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Dissertation

General anaesthetics do not deteriorate the β-amyloid (Aβ)-mediated cognitive deficits and Aβ plaque burden in a mouse model of Alzheimer`s disease (AD)

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Abstract

Several studies suggest that anaesthesia is associated with the development and progression of AD. Moreover, studies in cultured cells and animals show that commonly used inhalation anaesthetics such as desflurane and sevoflurane may induce changes consistent with AD neuropathogenesis, e.g., increased amyloid precursor protein (APP) processing and Aβ accumulation. $A\beta_{1-42}$ is thought to be the most pathogenic form and numerous studies have reported that soluble $A\beta_{1-42}$ oligomers affect N-methyl-D-aspartate (NMDA) receptor function and impair cognitive function. The gaseous anaesthetic xenon antagonizes NMDA receptors with low potency and has frequently been reported to be neuroprotective. Here we investigated the effects of xenon, sevoflurane and desflurane in the ArcAβ mouse model of AD.

Cognitive performance of 92 male mice aged 10-14 months were tested in the Water Cross Maze (WCM). The WCM forces the mice to make a right/left decision. Each mouse had to complete the WCM 6 times a day for 7 consecutive days. On the 8th day, anaesthesia was carried out and held stable for 2 hours and on the 9th day a first memory test was performed, which was repeated weekly for one month. Brain slices of the animals were methoxy-stained before analysing the size and number of Aβ Plaques with fluorescence microscopy and tested of significant differences using ANOVA. Using Western Blot, levels of the markers for mGluR5, caspase-3, GluN2B and TNFα were determined after anaesthesia and using ELISA, Aβ levels were determined. As expected, the TG mice of the AD disease model learned the task significantly later than the control mice, which can be explained by the Aβ-mediated AD symptoms of this mouse model. In the WCM no significant differences of accuracy between the groups treated with different anaesthetics, nor among the groups of control and TG mice treated with the same anaesthetic, were observable. In concordance with the behavioural experiments, anaesthesia with either sevoflurane, desflurane or xenon did not show any significant effect on Aβ plaque burden, the investigated western blot markers nor the Aβ levels. These findings indicate that the general anaesthetics of this study do not interact with the Aβ-mediated cognitive deficits, nor Aβ plaque burden in the AD mouse model.

Regarding anaesthesia and hence cognitive testing a direct transfer of the results of this study on human patients is difficult since it is impossible to apply a pure xenon anaesthesia to rodents under normo-baric conditions. Further investigations on that matter are urgently needed in order to provide AD patients with a scientifically based anaesthesia which is not aggravating their cognitive functions.

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I. Introduction

1. Alzheimer's disease (AD)

More than 46 million people in the world suffer from dementia, the vast majority of them of Alzheimer's disease (Mayeux and Stern, 2012). This disease, first described by the German neurologist Alois Alzheimer in 1907, which he referred to as the "illness of forgetfulness", mainly affects the memory and cognition of patients (Alzheimer et al., 1907).
In Germany, almost 1.6 million people are affected by this neurodegenerative disease and the numbers are rising rapidly, with 300,000 new cases every year (German Alzheimer's Association, 2016). As age is the biggest risk factor for AD, incidence rates increase dramatically with age (**Figure 1**).

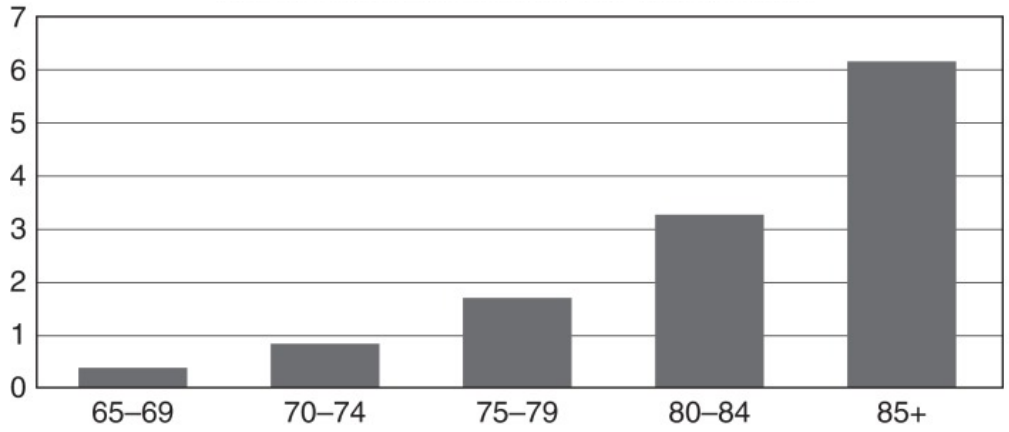

Figure 1 : Annual incidence rate of Alzheimer's disease *(per 100 person years) in the different age categories worldwide (Mayeux & Stern, 2012)*

Extrapolations have shown that by the year 2030, more than 3 million people in Germany will be affected by AD, for which demographic change can be held responsible, since there are every year more new cases than deaths (German Alzheimer's Association, 2016).

2. Symptoms and forms of Alzheimer's disease

The symptoms of AD vary in course of the disease. Cognitive abilities are typically decreasing constantly over time, until the cognitive deficits interfere with normal daily routines and patients are no longer able to perform familiar procedures or to recognize friends and family (Masters et al., 2015). In cases of advanced dementia, those affected are dependent on external help and can no longer cope with the daily routine without support. However, AD not only manifests itself in cognitive impairment, but also results in physical symptoms that can lead to weakening of the body and then to death (Bature et al., 2017). Muscular degeneration, urinary and faecal incontinence and language problems are just examples of a diverse spectrum of symptoms. Due to the relatively frequent occurrence of this neurodegenerative disease and its severe course, the need to find a safe and successful method of prevention and treatment is urgent.

Most patients (> 95%) suffer from the sporadic form of Alzheimer's disease, which usually begins at the age of 80-90 years, much later than the hereditary form, where the mean onset is 45 years (Masters et al., 2015). However, the course and symptoms of the two forms are similar.

The average duration of AD is 10-20 years before leading to death and according to the international Alzheimer's Association, the illness can be divided into 3 phases, which are not clearly distinguishable from each other:

In the early or mild phase, the patients are still self-sufficient, however, first mild memory problems occur, such as word finding disorders, forgetting of names of newly encountered persons or recently read texts and losing valuable items. This is mostly noticed by the relatives rather than by the patients themselves.

The middle or moderate phase typically lasts the longest. It is characterized by significant problems in everyday activities, but patients can still remember important details and persons of their lives. Typical symptoms of this phase include mood swings, poor spatial and temporal orientation, the tendency to wander, irregular sleep rhythms and the loss of episodic memories of the patient’s life.

In the advanced or late phase, it eventually comes to loss of responsiveness to the environment, loss of movement control and impaired speech ability. Typically, patients are dependent on constant help with everyday activities and have difficulty sitting, walking and swallowing in addition to mental symptoms. Increased bed restraint contributes to the worsening of the general condition and often results in infections such as pneumonia or ulcers, which can quickly lead to death.
(Alzheimer's Association, 2018)

To find a reliable method for the prevention of this until now incurable disease would be a big step for medical research, but unfortunately there is no cure in sight yet. High age, diabetes, hypertension, obesity, and high cholesterol are risk factors for Alzheimer's disease which risk patients need to avoid, but preventive drugs or even vaccines are not yet available (Bane and Cole, 2015). In recent studies some biomarkers are found to be related to Alzheimer’s disease and indicate that a long asymptomatic but already active phase before presentation of the first symptoms is taking course (Rafii and Aisen, 2015).
Therefore, further research into the causes and risk factors of the disease is very important for the prevention of AD and the anticipation of bad outcomes.

3. Neuropathology

The first scientific study of AD was carried out in 1907 by Alois Alzheimer, whose interest in the disease was aroused by a 51-year-old woman with severe impairment of memory and behavioural problems. Posthumously, Alzheimer examined the patient's brain and discovered conspicuous protein deposits ("plaques"), which he blamed for the symptoms (Alzheimer et al., 1907).
Although the exact causes and mechanisms of the disease are still unclear, the accumulation of plaques discovered by Alzheimer in 1907 (today also called ß-amyloid peptides), together with the protein tau, discovered half a century later, are still thought to be the two main causes of Alzheimer's disease (Arora et al., 2014).

3.1 Amyloid β

The accumulating amyloid-β (Aβ) peptides in patients with Alzheimer's disease are normal physiological metabolites of the brain with a probable trophic function (Dawkins and Small, 2014). The molecule from which the β-amyloid is produced is the amyloid precursor protein (APP) and was mapped to chromosome 21 (Robakis et al., 1987). In the healthy brain, α-secretases cleave the APP, resulting in small, neuroprotective proteins (so-called sAPPs) which are then further processed by γ-secretase into p3 peptides (Ganten and Greither, 2004). In Alzheimer's patients this process is disturbed. Resulting from the action of the two enzymes β-secretase and γ-secretase, a neurotoxic fragment, the β-amyloid is produced from the APP, which accumulates in the brain (Matsui et al., 2007).

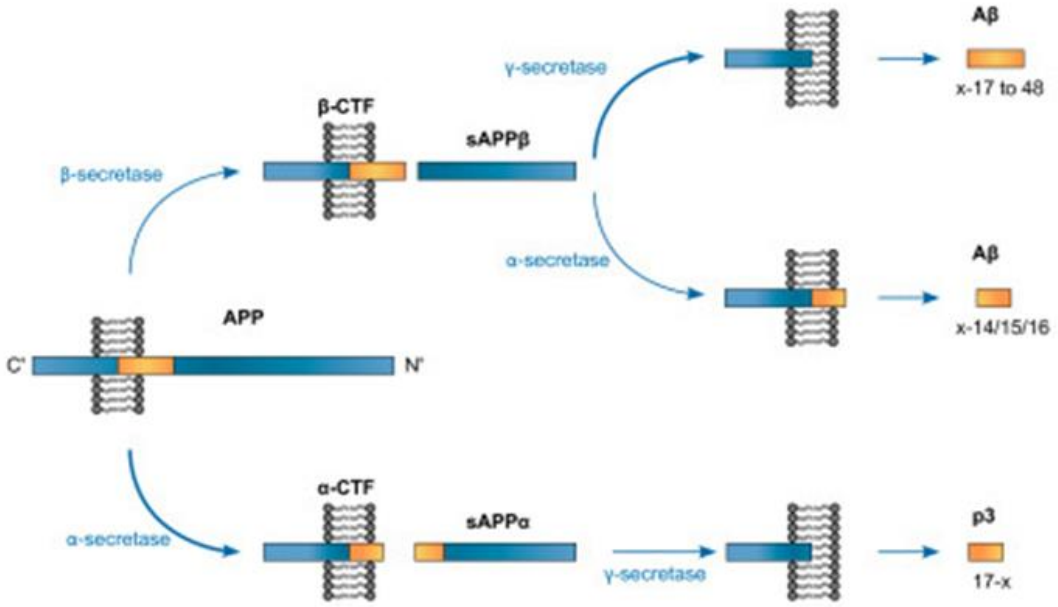

Figure 2: Generation of different amyloid-beta domain-derived peptides from the amyloid precursor protein *(Kummer & Heneka, 2014)*

The accumulations of β-amyloid in the brain of patients with AD are due to a disturbed production-elimination ratio and form indeed the major part of the plaques described by Alois Alzheimer (Arora et al., 2014). The abnormal accumulation of soluble Aβ oligomers affects *N*-methyl-d-aspartate (NMDA) receptor function and abolishes hippocampal long-term potentiation (LTP), therefore it is an important factor in AD pathology (Burge et al., 2019).

The second pathological phenomenon thought responsible for AD is the hyperphosphorylation and accumulation of the tau protein in nerve cells as neurofibrillary tangles ("tauopathy") (Ganten and Greither, 2004). Since tauopathy can be found not only in AD patients, but also in patients with other neurological diseases and dementias such as Pick's disease, dementia pugilistica or silver grain disease, the exact influence of tau is still unclear. Researchers suspect that tauopathy can be considered as a consequence of the pathological amyloid concentrations and suggest that Aβ aggregation is the sole known factor in the development of Alzheimer's disease (Ontiveros-Torres et al., 2016). The focus of this work lays therefore on Aβ pathology.

4. Therapeutic approaches

The treatment of Alzheimer's patients consists of both drug and non-drug therapy. The non-drug treatment can be used to facilitate the patient’s life in many ways. In order to reduce the psychiatric symptoms mainly behavioural therapy, memory training and occupational therapy are used, which promote the memory and competence to cope with daily life. Speech therapy is supposed to maintain the ability to speak and swallow, while physiotherapy is an established treatment to counteract the physical effects of the disease. Non-drug treatments is commonly accepted to have a positive effect on individual symptoms, but due to the weak study situation, no positive effect has been proven in the long term (German Neurological Society, 2016).

Scientifically better studied are the effects of a longer-term drug intake. The treatment focuses on the one hand on the maintenance of mental performance and the coping with everyday life and on the other hand to the attenuation of psychological symptoms and behavioural disorders, which is achieved by the usual psychotropic drugs such as risperidone (Kongpakwattana et al., 2018).

According to the S3 guideline for the treatment of dementias of the German Society of Neurology, acetylcholinesterase inhibitors such as donepezil and rivastigmine, as well as the non-competitive NMDA antagonist memantine, are currently approved for the reduction of the core symptoms of Alzheimer's disease. However, these therapeutic approaches, based on a change in neurotransmission, have had so far no proven positive influence on the course of the disease, but serve purely as a symptomatic therapy (German Alzheimer's Association, 2016).

5. Influence of anaesthesia on Alzheimer's disease

Since the available treatment of AD is so far purely symptomatically oriented, but cannot eliminate the cause of the disease, the affected patients must live to the end of their lives with this neurodegenerative impairment.
The likelihood that an Alzheimer's patient must undergo anaesthesia after the onset of the disease, possibly even before the diagnosis, is therefore quite high. In addition to falls and accidents that can be triggered by the medication or the disease itself, especially the advanced age and the associated multimorbidity of the patients is a factor that makes an operation with associated anaesthesia frequently necessary, since annually 66 Million patients in the age of 65 and above undergo surgery (Jiang and Jiang, 2015).

5.1 Postoperative cognitive dysfunction (POCD)

In addition to the usual risks after a surgical intervention such as bleeding, infection and pain, there may also be neurological side effects after a procedure. At hospital release 40% of over-60-year olds have cognitive performance limitations after surgery with anaesthesia, 3 months later still around 10% (Rundshagen, 2014). This so called "postoperative cognitive dysfunction" (POCD), which occurs more often in older age, was described as early as the 1950s (Bedford, 1955). The symptoms of POCD consist of postoperative newly developed deficits such as reduced memory, impaired ability to combine and reduced psychomotor dexterity. This leads to an increased risk of accidents in everyday life, such as in road traffic (Chung and Assmann, 2008).

The mechanisms that lead to POCD have not yet been conclusively clarified. In addition to immunological causes, an influence of the used anaesthetics is discussed, as studies have shown that a shorter time of the anaesthesia reduces the likelihood of POCD (Rundshagen, 2014). The influence of anaesthetics on neurocognition is, however, not clearly proven in humans, in contrast to the mouse model (Tang et al., 2017) . In twin studies on children for example, there has been no conclusive evidence of neurotoxicity (Stratmann, 2011).

Because there is no standardized clinical test available for the diagnosis of POCD, consistent data collection is difficult and sometimes various symptomatic complexes such as the demential syndrome, delirious syndrome, or the akinetic crisis are falsely referred to as POCD (Arora et al., 2014).
It is thought that POCD not only has temporary effects in patients with neurodegenerative diseases (especially Alzheimer's disease), but that it can also accelerate the course of the disease (Fong et al., 2009).
In a comparative study of magnetic resonance imaging (MRI) examinations 5-9 months after surgery, Alzheimer's disease patients showed increased degeneration of the gray matter, atrophic changes in the hippocampus, as well as enlargement of the lateral ventricles compared to the control group (Kline et al., 2012). Therefore, it is very important to take precautions to

minimize the occurrence of POCD in at-risk patients, as well as identify and avoid trigger factors which could be for example different inhalative anaesthetics (Inan and Ozkose Satirlar, 2015).
The preoperational precautions should consist of a standardized assessment of the patient's cognitive abilities by means of a mini-mental status test or similar measurement methods prior to the procedure and subsequent selection of the appropriate anaesthetic (Di Nino et al., 2010). The indication for anaesthesia should be viewed critically in high-risk patients. After performing anaesthesia, patients at risk should be monitored to diagnose POCD early on and initiate treatment (Rundshagen, 2014).

5.2 Sevoflurane, Desflurane and Xenon

In addition to injection anaesthetics such as propofol, inhalative narcotics are most commonly used in Germany to induce and maintain anaesthesia. While the use of nitrous oxide (N_2O) and xenon is possible, isoflurane, sevoflurane and desflurane are more commonly used. The high price of the NMDA receptor antagonist xenon and the fact that it can be used on humans only in combination with other inhalative narcotics because of its high minimal alveolar concentration (MAC), explains the rare use of xenon in clinical practice (Rundshagen, 2014). The formerly standard use of chloroform in the early stages of anaesthesia no longer meets current clinical standards. The choice of the appropriate way of anaesthesia (intravenous or inhaled) and the appropriate anaesthetic are adjusted to the circumstances of the proposed operation (Ortiz et al., 2014).

