repetitions or multi-center studies [study III]:

- Extinction of place preference: a nosepoke was needed to open an IntelliCage door. It is employed after the reversal place learning (note: a protocol including extinction of place preference has since been published).
- Novel object preference (preference for corners was analyzed for 2.5 h):
  - day 1, 2: water access was restricted to one drinking session interval, and the two corners on one of the long sides of the IntelliCage.
  - day 3: two different objects (paper baking cup, half of a rubber ball, aluminum candle support), were balanced between the cages and placed under the grid of the corners for 2.5 h. The mice did not have direct access to the objects. No prior preference existed for the objects used during the test.
  - day 4: retention interval.
  - day 5: one of the objects was replaced in each cage with a novel object.
- Novel smell (neophobia): in three of the cage corners, in the right bottles scented water (cloves, mint, almond) was presented. The bottles were kept in place for 5 h, from 24:00 to 05:00. The doors to all corners could be opened once per visit upon nosepoke.
- Air-puff in preferred corner: one corner for each mouse delivered a 3.5 bars air-puff when the animal performed visits with nosepokes to it.

3.2.2.2 AnimalGate protocols

- Free access: all the mice could freely access the external MII cage, as all 3 doors of the AnimalGate were open.
- Active AnimalGate: all the mice could pass through the AnimalGate, but access was not possible as long as another mouse was present outside (in the MII, add-on cage).
- Conditioned access: half of the mice in the IntelliCage (random assignment, balanced between strains) could pass through the AnimalGate.

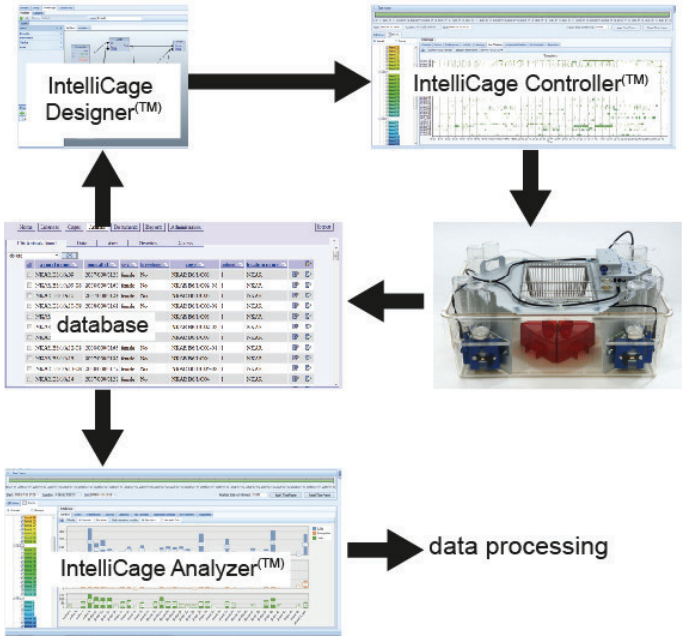

Fig. 1 Work chart flow for studies I and II. Following transponder implantation procedure, biological and behavioral data for each animal was recorded in a common database. The Intellicage Designer and Controller were used during the experimental phase of the studies. An independent collaborator processed the data acquired from the 4 participating labs.

3.3 CONVENTIONAL BEHAVIORAL TESTING

3.3.1 Elevated plus maze

The apparatus (Fig. 2) was made of grey PVC, raised 50 cm from the floor surface. The maze consisted of two open and two enclosed arms, equal in length (30 cm), and width (5 cm) arranged in the form of a cross. The closed arms were protected by 15 cm high, opaque side walls. The mice were placed in the central area of the

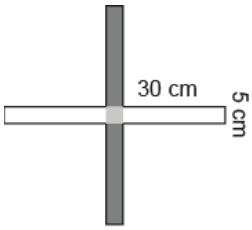

Fig. 2 Elevated plus maze

apparatus facing one of the open arms. The mice freely explored the maze for 5 minutes, while their behavior was videotaped. The records were processed off-line, using commercial software (TSE Systems®). The maze was cleaned with 70% Ethanol before the trials.

3.3.2 Open field

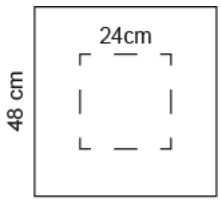

Fig. 3 Open field

A square arena (48 x 48 cm^2), enclosed by transparent plexiglas walls (32 cm high), was used for OF testing (Fig.3). The testing system consisted of 4 testing boxes (TSE-System®). Each OF was equipped with infrared photo beams and detectors, placed on two rows. On the lower row, the photocells were placed at a distance of 1.4 cm and detected the horizontal movements of the mice. Activity in the vertical axis (e.g. rearing, grooming and jumping) was detected by a second row of photo beams and detectors, placed 4 cm above the first one. The "central area" was defined as the central 24 x 24 cm^2 square of the arena, and the remaining surface was considered the "periphery". The mice were acclimatized to the experimental room for approximately 1h prior to behavioral testing. Then they were individually placed into the arena, facing one of the corners and allowed to explore for 30 minutes. The arena was cleaned with 70% Ethanol between the trials.

3.3.3 Rotarod

The apparatus was a five-lane Rotarod (TSE-Systems®), with a central plastic rod of 3 cm diameter. The mice were tested, one per lane, over two consecutive days, with three trials per day. For each trial, the mice were placed on the accelerating Rotarod. The speed of rotation increased from 4 to 40 rpm over a period of 5 minutes. The inter-trial interval was 60 minutes. The apparatus was cleaned with 70% Ethanol between the animals.

3.3.4 T-maze test

A white, T-shaped plexiglass arena was used (arm width 10 cm; arm length 30 cm; wall height 23 cm; Fig. 4). The method was adapted from (Deacon and Rawlins, 2006). In brief, the mice were placed at the end of the stem arm of the T maze and allowed to

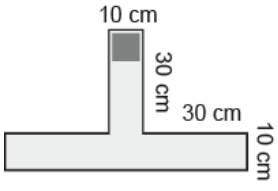

Fig. 4 T-maze

freely explore the maze. Once the mouse selected one of the left or right arms, after the intra-trial interval of 30 sec, a choice trial was administered. An alternation score of 1 implies that the animal selected, during the choice trial, the arm opposite to the arm visited during the sample phase. A score of 0 means the animal chose the same arm visited during the sample phase. The mice were tested two times per day for 3 consecutive days. A cut-off period of 5 min was used to stop a trial if the mouse did not make a choice. The maze was cleaned with 70% Ethanol between the animals.

3.3.5 Object recognition task

Two different protocols were used for the object recognition task, as follows:

[Study III]

The test was performed as previously described (Bevins and Besheer, 2006). In short, on day 1, the mice were allowed to freely explore in a medium size cage (21.5×37×18 cm), for 10 min, a pair of identical objects (Fig. 5). After a 24 h retention interval, one object was replaced with a novel one and each mouse was allowed to interact with the objects for 5min. Two distinct pairs of objects (A: brown porcelain coffee cups 6×6×6 cm and B: regular glass lab brown bottles 12×4×4 cm) were used to control for natural preferences for any of the objects. Therefore half of the mice were tested for preference of B over A, and the other half for preference of A over B. The number of approaches and time spent interacting with the respective objects was recorded (rearing on followed by sniffing above the objects was not counted as an approach). A discrimination ratio was calculated as follows: time spent for novel object interaction/total time interacting with both objects. A discrimination ratio above 0.5 was considered indicative of object recognition.

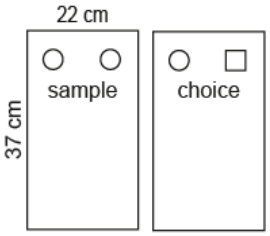

Fig. 5 Object recognition

[study IV]

A square shaped (45 x 45 cm^2); grey PVC arena, enclosed by 43 cm high walls, was used (Fig. 6). The mice were habituated to the arena on 3 consecutive days. On day 1: all mice in one cage were placed together in the arena and allowed to freely explore it for 15 min. On day 2, the mice from the same cage were allowed to explore the arena, in group, for 10 min. On day 3, each mouse explored the arena individually for 5 min.

On day 4, during the object exploration session (sample phase), each mouse was placed individually into the arena where two different wooden objects were placed in diagonal, at equal distance from each other and from the corners of the arena. The mice were allowed to explore for 5 min. The objects and arena were cleaned with 70% Ethanol before they were used and in between animals.

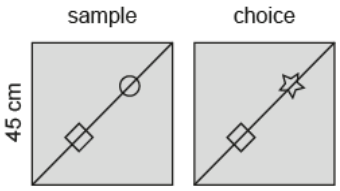

Fig. 6 Object recognition

One hour after the object exploration session, the object recognition session was performed. During the 3 min recognition session, a novel object (third object) was placed into the arena together with one of the previously presented objects (choice phase). The objects used were a cube: 3 cm side; a cone: radius 4 cm and height 4 cm; a pawn: base radius 3 cm and height 5.5 cm. Object exploration was evaluated automatically using the Videomot2 program from TSE-Systems(TM).

3.3.6 Fear conditioning

The test was carried out using an automated, commercially available operant conditioning system (TSE-Systems®). The training chamber (23 x 23 x 35 cm^3), had transparent walls. The floor of the chamber consisted of equally spaced (9mm), bars (4mm diameter). The training chamber was positioned inside a sound attenuated box (52 x 52 x 65 cm^3), where light intensity was set to 220 lux and background noise level at 40dB. The chamber was cleaned with 70% Ethanol before each animal was tested. The mice were tested on 2 consecutive days.

On *Day 1*, each mouse was placed inside the testing chamber and allowed to explore for 2 min (baseline). The conditioned stimulus (CS), an auditory cue of 2 kHz, 75dB, was presented for 15 sec at the end of the 2 min exposure time. During the last 2 sec of the auditory stimulus, a foot shock of 1.5 mA, the unconditioned stimulus (US), was administered. This procedure was repeated and the mice were removed from the chamber 30 sec after the end of the second US delivery (Fig. 7). On *Day 2*, (24 h after training), the mice were returned to the same chambers in which training occurred ("context test"), and freezing behavior was recorded automatically during a 5 min period. At the end of the 5 min context test, the mice were returned to their home cages. Approximately 1h later, freezing was recorded for 6 min


Fig. 7 Fear conditioning - day 1 training

in a modified chamber (black walls, cleaned with diluted detergent -D405). The initial 3 min period of the exposure to the modified chamber ("pre-CS"), were followed by 3 min of auditory cue presentation ("CS").

Freezing is defined as lack of movement except that required for respiration.

3.3.7 Morris water maze (MWM)

The MWM test was conducted in a black circular pool, 1 m in diameter (Fig. 8). Tap water (t = 22±1ºC) filled the pool to a depth of 20 cm. A transparent escape platform (8 cm diameter) was located in the southwest quadrant of the pool for all the trials, with the exception of the visual test. Posters with black, bold geometric symbols (e.g. a circle and a square) were fixed on the walls around the pool.

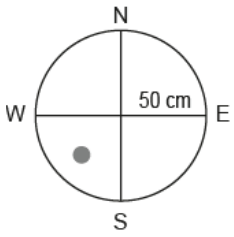

Fig. 8 Morris water maze

Hidden platform training: On each of four consecutive days, each mouse was subjected to three training trials. The sequence of start positions used was N-S-E for all the mice. A trial ended when the mouse found the platform or the cut-off period of 60 sec elapsed. The mice were allowed to rest for 10-15 sec on the platform. If the animal did not find the platform during one minute, the investigator gently guided the mouse to the platform.

Probe trial: On day 4, one hour after the last acquisition trial, a 30 sec probe trial. During this 30 sec trial, the platform was not available for escape.

The visible platform test: The mice were tested for their visual abilities at the end of the hidden platform navigation task. During two trials, the mice were released in the swimming pool where the escape platform was placed 0.5 cm above the water level, in the center of the pool, and flagged using a familiar object. The trial period was of 60 sec. Animals which did not find the platform within 1 min, were excluded from the study. A commercial tracking system (Kaminski; CS, Biomedical Research Systems) was used to record and analyze off-line the behavior of the mice during the MWM test.

3.4 GENE EXPRESSION AND TISSUE PREPARATION

Genotyping was performed as described before (Lord et al., 2006) with primers framing the basal promoter and the APP coding sequence (2577–2596 in M12379.1 and 541-522 in Y00264).

After the completion of behavioral experiments, the mice were anesthetized with 0.3 ml Avertin (25 mg/ml) and intracardially perfused with 0.9% saline solution. The brain was removed and sagitally divided in two halves. One hemisphere was frozen

on dry ice for biochemical analyses, and stored at – 80 until further use. The other hemisphere was immersed in 4% paraformaldehyde for 24 h and cryoprotected by sequential immersion in sucrose solutions (24 h in each of the following solutions 10%, 20% to 30% w/v).

3.5 ELISA AND IMMUNOHISTOCHEMISTRY

One brain hemisphere was homogenized in TBS (20 mMTris, 137 mM NaCl, pH 7.6) supplemented with Complete® protease inhibitor cocktail (Roche Diagnostics GmbH, Germany) with a tissue grinder (2×10 strokes on ice); tissue weight to extraction volume ratio was1:10.

The homogenates were centrifuged at 100000 × g, for 1 h, at +4°C in TBS. Soluble (extracellular and cytosolic) fractions were collected. Supernatants were stored in aliquots at −80°C and biochemical analyses were performed using the supernatants.

mAb158 protofibril ELISA

The mAb158 detects large Aβ oligomers (>100 kDa) and has been described in detail elsewhere (Englund et al., 2007). In brief, 96-well plates were coated with 200 ng/well of mAb158 in PBS over night at +4ºC and blocked with 1% BSA. The samples were centrifuged at 17900 rpm for 5 min prior to incubation. Samples were added to plates in duplicates and incubated for 2 h at room temperature (RT). Biotinylated mAb158 (0.5µg/ml) was added and incubated for 1 h at RT, followed by incubation with Streptavidin–horseradish peroxidase complex (SA-HRP, MabTech AB, Sweden), for 1 h at RT. K-blue aqueous (ANL-produkter AB, Sweden) HRP-substrate was used and the reaction was stopped with 1M H2SO4. All plates were washed three times between steps after blocking. All samples and antibodies were diluted in ELISA incubation buffer (PBS with 0.1%BSAand 0.02% Tween-20). The standard was generated by incubating wild type Aβ1–42 (American Peptide, Sunnyvale, CA) in 50 µM PBS for 30 min at +37 °C, followed by centrifugation at 16000×g for 5min. The supernatant was loaded on a Superdex 75 10/300 GL column resulting in a single high-molecular weight peak which was collected.

Total soluble Aβ ELISA

96-well plates were coated with 200 ng/well of 82E1antibody (human Amyloid N-terminal specific) in PBS, over night at +4°C and then blocked with 1% BSA. To measure total soluble Aβ the samples were supplemented with SDS (Sigma–Aldrich) to a final concentration of 0.2% and boiled at 95ºC for 5min. The samples were diluted in

ELISA incubation buffer, to a final SDS concentration of 0.02%, added to the plates as duplicates and incubated 2 h at RT. An incubation step followed consisting of 1 h at RT with 1 μg/ml biotinylated mAb27 (selective for Arctic Aβ, previously characterized (Lord et al., 2009b). Subsequent steps were performed in the same manner as for the mAb158 protofibril ELISA.

