Elucidating the diagnostic, therapeutic and mechanistic implications of stroke in Alzheimer's disease

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Contents

1. Abstract

By 2040, Alzheimer's disease (AD) will affect approximately 81 million people worldwide. Studies show that hypertension, diabetes, atherosclerosis, and obesity are risk factors for both vascular disorders such as stroke and AD. Risk of both stroke and AD increases with age. Emerging evidence shows that stroke increases the risk of developing AD and in return, AD is a risk factor for stroke. But exact mechanisms behind this correlation are unknown and remain to be investigated. To understand underlying mechanisms of stroke on AD pathophysiology and sex-specific differences, we investigated the effect of ischemic stroke on female and male double transgenic $APP_{SWE}/PS1_{\Delta E9}$ (AD) and C57BI/6 wild type (WT) mice from 3- months until 12-months of age. Mice were subjected to transient occlusion of the right middle cerebral artery (tMCAo) to induce an ischemic stroke. Before the stroke induction baseline measurement of general health parameters (e.g, body weight, blood pressure) and motor skills (e.g. activity, strength, coordination) were measured. After stroke induction, these measurements were repeated at several time points along with MRI measurements (e,g, rsfMRI, DTI, FAIR-ASL) to assess the effect of stroke on brain structure, function, and connectivity. $APP_{SWE}/PS1_{\Delta E9}$ mice and stroke mice showed impairments in physiological parameters, motor function, locomotion, and cognition. Our data show that stroke not only contributes significantly to AD but also exarcerbates the symptoms. $APP_{SWE}/PS1_{\Delta E9}$ stroke mice were more hyperactive and anxious than $APP_{SWE}/PS1_{\Delta E9}$ sham mice. Furthermore, male mice showed greater surgery effects than female mice, indicating strong sex differences in stroke and AD pathophysiology. Our findings suggest that there is a strong correlation between vascular risk factors, stroke and AD. We further showed that there is a great difference between sexes and genotypes, indicating any preventative or therapeutic approach should be personalized.

2. Introduction

a. Dementia, vascular dementia, and Alzheimer's disease

Dementia is a generalized term to describe progressive impairment of cognitive function that leads to loss of memory, disturbances in the performance of daily tasks and mood [1, 2]. Early signs of dementia are often mistaken for aging, but dementia and normal aging are not one and the same [2]. Declined cognitive processes are part of the aging process while dementia is progressive, interferes with the quality of life and is not part of growing old [2].

Vascular dementia (VaD) and Alzheimer disease (AD) are classified as the two most common types of dementia and have been classified as separate diseases [3, 4]. Clinical diagnosis criteria are different between these two diseases and demented patients with cerebrovascular diseases are diagnosed with VaD rather than AD [3, 4]. Recent studies show that vascular factors that are used as an exclusion criterion for AD, play an important role in the development of AD [3, 4]. Furthermore, there is considerable overlap in risk factors, symptoms and pathophysiology between these disorders. Clinical differentiation and diagnosis are complicated not because of shared factors but also because elderly people are affected not only by one disease [3, 4]. Therefore, mixed dementia diagnosis (dementia with vascular components) is becoming more predominant in the elderly since the pure form of AD and VaD are rare [3, 4].

According to the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN), VaD has two main criteria: existence of dementia and an underlying vascular cause [5]. Dementia caused by stroke is also identified as a prerequisite for a probable VaD [5]. But with increasing evidence that cerebrovascular diseases' contribution to cognitive dysfunction a broader term, vascular cognitive impairment (VCI), was introduced [6]. VCI recognizes a broader range of symptoms of vascular components on dementia and is useful in the clinical diagnosis of different subtypes [6].

AD is a heterogeneous, progressive neurodegenerative disorder that is characterized by cognitive impairment such as memory loss [7]. Worldwide 50 million people have dementia and it is estimated that the number of people affected will rise to 81 million by 2040 [7]. 70% of dementias are attributable to AD and it is the common cause of dementia among people 65 and older [8]. Although there are many hypotheses about the cause of AD exist, there are few that are predominantly accepted. Two of the hypothesis that are widely accepted are the amyloid beta (Aβ) hypothesis and the vascular hypothesis [3].