The inhalative anaesthetics commonly used for anaesthesia in Germany, such as isoflurane and desflurane, are thought to have an impact on the pathogenesis of Alzheimer's, both *in vivo* and *in vitro* (Inan and Ozkose Satirlar, 2015). The central cholinergic system is modulated by isoflurane and desflurane, which inhibits the release of acetylcholine and thus suppresses cholinergic synaptic transmission, leading to loss of consciousness and pain suppression (Fodale et al., 2006). Since the cholinergic system also appears to play a role in Alzheimer's pathogenesis, this association could be the reason for the increased incidence of POCD in Alzheimer's patients (Pratico et al., 2005).
In a mouse model with Alzheimer pathology could be shown that under repeated influence of isoflurane, the content of Aβ peptides in the brain and the mortality of mice increases and behavioural problems are more frequent (Run et al., 2009). Under sevoflurane anaesthesia it was shown in 2014 that patients with pre-existing impaired cognitive functioning experienced significant worsening of their neurocognition after spinal surgery (Hussain et al., 2014).

For anaesthesia with xenon, such effects on the pathology of Alzheimer's have been poorly studied in humans. A meta-analysis published in March 2016 by Law et al. in the journal "Anaesthesia and Analgesia", among other factors such as blood pressure and heart rate, also examined the influence of xenon on the orientation of patients directly after anaesthesia, which performed significantly better in comparison with sevoflurane and isoflurane. Furthermore, xenon has a very short recovery time, which is considered a positive factor for POCD

(Rundshagen, 2014). However, more studies in humans are needed to verify these results and to enable doctors to make an informed election of the appropriate anaesthetic for every individual patient. *In vitro* it was shown that NMDA receptor antagonists such as Xenon, at concentrations allowing physiological activation, can prevent Aβ-induced deficits in LTP and has been reported to be neuroprotective

More reliable data on the influence of xenon on cognitive abilities are available in mouse models and cell culture. Previous xenon treatment of mice under isoflurane anaesthesia could reduce memory loss after anaesthesia (Vizcaychipi et al., 2011). In 2019 it was shown that NMDA receptor antagonists such as Xenon, at concentrations allowing physiological activation, can prevent Aβ-induced deficits in LTP and has been reported to be neuroprotective partially restoring $A\beta_{1-42}$-induced impairment of LTP (Burge et al., 2019). These neuroprotective properties and the neurorestorative potential might make xenon an important alternative for anaesthesia in AD patients (Lavaur et al., 2016).

6. Outlook

Intensive research and above all a systematic comparison of the effects of different inhalative anaesthetics on the POCD symptoms in Alzheimer patients is necessary to find a scientifically supported alternative anaesthesia method, which is suitable for Alzheimer's patients. Here, in addition to reliable data on the mouse model, also clinical studies on patients are lacking. The uncertainty in the diagnosis, the long time interval between surgical intervention and diagnosis and the reduced physician contact after hospital release complicate the data collection in addition.
Standardized measurement methods should be developed to facilitate the diagnosis and thus ensure the accuracy of the studies. Also, research should approach the question if cognitive training before and after anaesthesia could reduce the symptoms. In addition to that, finding a better anaesthetic technique seems to be essential to prevent postoperative cognitive impairment in AD patients.

II. Thesis goal

Dealing with Alzheimer's disease in everyday clinical practice repeatedly presents physicians with problems and difficult decisions. Age is a major risk factor for the onset of Alzheimer's disease and people with Alzheimer's often require surgeries (Guerreiro and Bras, 2015). This is mostly due to the multimorbidity which often occurs in old age, but since AD is known to affect the function of the vestibular apparatus, also the disease itself can result in an increased risk of falling (Nakamagoe et al., 2015). The cognitive deterioration of many Alzheimer's patients following surgery suggests the need for well-adapted and well tolerated anaesthesia for these patients. The so far for cost reasons rarely used xenon could be a promising anaesthetic to prevent this deterioration and provide the patient with optimal anaesthesia. This thesis compares the influence of xenon anaesthesia with the influence of sevoflurane and desflurane anaesthesia in an Alzheimer's disease mouse model (ArcAβ), in order to estimate a possible use in the surgical field.

To demonstrate the influence of these inhalative anaesthetics on the disease, the memory of transgenic ArcAβ mice was tested after anaesthesia, by means of behavioural tests in the WCM. A laboratory analysis of the typical Alzheimer's disease-related Aβ-plaques in the brains of these mice was also performed.
Using Western blots and fluorescence microscopy of methoxy-stained brain sections, the number and size of the plaques were quantified and imaged, in order to investigate a possible change of plaque count or size after the anaesthesia with the different inhalative anaesthetics. Using ELISA, a change in the amount of the typical AD protein $A\beta_{1\text{-}42}$ was investigated.

The effects of the used anaesthetics on the behaviour and the brains were compared with each other and with a control group in order to see if xenon would be a reasonable alternative for anaesthesia in AD patients. Considering also the technical challenges that are imposed by the rodent xenon $MAC_{immobility}$ being hyperbaric, a mixture with xenon and sevoflurane was used in order to establish a stable anaesthesia at around 1 MAC.

III. Material and Methods

1. Behavioural experiments and anaesthesia

1.1 Animals

A total of 92 mice (48 wildtype ("WT") and 44 transgenic ArcAβ ("TG")) at the age of 10-14 months were used for the experiments. Only male mice were chosen for the research project to minimize the influence of hormonal fluctuations on the behaviour. The mice were kept in a 12h light / dark rhythm (light off at 2:00 pm) and caged individually at least 8 days before the start of the training phase. Standard laboratory conditions were established in the room (22 ° C, 60% humidity) and the animals had access to water and standard feed pellets ad libitum. All behavioural tests were performed in the dark phase of the circadian cycle, as this corresponds to the highest activity phase of the mice.
The mice were ordered from Charles River Laboratories International (Calco, Italy) and had at least 8 weeks to recover from transport.

The transgenic mice used are from the ArcAβ mouse line, which has both the Arctic mutation and the Swedish mutation. The Arctic mutation affects the Aβ sequence directly while the Swedish mutation affects the β-secretase, augmenting the Aβ levels in the mouse brain.(Hall and Roberson, 2012)
With these mutations, the ArcAß mice are a well-established mouse model for Alzheimer's disease (Figure 3).

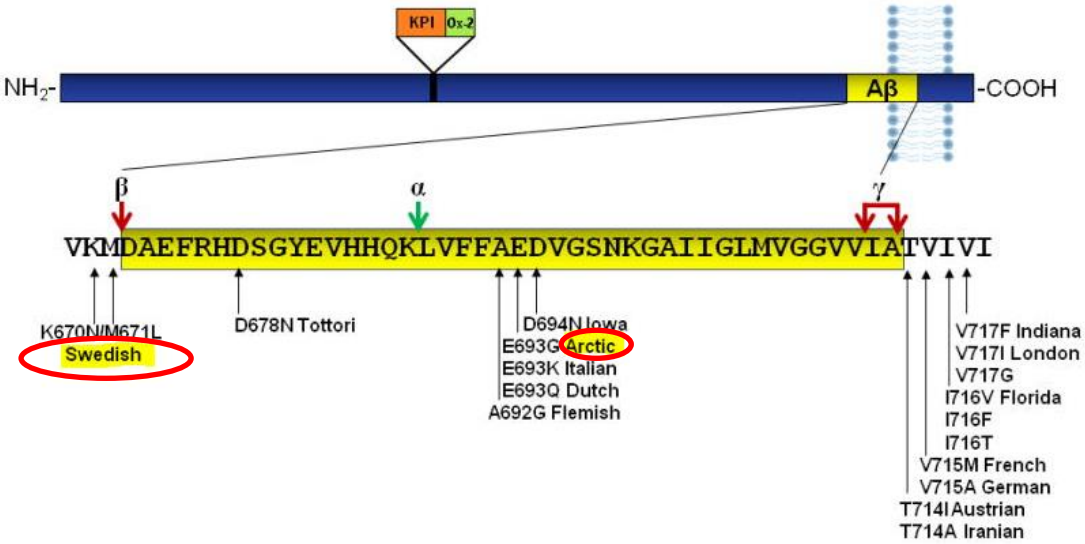

Figure 3: APP processing and APP mutations. *$A\beta_{1-42}$ is encoded by amino acids 672–713 of APP (numbered according to the longest isoform, APP770). Common APP mutations include the Swedish mutation at the ß-secretase cleavage site Intra-Aβ mutations including the Arctic mutation are also shown. Figure modulated after Hall and Roberson, 2012.*

All experiments in this dissertation were approved by the Ethics Committee of the Bavarian State Government.

1.2 Water Cross Maze (WCM)

The Water Cross Maze, which was used, is property and design of the Max Planck Institute for Psychiatry in Munich (Kleinknecht et al., 2012). The WCM consists of 0.5cm thick acrylic glass panes that form a cross. The transparent panes allow orientation of the test animals by looking at the environment in the room. The test room had enough items to allow orientation (sink, cupboard, door opening, pipes on the ceiling).
Each of the 4 arms is 50 cm long, 10 cm wide and 30 cm high. The arms are referred to clockwise as N, E, S, W to facilitate the distinction. The WCM was filled with fresh tap water every day to a height of 11 cm (23 +/- 1 ° C). In order to make the Cross Maze a T-Maze, a transparent acrylic glass disk was used to block the arm that was not needed (opposite the starting arm, **Figure 4** (Kleinknecht et al., 2012)).

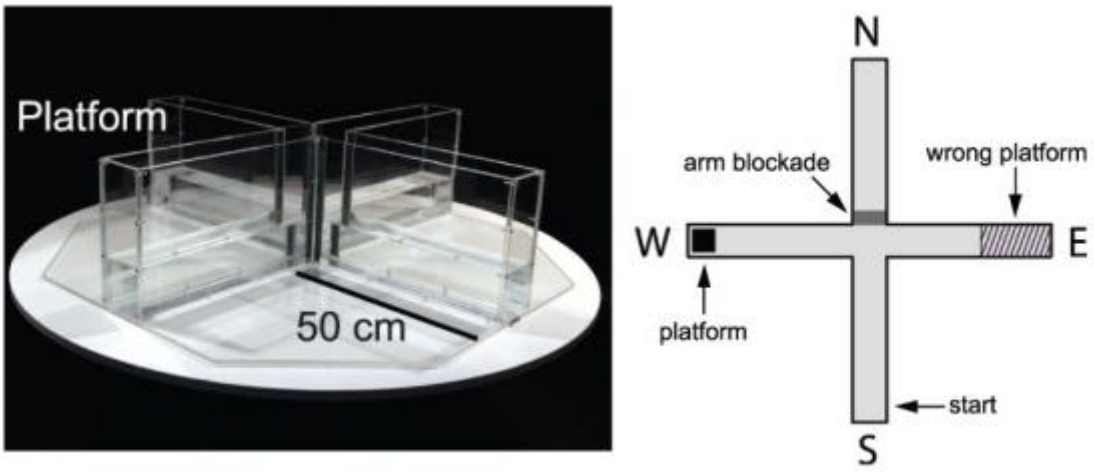

Figure 4: Photo and schematic drawing of the Water Cross Maze, *depicting an exemplary constellation of starting point, blocked arm, platform position, and wrong platform area oriented toward the four cardinal points [North (N), East (E), South (S), West (W)]. Note that a flexible disc was used for arm blockade of the arm opposite to the start arm, thus rendering the cross-maze a T-maze during each trial (Kleinknecht et al., 2012).*

This disc blocks access to arm N or S, depending on the protocol of the run that is being performed, forcing the mouse to choose between right and left.
At the end of the goal arm a platform of 8x8cm is placed (also transparent, made of acrylic glass), whereby the platform is 1cm below the surface of the water and therefore not visually recognizable for the mice (Kleinknecht et al., 2012).
After each run of the behavioural test, the animals were placed back into their cage with the aid of a metal grid attached to a pole (to inhibit direct contact with the experimenter) and placed in front of a heat lamp with red light to prevent hypothermia. Between the trials, water droplets on the walls of the WCM were removed with a cloth, the water mixed between the arms and the water cleaned of any soils with a sieve to preclude orientation of the mice by olfactory signs.

1.3 Behavioural experiments

The mice were trained in groups of 6 animals each. Each mouse had to complete the WCM 6 times a day for 7 consecutive days ("runs"). On the 8th day the narcosis took place and on the 9th the first memory test. The memory test was repeated for 4 weeks at intervals of 7 days (**Figure 5**).

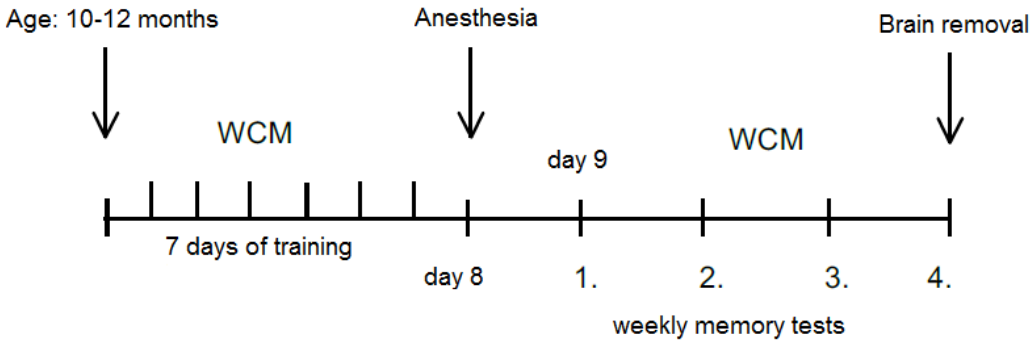

Figure 5: Graphical representation of the timeline of the behavioural experiments

The platform was placed in the same arm on all days and the starting arms of the 6 runs varied according to the following scheme: Days 1,3,5,7: 1.N, 2.S, 3.S, 4.N, 5.N , 6.S
Days 2,4,6: 1.S, 2.N, 3.N, 4.S, 5.S, 6.N

All 4 of the memory tests after the anaesthesia were carried out according to the scheme of the first day.

When starting a run, the mouse was placed in the water with its head facing the experimenter, who stood behind the start arm during the entire run. A run is completed when the animal has found the platform and climbed on it (where it stays for 10 seconds before it is put back in the cage) or at the latest after 30 seconds search time. If an animal remains motionless in the water after insertion (“floating”) it was "woken up" by snapping the glove against the forearm of the experimenter.

The following behaviours were observed and recorded:

a) "Arms visited": entry of the entire body of the mouse in one arm, except starting arm
b) "Wrong platform visits": The mouse enters the last third of the arm which is opposite the arm containing the platform
c) "Total time until platform": total time needed to find the platform, marked 31X if the platform was not found after 30 seconds.
d) Notes: the animal exhibits unusual behaviour (e.g. floated)

Based on the raised parameters, the "accuracy" could be determined: if the number of successfully completed runs in one day was at least 5 out of 6 (= 83%) the task was considered successful for this day and the accuracy was set as 1. If it was less than 5, the task was

considered failed for this day and the accuracy was set to zero.
A run was considered successfully completed if the platform in the target arm was directly found in time and the mouse did not visit any other arm.

1.4 Anaesthesia

The anaesthesia of the mice was performed by an anaesthesiologist of the university hospital "Rechts der Isar", of the Technical University Munich. Anaesthesia has been initiated by a volatile anaesthetic which was dribbled into a glass chamber and the mouse lost consciousness after a few minutes. The unconscious mouse was kept anesthetized for 2 hours by means of an anaesthetic machine securing the gas flow with tubes, while the temperature and heart rate were monitored during the entire time. The mouse was covered with a cloth and placed in front of a red-light lamp to preserve the body heat. The eyes were kept shut with Bepanthen- cream to keep them from drying out.
Every 15 minutes the temperature, heart rate, respiratory frequency and gas flow were recorded in a protocol. The anaesthesia depth was tested with a tail clamp and held stable at around 1 MAC by modifying the anaesthesia flow. To ensure the 1 MAC anaesthesia, pure sevoflurane anaesthesia was performed (mean = 11 %), pure desflurane anaesthesia (mean = 4 %) and a combination of xenon with sevoflurane anaesthesia (43 % / 3.1 %). The combination of xenon with sevoflurane is established because of the impossibility to anaesthetize mice with xenon alone at normobaric conditions, since the published human MAC of xenon for immobilization (MACimmobility) are 63 vol% or 71 vol% whereas the MACimmobility for rodents has been shown to be 1.61 atm and therefore hyperbaric (Cullen et al., 1969, Nakata et al., 2001, Koblin et al., 1998).

2. Laboratory

2.1 Sampling

To carry out the immunological tests, the brains of the test animals were taken one day after completion of the behavioural tests. The mice were anesthetized with 1-2 drops of isoflurane in a transparent glass case filled with paper towels. After loss of standing muscle tone and inter-toe reflex, the mice were decapitated using a table guillotine (World Precision Instruments Inc Sarasota, FL, USA).
The skull was opened along the fontanelle with scissors; the brain was dissected out of the calotte and stored at -80 ° C until further investigations.

All organ removal took place in accordance with EU Directive 2010/63 / EU for protection of the animals used for scientific purposes as well as the paragraph of the German animal protection law.

2.2 Preparation of brain sections

After removing the brains, the two hemispheres were separated with a razor blade. To prepare the sections for plaque staining, one hemisphere was glued on a plate with the cut surface facing upwards, using the tissue adhesive “Histoacryl” (B. Braun Melsungen AG, Tuttlingen, Germany). It was cut with a microtome (Leica VT 1000S, Leica Biosystems, Buffalo Grove, IL, USA) in 50 µm and 10 µm thick sections (alternately 18 sections in the respective thickness), putting three sections on each microscope slide. The sections selected for microscopy were slides 5, 6, 13, 14,15,16,17 and 18 to cover the broadest possible spectrum of the hippocampus (all of them 50µm thick). Of the three sections per slide, the best one was selected and visualized.
For the Western Blot and ELISA the hippocampus and cortex were separated manually with a scalpel.
The sections were stored at -80°C until further usage.