Immunohistochemistry

Coronal brain sections, 25 μm thick, were cut with a sledge microtome and stored at +4°C in PBS with 10mM NaN3. Individual sections were mounted on glass slides and antigen retrieval steps were performed by incubating the sections in pre-warmed citrate buffer (25mM, pH 7.3) at +85ºC followed by immersion in 70% formic acid. Endogenous peroxidase activity was quenched by incubation with 0.3% H_2O_2 in DAKO block/PBS.

Aβ deposits and amyloid cores were visualized using an Aβ40-specific antibody (Jensen et al., 2000) and Congo Red (Sigma–Aldrich) respectively. A secondary biotinylated goat anti-rabbit antibody (Vector Laboratories), Streptavidin–horseradish peroxidase complex (SA-HRP, MabTech AB, Sweden) and NOVATM (Vector Laboratories) reagents were used for visualization of immunosignals. Semi-quantitative image analysis was performed on three to five coronal sections from each animal (Bregma −1.22mm to−2.80mm) and Aβ plaque load in the cerebral cortex and hippocampus was measured in two image fields per section at 2 × magnification. Aβ and Congo red staining images were segmented with an auto threshold command (Image Pro-Plus, Cybernetics, USA). User-made macros in Image Pro Plus were designed as to measure area fraction (=stained area/total area) in the anatomic structure of interest. Tissue sections were incubated with a rabbit-anti CALB antibody (Swant – Switzerland, CB38, diluted at 1:15000, over night, at +4ºC) and with biotinylated secondary goat anti-rabbit antibody (Vector Laboratories). Heat retrieval and immersion in formic acid was omitted when sections were stained for CALB. Image Pro Plus custom macros were used to measure the optical density (OD) in the dentate gyrus (DG) for CALB. Images of histological staining were captured with a DXM 1200F Nikon microscope (Nikon Instruments Inc., USA) equipped with a digital camera and converted to greyscale.

3.6 STATISTICS

The data from each laboratory [studies I, II] was uploaded to a common databank, and analyzed using SAS software 9.13 (SAS Institute, Inc. 2006) by one of the partners in the study who did not perform experimental work.

[study I] Data were collected on 288 mice (six cages in four labs with four females per three strains). Normal distribution was evaluated and if both the Shapiro–Wilk and Kruskal–Wallis tests exhibited $p > 0.05$, the data was taken to be normally distributed. Several variables had to be transformed, and in a few cases extreme values had to be excluded, to induce residual variation to conform to the normality assumption. Untransformed data analyses did not give significant results for effects not significant with the transformed data. Place and Reversal Error rates could not be normalized as dispersion was too high. Therefore significance levels of inferences on cognitive differences are most probably conservative (under) estimates.

[study II] The activity data were square-rooted to approach normality; residuals remained nonnormally distributed (Kolmogoroff-Smirnov test, $p<0.05$). That implies significances levels for the activity to be too conservative. Sequential probability-ratio test (SPRT) statistics were computed to evaluate whether and when an individual exhibited significant preference for the rewarded corner in the IntelliCage.

The effects of protocol (Place learning or Pleace learning reversal), treatment, and strain on learning performance were evaluated in a non-parametric ANOVA model with the number of trials to criterion as dependent variable.

[study III, IV] Statistical analysis was performed using STATISTICA v8.0 software package (Stat Soft Inc, USA). The variables were considered normally distributed if the Kolmogoroff–Smirnov did not indicate a significant deviation from the normal ($p < 0.05$). For [study IV], several variables from conventional tests had a bimodal distribution and could not be normalized. Non-parametric statistics were used throughout.

Table 1 Overview of statistical models/tests used in the thesis

Study	Statistical model/tests
Study I	GLM, split-plot design $y_{ijk} = INT+Lab_i+Strain_j+Lab\times Strain_{ij}+ IC(Lab)_{k(i)}+Strain\times IC(Lab)_{jk(i)}+e_{jk(i)}$
Study II	GLM, split-plot for Activity

	$y_{ijk}=INT+Trt_i+Strain_j+Trt*Strain_{ij}+IC(Trt)_{k(i)}+Strain_{ij}*IC(Trt)_{jk(i)}+e_{jk(i)}$ Sequential probability ratio test for Learning followed by non-parametric ANOVA model (Brunner & Munzel, 2002)
Study III	t-test, Mann Whitney U for variables measured at one time point ANOVA for data followed over time (post-hoc N HSD and Tukey HSD) and Sign test Linear correlation analysis: - Parametric (Pearson correlation coefficient r) - Non-parametric test (Spearman rank, R)
Study IV	Linear correlation analysis - Non-parametric test (Spearman rank, R) Mann-Whitney U test Kruskal Wallis, and Friedman ANOVA Cluster analysis: Single linkage amalgamation rule and City-block (Manhattan) distances Projection to Latent Structures (PLS)

4 RESULTS AND DISCUSSION

4.1 INTELLICAGE AND INTELLIMAZE MULTI-CENTER STUDIES

Laboratory effects on IntelliCage behaviors

The *Lab x Strain* interaction effect did not cross the boundary of $p < 0.05$ significance level. As expected from the literature review, we found significant *Laboratory* effects [study I] both for unconditioned and conditioned *Activity* measures and measures of *Learning*. For the number of visits during the first 30 minutes ($F(3, 20) = 8.39$, $p< 0.0001$), the time necessary to visit all IntelliCage corners ($F(3, 20) = 5.97$, $p< 0.005$), 2 variables from the Nosepoking phase, (PokeProportion ($F(3, 20) = 9.81$, $p < 0.001$) as well as Poke duration ($F(3, 20) = 12.56$, $p < 0. 0001$), a strong *Laboratory* effect size was detected. Close to significant interaction effects for the variables highly affected by the *Laboratory* identity were seen.

In our study inter-laboratory consistency of behavioral data was attained using the IntelliCages. However, it should be noted that some variables are more sensitive to laboratory identity than others. The variability during initial phases of exposure to the cages could be explained by a high level of arousal triggered by new social partners as well as the novelty of the chamber itself. Mixed housing of experimental groups prior to the start of IntelliCage experiments as well as a standard procedure for releasing the mice to the cage could potentially reduce the variability during the initial stages.

[study II] The analysis of data from Control trials (IntelliCage only) confirmed that strain effects did not depend on laboratory identity, and the *laboratory* effect did not reach significance, either.

4.2 B6, D2 AND B6D2F1 BEHAVIOR IN THE INTELLICAGE

Activity measures: unconditioned access to water bottles

[study I] Following 5 days of adaptation (Adaptation phase last 2 days) to the cages, IntelliCage corner Visit Frequency was significantly different between strains, both during the dark phase ($F(2, 36) = 41.71$, $p<0.0001$) and the light phase (122.09, $p < 0.0001$). Visit Duration also differed between the strains ($F(2, 36) = 66.25$, $p < 0.0001$). D2 mice visited less frequently, but spent a longer time than the B6 mice inside the IntelliCage corners. For both variables the behavior of F1 mice was in between that of the parent strains. The difference between dark activity and light activity (Day pattern = (visits dark – visits day) / (visits dark + visits day)) was more

pronounced for the D2 mice which reduced the activity during the light phase more than the B6 mice ($F(2, 36) = 107.45$, $p < 0.0001$).

[study II] We replicated the finding of significant strain differences for both phases of the light-dark cycle. The effect size of the difference was higher for the light phase ($F(2, 38) = 145.85$, $p < 0.0001$) than for the dark phase of the adaptation ($F(2, 38) = 28.13$, $p < 0.0001$). The effects were explained by low activity of D2 mice, which were consistently followed by F1 mice and B6.

Activity measures: conditioned access to water bottles

[study I] During Nosepoke Adaptation (3 days), on measures for Activity (Visits per hour), significant differences between strains were obtained ($F(2, 40) = 20.25$, $p < 0.0001$). The activity was higher for the B6 than the D2 mice, while F1 ranked lowest. Nosepoke duration did not differ significantly between strains ($F(2, 40) = 0.30$, $p < 0.74$). D2 mice exhibited a higher proportion of visits with nosepokes than the other two strains. D2, but not F1, mice countered lower visiting rate by exhibiting an increased incidence and frequency of nosepokes per visit.

[study II] During light phases of Nosepoke Adaptation the frequency of visits to the IntelliCage corners was significantly different ($F(2, 40) = 8.41$, $p<0.001$) and strains ranked as during the Free adaptation phase. For the dark phases, the effect of strain was highly significant ($F(2, 40) = 52.98$, $p<0.0001$).

Learning measures

[study I] At the end of the Place learning phase (last 42 visits with nosepokes, after 3 days of learning), the error rate was significantly different between the strains ($F(2, 40) = 34.23$, $p < 0.0001$). B6 mice exhibited a lower rate of place learning errors. Following the switch to Reversal learning (first 42 visits with nosepokes) the strains were again discriminated by the number of reversal errors ($F(2, 40) = 12.12$, $p<0.0001$). B6 mice had the highest error rate. Both during the Place learning and reversal, the F1 had a higher error rate than the D2 mice.

[study II] The strain identity had also a significant effect i.e., D2 mice required fewer visits to acquire the preference as well as to reverse their preference for the rewarded corner. F1 mice behaved similarly to B6 during the place learning whereas in the reversal phase they behaved more similar to D2.

We further analyzed the number of visits during the Place reversal phase necessary for the mice to visit previously rewarded corners at approximately chance levels, as a measure of preference extinction. Thus, the B6 mice needed the highest number of corner visits to extinguish the acquired preference. The survival analysis confirmed the significant effect of strain (Wilcoxon Chi-square: 12.36, $p<0.001$).

The results from our studies were similar regarding the strain ranking for *activity* measures. For *learning*, the variables analyzed looked at different time points. While in the first study already learned preferences were analyzed, in the second study the number of visits necessary to reach a preference was analyzed. B6 mice discriminated more, whereas D2 acquired faster the preference in our study.

Classical studies on learning behavior of inbred strains have shown that D2 mice outperform the B6 mice when the test situation has an important procedural learning component (reviewed by Ammassari-Teule and Castellano, 2004). In what concerns spatial and contextual information processing, the B6 mice discriminate more efficiently than the D2 mice (Ammassari-Teule and Castellano, 2004). Building on this knowledge we can speculate that the IntelliCage place learning paradigm has a spatial component mirrored by variables addressing discrimination capacity in mice (Krackow et al. 2010), whereas the procedural component is better captured by variables addressing the speed of (re)learning (i.e. trials to criterion in this study, or re-learn score in our previous study).

4.3 INTELLICAGE APPLICATIONS FOR A TG-APP AD MODEL

The mice were tested twice, at young adult age (4 months old) and 10 months later. A total of 4 mice (2 tg-ArcSwe and 2 non-tg) did not contribute data points to the second behavioral experiment, as 3 mice (14 month old) did not drink inside the IntelliCages and 1 non-tg mouse died prior to the start of the second experiment.

The body weight of APP-ArcSwe mice was lower than the body weight of non-tg throughout the study.

Activity measures at 4 and 14 months

The frequency of corner visits during the initial 24 h of exposure to IntelliCage at 4 months, was higher for non-tg mice ($t(21) = 2.88$).

At 4 months there was a significant decrease over days in the number of visits the mice performed ($F(5, 110) = 8.3$, $p < 0.001$). Using the contrast estimate method, this difference was revealed to be due to the habituation between the first and the following days. No effects of genotype or genotype and time interaction were seen for the number of visits. Similarly, at 14 months a habituation effect for visits was detected for both groups ($F(5, 90) = 32.8$, $p < 0.001$). A significant time and genotype interaction effect for visits to IntelliCage corners was also revealed by repeated measures ANOVA ($F(5, 90) = 2.65$, $p < 0.05$). Separate analyses for the non-tg animals, showed a significant decrease in corner visits from the first to the second dark phase of exposure to the cages (Tukey HSD, $p < 0.05$). For the tg-ArcSwe group this difference was not significant. Using nonparametric Spearman R test, significant overall correlations were found for the number of visits during the first dark phase ($R = 0.44$, $p < 0.05$) and average number of visits during the active phases ($R = 0.45$, $p < 0.05$) between the two experiments.

At 4 months the amount of time spent drinking (lick duration) increased significantly from the first to the subsequent dark phases, both for tg-ArcSwe and non-tg mice ($F(5,110) = 6.05$, $p < 0.001$). Lick duration for the tg-ArcSwe mice was significantly lower than for the non-tg littermates, as shown by a main genotype effect in repeated measures ANOVA ($F(1, 22) = 5.65$, $p < 0.05$). Consistent with previous studies on diurnal activity of laboratory mice, our data show that close to 90% of visits to IntelliCage corners occur during the dark phase. Similarly, at 14 months the time spent drinking increased significantly over time for both groups, ANOVA ($F(5, 90) = 15.6$, $p < 0.001$). However, no difference in the duration of drinking between genotypes was detected at this age. No significant correlation for lick duration at 4 and 14 month was found.

Learning measures at 4 and 14 months

Place learning, place learning reversal and extinction of place preference

Training was done in 2 learning sessions per dark phase for 4 days. An error was defined as a visit to any of the corners where water was not available (unrewarded corner, yellow in Fig. 9). Because we observed low levels of activity during the second learning session (i.e. 04:00-05:00 AM), the change over time of "%Error" for the evening learning sessions (i.e. sessions between 23:00 and 24:00), was analyzed. The same analyses were applied for reversal learning phase.

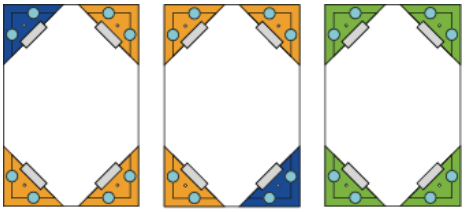

Fig. 9 Schematic depiction of the place learning protocol for IntelliCage

At 4 months, the percentage of "incorrect visits/errors" significantly decreased from approximately 68%, to 52%, repeated measures ANOVA ($F(3, 57) = 8.26$, $p < 0.001$). Both genotype groups had a similar percentage of visits to the corners rewarded during the Place learning (PL) and the Reversal learning. During the Extinction phase (12 h/first dark phase) when all corners of the cages became accessible again for drinking, the non-tg mice lost the preference for corners rewarded during the Reversal module. Tg-ArcSwe mice continued to significantly prefer them over the other three corners in the cage ($t(12) = 6.09$, $p < 0.001$). The percentage of visits to the last corner rewarded was significantly different between the two groups ($t(22) = 3.19$, $p < 0.01$), indicating response perseverance in the tg-ArcSwe mice. Both genotypes preferred to visit the rewarded corners (non-tg 58.0±8.4%; and tg-ArcSwe: 61.4±6.1%) to the other two corners in the cage ($t(23) = 6.59$, $p < 0.001$).

At 14 months, the percentage of errors (*%Error*) made during the evening learning sessions significantly decreased over days as shown by repeated measures ANOVA, ($F(3, 51) = 7.58$, $p < 0.001$). *%Error* decreased from an average 50% to 34% during 4 days. No significant overall difference due to genotype or, time by genotype interaction was detected for 14 months old animals. Nor was there a significant overall genotype difference during the reversal place learning sessions on *%Error*. Further analyses for the evening sessions on days 2 and 3 of reversal confirmed a significant difference for *%Error* between the genotypes on both days, ($t(17) = 2.43$, $p < 0.05$) and ($t(17) = 2.29$, $p < 0.05$). For the same sessions, tg-ArcSwe mice showed (a) on day 2, a trend for visiting more frequently the corners rewarded during place learning ($t(17) = 1.69$, $p < 0.12$) and (b) on day 3, a significantly higher preference for this corner ($t(17) = 2.92$, $p < 0.01$) as compared to the non-tg mice. During the first dark phase of the Place preference extinction module the summed preference for the rewarded corners was maintained by tg-ArcSwe mice ($t(10) = 4.55$, $p < 0.01$), but not by non-tg animals.

14 months: Passive avoidance in the IntelliCage and probe trial memory test

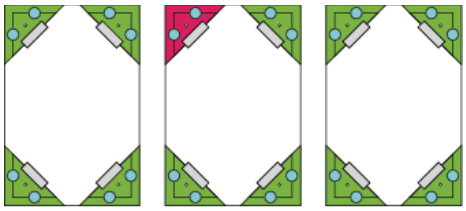

Fig. 10 Schematic depiction of the passive avoidance protocol for IntelliCage

At 4 month this test was not performed.

Prior to the start of the passive avoidance test, the mice did not show significant avoidance behavior towards the IntelliCage corners.

As compared to the baseline preference, both during training and probe trial, non-tg mice avoided significantly (Sign test, $p < 0.05$) the corners where air-puffs where delivered (red in Fig. 10). The change in preference from baseline was not significant for the tg-ArcSwe mice. A significant genotype effect on the percentage of visits to corners where air-puffs were delivered was detected during the training phase (Mann Whitney U test, $p < 0.05$).

4.4 INTELLICAGE BEHAVIOR AND CALB IMMUNOREACTIVITY

Significantly higher CALB immunoreactivity (Mann Whitney U, $p < 0.01$), in the granular layer of the dentate gyrus) for the tg-ArcSwe mice was found. The average values for tg-ArcSwe mice (0.26±0.05 OD; mean±stdev) were higher than in non-tg mice, (0.19±0.03 OD). In contrast, a lower level of CALB immunoreactivity in the polymorphic layer of the tested tg-ArcSwe mice compared to the non-tg ($t(16) = 2.13$, $p < 0.05$), was seen. In the overall group, the correlation between CALB and the Preference for the punished corner during training was significant at the $p < 0.05$, (Spearman $R = -0.49$).

In the cerebral cortex of 17 month old tg-ArcSwe, 4.7 ± 0.1% of the surface was occupied by plaques and in the hippocampus 4.00±2%. Protofibril levels were 9.3 ± 2.7 pg/mg tissue in tg-ArcSwe mice and total soluble from one hemisphere amounted to 700±200 pg/mg. No statistically significant correlation between the number of Aβ plaques in the cerebral cortex ($r = -0.38$, $p = 0.27$) or hippocampus ($r = 0.39$, $p = 0.26$), soluble Aβ ($r = -0.03$, $p = 0.92$) or Aβ protofibril levels ($r = -0.09$, $p = 0.79$) and learning behavior (visit preference for the punished corner during the training phase, 24 h) in the passive avoidance and probe trial memory test was found.

We thus described behavioral perseverance at 4 month and a deficit in passive avoidance learning at 14 month in IntelliCage for tg-ArcSwe mice. Impairments in

hippocampal dependent tests such as the MWM, Barnes Maze and Y-maze, as well as two - way active avoidance and O-maze exploration (Knobloch et al., 2007, Ronnback et al., 2011, Cheng et al., 2007) have been described for other APP transgenic lines harboring the Arctic mutation. Our findings add to the known impairment in spatial learning as assessed in the MWM (Lord et al., 2009a) in this strain. Hence, using the IntelliCage in the APP-tgArcSwe mice, complementary behavioral phenotypes were described. Interestingly, behavior of the tested mice could be related to a neuronal marker - CALB. Our study is limited by the semi quantitative approach and lack of non-tested control material. However, this is the first study to show elevated CALB in the granular layer of an AD mouse model. Previous work, using other APP mice (i.e. the APPSweInd or APPSwe/PS1) mice, reported a decrease of CALB expression at the same level (Palop et al., 2003). The differences between ours and previous studies include the transgene used, background strain, sex of the animals, housing conditions and the length/type of behavioral testing. At this stage, we can only speculate that under our experimental conditions, in the tg-ArcSwe model, an increase in CALB expression might compensate for deleterious effects of mutant human APP overexpression. Altered protein expression at the hippocampal level could be induced directly by APP activated pathological cascades or as part of the compensatory mechanisms (Palop et al., 2011). It has been proposed that the latter is a more likely explanation in light of the network imbalance induced by the presence of Aβ.

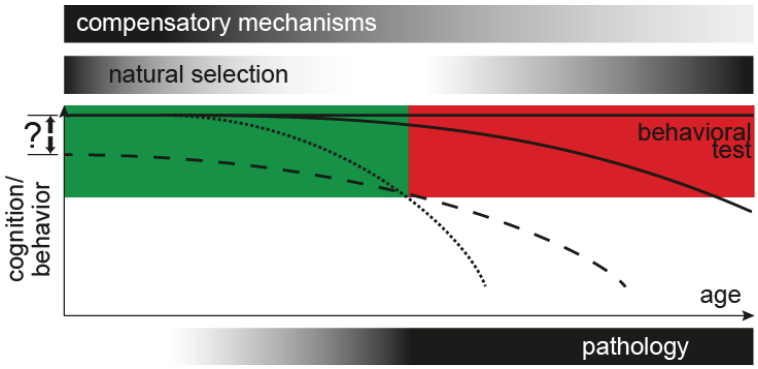

Fig. 11 Cognitive / behavioral trajectories in mice. Cognitive decline with aging is expected (solid line). Transgenic models should ideally develop cognitive decline (dashed line) which parallels pathology (dotted line). Early phenotypic differences

between the tg and non-tg AD mice (dashed line) have been commonly reported. These differences mirror several processes in the tg mouse brain: effects of transgene expression, accumulation of pathology as well as compensatory mechanisms. Furthermore, detection of cognitive and behavioral impairments critically relies upon test sensitivity, which differs between paradigms.

4.5 THE INTELLIMAZE/INTELLICAGE - AN ENRICHMENT DEVICE

[study II] Effects of add-on availability on:

Activity measures Only visit frequency during the dark phases of the Free adaptation (3 days) were significantly lower in the presence of add-ons ($F(1, 19) = 16.95$, $p < 0.001$). The other measures (activity during the light phases of the Free adaptation and both phases of Nosepoke adaptation) were not affected in the presence of add-ons. The difference between add-ons did not reach statistical significance ($F(1, 19) = 2.61$, $p>0.12$).

Learning measures During the place conditioning paradigms, the overall number of trials (visits with nosepokes) needed to reach the learning criterion, (30% visits to the rewarded corner), was lower in the presence of add-ons. The number of visits during the Place reversal phase necessary for the mice to visit previously rewarded corners at approx. chance levels was analyzed as a measure of preference extinction. The availability of add-ons did not have an effect on this variable.

Body weight For all groups of mice, under all experimental conditions, we observed an increase in average body weight over the 16 days of experimental trial period. The increase of body weight did not significantly depend on treatment in any of the cohorts.

[study IV] For the analyses the mice were grouped according to the housing condition in IntelliCage (Experiment 1). Following IntelliCage exposure, both systematic and discordant effects on subsequent behavioral tests were obtained. Thus, activity measures, as assessed in the OF and FC were lower for the IntelliCage tested mice Discordant results between the 2 groups of tested mice were obtained for *CA visits* in EPM, a variable considered to reflect locomotor activity, and in the Rotarod test (Day2 Trial A, Latency to fall). For both tests the ranking was the same (group1-IC > group2-IC). Both during Day 1 of Rotarod and the MWM tests, a significant learning effect for IC-tested mice was seen, even for small groups of mice (n=7). During the Rotarod test,

the dynamic of learning the task was different between tested and not-tested mice. In the MWM, the main difference came from behavior during the first exposure to the test situation when IC-tested mice swam longer distances than the not-tested ones. In previous work, mice that had been exposed to a battery test performed better in the Rotarod test (Voikar et al., 2004). Learning was also facilitated following exposure to enriched environments in tests with an important motor component (Madronal et al., 2010).

4.6 INTELLICAGE PHENOTYPES AND CONVENTIONAL TESTING PHENOTYPES

In order to test whether IntelliCage behaviors would predict behavior in conventional tests, we applied multivariate regression models (PLS). Variables from conventional tests, selected after Cluster analyses were considered "responses" in PLS analyses while "factors" were variables extracted from IntelliCage data.

In the first PLS model, IntelliCage variables for Free exploration and Habituation (Table 2) and variables reflecting locomotion, exploratory activity and anxiety-like behavior from conventional behavioral tests were used (Table 3).

PLS analysis identified one significant component (Q^2= 0.015), explaining 14.32 % of the sum of squares of the response variables. Variable importance > 2/Number of variables in the model are considered relevant for the model.

For the second PLS model, variables for Place learning and Place learning reversal modules the Percentage of errors for bins of 12 visits were extracted. In addition, for Place learning reversal the Percentage preference for the previously rewarded corner was also calculated (using 12 visit bins). These variables were 'factors' in the model. 'Responses" were variables form MWM (Mean daily path lengths and Probe trial variables), as well as Freezing % during all phases of the FC test. PLS identified one significant component (Q^2 = - 0.107), explaining 9.45% of the sum of squares of the response variables. Since Q^2 was < 0, this model was not discussed.

Several approaches can be used to clarify relationships between variables measured during different behavioral tests. Simple correlation analyses increase the probability of Type I errors and increase the risk of spurious results. Relationships between variables recorded during classical behavioral tests have been generally explored using Factor analysis (Whimbey and Denenberg, 1967, Lamberty and Gower, 1993, Rodgers and Johnson, 1995, Arendash and King, 2002). The variable space is thus reduced to

several underlying factors. However our data set has characteristics which make it less suitable for Factor analysis: a small sample size and bi - modal distribution for several variables (e.g. *OA arm visits* and *OA distance*). Therefore Cluster analysis was considered appropriate to explore the relationships between variables as it is less strict regarding the assumptions. Following Cluster analysis, a smaller number of variables for each test was retained for use in PLS analysis.

Only a relatively small part of the sum of squares in the PLS "responses", i.e. behavior in conventional tests, could be explained by IC behavioral "factors" in model 1. However, due to the exploratory nature of our study only several of the possible models were investigated, and further analyses are required to disentangle latent mechanisms.

Table 2 Input variables for PLS model 1

Variable name	Description (IC behavior)
Latency to first visitStartTimecode (s)	Time until the first visit is recorded
Latency to first lickStartTimecode	Time until the first lick is recorded
DARKFirst12VisitNumber	First 12h (dark phase) visit number
DARKFirst12VisitDuration	First 12h (dark phase) visit duration
DARKFirst12NosepokeNumber	First 12h (dark phase) nosepoke number
DARKFirst12NosepokeDuration	First 12h (dark phase) nosepoke duration
DARKFirst12LickNumber	First 12h (dark phase) lick number
DARKFirst12LickDuration	First 12h (dark phase) lick duration
habituation visits number	Overall habituation visit number
habit visits duration	Overall habituation visit duration
habit nosepoke number	Overall habituation nosepoke number
habituation nosepoke duration	Overall habituation nosepoke duration
habituation lick number	Overall habituation lick number
habituation lick duration	Overall habituation lick duration

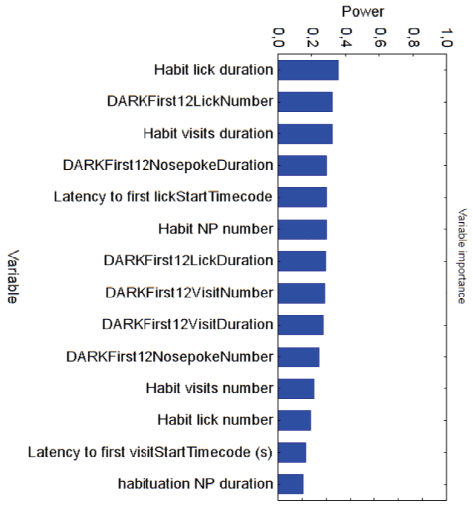

Fig.12 Variable importance in the PCA model for predictor factors.

Table 3 Response variables for PLS model 1

Variable name	Description
Closed arm distance (cm)	EPM distance traveled in the open arms
Closed arm visits count	EPM closed arm visits
Latency to visit an arm (ms)	EPM latency to visit an arm
Distance in center (cm = total-OA-CA)	EPM Distance in center
5 min Distance traversed (m)	5 min distance in OF
30 min Time in Center (s)	30 min distance in OF
Fecal boli	Fecal boli deposited during OF test
30 min vertical time (s)	30 min vertical activity time in OF
5 min Time in Center (s)	5 min horizontal activity

5 CONCLUSIONS

1. Behavioral measures of *activity* and *learning* obtained using the IntelliCage methodology are consistent between laboratories.
2. In the presence of additional devices (IntelliMaze) both *activity* and *learning* measures in the IntelliCage are modified. Thus, visit frequency to IntelliCage corners is reduced and discrimination in a place learning task enhanced.
3. The behavior of B6, D2 and F1 hybrid mice could be distinguished by the IntelliCage methodology. Our findings of higher visit frequency for the B6 mice and better place discrimination are in line with earlier work using conventional behavioral tests. Unpredicted behavioral differences between strains also emerged from the studies.
4. Differences in activity and learning between tg- and non-tg APP-ArcSwe mice, a model for AD can be detected using the IntelliCage.
5. Exposure to IntelliCage can influence behavior in subsequent tests.
6. For a comprehensive behavioral assessment, there is a need to further develop and validate IntelliCage protocols.

6 GENERAL REMARKS AND FUTURE PERSPECTIVES

In order to provide a valuable tool for behavioral research, the parameters measured in IntelliCage should be valid and replicable. Through multi-center studies, the replicability of measurements was confirmed, as we obtained results which are not interfered by laboratory identity. However, the limitation of our study arises from the limited number of strains we have employed. The validity of behavioral parameters obtained in the IntelliCage is currently evaluated. Several approaches have been undertaken, such as characterizing brain lesioned mice or inbred mice with known phenotypic traits. Administration of drugs with well characterized behavioral effects can also provide useful information. However, using the IntelliCage, new, replicable behavioral endpoints can emerge, as shown in our studies.