2.3 Plaque staining

2.3.1 Staining

Prior to the staining, the microscopy slides with the 50 µm brain sections were stored for at least 20 min at -20 ° C to avoid crystal formation.
For fixation, the slides were first incubated for 20 min in ice cold (-20 ° C) acetone-isopropanol solution (1: 1) and then washed twice with 1-fold phosphate buffered saline (PBS)-EtOH solution ("washing solution") for 10 minutes each.

The sections were then stained in the dark with cooled methoxy staining solution (see appendix) for 30 min on the 3D shaker and washed 3 times with the washing solution for 5-10 min each. Then the sections were washed 3 times for 10 min with distilled water.
The dyed slides were dried in the dark for at least 30min in an angled position on filter paper. To cover the sections with coverslips, a special fluorescent medium (Dako Flourescence Mounting Medium, Dako North America Inc., Carpintera CA USA) was used and the sections dried overnight in the dark. The microscopy was carried out in the first 2 weeks after staining, until then the sections were stored at 4 ° C in the dark to avoid premature excitation of the fluorescent dye.

2.3.2 Microscopy

The fluorescence microscopy of the stained sections was carried out with an Apotome AxioImager.M2 from Zeiss (Carl Zeiss, Oberkochen, Germany). With the 10x objective and a 4`, 6-diamidin-2-phenylindole (DAPI) reflector, image files of the hippocampus and cortex were carried out. For the processing of the pictures the program ZEN blue from Zeiss was used (**Figure 6**).
The image files were saved as JPEG and loaded into the program ImageJ for further evaluation.

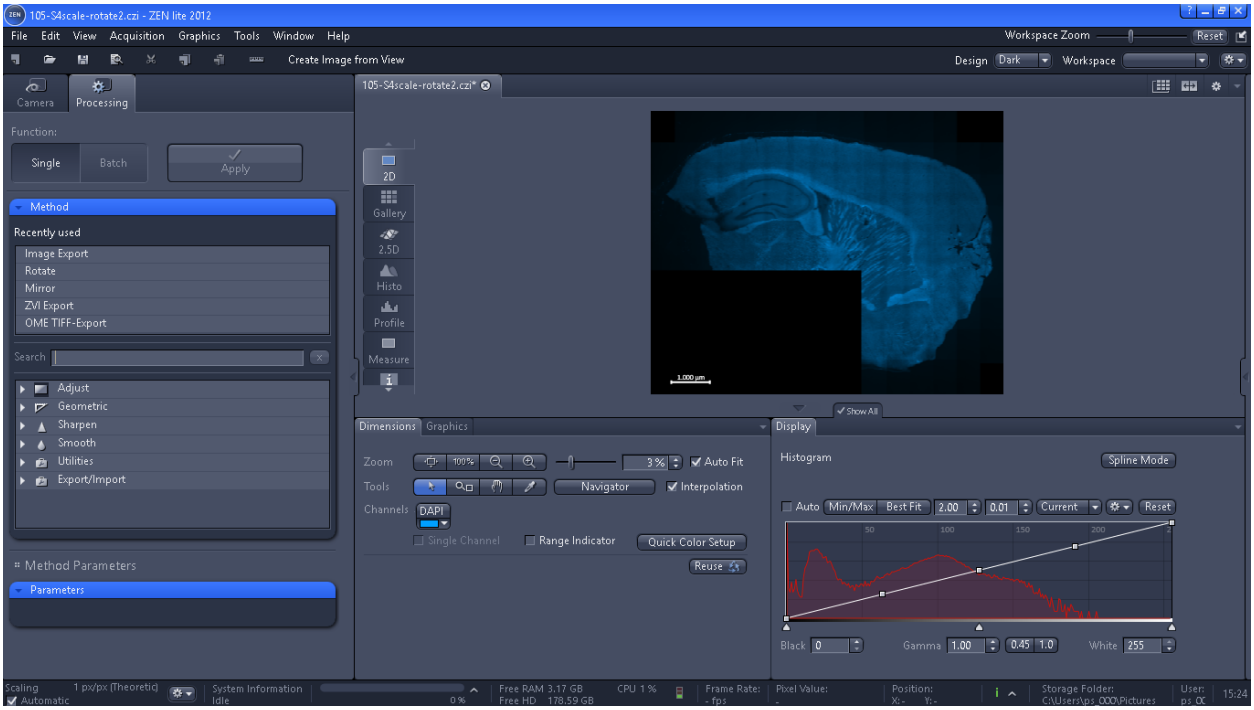

***Figure 6: Fluorescence microscopy of TG brain slices**. The images of the brain slices were taken and processed with the Apotome and the ZEN blue software (Zeiss).*

2.3.3 Evaluation of Aβ plaques with ImageJ

The following criteria were used to determine and assess the Aβ-load in the mouse brains of the different anaesthesia groups (wild-type vs. transgenic):

a) Number (s) of plaques in hippocampus and cortex
b) Aβ plaque size in pixels (px)
c) Percentage of the plaque area on the hippocampus and cortex area

The jpeg file produced with ZEN was opened with ImageJ and the brightness and contrast were adjusted if necessary. The cortex, prefrontal cortex and hippocampus were selected using the freehand selection tool, and the background subtracted using the function “subtract background - rolling ball radius 5 pixels-sliding paraboloid “. The determination of the brain regions was carried out with the help of the brain atlas "The mouse brain" by K. Franklin and G. Paxinos.

The colour threshold was set to brightness 70-255 using the colour threshold tool and the plaques detected by the program were selected.
The total area of the cortex and hippocampus as well as the area of the plaques were measured and the percentage of the plaque area on the total area was determined.

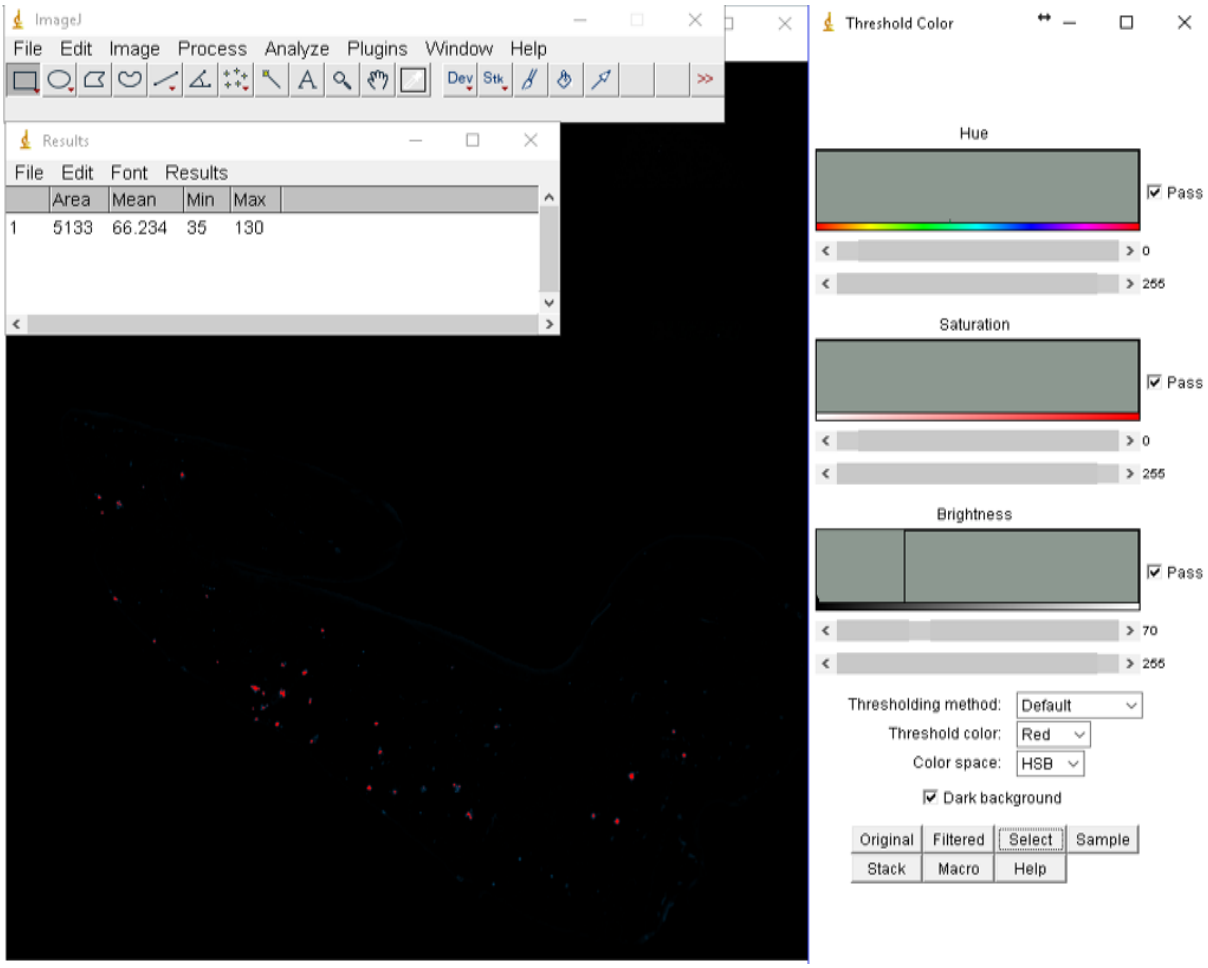

Figure 7: Evaluation of Aβ plaques with ImageJ, *the plaque area as well as the total area of cortex and hippocampus was determined and the percentage of plaque area on the total area was calculated.*

2.4 Western Blot

The Western Blot is an analytical laboratory technique that allows to detect and quantify proteins in a sample and be visualized on a membrane. First, the proteins are extracted from the cells, applied to a gel, and then separated according to their size by gel electrophoresis. The proteins are then transferred to a membrane where they are detected with specific antibodies and visualized (Eslami & Lujan, 2010).

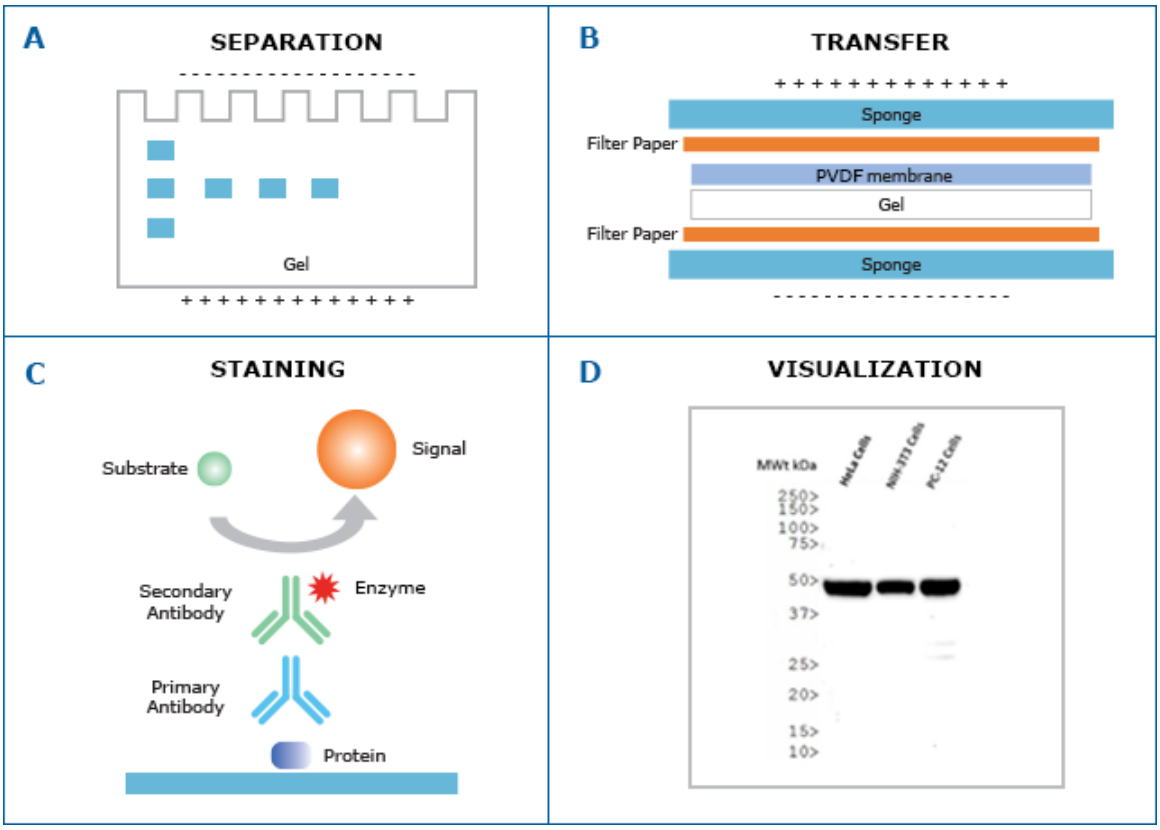

Figure 8: Conventional Western Blot. *(A) Proteins are separated by polyacrylamide gel electrophoresis (PAGE) and (B) transferred to a membrane for detection. (C) The membrane is probed with a primary antibody specific for the target protein and followed by a secondary antibody to detect the antibody-antigen complex., (D) the signal from the secondary antibody is then used to visualize the proteins (https://www.novusbio.com/application/western-blotting)*

2.4.1 Protein processing and extraction

To be able to detect the proteins in the Western blot, they were first extracted from the cells. To induce lysis of the cells, the hippocampus sample was placed in a 1.5 ml Eppendorf tube containing 200 µl (the cortex sample in 300µl) of radioimmunoprecipitation assay buffer (RIPA-buffer; Sigma-Aldrich, Saint-Louis, MO, USA, 20 µl / ml 50 × Complete, 1 mM PMSF and 1 µg / ml Pepstatin, Roche, Basel, Switzerland). The mixture was homogenized with a mortar on ice (Amersham Sample Grinding Kit (GE Healthcare Europe GmbH, Freiburg, Germany) and then centrifuged at 13000G for 30 min at 4 ° C (Biofuge fresco, Heraeus, Hanau, Germany), discarded and the supernatant transferred to a new Eppendorf tube and stored at -20°C.

2.4.2 Protein determination

After the protein extraction, the concentrations of the proteins in the samples were determined photometrically using a Bradford assay. Using this sensitive method, the protein concentration can be determined by taking advantage of the binding of the proteins to a blue stain (Bradford, 1976).
The samples were pipetted onto a 96-well plate together with a BSA standard series (Bovine Serum Albumin Standard Pre-Diluted Set, Life Technologies GmbH, Darmstadt, Germany), the BioRad DC Protein Assay (Bio-Rad Laboratories, Inc.,Hercules, CA, USA) was added and the resulting blue colour was quantified with photometry at 650 nm (sunrise ™ Reader, Tecan Trading AG Männedorf, Switzerland). The protein concentration could now be determined using the established standard curve.

2.4.3 Gelelektrophoresis

After the protein concentration had been determined, the proteins were diluted to 2 µg / µl with the loading buffer (NuPage LDS Sample Buffer, Invitrogen AG, Carlsbad, CA, USA). The loading buffer which is containing glycerol serves to increase the density of the samples and thus to sink into the pockets, as well as to provide colour markers using Coomassie G250. IN order to break the secondary and tertiary structure of the proteins, they were denatured by heating the samples for 5 minutes at 95 ° C in a heat block (Eppendorf AG, Hamburg, Germany).

For the protein separation 10% SDS gels were prepared in a BioRad Gel chamber (see appendix) and stored in the refrigerator until use. They consist of a stacking gel with pockets in order to bring all samples to the same level and a separating gel.

10 µl of the marker and 20 µl of each sample were placed in the pockets of the stacking gel and one pocket filled with loading buffer in order to control the running behaviour. Then for gel electrophoresis, 100 V were applied to the gel for 20 minutes to level the proteins in one line in the stacking gel. Subsequently, the voltage was increased to 200V in order to separate the proteins in the separating gel according to size.
A BioRad Chemi Doc XRS+ Molecular Imager was used to take a control image of the stain free gel.

2.4.4 Blotting

The gel with the separated proteins was transferred to a polyvinylidenfluorid blotting membrane (Amersham Hybond low fluorescence 0,2 µm PVDF, GE Healthcare Europe GmbH Freiburg, Deutschland) using blotting buffer (Blotting Buffer 10x: 247,93 mM Tris, 920 mM Glycin). To facilitate the transfer, a tank blot system (BioRad, Hercules, CA, USA) was used with 80 V during 60 min.

In order to be able to compare the amount of the protein of interest to the total amount of protein, an image of the stain free blot was taken directly after finishing the blotting process. The blotting membrane was then blocked for at least 60min at room temperature in blocking solution (see appendix) to inhibit unspecific bindings between the proteins and the primary antibodies (Table 1) which were put directly onto the membrane after the blocking and incubated overnight in the fridge on a shaker.

short name	Antibody	Dilution	Company
Casp3	Caspase -3	1:1000	Cell Signaling Technology, Inc., Danvers, MA, USA
mGluR5	anti-Metabotropic Glutamate Receptor 5	1:1000	Abcam, Cambridge, UK
GluN2B	NMDA Receptor 2B	1:1000	Cell Signaling Technology, Inc., Danvers, MA, USA
TNF-α	Tumor necrosis factor α	1:1000	ProSci, San Diego, CA, USA

Table 1: primary antibodies used for Western Blots

On the next day, the membrane was washed 3 times for 5min in Tris-buffered saline with Tween20 (TBS/T, pH=7.7) and then incubated for 60 min with the secondary antibody (Anti-rabbit IgG, HRP-linked Antibody, 1: 10 000, Cell Signaling Technology, Inc., Danvers, MA, USA) at room temperature on a 3D shaker.
After washing again 3 times for 5 min in TBS/T, the membrane was put 1min in Enhanced Chemiluminescence reagent (ECL, see appendix), before taking pictures.