Behavioral testing using automated methods, such as the IntelliCage, is still in its infancy. Detailed behavioral analyses in automated long lasting tests, as well as valid inter-laboratory results bring one step closer the possibility of acquiring relevant biological data in a reliable way on a large scale.

For AD mouse models though, further applications taking advantage of the short, already validated protocols (e.g. Passive avoidance test) seem a reasonable approach. However, care must be exercised to consider potential genotype-by-environment interactions. Development of future applications in the IntelliCage (such as drug testing) requires fast protocols with good discriminative value. To this end, modeling approaches using data from bigger experimental groups will provide a clearer answer regarding which behavioral domains are better predicted by IntelliCage results. To enable collaborative work on common data bases, harmonious development of protocols and nomenclature are required.

The amount of data available for analyses (although containing only 3 types of events) is suitable for data mining approaches. Relevant behavioral shifts and time points could be detected. Furthermore, the dynamics of social interactions in the IntelliCage have yet to be described.

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I tend to forget things; this is why I came to the KI-Alzheimer Disease Center, in fact. Therefore I will start by thanking each of the people who feel contributed to this work or to my other life experiences; I also thank those I felt were close, in any way. Please forgive me if I will fail to mention your name!

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The contribution of THE MICE is not forgotten!

7 APPENDIX

Table 1 Peer reviewed selected publications where the IntelliCage methodology was used.

	Reference	Animals	Protocol	Parameters analyzed/Notes
1	Galsworthy M. et al. 2005 Behav Brain Res	bank voles wood mice n=7/group male (separate groups)	Free adaptation („habituation“)- Corner preference learning: least preferred corner Reversal learning: new least preferred	- Cumulative number of corner visits (90 min) - Latency to first visit - Visits / hour during the first day - Visits / hour average for 8 days = habituated activity - Baseline corner preference
2	Knapska E. et al. 2006 Learn Mem.	B6 total 28 n = 4-5 x 6 cages female 8 weeks old (mixed groups)	Nosepoke adaptation 48 h Drinking session adapt (2 h/day) Place preference test – (10 % sucrose – least preferred) Aversive training – (air puff – most preferred)	- Percentage of corner visits (Results preference: increased after short training and after long training; higher after long training than after short training). (Results avoidance: the fast acquisition of response was seen; percentage of corner visits decreased both after short training and long training; lower after long training was than after short training)
3	Safi K. et al. 2006 Cognition, Brain, Behavior	B6 diazepam/saline TT n = 12 female 7 weeks old	Water deprivation 18 h Door opening training 2 days (water access in one corner/mouse). Deprivation Air-puff upon first NP (1/ 24 h) Deprivation +TT Air-puff upon first NP (1/ 24 h) Punished drinking 2 days Switch treatment groups repeat	-Latency to visit: time between first punished visit and the next visit -Number of visits: equivalent of activity -Duration of visits: time in the corner with water access (4 h) -Number of licks: total number of licks during the period animals had access to water -Duration of licks: total lick duration -Duration of nose pokes: measure of the total time animals tried to get access to the bottles by nosepoking (successful and unsuccessful).

4	Onishchenko N. et al. 2007 Toxicol. Sci.	ARE-hPAP tg on B6/Bkl TT = in utero exposure to MeHg n = 4 TT MeHg n =3 control	Adaptation Corner preference learning: (least preferred corner open). Reversal learning (reverse corner; corner open). Patrolling: - patrolling conditioning (2 d): all doors closed, any opens upon NP. If drink occured the green LED turned on. - patrolling: after each correct visit (green LED light on) the next corner, in a clock wise manner available for drinking	-Latency to enter the first corner -Visits during the first 30-min -Visits with and without drinking over the first day -Activity during ‘‘sunset’’ and ‘‘night’’ -Activity during dark period -% Correct visits -% Correct nosepokes
5	Kiryk A. et al. 2008 Neurotox. Res.	GLT1 KO mice (glutamate -1 transporters) n = 43 wt n = 40 GLT1 (+/-)	Habituation (48 h) Nose poke adaptation Place preference (10% sucrose)/ least preferred Place avoidance (1 bar air puff)/ most preferred	-Percentage of correct nosepokes (n.s.)

6	Mechan A.O. et al. 2009 J. of Neurosci.Meth.	C57B1/6J n = 12 / group middle aged (14 m.o.) young adult (2.5 m.o.) female note: different suppliers	Exploration – (all doors open, 6 d) Nosepoke adaptation (2 d; nosepoke opens door for 7 sec) Place learning I (least preferred corner / mouse) Reversal place learning I-diagonally opposite corner of least preferred corner open, Behavioural extinction-all doors open (10 days) Novelty induced exploration-(Eppendorf below corner) Reversal place learning II – (least preferred corner open; air-puff if visits to“incorrect” corners; 1-2-bar 2 sec)	-Corner visits number (reduced with age) -Licks / visit (higher in aged mice) -% Corner preference (number of “incorrect” corner visits/total number of corner visits) × 100. - % Visits to novel corners ([(visits to “novel object corner”)/total number of corner visits)×100] (Note: in the presence of punishment improved discrimination)
7	Jaholkowski P. et al. 2009 Learning & Memory	D2 KO mice (cyclin D2 lacking adult brain neurogenesis) Place preference n = 17/group Place avoidance n = 10/group	Adaptation (Open gate) Nosepoking Place preference with (Open Gate / Nosepoke) Nose poke Place avoidance	-Corner visits number -Visits to correct cornet -Visits to punished corner -Nosepoke number -Nosepoke to correct corner

8	Rudenko O. et al. 2009 Behav Brain Res.	R6/2 transgenic and littermates n =18 n=14 4-5 weeks/ 12 weeks female	Adaptation (doors open; LED light; nosepoke) Place learning (least preferred) (+LED light) Reversal learning (new least preferred) Place avoidance (0.4 bar, 4sec) Patrolling (clock-wise manner) Side alternation task	-Visit number (first 3 h) -Latency to visit, nosepoke, lick corner -Nuber of visits without drink/total number of visits -Lick duration, lick number -Nosepokes per visit -Preference for the correct corner -Drinking times -Percentage of visits to punished corner after air puff -Correct corner preference
9	Barlind A. et al. 2010 Exp Brain Res	B6 control /irradiation two litters 8-10 mice/cage 8 weeks male	Adaptation One corner randomly assigned – nose poke evaluation daily	-Ratio and number of nose pokes per correct visit
10	Zhu, C. et al. 2010 J Cereb Blood Flow Metab.	B6/J Isoflurane anesthesia 35min/4 days n=16 (tt) n=15 (ctrl) male	Adaptation (acclimatization) Nosepoke adaptation Corner learning: (east preferred reinforced with water and fructose and errors punished with air-puff) Reversal learning (least preferred) Same as the task above, but the corner was switched to the new least preferred	-Incorrect visit ratio -Number of visits -Recognition index -Time in the correct corner -Correct/incorrect nosepokes -Nopepoke per visit in correct corner

11	Branchi I. et al. 2010 Psychoneuroendocrinol.	Outbred CD-1, Swiss-derived strain (ICR) n = 8 standard nesting n = 8 communal nesting 4 SN + 4 CN/Intellicage male 5 months	Sucrose 4% sol 1 bottle/corner Accomodation Basal monitoring Social stress (Mixing mice in different Intellicages) No social stress Social stress II	-Drinking sucrose % lick from baseline -Nosepokes/exploratory activity
12	Krackow S. et al. 2010 Genes Brain Behav.	B6/ D2 B6D2F1 n = 4 n = 4 n = 4 mixed/per cage x 4 laboratories x 2 cages each	Free adaptation (doors open) Nosepoke adaptation (nosepoke for access to water) Drinking session adaptation (2 h/night) Place learning Water available in one corner per animal Reversal Learning Water access shifted to another corner	Study I, included

13	Voikar V. et al. 2010 Behav Brain Res.	B6/NCrl D2 B6D2 F1 Hippocampal lesioned Striatum lesioned Multiple groups for test/retest validation	Adaptation (free adaptation) Nosepoke adaptation (all doors open once per visit, for 5 sec, unpon NP) Corner avoidance learning (one randomly selected corner for each animal delivered short/long 0.8 barr air-puff) ITI – regular home cage setting, water deprived for 18h Probe trial – same as nosepoke adaptation	Nosepoke response suppression
14	Sekiguchi K et al. Phytother Res. 2010	ddY mice i.c.v injection of Aβ i.p saline i.p. donepezil i.p.yokukansan n=8/group 6 week old male	Adaptation Nosepoke adaptation Place learning Reversal learning	-Correct-choice ratio (Note Aβ group lower than control. Ameliorated in both yokukansan and donepezil groups).
15	Konopka W. et al. 2010 J Neurosci.	B6 inducible disruption of the Dicer1 gene n=11-12/cage female	Adaptation open doors Adaptation sweetened water (10 % sucrose) –least preferred (Place learning)	-Correct visits/total number of visits

16	Karlsson N. et al. 2011 Radiation Research	B6 (irradiated/control) n = 10/cage 16 week old males mixed	Acclimatization and preference for 0.1 (%) fructose Place learning Reversal learning I Reversal learning II Negative (air-puff) and positive (LED lights) separate experiments Social boxes annexes	-Number of licks to sweetened water bottles -Total number of visits -Total number of nosepokes -Time until all four corners are visited -Nosepokes/day -Nosepoke/ incorrect visit /previously correct visit -Incorrect visit ratio -Incorrect nosepoke ratio social preference in SocialBox add-on
17	Endo T. et al. 2011 Behav Brain Res.	B6 D2 ICR n = 8-15/group different ags (3, 6, 8, 12 mo) male (3 centers) (2 providers)	Adaptation 3 days Nosepoke (6 days/ 4 secs) Drink session nosepoke (3 h/24 h) Behavioral sequencing task (2 opposite rewarding corners) Serial reversal learning	-Discrimination error rate= incorrect visits within the first 100 visits/session (never rewarded corners) -Alternation score=percentage of alternate visits in specific diagonals (rewarded or never-rewarded)within total visits in a session or choice block -Cumulative errors=a cumulative number of corner visits to never-rewarded corners -Discrimination index = [10 − (number of errors within 10 choices) × 10].

8 REFERENCES

AISEN, P. S., PETERSEN, R. C., DONOHUE, M. C., GAMST, A., RAMAN, R., THOMAS, R. G., WALTER, S., TROJANOWSKI, J. Q., SHAW, L. M., BECKETT, L. A., JACK, C. R., JR., JAGUST, W., TOGA, A. W., SAYKIN, A. J., MORRIS, J. C., GREEN, R. C. & WEINER, M. W. 2010. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. *Alzheimers Dement,* 6**,** 239-46.

AMBREE, O., TOUMA, C., GORTZ, N., KEYVANI, K., PAULUS, W., PALME, R. & SACHSER, N. 2006. Activity changes and marked stereotypic behavior precede Abeta pathology in TgCRND8 Alzheimer mice. *Neurobiol Aging,* 27**,** 955-64.

AMMASSARI-TEULE, M. & CASTELLANO, C. 2004. Strains of rodents and the pharmacology of learning and memory. *Neural Plast,* 11**,** 205-16.

AMMASSARI-TEULE, M., RESTIVO, L. & PASSINO, E. 2000. Contextual-dependent effects of nucleus accumbens lesions on spatial learning in mice. *Neuroreport,* 11**,** 2485-90.

ANDO, K., LEROY, K., HERAUD, C., KABOVA, A., YILMAZ, Z., AUTHELET, M., SUAIN, V., DE DECKER, R. & BRION, J. P. 2010. Deletion of murine tau gene increases tau aggregation in a human mutant tau transgenic mouse model. *Biochem Soc Trans,* 38**,** 1001-5.

ANDORFER, C., KRESS, Y., ESPINOZA, M., DE SILVA, R., TUCKER, K. L., BARDE, Y. A., DUFF, K. & DAVIES, P. 2003. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem,* 86**,** 582-90.

ARENDASH, G. W., GORDON, M. N., DIAMOND, D. M., AUSTIN, L. A., HATCHER, J. M., JANTZEN, P., DICARLO, G., WILCOCK, D. & MORGAN, D. 2001. Behavioral assessment of Alzheimer's transgenic mice following long-term Abeta vaccination: task specificity and correlations between Abeta deposition and spatial memory. *DNA Cell Biol,* 20**,** 737-44.

ARENDASH, G. W. & KING, D. L. 2002. Intra- and intertask relationships in a behavioral test battery given to Tg2576 transgenic mice and controls. *Physiol Behav,* 75**,** 643-52.

ARRIAGADA, P. V., GROWDON, J. H., HEDLEY-WHYTE, E. T. & HYMAN, B. T. 1992a. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology,* 42**,** 631-9.

ARRIAGADA, P. V., MARZLOFF, K. & HYMAN, B. T. 1992b. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology,* 42**,** 1681-8.

BACKMAN, L., JONES, S., BERGER, A. K., LAUKKA, E. J. & SMALL, B. J. 2005. Cognitive impairment in preclinical Alzheimer's disease: a meta-analysis. *Neuropsychology,* 19**,** 520-31.

BADDELEY, A. 2010. Working memory. *Curr Biol,* 20**,** R136-40.

BAILEY, K. R. & CRAWLEY, J. N. 2009. Anxiety-Related Behaviors in Mice.

BALLARD, C. G., GAUTHIER, S., CUMMINGS, J. L., BRODATY, H., GROSSBERG, G. T., ROBERT, P. & LYKETSOS, C. G. 2009. Management of agitation and aggression associated with Alzheimer disease. *Nat Rev Neurol,* 5**,** 245-55.

BARTKE, A. & BROWN-BORG, H. 2004. Life extension in the dwarf mouse. *Curr Top Dev Biol,* 63**,** 189-225.

BELZUNG, C. & LE PAPE, G. 1994. Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiol Behav,* 56**,** 623-8.

BERGMANS, B. A., SHARIATI, S. A., HABETS, R. L., VERSTREKEN, P., SCHOONJANS, L., MULLER, U., DOTTI, C. G. & DE STROOPER, B. 2010. Neurons generated from APP/APLP1/APLP2 triple knockout embryonic stem cells behave normally in vitro and in vivo: lack of evidence for a cell autonomous role of the amyloid precursor protein in neuronal differentiation. *Stem Cells,* 28**,** 399-406.

BEVINS, R. A. & BESHEER, J. 2006. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nat Protoc,* 1**,** 1306-11.

BILLINGS, L. M., ODDO, S., GREEN, K. N., MCGAUGH, J. L. & LAFERLA, F. M. 2005. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron,* 45**,** 675-88.

BIRNEY. 2004. *http://mouse.ensembl.org/* [Online]. Available: http://mouse.ensembl.org/ [Accessed].

BOTHE, G. W., BOLIVAR, V. J., VEDDER, M. J. & GEISTFELD, J. G. 2004. Genetic and behavioral differences among five inbred mouse strains commonly used in the production of transgenic and knockout mice. *Genes Brain Behav,* 3**,** 149-57.

BRANCHI, I. & ALLEVA, E. 2006. Communal nesting, an early social enrichment, increases the adult anxiety-like response and shapes the role of social context in modulating the emotional behavior. *Behav Brain Res,* 172**,** 299-306.

BRANCHI, I., D'ANDREA, I., CIRULLI, F., LIPP, H. P. & ALLEVA, E. 2010. Shaping brain development: mouse communal nesting blunts adult neuroendocrine and behavioral response to social stress and modifies chronic antidepressant treatment outcome. *Psychoneuroendocrinology,* 35**,** 743-51.

BRANCHI, I. & RICCERI, L. 2004. Refining learning and memory assessment in laboratory rodents. An ethological perspective. *Ann Ist Super Sanita,* 40**,** 231-6.

BRANCHI, I., SANTUCCI, D., PUOPOLO, M. & ALLEVA, E. 2004. Neonatal behaviors associated with ultrasonic vocalizations in mice (mus musculus): A slow-motion analysis. *Dev Psychobiol,* 44**,** 37-44.

BROWN, S. D., CHAMBON, P. & DE ANGELIS, M. H. 2005. EMPReSS: standardized phenotype screens for functional annotation of the mouse genome. *Nat Genet,* 37**,** 1155.

BROWN, S. D. & HANCOCK, J. M. 2006. The mouse genome. *Genome Dyn,* 2**,** 33-45.

BROWN, S. D., WURST, W., KUHN, R. & HANCOCK, J. M. 2009. The functional annotation of mammalian genomes: the challenge of phenotyping. *Annu Rev Genet,* 43**,** 305-33.

BRYANT, C. D., ZHANG, N. N., SOKOLOFF, G., FANSELOW, M. S., ENNES, H. S., PALMER, A. A. & MCROBERTS, J. A. 2008. Behavioral differences among C57BL/6 substrains: implications for transgenic and knockout studies. *J Neurogenet,* 22**,** 315-31.

BURCH, R. L. 2009. The progress of humane experimental technique since 1959: a personal view. *Altern Lab Anim,* 37**,** 269-75.

BURGESS, N., MAGUIRE, E. A. & O'KEEFE, J. 2002. The human hippocampus and spatial and episodic memory. *Neuron,* 35**,** 625-41.

CARRO, E., TREJO, J. L., GERBER, A., LOETSCHER, H., TORRADO, J., METZGER, F. & TORRES-ALEMAN, I. 2006. Therapeutic actions of insulin-like growth factor I on APP/PS2 mice with severe brain amyloidosis. *Neurobiol Aging,* 27**,** 1250-7.

CASERTA, M. T., BANNON, Y., FERNANDEZ, F., GIUNTA, B., SCHOENBERG, M. R. & TAN, J. 2009. Normal brain aging clinical, immunological, neuropsychological, and neuroimaging features. *Int Rev Neurobiol,* 84**,** 1-19.

CASTAGNE, V., MOSER, P., ROUX, S. & PORSOLT, R. D. 2011. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci,* Chapter 8**,** Unit8 10A.

CASTELLANI, R. J., ROLSTON, R. K. & SMITH, M. A. 2010. Alzheimer disease. *Dis Mon,* 56**,** 484-546.

CHADMAN, K. K., YANG, M. & CRAWLEY, J. N. 2009. Criteria for validating mouse models of psychiatric diseases. *Am J Med Genet B Neuropsychiatr Genet,* 150B**,** 1-11.

CHENG, I. H., SCEARCE-LEVIE, K., LEGLEITER, J., PALOP, J. J., GERSTEIN, H., BIEN-LY, N., PUOLIVALI, J., LESNE, S., ASHE, K. H., MUCHOWSKI, P. J. & MUCKE, L. 2007. Accelerating amyloid-beta fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J Biol Chem,* 282**,** 23818-28.

CHIA, R., ACHILLI, F., FESTING, M. F. & FISHER, E. M. 2005. The origins and uses of mouse outbred stocks. *Nat Genet,* 37**,** 1181-6.

CIRULLI, F., BERRY, A., BONSIGNORE, L. T., CAPONE, F., D'ANDREA, I., ALOE, L., BRANCHI, I. & ALLEVA, E. 2010. Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels. *Neurosci Biobehav Rev,* 34**,** 808-20.

CIRULLI, F., SANTUCCI, D., LAVIOLA, G., ALLEVA, E. & LEVINE, S. 1994. Behavioral and hormonal responses to stress in the newborn mouse: effects of maternal deprivation and chlordiazepoxide. *Dev Psychobiol,* 27**,** 301-16.

CLAYTON, N. S. & DICKINSON, A. 1998. Episodic-like memory during cache recovery by scrub jays. *Nature,* 395**,** 272-4.

CODITA, A., WINBLAD, B. & MOHAMMED, A. H. 2006. Of mice and men: more neurobiology in dementia. *Curr Opin Psychiatry,* 19**,** 555-63.

COLLE, M. A., HAUW, J. J., CRESPEAU, F., UCHIHARA, T., AKIYAMA, H., CHECLER, F., PAGEAT, P. & DUYKAERTS, C. 2000. Vascular and parenchymal Abeta deposition in the aging dog: correlation with behavior. *Neurobiol Aging,* 21**,** 695-704.

COLTON, C. A., VITEK, M. P., WINK, D. A., XU, Q., CANTILLANA, V., PREVITI, M. L., VAN NOSTRAND, W. E., WEINBERG, J. B. & DAWSON, H. 2006. NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A,* 103**,** 12867-72.

COSTA, D. A., CRACCHIOLO, J. R., BACHSTETTER, A. D., HUGHES, T. F., BALES, K. R., PAUL, S. M., MERVIS, R. F., ARENDASH, G. W. & POTTER, H. 2007. Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiol Aging,* 28**,** 831-44.

COTEL, M. C., JAWHAR, S., CHRISTENSEN, D. Z., BAYER, T. A. & WIRTHS, O. 2010. Environmental enrichment fails to rescue working memory deficits, neuron loss, and neurogenesis in APP/PS1KI mice. *Neurobiol Aging*.

CRACCHIOLO, J. R., MORI, T., NAZIAN, S. J., TAN, J., POTTER, H. & ARENDASH, G. W. 2007. Enhanced cognitive activity--over and above social or physical activity--is required to protect Alzheimer's mice against cognitive impairment, reduce Abeta deposition, and increase synaptic immunoreactivity. *Neurobiol Learn Mem,* 88**,** 277-94.

CRAWLEY, J. N. 2004. Designing mouse behavioral tasks relevant to autistic-like behaviors. *Ment Retard Dev Disabil Res Rev,* 10**,** 248-58.

CRAWLEY, J. N. 2007. Mouse behavioral assays relevant to the symptoms of autism. *Brain Pathol,* 17**,** 448-59.

CRAWLEY, J. N., BELKNAP, J. K., COLLINS, A., CRABBE, J. C., FRANKEL, W., HENDERSON, N., HITZEMANN, R. J., MAXSON, S. C., MINER, L. L., SILVA, A. J., WEHNER, J. M., WYNSHAW-BORIS, A. & PAYLOR, R. 1997. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl),* 132**,** 107-24.

DARMOPIL, S., PETANJEK, Z., MOHAMMED, A. H. & BOGDANOVIC, N. 2009. Environmental enrichment alters dentate granule cell morphology in oldest-old rat. *J Cell Mol Med,* 13**,** 1845-56.

DE GREEVE, P. C. M., HAMPSON, J. & ZUTPHEN, L. F. M. 2001. Legislation and animal experimentation. *In:* ZUTPHEN, L. F. M., BAUMANS, V. & BEYNEN, A. (eds.) *Principles of Laboratory Animal Science. A contribution to the humane use and care of animals and the quality of experimental results.* Elsevier.

DE SOUSA, R. Available: <http://plato.stanford.edu/archives/spr2010/entries/emotion/>. by Standford http://plato.stanford.edu/entries/emotion/ [Accessed Apr 2011].

DE VISSER, L., VAN DEN BOS, R., KUURMAN, W. W., KAS, M. J. & SPRUIJT, B. M. 2006. Novel approach to the behavioural characterization of inbred mice: automated home cage observations. *Genes Brain Behav,* 5**,** 458-66.

DE VISSER, L., VAN DEN BOS, R. & SPRUIJT, B. M. 2005. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. *Behav Brain Res,* 160**,** 382-8.
DEACON, R. M. 2006a. Assessing hoarding in mice. *Nat Protoc,* 1**,** 2828-30.
DEACON, R. M. 2006b. Assessing nest building in mice. *Nat Protoc,* 1**,** 1117-9.
DEACON, R. M. 2006c. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. *Nat Protoc,* 1**,** 122-4.
DEACON, R. M. 2006d. Housing, husbandry and handling of rodents for behavioral experiments. *Nat Protoc,* 1**,** 936-46.
DEACON, R. M. 2009. Burrowing: a sensitive behavioural assay, tested in five species of laboratory rodents. *Behav Brain Res,* 200**,** 128-33.
DEACON, R. M., CHOLERTON, L. L., TALBOT, K., NAIR-ROBERTS, R. G., SANDERSON, D. J., ROMBERG, C., KOROS, E., BORNEMANN, K. D. & RAWLINS, J. N. 2008. Age-dependent and -independent behavioral deficits in Tg2576 mice. *Behav Brain Res,* 189**,** 126-38.
DEACON, R. M., KOROS, E., BORNEMANN, K. D. & RAWLINS, J. N. 2009. Aged Tg2576 mice are impaired on social memory and open field habituation tests. *Behav Brain Res,* 197**,** 466-8.
DEACON, R. M. & RAWLINS, J. N. 2005. Hippocampal lesions, species-typical behaviours and anxiety in mice. *Behav Brain Res,* 156**,** 241-9.
DEACON, R. M. & RAWLINS, J. N. 2006. T-maze alternation in the rodent. *Nat Protoc,* 1**,** 7-12.
DENENBERG, V. H. 1969. Open-field behavior in the rat: what does it mean? *Ann N Y Acad Sci,* 159**,** 852-9.
DEVITO, L. M. & EICHENBAUM, H. 2010. Distinct contributions of the hippocampus and medial prefrontal cortex to the "what-where-when" components of episodic-like memory in mice. *Behav Brain Res,* 215**,** 318-25.
DHAHBI, J. M., KIM, H. J., MOTE, P. L., BEAVER, R. J. & SPINDLER, S. R. 2004. Temporal linkage between the phenotypic and genomic responses to caloric restriction. *Proc Natl Acad Sci U S A,* 101**,** 5524-9.
DIAMOND, M. C. 1988. Can we change our brains ? *Enriching Heredity. The Impact of the Environment on the Anatomy of the Brain.* New York: The Free Press. A Division of Macmillan, Inc.
DIAMOND, M. C. 2001. Response of the brain to enrichment. *An Acad Bras Cienc,* 73**,** 211-20.
DIAMOND, M. C., INGHAM, C. A., JOHNSON, R. E., BENNETT, E. L. & ROSENZWEIG, M. R. 1976. Effects of environment on morphology of rat cerebral cortex and hippocampus. *J Neurobiol,* 7**,** 75-85.
DINELEY, K. T., XIA, X., BUI, D., SWEATT, J. D. & ZHENG, H. 2002. Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins. *J Biol Chem,* 277**,** 22768-80.
DOETSCHMAN, T. 2009. Influence of genetic background on genetically engineered mouse phenotypes. *Methods Mol Biol,* 530**,** 423-33.
DUBOIS, B., FELDMAN, H. H., JACOVA, C., DEKOSKY, S. T., BARBERGER-GATEAU, P., CUMMINGS, J., DELACOURTE, A., GALASKO, D., GAUTHIER, S., JICHA, G., MEGURO, K., O'BRIEN, J., PASQUIER, F., ROBERT, P., ROSSOR, M., SALLOWAY, S., STERN, Y., VISSER, P. J. & SCHELTENS, P. 2007. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol,* 6**,** 734-46.
ENGLUND, H., SEHLIN, D., JOHANSSON, A. S., NILSSON, L. N., GELLERFORS, P., PAULIE, S., LANNFELT, L. & PETTERSSON, F. E. 2007. Sensitive ELISA detection of amyloid-beta protofibrils in biological samples. *J Neurochem,* 103**,** 334-45.
FAGAN, A. M., WATSON, M., PARSADANIAN, M., BALES, K. R., PAUL, S. M. & HOLTZMAN, D. M. 2002. Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol Dis,* 9**,** 305-18.
FANSELOW, M. S. 2000. Contextual fear, gestalt memories, and the hippocampus. *Behav Brain Res,* 110**,** 73-81.

FANSELOW, M. S. 2010. From contextual fear to a dynamic view of memory systems. *Trends Cogn Sci,* 14**,** 7-15.
FERCHMIN, P. A. & ETEROVIC, V. A. 1986. Forty minutes of experience increase the weight and RNA content of cerebral cortex in periadolescent rats. *Dev Psychobiol,* 19**,** 511-9.
FESTING, M. F. 2010. Inbred strains should replace outbred stocks in toxicology, safety testing, and drug development. *Toxicol Pathol,* 38**,** 681-90.
FEUSNER, J. D., HEMBACHER, E. & PHILLIPS, K. A. 2009. The mouse who couldn't stop washing: pathologic grooming in animals and humans. *CNS Spectr,* 14**,** 503-13.
FLANAGAN, K. A., WEBB, W. & STOWERS, L. 2011. Analysis of male pheromones that accelerate female reproductive organ development. *PLoS One,* 6**,** e16660.
FRATIGLIONI, L., DE RONCHI, D. & AGUERO-TORRES, H. 1999. Worldwide prevalence and incidence of dementia. *Drugs Aging,* 15**,** 365-75.
FRATIGLIONI, L. & QIU, C. 2009. Prevention of common neurodegenerative disorders in the elderly. *Exp Gerontol,* 44**,** 46-50.
FREDENS, K. 1981. Genetic variation in the histoarchitecture of the hippocampal region of mice. *Anat Embryol (Berl),* 161**,** 265-81.
FRICK, K. M., STILLNER, E. T. & BERGER-SWEENEY, J. 2000. Mice are not little rats: species differences in a one-day water maze task. *Neuroreport,* 11**,** 3461-5.
FUCHS, T., IACOBUCCI, P., MACKINNON, K. M. & PANKSEPP, J. 2010. Infant-mother recognition in a social rodent (Octodon degus). *J Comp Psychol,* 124**,** 166-75.
GAMES, D., ADAMS, D., ALESSANDRINI, R., BARBOUR, R., BERTHELETTE, P., BLACKWELL, C., CARR, T., CLEMENS, J., DONALDSON, T., GILLESPIE, F. & ET AL. 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature,* 373**,** 523-7.
GANEA, K., LIEBL, C., STERLEMANN, V., MULLER, M. B. & SCHMIDT, M. V. 2007. Pharmacological validation of a novel home cage activity counter in mice. *J Neurosci Methods,* 162**,** 180-6.
GATES, H., MALLON, A. M. & BROWN, S. D. 2011. High-throughput mouse phenotyping. *Methods,* 53**,** 394-404.
GELDMACHER, D. S. 2007. Treatment guidelines for Alzheimer's disease: redefining perceptions in primary care. *Prim Care Companion J Clin Psychiatry,* 9**,** 113-21.
GIMENEZ-LLORT, L., BLAZQUEZ, G., CANETE, T., JOHANSSON, B., ODDO, S., TOBENA, A., LAFERLA, F. M. & FERNANDEZ-TERUEL, A. 2007. Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: a role for intraneuronal amyloid. *Neurosci Biobehav Rev,* 31**,** 125-47.
GOEDERT, M., WISCHIK, C. M., CROWTHER, R. A., WALKER, J. E. & KLUG, A. 1988. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci U S A,* 85**,** 4051-5.
GOTZ, J., GLADBACH, A., PENNANEN, L., VAN EERSEL, J., SCHILD, A., DAVID, D. & ITTNER, L. M. 2010. Animal models reveal role for tau phosphorylation in human disease. *Biochim Biophys Acta,* 1802**,** 860-71.
GOTZ, J. & ITTNER, L. M. 2008. Animal models of Alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci,* 9**,** 532-44.
GOTZ, J., SCHONROCK, N., VISSEL, B. & ITTNER, L. M. 2009. Alzheimer's disease selective vulnerability and modeling in transgenic mice. *J Alzheimers Dis,* 18**,** 243-51.
GREEN, T. A., ALIBHAI, I. N., ROYBAL, C. N., WINSTANLEY, C. A., THEOBALD, D. E., BIRNBAUM, S. G., GRAHAM, A. R., UNTERBERG, S., GRAHAM, D. L., VIALOU, V., BASS, C. E., TERWILLIGER, E. F., BARDO, M. T. & NESTLER, E. J. 2010. Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens. *Biol Psychiatry,* 67**,** 28-35.