2.4.5 Evaluation of Western Blot data

The evaluation of the Western Blot data was performed with ImageLab (BioRad Laboratories, Inc, CA, USA). The amount of the protein of interest was determined by measuring the intensity (grey value) of each band. The value was normalized with the total protein of the sample. It was possible to compare the different plots by means of a standard protein sample on each membrane which was used to normalize the protein amounts.

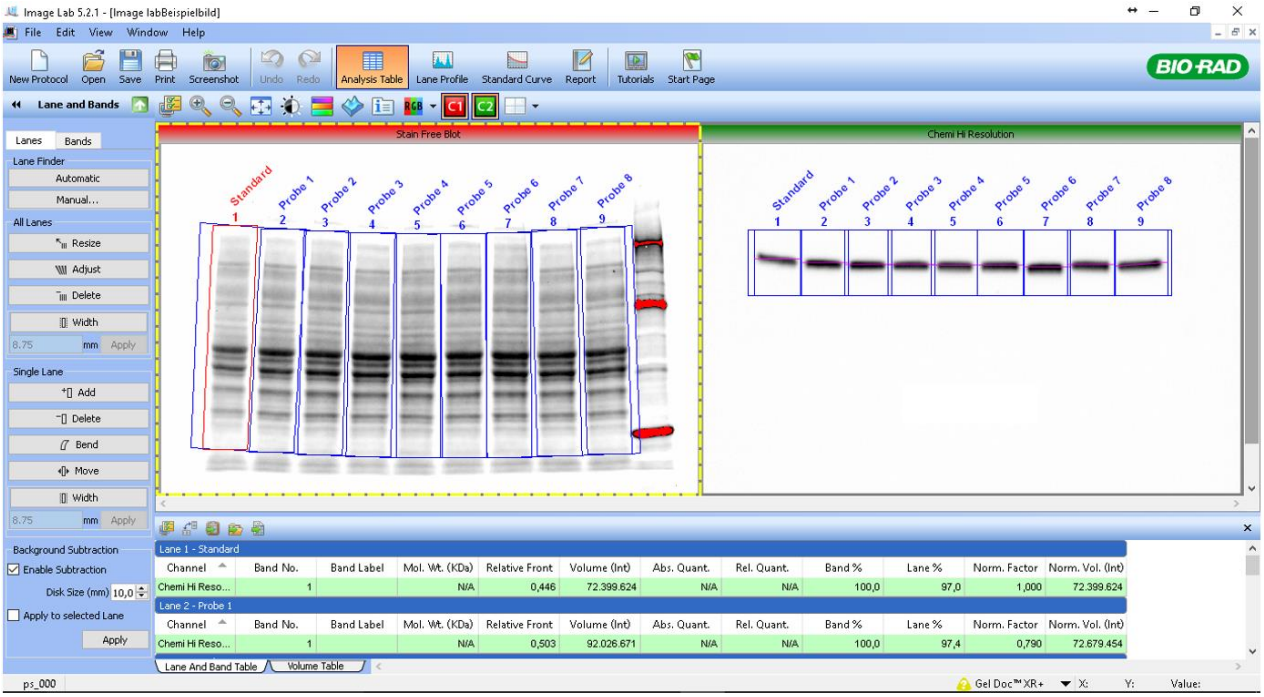

Figure 9: Evaluation of Western Blot results, *the intensity of each band corresponds to the amount of the protein of interest (right) and was then compared to the total protein amount of each sample.*

2.5 Enzyme-Linked-Immunosorbent-Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a serological method used for protein detection, where antibodies are detected using an enzymatic colour reaction. There exist different ELISA-techniques, for the detection of the protein of interest in this dissertation, the sandwich ELISA was used, which is based on an antibody-antigen-antibody complex.
The coat antibody that binds to the protein of interest is bound to a microtiter plate. The added HRP-conjugated detection antibody binds to the by the coat antibody bound protein and a secondary antibody binds the detection antibody. This triggers a colour reaction with the substrate solution, by means of which the amount of protein sought can be determined photometrically (Xia et al., 2009).

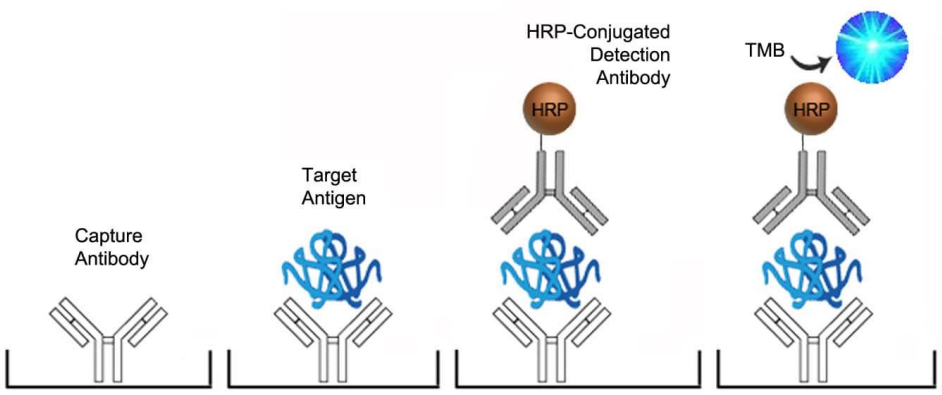

Figure 10: principle of the sandwich ELISA. *The protein of interest is bound by an antibody in a coated microtiter plate. A second antibody binds the first antibody and the amount of protein can be determined through a colour reaction. (LSBio, 2019)*

2.5.1 Sample preparation

From each sample, a piece of hippocampus and a piece of cortex were separately weighed, homogenized with 10 times its weight of guanidine buffer (see appendix) and incubated on the 3D shaker for 3.5 h at room temperature. Thereafter, the 10-fold weight of casein buffer (see appendix) was added and the mix was centrifuged at 4 ° C for 20 min at 13,000 rpm. The supernatant was transferred to a new Eppendorf tube and stored at -80 ° C until further use with a Human $A\beta_{1-42}$ ELISA Kit (Invitrogen, CA, USA).

2.5.2 Detection of the human $A\beta_{1-42}$

50 µl of the standard and each probe was added to the appropriate wells (every probe was determined 2 times to determine a mean concentration). 50 µl of HU $A\beta_{1-42}$ Detection Antibody was added and the mix was incubated over night at 4° C. Then the solution was aspired and the wells washed 4 times with 1X Wash Buffer. 100 µl Anti-Rabbit IgG HRP was added and incubated for 30 min at room temperature. After adding 100 µl of stabilized Chromogen to the wells, the plate was incubated for 30 min at room temperature in the dark. 100 µl of Stop Solution were added and the absorbance was read at 450 nm. For every probe the absorbance was detected two times and the mean concentration was determined by comparing the results of the absorbance to a standard curve, which was newly established for each plate.

2.6 Statistical Methods

For the statistical analysis the statistics program SPSS Version 11.5 for Microsoft Windows (SPSS Inc., Chicago, IL, USA) was used. The behavioural tests, Western Blot and ELISA data were investigated for significant differences on a $p<0.05$ level using ANOVA, while a Kruskal Wallis test was performed with the microscopy data for lack of normal data distribution. The figures were generated using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA).

IV. Results

1.1 Behavioural experiments

1.1.1 Learning capacity

In order to compare the ability to learn to find the platform in the WCM after anaesthesia, the mice were trained on 7 consecutive days with 6 runs per day to learn the task.
The WT mice learned the task successfully (<86% of runs completed) on day 2.48 (mean), while TG mice managed to learn the task successfully on day 4.05 (mean), which is significantly later ($p<0.05$, Figure 11).

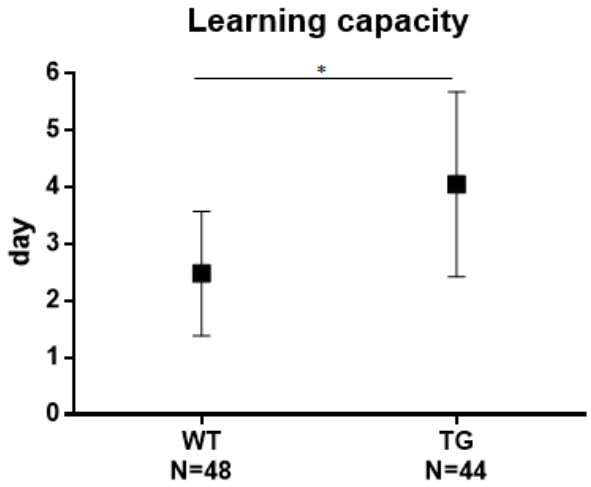

***Figure 11: Comparison of the learning capacity of wildtype (WT) and transgenic (TG) mice**. Shown is the mean of the first day when the mice learned to complete the task successfully (≥86% of runs completed). WT mice learned the task in the mean on day 2.48, TG mice on day 4.05. The difference is significant on a $p<0.05$ level.*

Only mice that managed to learn the task successfully in the 7-day training period were anesthetized and included in the memory testing.

1.1.2 Accuracy in the WCM

In order to detect a possible inhibition of cognitive function, the accuracy of the mice in the WCM was determined. First, the accurate executors of every group were determined by counting the mice who successfully completed at least 5 out of 6 runs of each day (=83%) and expressed as percentage of all anesthetized mice of every group (Figure 12, Figure 13).
On the last training day (d7), the mice in every group who completed the task successfully (100 % accurate executors) were included in the experiment. On day 8 the mice were

anaesthetized or sham anesthetized and tested from day 9 onwards weekly to investigate a possible cognitive impairment.

First, we analyzed the accurate executors of WT and ArcAβ mice after anesthesia expressed as the percentage of accurate learners per day. This analysis indicates that the mutation and applied anesthesia affects the individual animals in a uniform way, not producing severe deficits in only a few animals. On the first testing day (T1, one day after anaesthesia) and the weekly testing afterwards (T2-T4), all of the control WT mice were able to complete the task (100 % accurate executors d7-T4, Figure 12). The anesthetized WT mice had a slight decrease in the percentage of accurate executors on testing days T1-T3, but all groups achieved again 100 % accurate executors on T4. The most pronounced decrease of accurate executors was shown for the WT mice anesthetized with sevoflurane, which had 92 % of accurate executors on testing day T2 and 75 % on day T3. The WT mice anesthetized with Desflurane showed a less pronounced decrease of accurate executors, from 100 % on T1 to 92 % on T2 and 83 % on T3. The with xenon anesthetized WT mice had 90 % of accurate executors on day T1 and T3, also rising to 100 % on T4. While these differences are not significant on a $p<0.05$ level, a tendency in decrease of accurate executors compared to the control group in which every mouse completed the task on every day successfully, is visible.

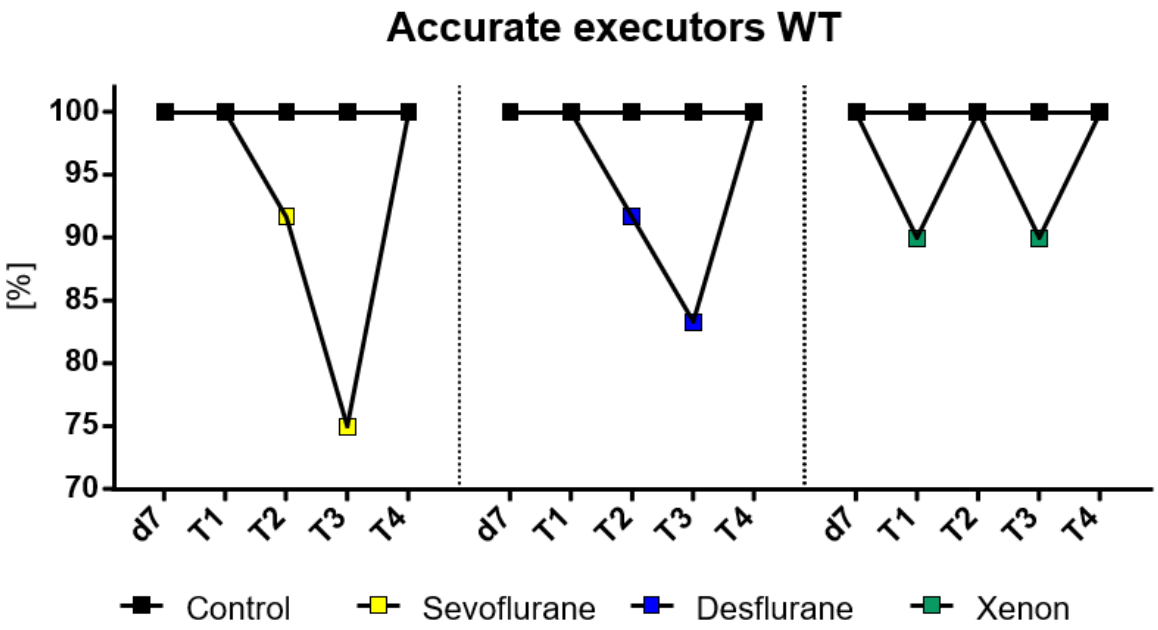

Figure 12: Accuracy of WT mice treated with different anaesthetics, expressed as the percentage of accurate runs per day. *Control: N=14, Sevoflurane: N=12, Desflurane: N= 12, Xenon: N=10. No significant differences between the groups on a p<0.05 level*

While in the WT control group the percentage of accurate executors stayed at 100 % during the entire experiment, the TG control group had a decrease of accurate runs on T1 (93 %) and T3 (86 %, Figure 13). Same as in the WT groups, every TG group apart from the xenon treated group, accomplished a 100% of accurate executors on T4. The TG mice anesthetized with sevoflurane had a decrease of accurate executors on T2 (73 %), with 100 % accurate executors during the rest of the behavioural testing, while the with desflurane treated TG group had a decrease from 100 % accurate executors (T1) to 82 % (T2, T3) and then an increase to 100 %

at T4 again. The TG mice treated with xenon had a decrease of the accurate executors to 88 % over the entire testing time, which corresponded to one mouse which what not able to complete the task at T1-T4.

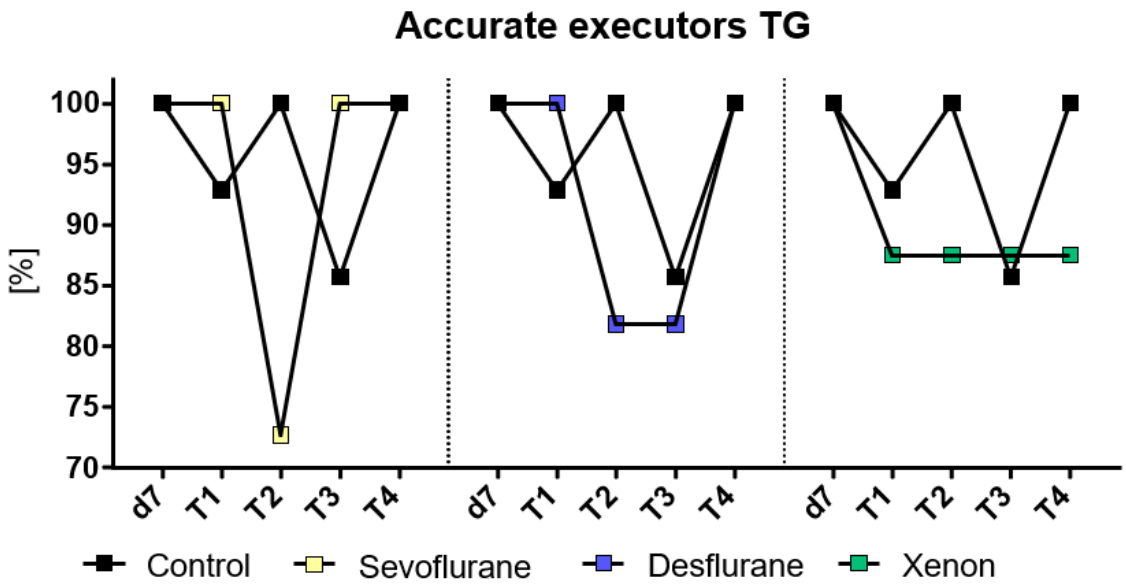

Figure 13: Accuracy of TG mice treated with different anaesthetics, expressed as the percentage of accurate executors per day*, Control: N=14, Sevoflurane: N=11, Desflurane: N=11, Xenon: N=8. No significant differences between the groups on a p<0.05 level.*

While the differences in the groups were not significant on a $p<0.05$ level, a tendency of decrease of accurate executors on T2 or T3 in almost all groups apart from the WT control is visible.

In order to provide a more detailed view at these effects and see if the 83% definition of a successfully executed task was reasonable, the percentage of accurate trials on all trials per day was calculated and called “accuracy” (Figure 14, Figure 15). On the first testing day (T1, one day after anaesthesia) and the weekly testing afterwards (T2-T4), all the control WT mice were able to complete the task (96 - 99 % accuracy d7-T4). The anesthetized WT mice had a slight decrease in the percentage of accuracy on testing days T1-T3, but all groups rose again to 99 - 100 % accuracy on T4. The most pronounced decrease of accuracy was shown for the WT mice anesthetized with sevoflurane, which had a mean accuracy of 94 % on T2 and 86 % on T3. The WT mice anesthetized with desflurane showed a less pronounced decrease of accuracy, from 100 % on T1 to 97 % on T2 and 92 % on T3. The xenon anesthetized WT mice had 97 % of accuracy on T3, also rising to 100 % on T4. These differences are not significant on a $p<0.05$ level.