GRESACK, J. E. & FRICK, K. M. 2003. Male mice exhibit better spatial working and reference memory than females in a water-escape radial arm maze task. *Brain Res,* 982**,** 98-107.
GRIFFITHS, D., DICKINSON, A. & CLAYTON, N. 1999. Episodic memory: what can animals remember about their past? *Trends Cogn Sci,* 3**,** 74-80.
GRILL, J. D. & CUMMINGS, J. L. 2010. Current therapeutic targets for the treatment of Alzheimer's disease. *Expert Rev Neurother,* 10**,** 711-28.
GUENET, J. L. & BONHOMME, F. 2003. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet,* 19**,** 24-31.
HALUK, D. M. & WICKMAN, K. 2010. Evaluation of study design variables and their impact on food-maintained operant responding in mice. *Behav Brain Res,* 207**,** 394-401.
HAMPEL, H., BURGER, K., TEIPEL, S. J., BOKDE, A. L., ZETTERBERG, H. & BLENNOW, K. 2008. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimers Dement,* 4**,** 38-48.
HARDY, J. 2006. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. *J Alzheimers Dis,* 9**,** 151-3.
HARRIS, J. A., DEVIDZE, N., VERRET, L., HO, K., HALABISKY, B., THWIN, M. T., KIM, D., HAMTO, P., LO, I., YU, G. Q., PALOP, J. J., MASLIAH, E. & MUCKE, L. 2010. Transsynaptic progression of amyloid-beta-induced neuronal dysfunction within the entorhinal-hippocampal network. *Neuron,* 68**,** 428-41.
HAVENAAR, R., MEIJER, J. C., MORTON, D. B., RITSKES-HOITINGA, J. & P., Z. 2001. Biology and husbandry of laboratory animals *In:* ZUTPHEN, L. F. M., BAUMANS, V. & BEYNEN, A. C. (eds.) *Principles of Laboratory Animal Science. A contribution to the humane use and care of animals and to the quality of experimental results - revised edition* Elsevier
HELMEKE, C., SEIDEL, K., POEGGEL, G., BREDY, T. W., ABRAHAM, A. & BRAUN, K. 2009. Paternal deprivation during infancy results in dendrite- and time-specific changes of dendritic development and spine formation in the orbitofrontal cortex of the biparental rodent Octodon degus. *Neuroscience,* 163**,** 790-8.
HEYSER, C. J. 2004. Assessment of developmental milestones in rodents. *Curr Protoc Neurosci,* Chapter 8**,** Unit 8 18.
HIGUCHI, K., NAIKI, H., KITAGAWA, K., HOSOKAWA, M. & TAKEDA, T. 1991. Mouse senile amyloidosis. ASSAM amyloidosis in mice presents universally as a systemic age-associated amyloidosis. *Virchows Arch B Cell Pathol Incl Mol Pathol,* 60**,** 231-8.
HILLEN, H., BARGHORN, S., STRIEBINGER, A., LABKOVSKY, B., MULLER, R., NIMMRICH, V., NOLTE, M. W., PEREZ-CRUZ, C., VAN DER AUWERA, I., VAN LEUVEN, F., VAN GAALEN, M., BESPALOV, A. Y., SCHOEMAKER, H., SULLIVAN, J. P. & EBERT, U. 2010. Generation and therapeutic efficacy of highly oligomer-specific beta-amyloid antibodies. *J Neurosci,* 30**,** 10369-79.
HOLCOMB, L. A., GORDON, M. N., JANTZEN, P., HSIAO, K., DUFF, K. & MORGAN, D. 1999. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. *Behav Genet,* 29**,** 177-85.
HOLMES, A. & HARIRI, A. R. 2003. The serotonin transporter gene-linked polymorphism and negative emotionality: placing single gene effects in the context of genetic background and environment. *Genes Brain Behav,* 2**,** 332-5.
HSIAO, K. 1998. Strain dependent and invariant features of transgenic mice expressing Alzheimer amyloid precursor proteins. *Prog Brain Res,* 117**,** 335-41.
HSIAO, K. K., BORCHELT, D. R., OLSON, K., JOHANNSDOTTIR, R., KITT, C., YUNIS, W., XU, S., ECKMAN, C., YOUNKIN, S., PRICE, D. & ET AL. 1995. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron,* 15**,** 1203-18.
HU, X. L., BERGSTROM, S. A., BRINK, M., RONNBACK, A. & DAHLQVIST, P. 2010. Enriched environment increases spinophilin mRNA expression and spinophilin immunoreactive dendritic spines in hippocampus and cortex. *Neurosci Lett,* 476**,** 79-83.

HUITRON-RESENDIZ, S., SANCHEZ-ALAVEZ, M., GALLEGOS, R., BERG, G., CRAWFORD, E., GIACCHINO, J. L., GAMES, D., HENRIKSEN, S. J. & CRIADO, J. R. 2002. Age-independent and age-related deficits in visuospatial learning, sleep-wake states, thermoregulation and motor activity in PDAPP mice. *Brain Res,* 928**,** 126-37.

ICKES, B. R., PHAM, T. M., SANDERS, L. A., ALBECK, D. S., MOHAMMED, A. H. & GRANHOLM, A. C. 2000. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp Neurol,* 164**,** 45-52.

INESTROSA, N. C., REYES, A. E., CHACON, M. A., CERPA, W., VILLALON, A., MONTIEL, J., MERABACHVILI, G., ALDUNATE, R., BOZINOVIC, F. & ABOITIZ, F. 2005. Human-like rodent amyloid-beta-peptide determines Alzheimer pathology in aged wild-type Octodon degu. *Neurobiol Aging,* 26**,** 1023-8.

ITTNER, L. M. & GOTZ, J. 2007. Pronuclear injection for the production of transgenic mice. *Nat Protoc,* 2**,** 1206-15.

JANKOWSKY, J. L., MELNIKOVA, T., FADALE, D. J., XU, G. M., SLUNT, H. H., GONZALES, V., YOUNKIN, L. H., YOUNKIN, S. G., BORCHELT, D. R. & SAVONENKO, A. V. 2005. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci,* 25**,** 5217-24.

JANKOWSKY, J. L., XU, G., FROMHOLT, D., GONZALES, V. & BORCHELT, D. R. 2003. Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *J Neuropathol Exp Neurol,* 62**,** 1220-7.

JENSEN, M., HARTMANN, T., ENGVALL, B., WANG, R., ULJON, S. N., SENNVIK, K., NASLUND, J., MUEHLHAUSER, F., NORDSTEDT, C., BEYREUTHER, K. & LANNFELT, L. 2000. Quantification of Alzheimer amyloid beta peptides ending at residues 40 and 42 by novel ELISA systems. *Mol Med,* 6**,** 291-302.

JHUANG, H., GARROTE, E., EDELMAN, N., POGGIO, T., STEELE, A. & SERRE, T. 2010. Trainable, Vision-Based Automated Home Cage Behavioral Phenotyping. *Proceedings of Measuring Behavior 2010,* (Eindhoven, The Netherlands, August 24-27, 2010), Eds. A.J. Spink, F. Grieco, O.E. Krips, L.W.S. Loijens, L.P.J.J. Noldus, and P.H. Zimmerman**,** 149-152.

JI, Q., LUO, Z. X., YUAN, C. X., WIBLE, J. R., ZHANG, J. P. & GEORGI, J. A. 2002. The earliest known eutherian mammal. *Nature,* 416**,** 816-22.

JICHA, G. A. & CARR, S. A. 2010. Conceptual evolution in Alzheimer's disease: implications for understanding the clinical phenotype of progressive neurodegenerative disease. *J Alzheimers Dis,* 19**,** 253-72.

KAROLCHIK. 2003. *http://genome.ucsc.edu/* [Online]. Available: http://genome.ucsc.edu/ [Accessed].

KEMPERMANN, G., FABEL, K., EHNINGER, D., BABU, H., LEAL-GALICIA, P., GARTHE, A. & WOLF, S. A. 2010. Why and how physical activity promotes experience-induced brain plasticity. *Front Neurosci,* 4**,** 189.

KILGORE, M., MILLER, C. A., FASS, D. M., HENNIG, K. M., HAGGARTY, S. J., SWEATT, J. D. & RUMBAUGH, G. 2010. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology,* 35**,** 870-80.

KISELYCZNYK, C. & HOLMES, A. 2011. All (C57BL/6) Mice are not Created Equal. *Front Neurosci,* 5**,** 10.

KIVIPELTO, M., ROVIO, S., NGANDU, T., KAREHOLT, I., ESKELINEN, M., WINBLAD, B., HACHINSKI, V., CEDAZO-MINGUEZ, A., SOININEN, H., TUOMILEHTO, J. & NISSINEN, A. 2008. Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: a population-based study. *J Cell Mol Med,* 12**,** 2762-71.

KNOBLOCH, M., KONIETZKO, U., KREBS, D. C. & NITSCH, R. M. 2007. Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. *Neurobiol Aging,* 28**,** 1297-306.

KRACKOW, S., VANNONI, E., CODITA, A., MOHAMMED, A. H., CIRULLI, F., BRANCHI, I., ALLEVA, E., REICHELT, A., WILLUWEIT, A., VOIKAR, V., COLACICCO, G., WOLFER, D. P., BUSCHMANN, J. U., SAFI, K. &

LIPP, H. P. 2010. Consistent behavioral phenotype differences between inbred mouse strains in the IntelliCage. *Genes Brain Behav,* 9**,** 722-31.
KUDRYAVTSEVA, N. N., BAKSHTANOVSKAYA, I. V. & KORYAKINA, L. A. 1991. Social model of depression in mice of C57BL/6J strain. *Pharmacol Biochem Behav,* 38**,** 315-20.
KURIEN, B. T., GROSS, T. & SCOFIELD, R. H. 2005. Barbering in mice: a model for trichotillomania. *BMJ,* 331**,** 1503-5.
LALONDE, R., KIM, H. D. & FUKUCHI, K. 2004. Exploratory activity, anxiety, and motor coordination in bigenic APPswe + PS1/DeltaE9 mice. *Neurosci Lett,* 369**,** 156-61.
LALONDE, R., KIM, H. D., MAXWELL, J. A. & FUKUCHI, K. 2005. Exploratory activity and spatial learning in 12-month-old APP(695)SWE/co+PS1/DeltaE9 mice with amyloid plaques. *Neurosci Lett,* 390**,** 87-92.
LALONDE, R., QIAN, S. & STRAZIELLE, C. 2003. Transgenic mice expressing the PS1-A246E mutation: effects on spatial learning, exploration, anxiety, and motor coordination. *Behav Brain Res,* 138**,** 71-9.
LAMBERTY, Y. & GOWER, A. J. 1993. Spatial processing and emotionality in aged NMRI mice: a multivariate analysis. *Physiol Behav,* 54**,** 339-43.
LAZAROV, O., ROBINSON, J., TANG, Y. P., HAIRSTON, I. S., KORADE-MIRNICS, Z., LEE, V. M., HERSH, L. B., SAPOLSKY, R. M., MIRNICS, K. & SISODIA, S. S. 2005. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell,* 120**,** 701-13.
LEDOUX, J. E. 2000. Emotion circuits in the brain. *Annu Rev Neurosci,* 23**,** 155-84.
LEMERE, C. A., BEIERSCHMITT, A., IGLESIAS, M., SPOONER, E. T., BLOOM, J. K., LEVERONE, J. F., ZHENG, J. B., SEABROOK, T. J., LOUARD, D., LI, D., SELKOE, D. J., PALMOUR, R. M. & ERVIN, F. R. 2004. Alzheimer's disease abeta vaccine reduces central nervous system abeta levels in a non-human primate, the Caribbean vervet. *Am J Pathol,* 165**,** 283-97.
LEMERE, C. A., MAIER, M., JIANG, L., PENG, Y. & SEABROOK, T. J. 2006. Amyloid-beta immunotherapy for the prevention and treatment of Alzheimer disease: lessons from mice, monkeys, and humans. *Rejuvenation Res,* 9**,** 77-84.
LESNE, S., KOH, M. T., KOTILINEK, L., KAYED, R., GLABE, C. G., YANG, A., GALLAGHER, M. & ASHE, K. H. 2006. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature,* 440**,** 352-7.
LINE, S. J., BARKUS, C., COYLE, C., JENNINGS, K. A., DEACON, R. M., LESCH, K. P., SHARP, T. & BANNERMAN, D. M. 2011. Opposing alterations in anxiety and species-typical behaviours in serotonin transporter overexpressor and knockout mice. *Eur Neuropsychopharmacol,* 21**,** 108-16.
LLOYD, S. E., MAYTHAM, E. G., POTA, H., GRIZENKOVA, J., MOLOU, E., UPHILL, J., HUMMERICH, H., WHITFIELD, J., ALPERS, M. P., MEAD, S. & COLLINGE, J. 2009a. HECTD2 is associated with susceptibility to mouse and human prion disease. *PLoS Genet,* 5**,** e1000383.
LLOYD, S. E., ROSSOR, M., FOX, N., MEAD, S. & COLLINGE, J. 2009b. HECTD2, a candidate susceptibility gene for Alzheimer's disease on 10q. *BMC Med Genet,* 10**,** 90.
LOGUE, S. F., PAYLOR, R. & WEHNER, J. M. 1997. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci,* 111**,** 104-13.
LORD, A., ENGLUND, H., SODERBERG, L., TUCKER, S., CLAUSEN, F., HILLERED, L., GORDON, M., MORGAN, D., LANNFELT, L., PETTERSSON, F. E. & NILSSON, L. N. 2009a. Amyloid-beta protofibril levels correlate with spatial learning in Arctic Alzheimer's disease transgenic mice. *FEBS J,* 276**,** 995-1006.
LORD, A., GUMUCIO, A., ENGLUND, H., SEHLIN, D., SUNDQUIST, V. S., SODERBERG, L., MOLLER, C., GELLERFORS, P., LANNFELT, L., PETTERSSON, F. E. & NILSSON, L. N. 2009b. An amyloid-beta protofibril-selective antibody prevents amyloid formation in a mouse model of Alzheimer's disease. *Neurobiol Dis,* 36**,** 425-34.
LORD, A., KALIMO, H., ECKMAN, C., ZHANG, X. Q., LANNFELT, L. & NILSSON, L. N. 2006. The Arctic Alzheimer mutation facilitates early

intraneuronal Abeta aggregation and senile plaque formation in transgenic mice. *Neurobiol Aging,* 27**,** 67-77.