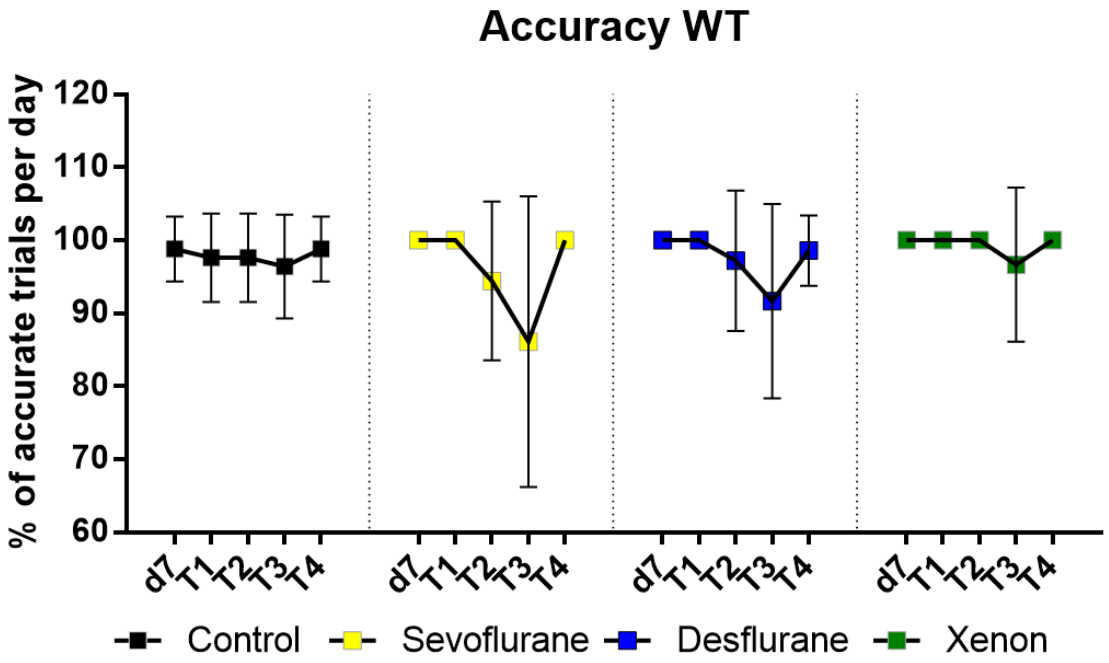

Figure 14: Accuracy of WT mice treated with different anaesthetics, expressed as the percentage of accurate trials on every trial performed per day (mean ± SD). *Control: N=14, Sevoflurane: N=12, Desflurane: N= 12, Xenon: N=10. No significant differences between the groups on a p<0.05 level*

While in the WT control group the accuracy stayed at nearly 100 % during the entire experiment, the TG control group had a slight decrease of accuracy on T3 (90 %, Figure 3). Same as in the WT groups, the control and sevoflurane treated TG groups accomplished a near 100% of accuracy on T4. The TG mice anesthetized with sevoflurane had a decrease of accuracy on T2 (86 %), with near 100 % accuracy during the rest of the behavioural testing, while the with desflurane treated TG group had a decrease from near 100 % accuracy (T1) to 92 % (T2) and 94 % (T3) and then 95 % at T4. The TG mice treated with xenon had a decrease of the accuracy to 94 – 95 % over the entire testing time. All differences were not significant on a $p<0.05$ level.

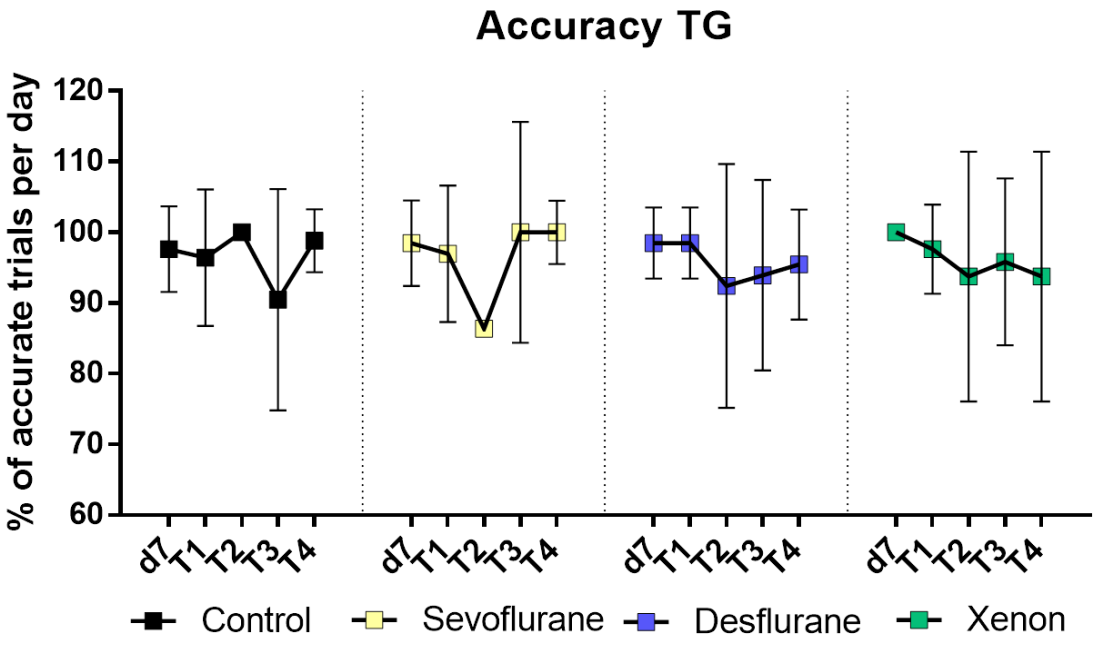

Figure 15: Accuracy of TG mice treated with different anaesthetics, expressed as the percentage of accurate trials on every trial performed per day (mean ± SD). *Control: N=14, Sevoflurane: N=11, Desflurane: N=11, Xenon: N=8. No significant differences between the groups on a p<0.05 level*

1.1.3 Latency in the WCM

To further investigate the nature of the behaviour after anaesthesia and detect a possible locomotion or perception deficit in TG mice, the time until the mice found and climbed upon the platform was measured during every run and averaged over the groups (Figure 16, Figure 17).

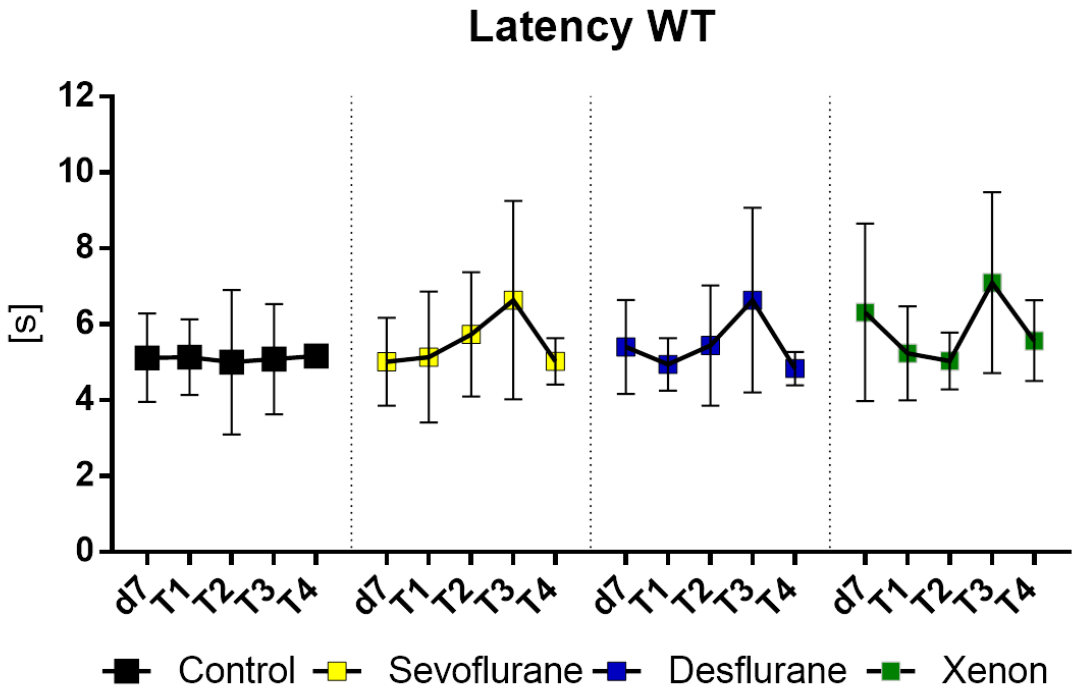

Figure 16: Latency as time in seconds until WT mice climbed onto the platform, mean of all trials per day (mean ±SD). *Control: N=14, Sevoflurane: N=12, Desflurane: N= 12, Xenon: N=10. No significant differences between the groups on a $p<0.05$ level*

The WT control mice needed around 5 s to complete the task, not showing any differences throughout the experiment (Figure 16). The anesthetized WT mice showed a bigger time range, all groups apart from the control showing tendency of increase in latency at T2 and T3. TG mice showed an average latency of 4-8 s, without significant differences in between the groups (Figure 17). The standard deviation of TG mice was greater than for WT mice.

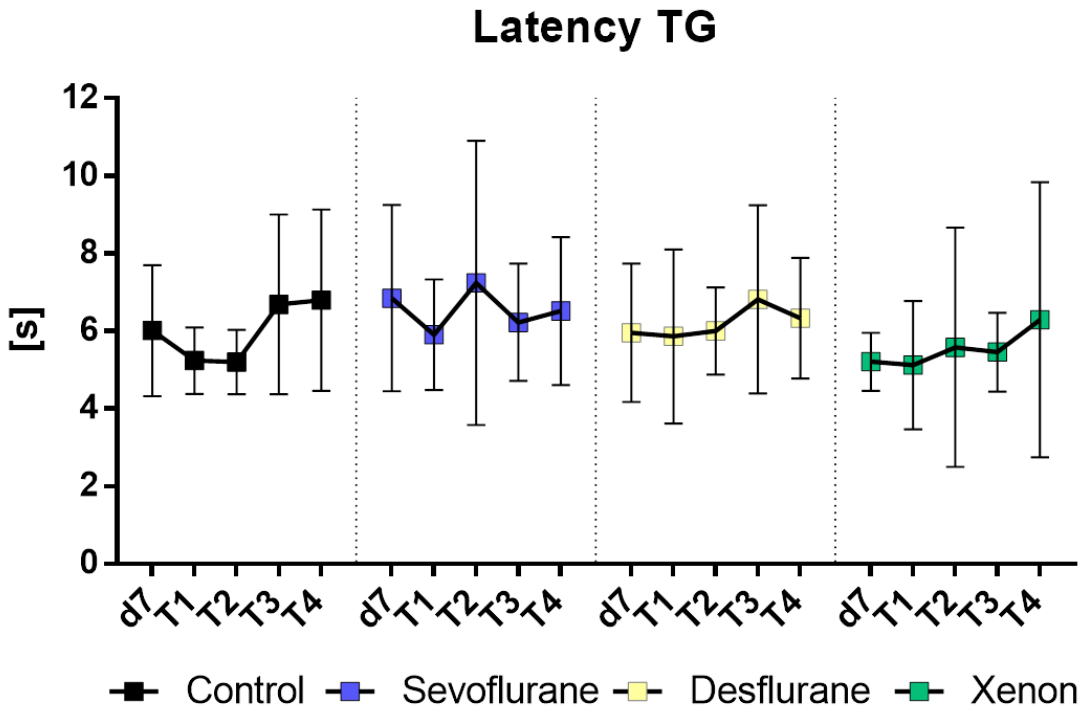

Figure 17: Latency as time in seconds until TG mice climbed onto the platform, average of all trials per day (mean ±SD). *Control: N=14, Sevoflurane: N=11, Desflurane: N= 11, Xenon: N=8. No significant differences between the groups on a p<0.05 level*

There are no significant differences to be found in the latency between the WT and TG groups, but the average time until climbing upon the platform shows a tendency to increase slightly on day T2 or T3 for all groups except the WT control.

1.2 Laboratory analysis

1.2.1 Fluorescent Microscopy

Microscopy of the methoxy-stained brain slices was performed, in order to investigate a possible influence of anaesthesia on Aβ plaque burden in the cortex and hippocampus of TG mice. WT mice served as negative control and did not show any plaques (Figure 18).
The methoxy-stained brain slices were further investigated with the Apotome (2.3.2). and the images obtained were evaluated with ImageJ. The goal of this analysis was to compare the plaque size measuring the area of the plaques in pixel, the percentage of the plaque area on the hippocampus and isocortex area and the number of plaques in the brains of the different experimental groups after anaesthesia.

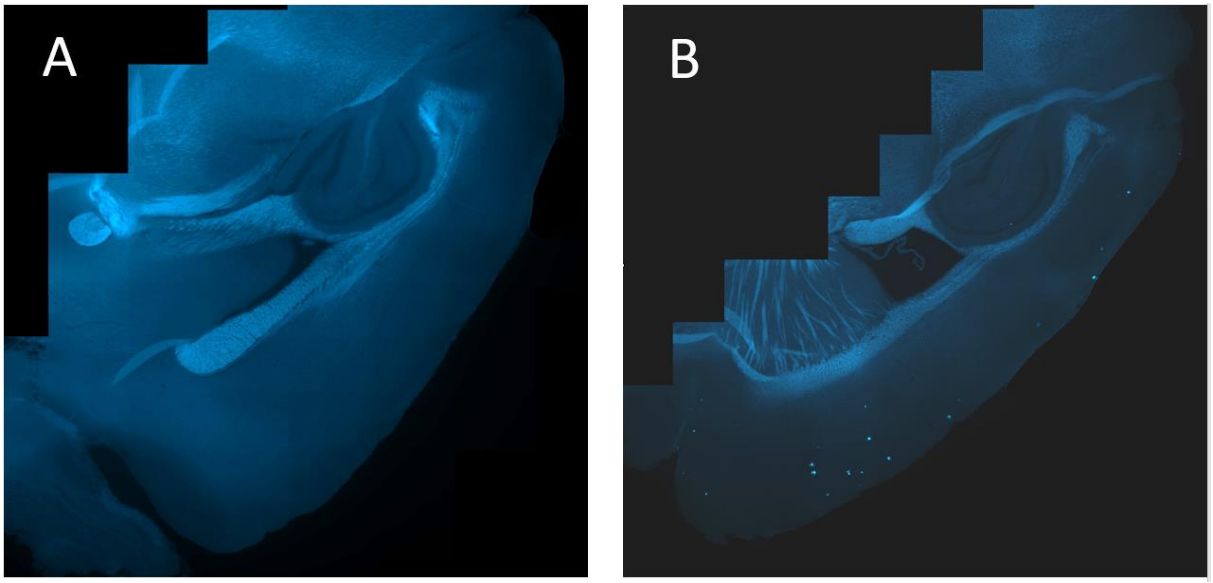

Figure 18: Fluorescent microscopy of cortex and hippocampus*, A: WT mouse control, methoxy-staining, no plaques to be seen. B: TG mouse, methoxy-staining, plaques clearly visible*

The plaque size was similar for all experimental groups, showing no significant difference of pixel count of the brains of anesthetized mice compared to the control group (Figure 19). The median plaque size was between 70 and 90 pixels for all groups.

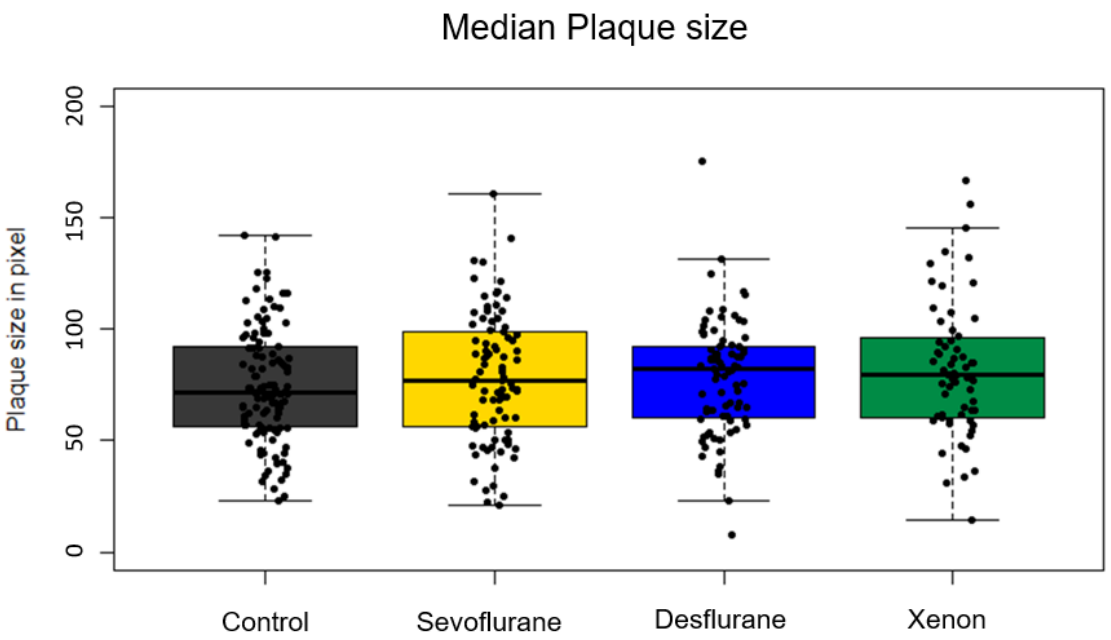

***Figure 19: Plaque size in TG mice after treatment with different anaesthetics**, represented in the boxplots is the median, 1. & 3. quartile as well as the minimum and maximum of every group. No significant differences between the groups on a $p<0.05$ level.*

Similar results were obtained for the percentage of the plaque area on the area of hippocampus and cortex of the TG mice. The median percentage was between 0.008 and 0.013 % for all experimental groups (Figure 20).

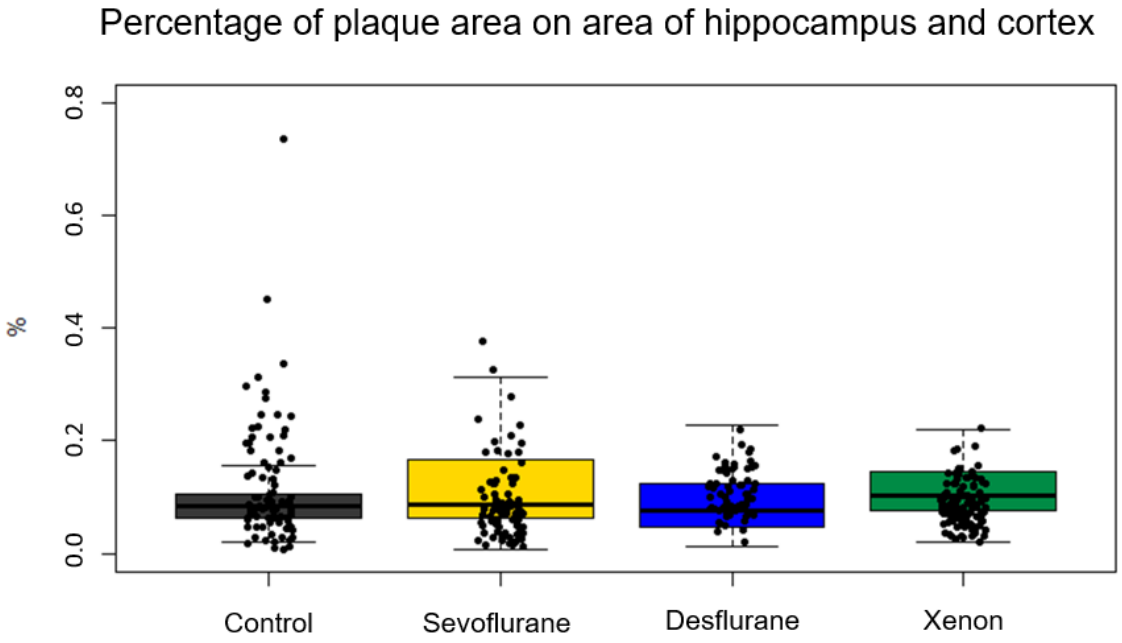

***Figure 20: Percentage of plaque area on area of hippocampus and cortex in TG mice after treatment with different anaesthetics,** represented in the boxplots are the median, 1. & 3. quartile as well as the minimum and maximum of every group. No significant differences between the groups on a $p<0.05$ level.*

The median plaque count was for all experimental groups between 45 and 55 (Figure 21). While the control and xenon anesthetized mice had a close range of plaque counts in all evaluated brain slices, the sevoflurane and desflurane anesthetized mice varied more widely in the plaque count. Neither plaque size, the percentage of plaques on the total area nor the plaque count showed any significant differences in the brains of all TG mice.

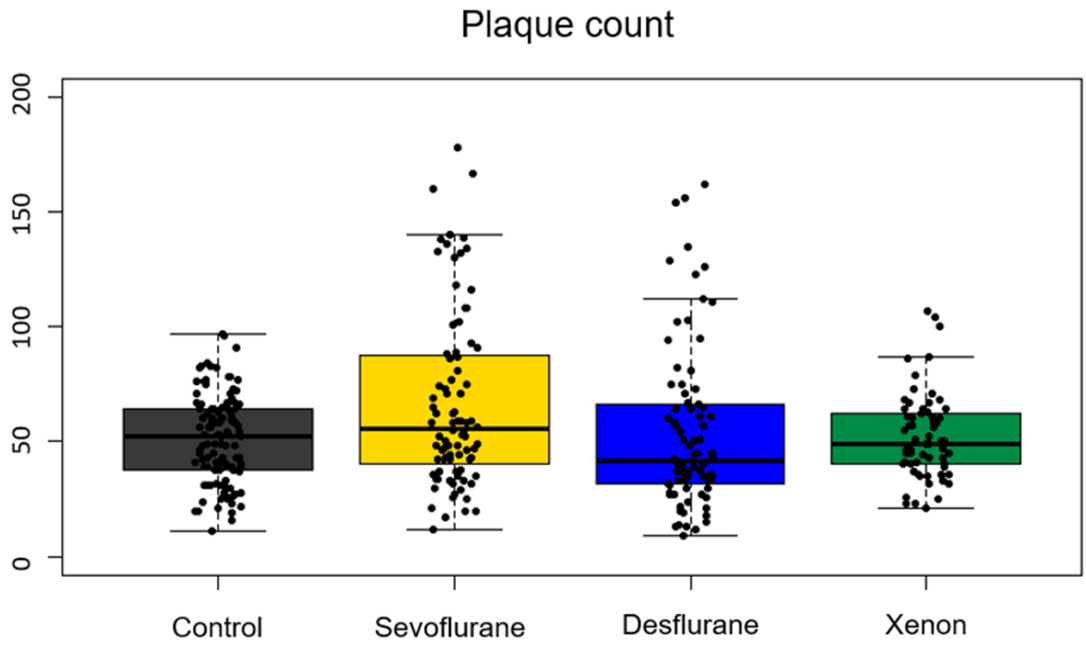

***Figure 21: Plaque count in TG mice brain slices after treatment with different anaesthetics**, represented in the boxplots are the median, 1. & 3. quartile as well as the minimum and maximum of every group. No significant differences between the groups on a $p<0.05$ level.*

1.2.2 Western Blot

To assess the expression levels of different markers which could be affected by the anaesthesia, western blots of the homogenized hippocampus and cortex brain tissue obtained from all experimental groups were performed.
The markers chosen for western blotting were mGluR5, the apoptosis marker Casp3, the GluN2B subunit of the NMDA receptor and the inflammation marker TNFα.

The mGluR5 protein levels in all experimental groups were not significantly different in the TG mice compared to the WT mice (Figure 22). The mean normalized volume of the mGLuR5 in the cortex tissue of WT mice was determined as 1.78 x 10^7 in the control group, 2.01 x 10^7 in the sevoflurane group, 2.15 x 10^7 in the desflurane group and 1.14 x 10^7 in the xenon group. In comparison to these values, the mean mGluR5 volume values for the TG mice cortex tissues were determined as 1.17 x 10^7 (control), 1.34 x 10^7 (sevoflurane), 1.11 x 10^7 (desflurane) and 1.19 x 10^7 (xenon).

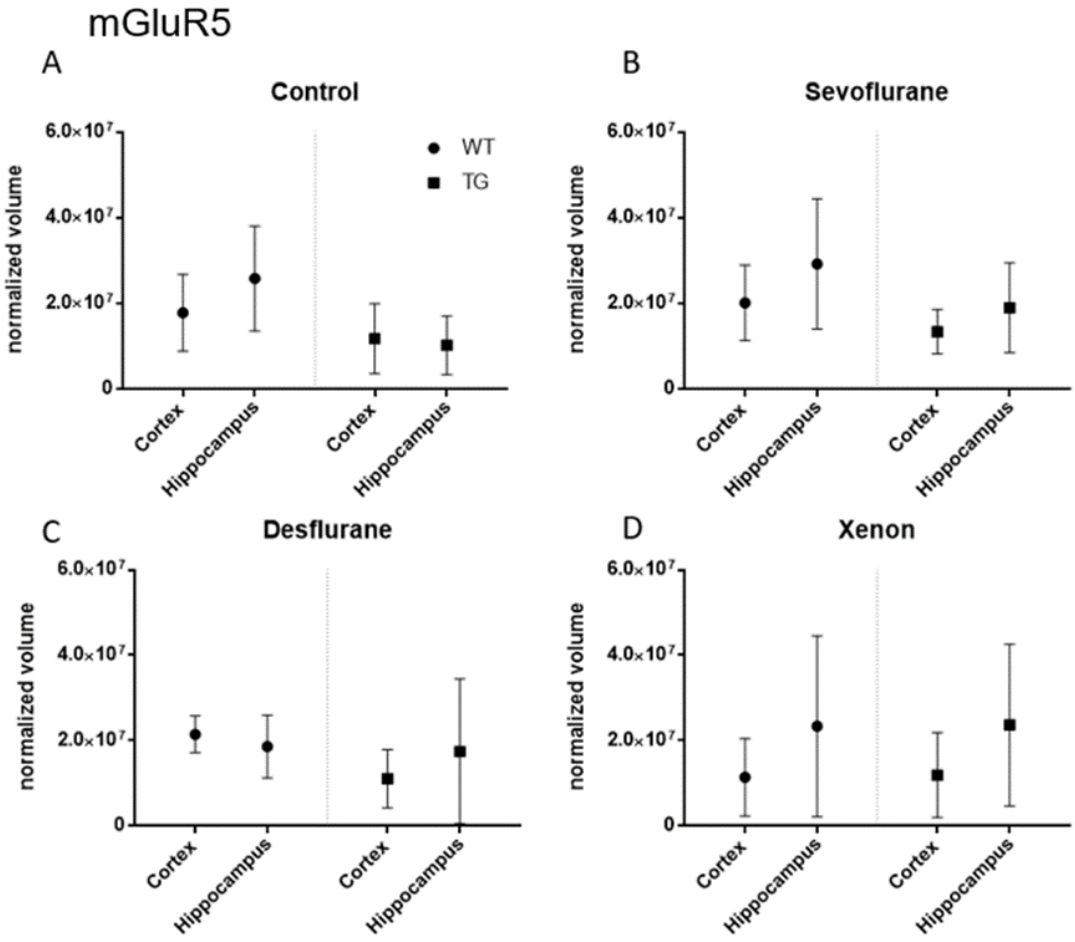

Figure 22: Detection of mGluR5 in Cortex and Hippocampus for WT and TG four weeks after anaesthesia with Western Blotting*, measured with ImageLab and normalized to standard. A: Control, B: Sevoflurane, C: Desflurane, D: Xenon*

For the hippocampus tissues, the mGluR5 protein levels were determined as 2.58 x 10^7 (WT control), 1.02 x 10^7 (TG control), 2.92 x 10^7 (WT sevoflurane), 1.89 x 10^7 (TG sevoflurane),

1.86 x 10^7 (WT desflurane), 1.74 x 10^7 (TG desflurane), 2.33 x 10^7 (WT xenon) and 2.36 x 10^7 (TG xenon).

In a next step, the mean normalized volume of the GluN2B subunit of the NMDA receptor was determined and the values compared in all groups (Figure 23).
For the cortex tissues, the GluN2B levels were determined as 2.26 x 10^7 (WT control), 2.22 x 10^7 (TG control), 4.03 x 10^7 (WT sevoflurane), 2.18 x 10^7 (TG sevoflurane), 3.11 x 10^7 (WT desflurane), 2.34 x 10^7 (TG desflurane), 1.95 x 10^7 (WT xenon) and 3.67 x 10^7 (TG xenon). There were no significant differences to be seen between all experimental groups in the GluN2B levels, neither in the cortex nor in the hippocampus tissues, where the GluN2B levels were determined as 2.28 x 10^7 (WT control), 8.87 x 10^6 (TG control), 1.19 x 10^7 (WT sevoflurane), 1.69 x 10^7 (TG sevoflurane), 7.85 x 10^6 (WT desflurane), 1.16 x 10^7 (TG desflurane), 1.44 x 10^7 (WT xenon) and 1.54 x 10^7 (TG xenon).

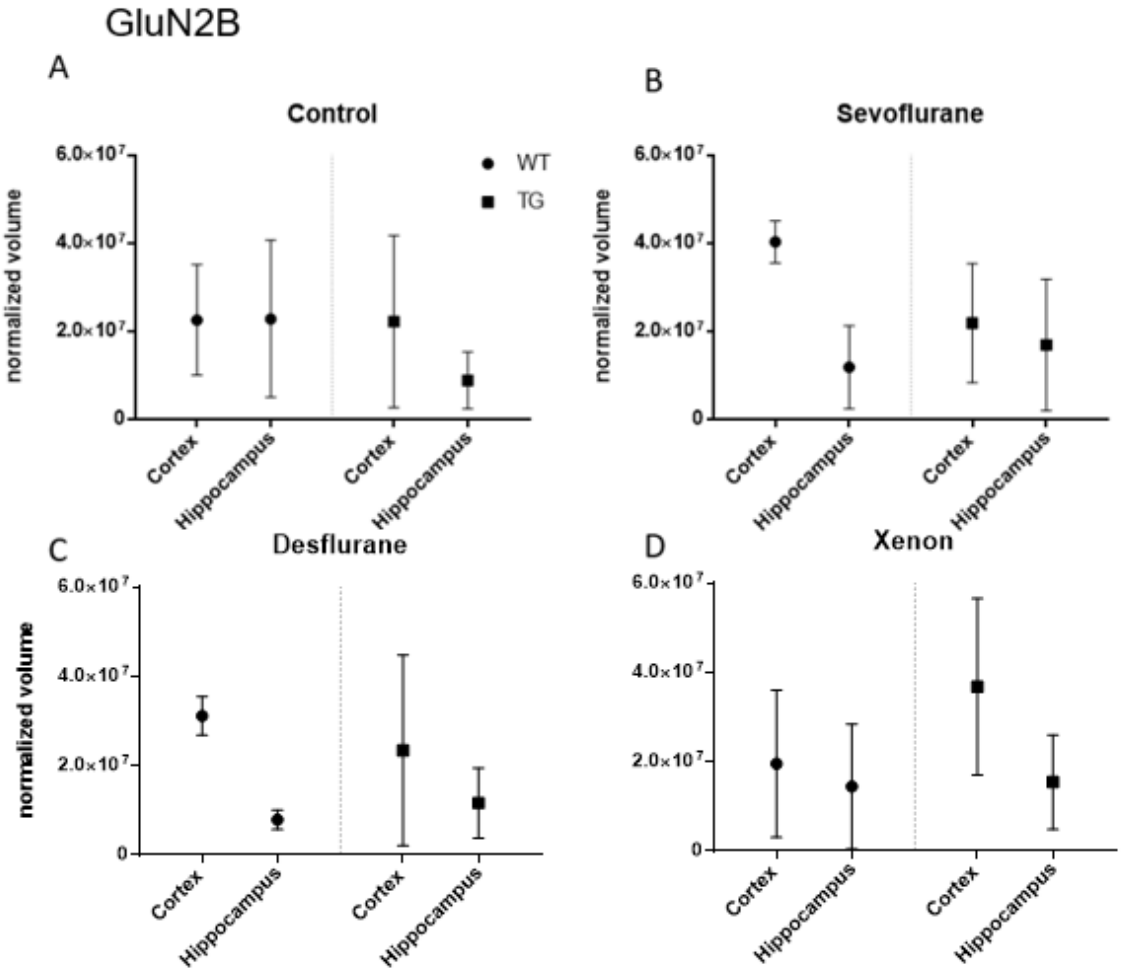

Figure 23: Detection of GluN2B in Cortex and Hippocampus for WT and TG four weeks after anaesthesia with Western Blotting, *measured with ImageLab and normalized to standard. A: Control, B: Sevoflurane, C: Desflurane, D: Xenon*

For the TNFα values (Figure 24), the mean cortex values were determined as 2.46 x 10^7 (WT control), 2.51 x 10^7 (TG control), 2.64 x 10^7 (WT sevoflurane), 2.20 x 10^7 (TG sevoflurane),

2.73 x 10^7 (WT desflurane), 2.39 x 10^7 (TG desflurane), 2.63 x 10^7 (WT xenon) and 2.53 x 10^7 (TG xenon).
The mean hippocampus values were determined as 1.52 x 10^7 (WT control), 2.23 x 10^7 (TG control), 1.21 x 10^7 (WT sevoflurane), 1.36 x 10^7 (TG sevoflurane), 1.31 x 10^7 (WT desflurane), 2.08 x 10^7 (TG desflurane), 1.01 x 10^7 (WT xenon) and 2.40 x 10^7 (TG xenon).
For all TNFα levels, no significant differences were found on a $p<0.05$ level, nor for the casp3 levels which were determined using western blotting as well (Figure 25).