MADRONAL, N., LOPEZ-ARACIL, C., RANGEL, A., DEL RIO, J. A., DELGADO-GARCIA, J. M. & GRUART, A. 2010. Effects of enriched physical and social environments on motor performance, associative learning, and hippocampal neurogenesis in mice. *PLoS One,* 5**,** e11130.

MALONEY, B., GE, Y. W., GREIG, N. & LAHIRI, D. K. 2004. Presence of a "CAGA box" in the APP gene unique to amyloid plaque-forming species and absent in all APLP-1/2 genes: implications in Alzheimer's disease. *FASEB J,* 18**,** 1288-90.

MANDILLO, S., TUCCI, V., HOLTER, S. M., MEZIANE, H., BANCHAABOUCHI, M. A., KALLNIK, M., LAD, H. V., NOLAN, P. M., OUAGAZZAL, A. M., COGHILL, E. L., GALE, K., GOLINI, E., JACQUOT, S., KREZEL, W., PARKER, A., RIET, F., SCHNEIDER, I., MARAZZITI, D., AUWERX, J., BROWN, S. D., CHAMBON, P., ROSENTHAL, N., TOCCHINI-VALENTINI, G. & WURST, W. 2008. Reliability, robustness, and reproducibility in mouse behavioral phenotyping: a cross-laboratory study. *Physiol Genomics,* 34**,** 243-55.

MANGIALASCHE, F., SOLOMON, A., WINBLAD, B., MECOCCI, P. & KIVIPELTO, M. 2010. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol,* 9**,** 702-16.

MARQUES, J. M., OLSSON, I. A., OGREN, S. O. & DAHLBORN, K. 2008. Evaluation of exploration and risk assessment in pre-weaning mice using the novel cage test. *Physiol Behav,* 93**,** 139-47.

MATSUO, N., TAKAO, K., NAKANISHI, K., YAMASAKI, N., TANDA, K. & MIYAKAWA, T. 2010. Behavioral profiles of three C57BL/6 substrains. *Front Behav Neurosci,* 4**,** 29.

MCILWAIN, K. L., MERRIWEATHER, M. Y., YUVA-PAYLOR, L. A. & PAYLOR, R. 2001. The use of behavioral test batteries: effects of training history. *Physiol Behav,* 73**,** 705-17.

MCOMISH, C. E. & HANNAN, A. J. 2007. Enviromimetics: exploring gene environment interactions to identify therapeutic targets for brain disorders. *Expert Opin Ther Targets,* 11**,** 899-913.

MECHAN, A. O., WYSS, A., RIEGER, H. & MOHAJERI, M. H. 2009. A comparison of learning and memory characteristics of young and middle-aged wild-type mice in the IntelliCage. *J Neurosci Methods,* 180**,** 43-51.

MEKADA, K., ABE, K., MURAKAMI, A., NAKAMURA, S., NAKATA, H., MORIWAKI, K., OBATA, Y. & YOSHIKI, A. 2009. Genetic differences among C57BL/6 substrains. *Exp Anim,* 58**,** 141-9.

MENARD, C., VALASTRO, B., MARTEL, M. A., MARTINOLI, M. G. & MASSICOTTE, G. 2004. Strain-related variations of AMPA receptor modulation by calcium-dependent mechanisms in the hippocampus: contribution of lipoxygenase metabolites of arachidonic acid. *Brain Res,* 1010**,** 134-43.

MENDL, M., BURMAN, O. H. & PAUL, E. S. 2010. An integrative and functional framework for the study of animal emotion and mood. *Proc Biol Sci,* 277**,** 2895-904.

MILNER, B. 2005. The medial temporal-lobe amnesic syndrome. *Psychiatr Clin North Am,* 28**,** 599-611, 609.

MINKEVICIENE, R., BANERJEE, P. & TANILA, H. 2004. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. *J Pharmacol Exp Ther,* 311**,** 677-82.

MODIGH, K. 1974. Effects of social stress on the turnover of brain catecholamines and 5-hydroxytryptamine in mice. *Acta Pharmacol Toxicol (Copenh),* 34**,** 97-105.

MOECHARS, D., GILIS, M., KUIPERI, C., LAENEN, I. & VAN LEUVEN, F. 1998. Aggressive behaviour in transgenic mice expressing APP is alleviated by serotonergic drugs. *Neuroreport,* 9**,** 3561-4.

MOHAJERI, M. H. & WOLFER, D. P. 2009. Neprilysin deficiency-dependent impairment of cognitive functions in a mouse model of amyloidosis. *Neurochem Res,* 34**,** 717-26.

MOHAMMED, A. H., ZHU, S. W., DARMOPIL, S., HJERLING-LEFFLER, J., ERNFORS, P., WINBLAD, B., DIAMOND, M. C., ERIKSSON, P. S. & BOGDANOVIC, N. 2002. Environmental enrichment and the brain. *Prog Brain Res,* 138**,** 109-33.

MORGAN, D., DIAMOND, D. M., GOTTSCHALL, P. E., UGEN, K. E., DICKEY, C., HARDY, J., DUFF, K., JANTZEN, P., DICARLO, G., WILCOCK, D., CONNOR, K., HATCHER, J., HOPE, C., GORDON, M. & ARENDASH, G. W. 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature,* 408**,** 982-5.

MORSE, H. C. 2007. Building a Better Mouse: One Hundred Years of Genetics and Biology. *In:* FOX, J. G., DAVISSON, M. T., QUIMBY, F. W., BARTHOLD, S. W., NEWCOMER, C. E. & SMITH, A. L. (eds.) *The Mouse in Biomedical Research, 2nd Edition.* 2 ed.: Academic Press, Elsevier.

MOTT, R. 2007. A haplotype map for the laboratory mouse. *Nat Genet,* 39**,** 1054-6.

NESTLER, E. J. & HYMAN, S. E. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci,* 13**,** 1161-9.

NGUYEN, P. V., ABEL, T., KANDEL, E. R. & BOURTCHOULADZE, R. 2000. Strain-dependent differences in LTP and hippocampus-dependent memory in inbred mice. *Learn Mem,* 7**,** 170-9.

NOLAN, P. M., PETERS, J., STRIVENS, M., ROGERS, D., HAGAN, J., SPURR, N., GRAY, I. C., VIZOR, L., BROOKER, D., WHITEHILL, E., WASHBOURNE, R., HOUGH, T., GREENAWAY, S., HEWITT, M., LIU, X., MCCORMACK, S., PICKFORD, K., SELLEY, R., WELLS, C., TYMOWSKA-LALANNE, Z., ROBY, P., GLENISTER, P., THORNTON, C., THAUNG, C., STEVENSON, J. A., ARKELL, R., MBURU, P., HARDISTY, R., KIERNAN, A., ERVEN, A., STEEL, K. P., VOEGELING, S., GUENET, J. L., NICKOLS, C., SADRI, R., NASSE, M., ISAACS, A., DAVIES, K., BROWNE, M., FISHER, E. M., MARTIN, J., RASTAN, S., BROWN, S. D. & HUNTER, J. 2000. A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet,* 25**,** 440-3.

O'SULLIVAN, G. J., O'TUATHAIGH, C. M., CLIFFORD, J. J., O'MEARA, G. F., CROKE, D. T. & WADDINGTON, J. L. 2006. Potential and limitations of genetic manipulation in animals. *Drug Discovery Today: Technologies,* 3**,** 173-180.

ODDO, S., CACCAMO, A., SHEPHERD, J. D., MURPHY, M. P., GOLDE, T. E., KAYED, R., METHERATE, R., MATTSON, M. P., AKBARI, Y. & LAFERLA, F. M. 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron,* 39**,** 409-21.

OLSSON, I. A. & DAHLBORN, K. 2002. Improving housing conditions for laboratory mice: a review of "environmental enrichment". *Lab Anim,* 36**,** 243-70.

PALLIER, P. N., DREW, C. J. & MORTON, A. J. 2009. The detection and measurement of locomotor deficits in a transgenic mouse model of Huntington's disease are task- and protocol-dependent: influence of non-motor factors on locomotor function. *Brain Res Bull,* 78**,** 347-55.

PALOP, J. J., JONES, B., KEKONIUS, L., CHIN, J., YU, G. Q., RABER, J., MASLIAH, E. & MUCKE, L. 2003. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proc Natl Acad Sci U S A,* 100**,** 9572-7.

PALOP, J. J., MUCKE, L. & ROBERSON, E. D. 2011. Quantifying biomarkers of cognitive dysfunction and neuronal network hyperexcitability in mouse models of Alzheimer's disease: depletion of calcium-dependent proteins and inhibitory hippocampal remodeling. *Methods Mol Biol,* 670**,** 245-62.

PANKSEPP, J. 2010. Affective consciousness in animals: perspectives on dimensional and primary process emotion approaches. *Proc Biol Sci,* 277**,** 2905-7.

PASSER, M., SMITH, R., HOLT, A., BREMNER, A., SUTHERLAND, E. & VLIEK, M. L. W. 2009. Psychology: The Science of Mind and Behaviour. *In:* PASSER, M., SMITH, R., HOLT, A., BREMNER, A., SUTHERLAND, E. & VLIEK, M. L. W. (eds.).

PAYLOR, R., MORRISON, S. K., RUDY, J. W., WALTRIP, L. T. & WEHNER, J. M. 1992. Brief exposure to an enriched environment improves performance on the Morris water task and increases hippocampal cytosolic protein kinase C activity in young rats. *Behav Brain Res,* 52**,** 49-59.

PELLOW, S., CHOPIN, P., FILE, S. E. & BRILEY, M. 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods,* 14**,** 149-67.

PENDSE, A. A., ARBONES-MAINAR, J. M., JOHNSON, L. A., ALTENBURG, M. K. & MAEDA, N. 2009. Apolipoprotein E knock-out and knock-in mice: atherosclerosis, metabolic syndrome, and beyond. *J Lipid Res,* 50 Suppl**,** S178-82.

PEREZ, M., RIBE, E., RUBIO, A., LIM, F., MORAN, M. A., RAMOS, P. G., FERRER, I., ISLA, M. T. & AVILA, J. 2005. Characterization of a double (amyloid precursor protein-tau) transgenic: tau phosphorylation and aggregation. *Neuroscience,* 130**,** 339-47.

PERL, D. P. 2010. Neuropathology of Alzheimer's disease. *Mt Sinai J Med,* 77**,** 32-42.

PHAM, T. M., ICKES, B., ALBECK, D., SODERSTROM, S., GRANHOLM, A. C. & MOHAMMED, A. H. 1999. Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. *Neuroscience,* 94**,** 279-86.

PHILIPSON, O., LORD, A., GUMUCIO, A., O'CALLAGHAN, P., LANNFELT, L. & NILSSON, L. N. 2010. Animal models of amyloid-beta-related pathologies in Alzheimer's disease. *FEBS J,* 277**,** 1389-409.

POPOVIC, N., BANO-OTALORA, B., ROL, M. A., CABALLERO-BLEDA, M., MADRID, J. A. & POPOVIC, M. 2009. Aging and time-of-day effects on anxiety in female Octodon degus. *Behav Brain Res,* 200**,** 117-21.

PRICE, J. L., DAVIS, P. B., MORRIS, J. C. & WHITE, D. L. 1991. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol Aging,* 12**,** 295-312.

PUGH, P. L., RICHARDSON, J. C., BATE, S. T., UPTON, N. & SUNTER, D. 2007. Non-cognitive behaviours in an APP/PS1 transgenic model of Alzheimer's disease. *Behavioural Brain Research,* 178**,** 18–28.

REAUME, A. G., HOWLAND, D. S., TRUSKO, S. P., SAVAGE, M. J., LANG, D. M., GREENBERG, B. D., SIMAN, R. & SCOTT, R. W. 1996. Enhanced amyloidogenic processing of the beta-amyloid precursor protein in gene-targeted mice bearing the Swedish familial Alzheimer's disease mutations and a "humanized" Abeta sequence. *J Biol Chem,* 271**,** 23380-8.

REISERER, R. S., HARRISON, F. E., SYVERUD, D. C. & MCDONALD, M. P. 2007. Impaired spatial learning in the APPSwe + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease. *Genes Brain Behav,* 6**,** 54-65.

REZNIKOVA, Z. 2007. What is memory for an intelligent animal ? *Animal Intelligence from individual to social cognition*
Cambridge university press.

RIBE, E. M., PEREZ, M., PUIG, B., GICH, I., LIM, F., CUADRADO, M., SESMA, T., CATENA, S., SANCHEZ, B., NIETO, M., GOMEZ-RAMOS, P., MORAN, M. A., CABODEVILLA, F., SAMARANCH, L., ORTIZ, L., PEREZ, A., FERRER, I., AVILA, J. & GOMEZ-ISLA, T. 2005. Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice. *Neurobiol Dis,* 20**,** 814-22.

RICHTER, H., AMBREE, O., LEWEJOHANN, L., HERRING, A., KEYVANI, K., PAULUS, W., PALME, R., TOUMA, C., SCHABITZ, W. R. & SACHSER, N. 2008. Wheel-running in a transgenic mouse model of Alzheimer's disease: protection or symptom? *Behav Brain Res,* 190**,** 74-84.

RING, S., WEYER, S. W., KILIAN, S. B., WALDRON, E., PIETRZIK, C. U., FILIPPOV, M. A., HERMS, J., BUCHHOLZ, C., ECKMAN, C. B., KORTE, M., WOLFER, D. P. & MULLER, U. C. 2007. The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. *J Neurosci,* 27**,** 7817-26.

RITSKES-HOTINGA, M. & CHWALIBOG, A. 2003. Nutrient Requirements, Experimental Design and Feeding Schedules in Animal Experimentation. *In:* HAU, J. & VAN HOOSIER, G. L. J. (eds.) *Handbook of Laboratory animal Science Second Edition.* CRC Press LLC.

ROCKENSTEIN, E., MALLORY, M., MANTE, M., SISK, A. & MASLIAHA, E. 2001. Early formation of mature amyloid-beta protein deposits in a mutant APP transgenic model depends on levels of Abeta(1-42). *J Neurosci Res,* 66**,** 573-82.