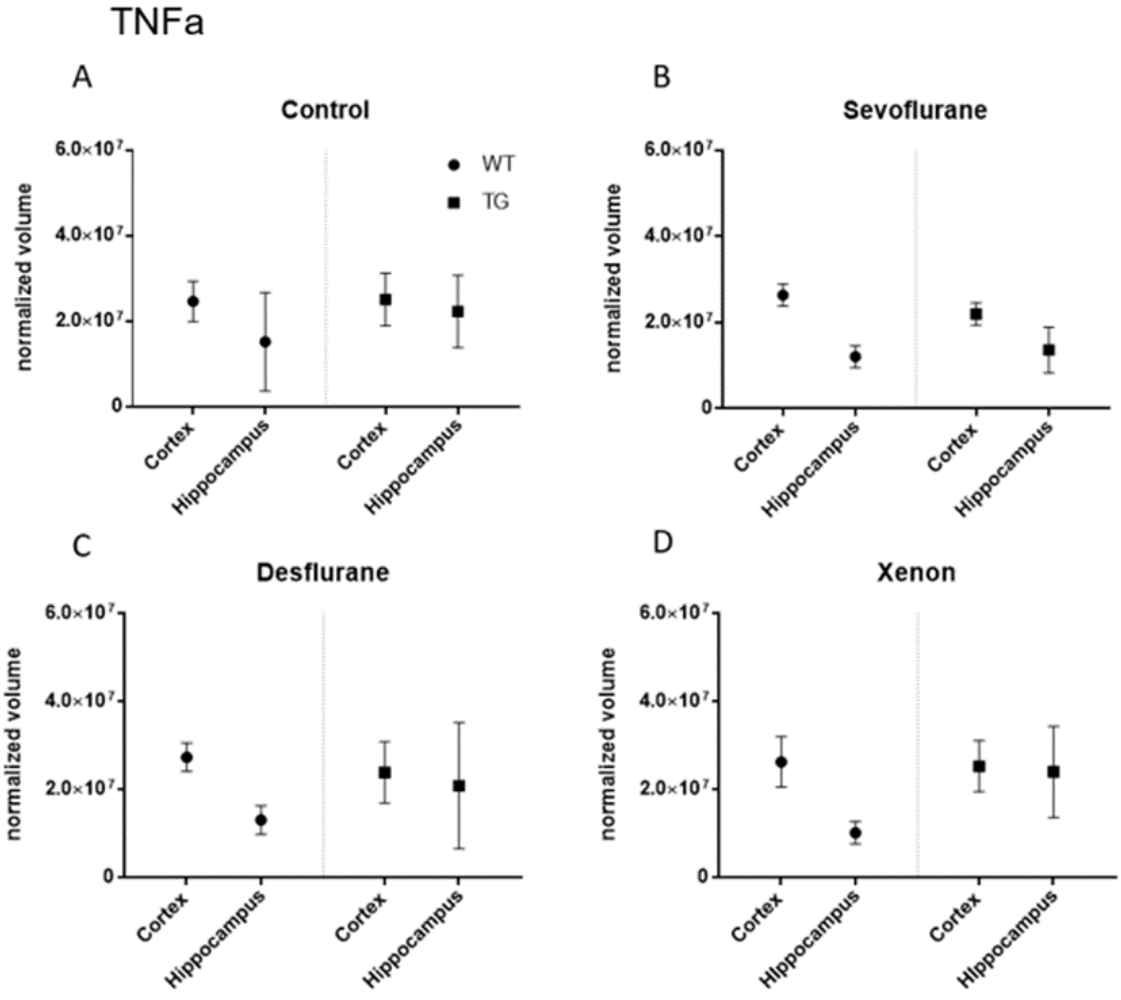

Figure 24: Detection of TNFα in Cortex and Hippocampus for WT and TG four weeks after anaesthesia with Western Blotting, *measured with ImageLab and normalized to standard. A: Control, B: Sevoflurane, C: Desflurane, D: Xenon*

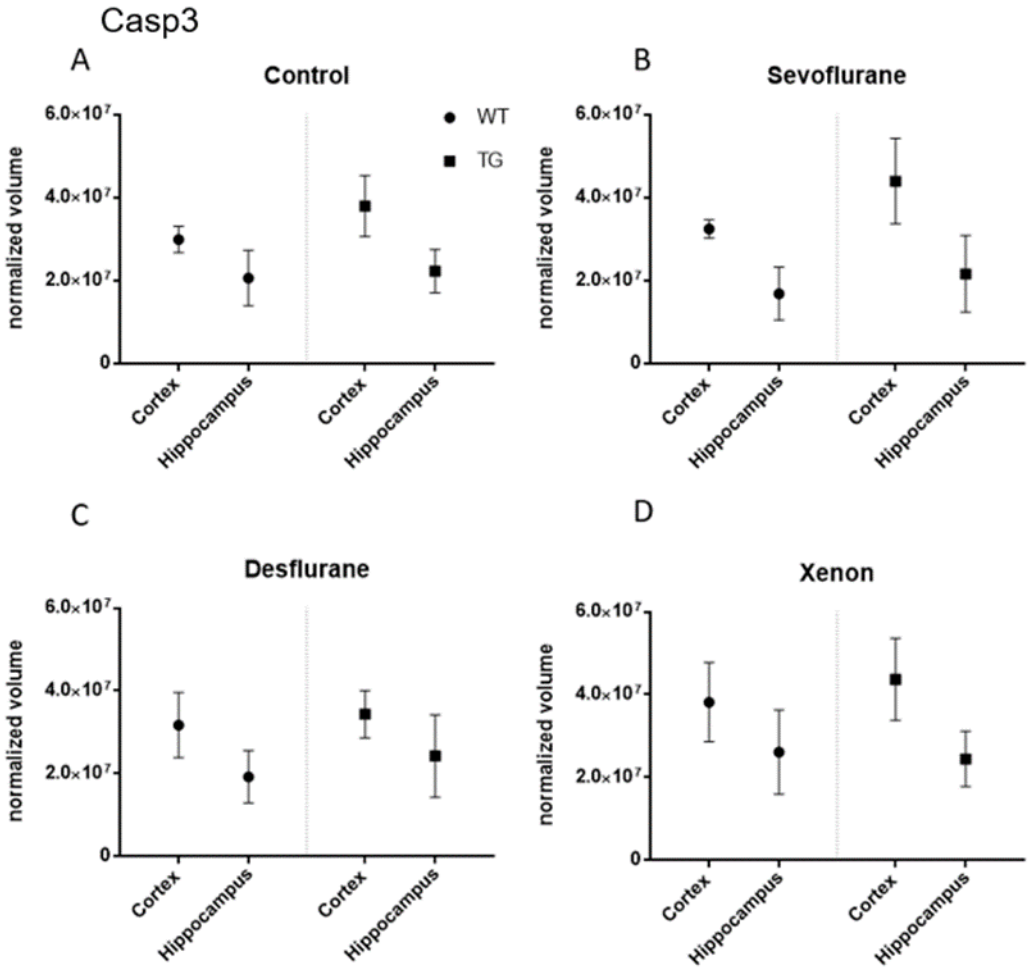

Figure 25: Detection of Casp3 in Cortex and Hippocampus for WT and TG four weeks after anaesthesia with Western Blotting, *measured with ImageLab and normalized to standard. A: Control, B: Sevoflurane, C: Desflurane, D: Xenon*

The mean cortex values of casp3 were determined as 2.99 x 10^7 (WT control), 3.79 x 10^7 (TG control), 3.25 x 10^7 (WT sevoflurane), 4.40 x 10^7 (TG sevoflurane), 3.17 x 10^7 (WT desflurane), 3.43 x 10^7 (TG desflurane), 3.81 x 10^7 (WT xenon) and 4.37 x 10^7 (TG xenon), while the mean hippocampus levels were determined as 2.06 x 10^7 (WT control), 2.23 x 10^7 (TG control), 1.48 x 10^7 (WT sevoflurane), 2.16 x 10^7 (TG sevoflurane), 1.92 x 10^7 (WT desflurane), 2.42 x 10^7 (TG desflurane), 2.60 x 10^7 (WT xenon) and 2.48 x 10^7 (TG xenon).

1.2.3 ELISA

In order to perform a quantification of the soluble Aβ, a $A\beta_{1-42}$-senisitive ELISA was used. The total amount of $A\beta_{1-42}$ levels in TG mice was dramatically increased (Figure 26). The $A\beta_{1-42}$ amount in the cortex was determined for WT mice as 127,5 pg/ml in average, while for TG mice the amount was with 1505,33 pg/ml more than 10-fold as high. In the hippocampus, the WT mice showed an average of 115,44 pg/ml, while the average amount of $A\beta_{1-42}$ in TG mice was determined as 645,88 pg/ml. When comparing the hippocampus and cortex values of $A\beta_{1-42}$ for every mouse directly, all mice showed more $A\beta_{1-42}$ in the cortex than in the hippocampus.

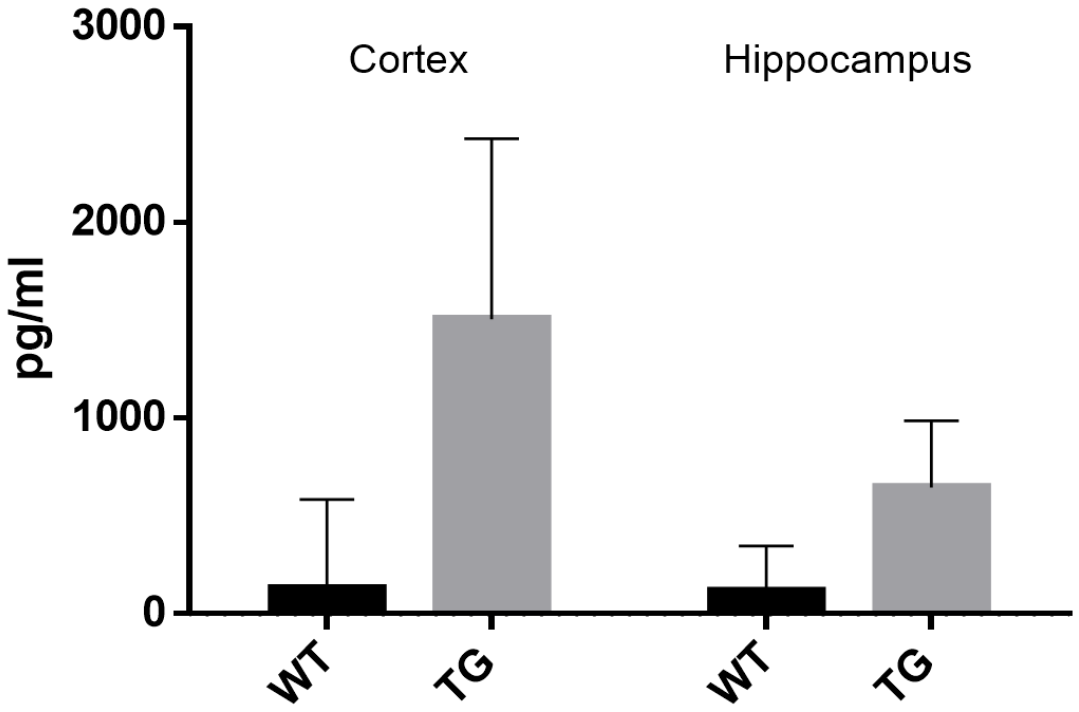

Figure 26: Total amount of $A\beta_{1-42}$ in hippocampus and cortex of WT and TG mice determined by ELISA. *In WT mice the amount of $A\beta_{1-42}$ was a mean of 127,5 pg/ml in the cortex and 115,44 pg/ml in the hippocampus. In TG mice the mean amount in the cortex was 1505,33pg/ml and 645,88 pg/ml in the Hippocampus.*

When comparing the different experimental groups, the amount of $A\beta_{1-42}$ was not changed significantly neither in the cortex nor in the hippocampus tissue of TG mice (Figure 27). The mean amount of $A\beta_{1-42}$ was in the cortex determined as 1520.8 pg/ml for the control group, having a similar value to the sevoflurane (1088.37 pg/ml). The desflurane experimental group showed a $A\beta_{1-42}$ value of 631.4 pg/ml. For xenon, the mean value was with 2780 pg/ml higher than in the other groups but varying widely.

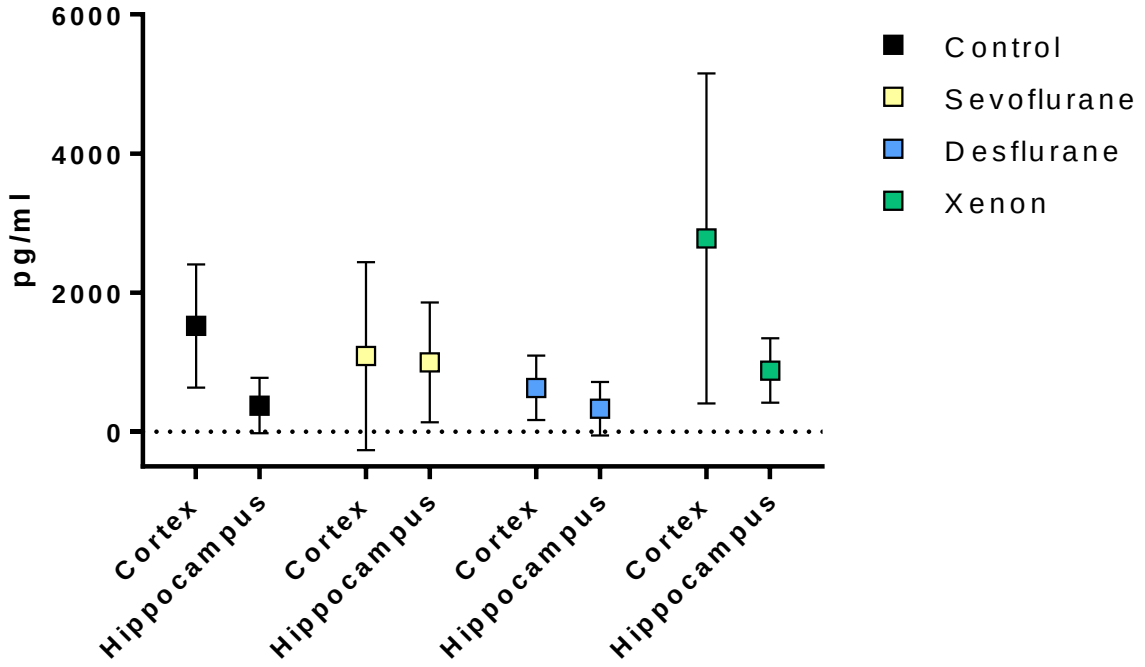

Figure 27: Amount of Aβ$_{1-42}$ in Hippocampus and Cortex of TG mice determined by ELISA 4 weeks after the anaesthesia. *There were no significant differences to be seen on a p< 0.05 level*

For the hippocampus the Aβ$_{1-42}$ values were more similar. For the control group the amount of Aβ$_{1-42}$ was determined as 376.5 pg/ml, for the sevoflurane anesthetized group as 996.5 pg/ml and for the desflurane anesthetized group as 330.7 pg/ml. In the hippocampus of xenon anesthetized mice, the amount of Aβ$_{1-42}$ was determined as 879.8 pg/ml. The differences to be seen in all determined Aβ$_{1-42}$ values are not significant on a p< 0.05 level.

V. Discussion

Several studies suggest that anaesthesia is associated with the development and progression of AD (Fodale et al., 2006). Moreover, studies in cultured cells and animals show that commonly used inhalation anaesthetics such as desflurane and sevoflurane may induce changes consistent with AD neuropathogenesis, e.g., increased amyloid precursor protein (APP) processing and Aβ accumulation (Mandal and Fodale, 2009, Yu et al., 2019). $A\beta_{1-42}$ is thought to be the most pathogenic form and numerous studies have reported that soluble $A\beta_{1-42}$ oligomers affect N-methyl-D-aspartate (NMDA) receptor function and impair cognitive function (Dawkins and Small, 2014). The gaseous anaesthetic xenon antagonizes NMDA receptors with low potency and has frequently been reported to be neuroprotective (Lavaur et al., 2016). In this dissertation the effects of xenon, sevoflurane and desflurane in the ArcAβ mouse model of AD were investigated using behavioural experiments. The brains of the anaesthetized mice were analysed using Western Blot, ELISA and fluorescence microscopy.

In the behavioural experiments in this study, the WT mice learned the task in the WCM significantly faster than the TG mice, therefore the WCM seems to be a well-chosen tool to distinguish behavioural differences between the WT and TG mice with the present mutations (Figure 11). In contrast to the Morris water maze (MWM), which is the most widely used behavioural testing maze first established by Richard G. Morris in 1981, the WCM demands a simple right/left decision (Bromley-Brits et al., 2011). In the Morris Maze, the mouse is put inside a circular pool where it must find a platform in a 60 s timespan. Here, the mouse can apply too many different escape strategies, since direct swimming, spiralling, parallel swimming and many other search strategies may result in a successful run (Garthe et al., 2009). The WCM used in the present work has therefore an explicit advantage, it combines the high motivation of a wet labyrinth (escape of water is considered a higher motivation than the search for food or shelter) with a simple single decision of a T-Maze (Pistell and Ingram, 2010).
The WT mice learned to find the platform after a mean of 2.48 days, which is within the expected time frame, since a study from 2012 showed similar results for the here also applied place learning, enabling the mice to orient themselves with the help of visual keys (Kleinknecht et al., 2012). The TG mice in this present study learned the task in average on day 4.05, which is significantly later than the WT mice. This confirms a cognitive impairment provoked by the inflicted mutations. Thus, this particular transgenic mouse strain seems to be an appropriate mean to investigate Aβ inflicted cognitive impairment *in vivo*.

In order to investigate the effects of sevoflurane, desflurane and xenon on the cognitive performance of the ArcAβ mouse model of AD, the accuracy of the mice in the WCM was determined on the testing days after anaesthesia. Here the threshold of 83% (≥5 out of 6) successfully completed runs seems to be reasonable, since there was no significant difference to be seen between the overall accuracy and the accurate executors. Additionally, the lack of a difference between the accuracy and the accurate executors shows, that the accuracy was not influenced by one or two mice which were affected more severely by the mutation, but that the mutations affected all mice in a uniform way.

The time which the mice needed to complete the WCM was not significantly altered nor by the anaesthesia nor between the WT and TG groups (Figure 16, Figure 17). This indicates that the inflicted mutation in the TG mice is not provoking any significant motor or perception deficits nor increased stress load. Furthermore there could not be seen an increased locomotor activity which can be found in many mouse models (Webster et al., 2014).