RODGERS, R. J., BOULLIER, E., CHATZIMICHALAKI, P., COOPER, G. D. & SHORTEN, A. 2002. Contrasting phenotypes of C57BL/6JOlaHsd, 129S2/SvHsd and 129/SvEv mice in two exploration-based tests of anxiety-related behaviour. *Physiol Behav,* 77**,** 301-10.

RODGERS, R. J. & JOHNSON, N. J. 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav,* 52**,** 297-303.

ROFINA, J. E., VAN EDEREN, A. M., TOUSSAINT, M. J., SECREVE, M., VAN DER SPEK, A., VAN DER MEER, I., VAN EERDENBURG, F. J. & GRUYS, E. 2006. Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. *Brain Res,* 1069**,** 216-26.

ROGAEV, E. I., SHERRINGTON, R., ROGAEVA, E. A., LEVESQUE, G., IKEDA, M., LIANG, Y., CHI, H., LIN, C., HOLMAN, K., TSUDA, T. & ET AL. 1995. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature,* 376**,** 775-8.

ROGERS, D. C., FISHER, E. M., BROWN, S. D., PETERS, J., HUNTER, A. J. & MARTIN, J. E. 1997. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome,* 8**,** 711-3.

RONNBACK, A., ZHU, S., DILLNER, K., AOKI, M., LILIUS, L., NASLUND, J., WINBLAD, B. & GRAFF, C. 2011. Progressive neuropathology and cognitive decline in a single Arctic APP transgenic mouse model. *Neurobiol Aging,* 32**,** 280-92.

ROSEN, R. F., WALKER, L. C. & LEVINE, H., 3RD 2011. PIB binding in aged primate brain: enrichment of high-affinity sites in humans with Alzheimer's disease. *Neurobiol Aging,* 32**,** 223-34.

ROSENZWEIG, M. R. & BENNETT, E. L. 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res,* 78**,** 57-65.

RUSSELL, W. M. 1995. The development of the three Rs concept. *Altern Lab Anim,* 23**,** 298-304.

RUSTAY, N. R., CRONIN, E. A., CURZON, P., MARKOSYAN, S., BITNER, R. S., ELLIS, T. A., WARING, J. F., DECKER, M. W., RUETER, L. E. & BROWMAN, K. E. 2010. Mice expressing the Swedish APP mutation on a 129 genetic background demonstrate consistent behavioral deficits and pathological markers of Alzheimer's disease. *Brain Res,* 1311**,** 136-47.

SAHGAL, A. 1993. Behavioural Neuroscience. A practical approach. *In:* SAHGAL, A. (ed.). Oxford University Press.

SARASA, M. & PESINI, P. 2009. Natural non-trasgenic animal models for research in Alzheimer's disease. *Curr Alzheimer Res,* 6**,** 171-8.

SAVONENKO, A., XU, G. M., MELNIKOVA, T., MORTON, J. L., GONZALES, V., WONG, M. P., PRICE, D. L., TANG, F., MARKOWSKA, A. L. & BORCHELT, D. R. 2005. Episodic-like memory deficits in the APPswe/PS1dE9 mouse model of Alzheimer's disease: relationships to beta-amyloid deposition and neurotransmitter abnormalities. *Neurobiol Dis,* 18**,** 602-17.

SAYLER, A. & SALMON, M. 1969. Communal nursing in mice: influence of multiple mothers on the growth of the young. *Science,* 164**,** 1309-10.

SCATTONI, M. L., CRAWLEY, J. & RICCERI, L. 2009. Ultrasonic vocalizations: a tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. *Neurosci Biobehav Rev,* 33**,** 508-15.

SCHWACH G., BAUER U., HUTTER-PAIER B., PROKESCH M., RAUTER G., D'ARRIGO A., KOSTNER G . & M., W. 2006. Establishment of a human p75NTR Transgenic Mouse Model. *Poster, ICAD* JSW research.

SCHWEIZER, M. C., HENNIGER, M. S. & SILLABER, I. 2009. Chronic mild stress (CMS) in mice: of anhedonia, 'anomalous anxiolysis' and activity. *PLoS One,* 4**,** e4326.

SELKOE, D. J. 2008. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. *Behav Brain Res,* 192**,** 106-13.

SHANKAR, G. M., LEISSRING, M. A., ADAME, A., SUN, X., SPOONER, E., MASLIAH, E., SELKOE, D. J., LEMERE, C. A. & WALSH, D. M. 2009. Biochemical and immunohistochemical analysis of an Alzheimer's disease mouse model reveals the presence of multiple cerebral Abeta assembly forms throughout life. *Neurobiol Dis,* 36**,** 293-302.

SHERRINGTON, R., ROGAEV, E. I., LIANG, Y., ROGAEVA, E. A., LEVESQUE, G., IKEDA, M., CHI, H., LIN, C., LI, G., HOLMAN, K., TSUDA, T., MAR, L., FONCIN, J. F., BRUNI, A. C., MONTESI, M. P., SORBI, S., RAINERO, I., PINESSI, L., NEE, L., CHUMAKOV, I., POLLEN, D., BROOKES, A., SANSEAU, P., POLINSKY, R. J., WASCO, W., DA SILVA, H. A., HAINES, J. L., PERKICAK-VANCE, M. A., TANZI, R. E., ROSES, A. D., FRASER, P. E., ROMMENS, J. M. & ST GEORGE-HYSLOP, P. H. 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature,* 375**,** 754-60.

SINGLETON, G. R. & KREBS, C. J. 2007. The Secret World of Wild Mice. *In:* FOX, J. G., DAVISSON, M. T., QUIMBY, F. W., BARTHOLD, S. W., NEWCOMER, C. E. & SMITH, A. L. (eds.) *The Mouse in Biomedical Research.* Academic Press, Elsevier.

SLOVITER, R. S. 1989. Calcium-binding protein (calbindin-D28k) and parvalbumin immunocytochemistry: localization in the rat hippocampus with specific reference to the selective vulnerability of hippocampal neurons to seizure activity. *J Comp Neurol,* 280**,** 183-96.

SMITS, B. M. & GOULD, M. N. 2009. "Gene targeting in the rat? Cut it out!". *Mol Interv,* 9**,** 226-9.

SQUIRE, L. R. 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev,* 99**,** 195-231.

SYDOW, A. & MANDELKOW, E. M. 2010. 'Prion-like' propagation of mouse and human tau aggregates in an inducible mouse model of tauopathy. *Neurodegener Dis,* 7**,** 28-31.

TERRY, A. V., JR., BUCCAFUSCO, J. J. & WILSON, C. 2008. Cognitive dysfunction in neuropsychiatric disorders: selected serotonin receptor subtypes as therapeutic targets. *Behav Brain Res,* 195**,** 30-8.

TERRY, A. V., JR., CALLAHAN, P. M., HALL, B. & WEBSTER, S. J. 2011. Alzheimer's disease and age-related memory decline (preclinical). *Pharmacol Biochem Behav*.

TOSCANO, C. D., MCGLOTHAN, J. L. & GUILARTE, T. R. 2006. Experience-dependent regulation of zif268 gene expression and spatial learning. *Exp Neurol,* 200**,** 209-15.

TUCKER, K. P. 2007. Systematics of the Genus Mus. *In:* FOX, J. G., DAVISSON, M. T., QUIMBY, F. W., BARTHOLD, S. W., NEWCOMER, C. E. & SMITH, A. L. (eds.) *The Mouse in Biomedical Research 2nd Edition.* Elsevier, Academic Press.

TULVING, E. & MARKOWITSCH, H. J. 1998. Episodic and declarative memory: role of the hippocampus. *Hippocampus,* 8**,** 198-204.

URLINGER, S., BARON, U., THELLMANN, M., HASAN, M. T., BUJARD, H. & HILLEN, W. 2000. Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. *Proc Natl Acad Sci U S A,* 97**,** 7963-8.

UTOMO, A. R., NIKITIN, A. Y. & LEE, W. H. 1999. Temporal, spatial, and cell type-specific control of Cre-mediated DNA recombination in transgenic mice. *Nat Biotechnol,* 17**,** 1091-6.

VAN DER STAAY, F. J., ARNDT, S. S. & NORDQUIST, R. E. 2009. Evaluation of animal models of neurobehavioral disorders. *Behav Brain Funct,* 5**,** 11.
VAN GROEN, T., KADISH, I., POPOVIC, N., POPOVIC, M., CABALLERO-BLEDA, M., BANO-OTALORA, B., VIVANCO, P., ROL, M. A. & MADRID, J. A. 2009. Age-related brain pathology in Octodon degu: Blood vessel, white matter and Alzheimer-like pathology. *Neurobiol Aging*.
VAN PRAAG, H., KEMPERMANN, G. & GAGE, F. H. 2000. Neural consequences of environmental enrichment. *Nat Rev Neurosci,* 1**,** 191-8.
VAN ZUTPHEN, L. F. M., HEDRICH, H. J., VAN LITH, H. & PRINS, J. B. 2001. Genetic standardization. *In:* VAN ZUTPHEN, B., BAUMANS, V. & BEYNEN, A. (eds.) *Principles of Laboratory Animal Science. A contribution to the humane use and care of animals and to the quality of experimental results.*: Elsevier.
VASILEVKO, V. & HEAD, E. 2009. Immunotherapy in a natural model of Abeta pathogenesis: the aging beagle. *CNS Neurol Disord Drug Targets,* 8**,** 98-113.
VEYRUNES, F., DOBIGNY, G., YANG, F., O'BRIEN, P. C., CATALAN, J., ROBINSON, T. J. & BRITTON-DAVIDIAN, J. 2006. Phylogenomics of the genus Mus (Rodentia; Muridae): extensive genome repatterning is not restricted to the house mouse. *Proc Biol Sci,* 273**,** 2925-34.
VOIKAR, V., COLACICCO, G., GRUBER, O., VANNONI, E., LIPP, H. P. & WOLFER, D. P. 2010. Conditioned response suppression in the IntelliCage: assessment of mouse strain differences and effects of hippocampal and striatal lesions on acquisition and retention of memory. *Behav Brain Res,* 213**,** 304-12.
VOIKAR, V., VASAR, E. & RAUVALA, H. 2004. Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: implications for phenotyping screens. *Genes Brain Behav,* 3**,** 27-38.
VORHEES, C. V. & WILLIAMS, M. T. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc,* 1**,** 848-58.
WAHLSTEN, D. 1975. Genetic variation in the development of mouse brain and behavior: evidence from the middle postnatal period. *Dev Psychobiol,* 8**,** 371-80.
WALF, A. A. & FRYE, C. A. 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc,* 2**,** 322-8.
WEHNER, J. M., SLEIGHT, S. & UPCHURCH, M. 1990. Hippocampal protein kinase C activity is reduced in poor spatial learners. *Brain Res,* 523**,** 181-7.
WHEELER. 2004. *http://www.ncbi.nlm.nih.gov/genome/guide/mouse/* [Online]. Available: http://www.ncbi.nlm.nih.gov/genome/guide/mouse/ [Accessed].
WHIMBEY, A. & DENENBERG, V. 1967. Two independent behavioral dimensions in open-field performance. *J Comp Physiol Psychol* 63**,** 4.
WHISHAW, I. Q. & TOMIE, J. 1996. Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiol Behav,* 60**,** 1191-7.
WILLIAMS, B. M., LUO, Y., WARD, C., REDD, K., GIBSON, R., KUCZAJ, S. A. & MCCOY, J. G. 2001. Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity. *Physiol Behav,* 73**,** 649-58.
WILLIS-OWEN, S. A. & FLINT, J. 2007. Identifying the genetic determinants of emotionality in humans; insights from rodents. *Neurosci Biobehav Rev,* 31**,** 115-24.
WILLNER, P. 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology,* 52**,** 90-110.
WILLNER, P., MUSCAT, R. & PAPP, M. 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev,* 16**,** 525-34.
WILSON, S. C. 1982. Contact-promoting behavior, social development, and relationship with parents in sibling juvenile degus (Octodon degus). *Dev Psychobiol,* 15**,** 257-68.
WIMO, A., JONSSON, L., GUSTAVSSON, A., MCDAID, D., ERSEK, K., GEORGES, J., GULACSI, L., KARPATI, K., KENIGSBERG, P. &

VALTONEN, H. 2010a. The economic impact of dementia in Europe in 2008-cost estimates from the Eurocode project. *Int J Geriatr Psychiatry*.

WIMO, A., WINBLAD, B. & JONSSON, L. 2010b. The worldwide societal costs of dementia: Estimates for 2009. *Alzheimers Dement,* 6**,** 98-103.

WOLF, S. A., KRONENBERG, G., LEHMANN, K., BLANKENSHIP, A., OVERALL, R., STAUFENBIEL, M. & KEMPERMANN, G. 2006. Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. *Biol Psychiatry,* 60**,** 1314-23.

WOLFER, D. P., CRUSIO, W. E. & LIPP, H. P. 2002. Knockout mice: simple solutions to the problems of genetic background and flanking genes. *Trends Neurosci,* 25**,** 336-40.

YANG, H., BELL, T. A., CHURCHILL, G. A. & PARDO-MANUEL DE VILLENA, F. 2007. On the subspecific origin of the laboratory mouse. *Nat Genet,* 39**,** 1100-7.

YOSHIKI, A. & MORIWAKI, K. 2006. Mouse phenome research: implications of genetic background. *ILAR J,* 47**,** 94-102.

ZHAN, S. S., VEERHUIS, R., KAMPHORST, W. & EIKELENBOOM, P. 1995. Distribution of beta amyloid associated proteins in plaques in Alzheimer's disease and in the non-demented elderly. *Neurodegeneration,* 4**,** 291-7.

ZHU, S. W., CODITA, A., BOGDANOVIC, N., HJERLING-LEFFLER, J., ERNFORS, P., WINBLAD, B., DICKINS, D. W. & MOHAMMED, A. H. 2009. Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. *Behav Brain Res,* 197**,** 339-46.

ZIYATDINOVA, S., GUREVICIUS, K., KUTCHIASHVILI, N., BOLKVADZE, T., NISSINEN, J., TANILA, H. & PITKANEN, A. 2011. Spontaneous epileptiform discharges in a mouse model of Alzheimer's disease are suppressed by antiepileptic drugs that block sodium channels. *Epilepsy Res,* 94**,** 75-85.

ZOTA, V., NEMIROVSKY, A., BARON, R., FISHER, Y., SELKOE, D. J., ALTMANN, D. M., WEINER, H. L. & MONSONEGO, A. 2009. HLA-DR alleles in amyloid beta-peptide autoimmunity: a highly immunogenic role for the DRB1*1501 allele. *J Immunol,* 183**,** 3522-30.

ZURITA, E., CHAGOYEN, M., CANTERO, M., ALONSO, R., GONZALEZ-NEIRA, A., LOPEZ-JIMENEZ, A., LOPEZ-MORENO, J. A., LANDEL, C. P., BENITEZ, J., PAZOS, F. & MONTOLIU, L. 2010. Genetic polymorphisms among C57BL/6 mouse inbred strains. *Transgenic Res*.