The anaesthesia in these experiments did not cause significant cognitive deficits in this AD mouse model nor in the WT mice in the WCM (Figure 14, Figure 15).
Similar studies with isoflurane and halothane, testing the cognitive function of mice with the APP Swedish mutation, showed also no influence on the cognitive function of the TG mice tested with the MWM 2 days after anaesthesia, but on the WT mice (Bianchi et al., 2008).

Sevoflurane was found to induce apoptosis and alter Aβ levels in human glioma cells *in vitro* (Dong et al., 2009). In 2015 sevoflurane was found to interact with Aβ, leading to its accumulation in human neuroglial cells (Tian et al., 2015) and to induce the apoptosis activating caspase-3, which can be attenuated by the γ-secretase inhibitor L-685,458 (Dong et al., 2009). The effect of sevoflurane anaesthesia on rodents' cognitive performance is not yet finally settled. In a study of 2012 it was found that sevoflurane anaesthesia did not impair acquisition learning and retention memory in rats tested in the MWM in a comparable timeframe to this study (Callaway et al., 2012). This finding could be confirmed in the experiments performed in this study, for mice as well. On the other hand, other studies did show a cognitive impairment in rats 48 h after sevoflurane anaesthesia (Xiong et al., 2013), as well as an decrease in cognitive function in rats one week after sevoflurane anaesthesia (Cui et al., 2018). These results in rats though are not transmittable directly on mice since there exist interspecies differences in navigational strategies used to solve spatial tasks (Hok et al., 2016). Furthermore, the first testing day in this study could have been chosen too long after the anaesthesia, not catching a cognitive impairment which could have been occurring before the first testing day.
For mice, a study in 2019 found a cognitive impairment after sevoflurane anaesthesia of 5 % for 4 h (Yu et al., 2019) which is a much higher sevoflurane concentration than in the present study. The higher concentration and the shorter time frame of testing after anaesthesia (24 h) could be the reason for the different results compared to the present study.

For desflurane it was found that memory impairment was age and dose dependent, showing in a study in 2015 that 4 weeks post-exposure 1.0 MAC desflurane-exposed 2 year old rats performed the same as sham-exposed controls, but showed cognitive impairment after 1 week (Callaway et al., 2015). This result is directly comparable to the results in this study, where 1.0 MAC desflurane anaesthesia did not have any significant influence on the performance of the mice in the WCM, so seemingly the results for different rodents can be diverse. In another study, where the effect of desflurane on learning and memory was compared to the effects of isoflurane, it was shown that desflurane did not induce memory impairment in mice, which supports the results of the present study (Zhang et al., 2012). So, for desflurane as well as sevoflurane, there is no final conclusion possible regarding possible memory impairment in mice.

Xenon was recently proposed as alternative anaesthesia for patients with cognitive impairment, since it was found to have a neuroprotective effect *in vitro* in various studies (Koziakova et al., 2019, Vizcaychipi et al., 2011, Liu et al., 2019). In 2019 it was shown, that xenon partially restores an Aβ-induced impairment in murine hippocampal synaptic plasticity (Burge et al., 2019).
In vivo studies could show a positive effect of xenon on neuronal injuries in subanaesthetic concentrations (Wilhelm et al., 2002), as well as an improvement of long term cognitive function after traumatic brain injury in mice (Campos-Pires et al., 2019).
A study in 2014 showed that xenon impaired reconsolidation of fear memories in a rat model (Meloni et al., 2014), while another study in 2018 proposed inhaled xenon washout as a biomarker of AD, because the xenon seemed to wash out more slowly in AD patients (Hane et al., 2018).

These findings were promising a possible positive effect of xenon on the cognitive performance of the mice of this study in the WCM.
The results of the behavioural testing in this study showed that xenon did not show any significant improvement or worsening of the performance in the WCM.

Several difficulties in the experimental design make an easy interpretation of these results difficult. The mice of this work could be able to cope with a possible inflicted cognitive impairment and therefore the orientation in the WCM could have been too easy for them, making it impossible to test of a minimal cognitive impairment. Another great limitation of this study is that it is impossible to apply a pure xenon anaesthesia at normobaric conditions to rodents since the $MAC_{immobility}$ for rodents has been shown to be hyperbaric with 1.61 atm (Koblin et al., 1998), while the human MAC of xenon for immobilization is 71 vol% (Nakata et al., 2001). This is the reason why in this study, a xenon/ sevoflurane mixture of 43% / 3.1% was applied to guarantee an anaesthesia at around 1 MAC (¼ xenon MAC, ¾ sevoflurane MAC). This modification was already tested and recommended in clinical trials but could be a possible reason for the lack of difference between the xenon anesthetized mice and the sevoflurane/desflurane anesthetized mice (Devroe et al., 2015). Another advantage of the xenon/sevoflurane anaesthesia combination is the possibility to introduce more oxygen to the mouse during anaesthesia, allowing more protection from hypoxia-induced neuronal damage (Ruder et al., 2014)

Another critical aspect of the design of this study is a possible relearning effect of the task in the testing weeks, masking a possibly more pronounced decrease in accuracy after the anaesthesia, because the relearning curve of tasks in water mazes in rodents is happening at a faster rate than the original learning of the task (Buccafusco, 2000). Here a repetition of the experiments with more groups and a testing only once after anaesthesia at the different time points would be a helpful insight into the relearning behaviour.

Despite these experimental difficulties it seems to be more probable that the anaesthetics used in this work just did not have any significant effects on the performance of TG mice used in this study in the WCM.

Similar problems as appeared in the behavioural testing are concerning the results of the plaque analysis in the TG brains, complicating their interpretation. Sevoflurane, desflurane or xenon did not have any significant effect on the Aβ plaque count or size of the ArcAβ mouse model in this work. This could be a result of the modified anaesthesia, adapted to the mouse MAC, since for inhalative anaesthetics such as sevoflurane and desflurane an interaction with $A\beta_{1\text{-}42}$ has been demonstrated *in vitro* (Bianchi et al., 2010, Mandal and Fodale, 2009). On the other hand, *in vitro* experiments are not always replicable *in vivo* since the surroundings of the cells can have an important impact on the behaviour of all cell components. The time of analysis could also be of importance, since all mice had again an increase in accuracy in the 4th week, maybe differences would have been visible just some days after the anaesthesia when a slight decrease in accuracy took place. This should be subject of further investigation, since a possible influence at these time points cannot be excluded, maybe imaging the Aβ plaques with positron emission tomography (PET), since Methoxy-X04 can be used as an amyloid-imaging agent for *in vivo* studies (Klunk et al., 2002).
All these influences, complicating the interpretation of the results have to be taken into consideration when trying to transfer the results of this study into the clinical practice.
The lack of influence of the here investigated inhalative anaesthetics on the Aβ plaque burden in the ArcAβ mouse model does not mean that in human patients similar results are guaranteed.

In order to assess the expression levels of different markers which could be affected by the anaesthesia with sevoflurane, desflurane and xenon, western blots of the homogenized hippocampus and cortex brain tissue obtained from all experimental groups were performed.
The markers chosen for western blotting were mGluR5, the apoptosis marker Casp3, the GluN2B subunit of the NMDA receptor and the inflammation marker TNFα.

mGluR5 is a metabotropic glutamatergic receptor which has been shown to play an important role in neuronal development and processes requiring synaptic plasticity, such as learning and memory (Kumar et al., 2015). It is thought to take part in the pathology of AD, since Aβ oligomers were found to induce the abnormal accumulation and overstabilization of mGluR5, thus providing a mechanistic and molecular basis for Aβ oligomer-induced early synaptic failure (Renner et al., 2010).
Glutamate receptor antagonists delivered at concentrations which allow physiological activities *in vitro*, are able to prevent $A\beta_{1\text{–}42}$ oligomer-induced synaptic toxicity (Rammes et al., 2011), for this reason mGluR5 was chosen as marker for western blotting in this study.
Here, for neither of the applied anaesthetics could be seen any significative effect on the mGluR5 levels in the hippocampus or cortex.
Sevoflurane anaesthesia was found to impair metabotropic glutamate receptor-dependent long-term depression and cognitive function in mice after 4 h of 5 % sevoflurane anaesthesia (Yu et al., 2019), so an influence of sevoflurane on the mGluR5 levels in the brains of the mice in this study would not have been surprising. Here the shorter anaesthesia with 2 h could be the reason for the different results in this study. In general, inhalative anaesthetics were found to inhibit glutamate receptors (Forkin and Nemergut, 2016), but it seems that the mGLuR5 levels in the brain cells remain unchanged in the mice of this experiment.

The NMDA receptor is a glutamate receptor and ion channel in nerve cells. It consists of 3 subunits: GluN1, GluN2 and GluN3. GluN2B is one of the 4 subunits of GluN2 in vertebrates, referred to as GluN2 A-D. GluN2B is mainly present in immature neurons and in extrasynaptic locations, and contains the binding-site for the selective inhibitor ifenprodil. (Laube et al., 1997)

Aβ mediated dysregulation of NMDA receptors has been hypothesized as a potential mechanism in the pathophysiology of AD (Miguel-Hidalgo et al., 2002). Preventing excessive extrasynaptic NMDA receptor activation may provide therapeutic benefit, particularly in Alzheimer disease and Huntington disease (Parsons and Raymond, 2014). Increased extrasynaptic GluN2B expression is involved in cognitive impairment after isoflurane anaesthesia (Li et al., 2016) which is why GluN2B levels in the hippocampal and cortex cells of the mice in this study were examined with using western blotting. The GluN2B levels in all probes were not significantly altered for neither of the anaesthetics of this study. Sevoflurane was shown to increase GluN2B expression in mice 24 h and 2 weeks post-anaesthesia with 2.5 % sevoflurane (Liu et al., 2015), while it was shown in 2001 that volatile anaesthetics (especially sevoflurane and desflurane) inhibit functioning of NMDA receptors reversibly *in vitro* (Hollmann et al., 2001). If there was an effect of the anaesthetics on NMDA receptors in this study, it seems that it was or transitory or without direct lasting effect on the GluN2B levels.

Another marker chosen for western blot analysis was casp3, which is a protein which forms part of the cysteine-aspartic acid protease (Caspase) family, a family of proteins which play an important role in apoptosis (Alnemri et al., 1996). Casp3 is involved in normal brain development as well as apoptosis, where it is found to be responsible for chromatin condensation and DNA fragmentation (Porter and Janicke, 1999). In AD patients, casp3 is increased in post-synaptic densities and therefore suggested to play a role in synapse degeneration during disease progression (Louneva et al., 2008). In this study, the casp3 levels in the mouse brains were not significantly altered by neither of the applied anaesthetics. This suggests that none of the applied anaesthetics has an influence on the casp3 pathways in the brains of the WT or TG mice. A study on rats showed in 2018 that casp3 was upregulated in the hippocampus of rats after sevoflurane anaesthesia (Cui et al., 2018), as well as a study in 2009, where sevoflurane was suggested to induce apoptosis, showing an sevoflurane-induced casp3 activation *in vitro* in human neuroglia cells as well as *in vivo* in mice (Dong et al., 2009). This stands in contrast to the results of the present study. A possible explanation for the different results could be the applied sevoflurane MAC (2.5 % vs 4 % in this study) and the time of evaluation of the brains which was with 6-24 h after anaesthesia much shorter than in the present study. Another possible explanation could be that sevoflurane simply does not have any effect on this particular mouse model.
For desflurane it is not surprising that no influence on casp3 levels was found in this work since a study in 2008 found that desflurane can only induce caspase activation in combination with hypoxia *in vitro* (Zhang et al., 2008), so these findings can be supported by the *in vivo* results of this study.
The casp3 levels after xenon anaesthesia remained unchanged as well in the present study. Here, few studies on mice have been executed, which makes a direct comparison difficult. A study in 2013 showed that there was no significant increase in number of casp3- positive and therefore

apoptotic cells after xenon treatment with a 0.55 MAC in a porcine myocardial infarction model compared to the control, so it is not surprising that xenon did not show any significant influence on the casp3 levels in the present mouse model (Sopka et al., 2013).

In order to investigate a possible influence of the sevoflurane, desflurane and xenon anaesthesia on inflammation, TNFα levels were determined using western blot as well. The role of TNFα and inflammation in AD has been subject to many discussions. Lately, studies could build more evidence for an involvement of TNFα in the pathogenesis of AD (Perry et al., 2001). TNFα is a cell signalling molecule (cytokine) and plays an important role in systemic inflammation and acute phase reaction (Makhatadze, 1998). IN AD patients, TNFα signalling seems to play a role in the Aβ accumulation in the brain, therefore targeting TNFα is thought to be a possible target for AD treatment (Decourt et al., 2017).
In this study, neither of the anaesthetics showed an influence on the TNFα levels in the brain of the anaesthetized mice. For sevoflurane this was an unexpected result, since a study in 2010 showed that sevoflurane increased TNFα levels in neonatal AD transgenic mice following a 3 % anaesthesia for 6 hours (Lu et al., 2010). Another study in 2018 showed that TNFα was upregulated in the hippocampus after sevoflurane anaesthesia in rats (Cui et al., 2018), so the increase of TNFα seems to occur in different rodents. Maybe the anaesthesia time of 2 hours in this study was not sufficient to induce such an increase of TNFα and sevoflurane could be used for short surgical interventions in AD patients.
TNFα levels did not differ after desflurane anaesthesia in this study, this is already known for human patients and was therefore expected (Baki et al., 2013, Chang et al., 2017). *In vitro*, no effect of xenon on TNFα levels is known (de Rossi et al., 2004).

In order to perform a quantification of the soluble Aβ, a $A\beta_{1-42}$-senisitive ELISA was used to determine the total amount of $A\beta_{1-42}$ levels in TG mice, which were dramatically increased in TG mice compared to the WT mice.
However, neither of the anaesthetics used in this study showed a significant influence on the Aβ levels measured by the ELISA.
This is an interesting result, considering that a study in 2009 found that sevoflurane increases the Aβ levels *in vivo* in young WT mice, so maybe sevoflurane is only taking part in the induction and early stage of AD without influencing elderly mice or mice with already elevated levels of Aβ (Dong et al., 2009). For desflurane it was shown in 2008 that desflurane increases Aβ levels only under hypoxic conditions (Zhang et al., 2008). Therefore, for desflurane it was expected that no influence on the Aβ levels was shown.
Xenon was shown to partially restore an Aβ-induced impairment in murine hippocampal synaptic plasticity (Burge et al., 2019), administered at subanaesthetic doses to brain slices. Here the results of the *in vitro* to the *in vivo* results seem to differ and xenon is not influencing the Aβ levels in ArcAβ mice.

Conclusion

The results of the experiments in this study show, that the ArcAβ mouse model is a well-chosen mean of investigation of Alzheimer's pathology *in vivo*. The WCM helps here to detect behavioural changes and shows, that neither of the inhalative anaesthetics of this study have an influence on the behaviour, plaque burden of Aβ and the chosen western blot markers. Here the greatest limitation is the impossibility of a pure xenon anaesthesia in mice which makes an exact transmission of the present results to human clinical trials difficult. More studies on different mouse models, with different anaesthesia depths and times as well as experimental trials in AD patients are needed to enable a scientifically based recommendation for anaesthesia in AD patients. If xenon is a good alternative for the currently used anaesthesia could not be clarified irrevocably in this work and a holistic medical approach, investigating the role of anaesthesia as well as surgery in the AD pathology after medical interventions needs to be established and investigated more thoroughly, given the inconsistent evidence in this field.

VI. Figures

VII. Abbreviations

Aβ : Amyloid β

AD: Alzheimer's Disease

APP: Amyloid-Precursor-Protein

Casp3: Caspase 3

MAC: Minimal alveolar concentration

MWM: Morris Water Maze

NMDA: N-Methyl--Aspartate

TG: transgenic mice, Arcaβ

V: Volt

WCM: Water Cross Maze

WT: Wildtype

VIII. Appendix

1. Microscopy

Methoxy staining solution: solving 10 mg Methoxy-Xo4 in 100 µl DMSO with 450 µl Isopropanol, 10 µl 4M NaOH and 450 µl PBS (1x). 800 µl of this solution were mixed with 100 ml EtOH and 100 ml PBS (1x).

2. Western Blot

stacking gel	30% PAA	25 ml
	1.5M Tris (pH8,8)	18.75 ml
	ddH $_2$O	29.75 ml
	10% SDS	0.75 ml
	10% TCR	0.75 ml
	TEMED	75µl
	10% APS	375µl
Separating gel	30% PAA	4.2ml
	1.25M Tris (pH8,8)	2.5ml
	ddH $_2$O	17.5 ml
	10% SDS	0.25 ml
	TEMED	50µl
	10% APS	250µl

Running buffer: 2l ml dH $_2$O, 6 g Tris, 28.8 g Glycin, 2 g SDS
Blotting buffer 10x: 247,93 mM Tris, 920 mM Glycin
Blocking solution: 5% BSA (Albumin Fraktion V, ROTH, Karlsruhe, GER), 2.5g in 50 ml H_2O
TBS/T (pH 7.7): 2l ml dH $_2$O, 6 g Tris, 22.2 g NaCl, 2 ml Tween 20 (Sigma-Aldrich Saint-Louis, MO, USA)
ECL reagent: 18 ml dH $_2$O, 2 ml 1M Tris (pH 8.5), 89 µl cumaric acid (CAS 501-98-4), 200 µl Luminol (CAS 521-31-3), 6.1µl H_2O_2 (CAs 7722-84-1)

3. ELISA

Guanidine buffer: 5,0 M Guanidine, 50 mM Tris-Cl at pH 8,0
Caseine buffer: 0,25 % Casein, 0.05 % Natriumazid, 20µg/ml Aprotinin, 5mM EDTA, 10µg/ml Leupeptin in PBS at pH 8,0

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X. Literature

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