The amyloid beta hypothesis states that there are two main pathological hallmarks of AD: amyloid beta tangles and neurofibrillary tangles [7, 9, 10]. $A\beta_{1-40}$ are short peptides are formed by the proteolytic cleavage of amyloid β precursor protein (AβPP) by β- and γ-secretases [7, 9, 10]. However, when γ-secretase cleaves after β secretase then the final fragment is called $A\beta_{1-42}$ which aggregates faster [7, 9, 10]. This, in turn, causes neuronal loss and to neurodegeneration. Presenilins1 and presenilins2 (PSEN1&PSEN2) genes play a crucial role in controlling the activity of γ secretase which responsible for cleavage of AβPP [7, 9, 10]. Mutations in AβPP, PSEN1, and PSEN2 changes the Aβ protein levels [7, 9, 10]. Although much research has been conducted and many trials tried to work on a treatment based on this hypothesis, many failed [11]. An association between AD and Aβ is established but it is not coherent and there is no effective treatment yet. Lack of consistency in causality between Aβ and AD remains. Amyloid beta hypothesis fails to explain for several points: Aβ deposition do not correlate with the severity of dementia, not every person with Aβ deposition develops AD or not all AD patients have increased Aβ depositions [3, 11-13]. With increasing questions on Aβ hypothesis, many recent studies now focus more and more on the vascular hypothesis [3, 11-13].

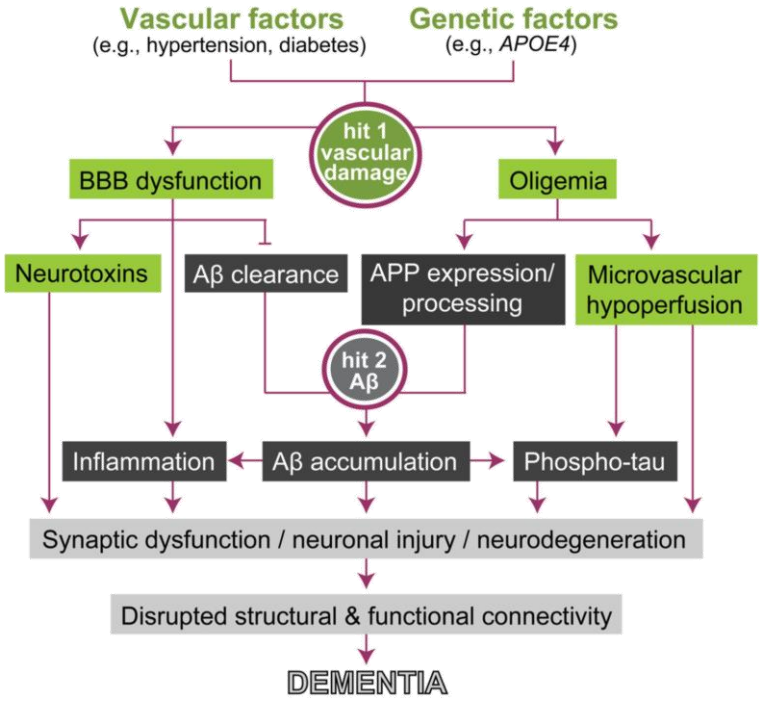

Figure 1. Two hit hypothesis of AD. [14]

Essential nutrients, oxygen, and glucose are delivered, and metabolic waste is removed from the central nervous system via the blood vessels, therefore regulation of cerebral blood flow (CBF) is crucial [14]. Improper CBF and cerebrovascular dysfunction damage brain function and structure [14]. Moreover, increasing evidence from recent studies shows that cerebrovascular dysfunction and dementia are linked [14]. In the case of AD, the two hit vascular hypothesis states that the first hit is the cerebrovascular damage which is enough to cause neurodegeneration alone [14]. Also, cerebrovascular damage can cause improper Aβ clearance from the brain and thus lead to increased Aβ aggregation which is the second hit (Fig. 1) [14]. Vascular risk factors can increase the accumulation of Aβ or decreases the Aβ clearance or contributes to both.[4, 15-17]. Ultimately, microvascular changes in the brain are dependent on the amyloid cascade to a certain degree [11].

b. Common risk factors for VaD and AD

Clinical studies repeatedly demonstrated the crucial interaction between vascular risk factors and AD [4, 11, 14-21]. VaD and AD share common vascular risk factors such as stroke, midlife hypertension, atherosclerosis, diabetes mellitus, obesity, hypocholesterolemia, improper CBF, coronary diseases, and smoking [4, 11, 14-21]. Furthermore, lifestyle factors such as diet, exercise, sleep, and education play an important role (Fig. 2) [4, 11, 14-21].

Vascular changes and AD pathology are dependent on age. Increased age has several effects on the vascular system such as changes in the elasticity of the veins, changes in membrane thickness and amyloid accumulation on artery walls [16-18, 21, 22]. Changes in blood pressure (BP) are also age dependent. While low BP (hypotension) later in life is associated with increased risk of dementia, midlife hypertension stands out as the most important risk factor [21-23]. When systolic blood pressure (SBP) is higher than 140mmHg and diastolic blood pressure (DBP) is higher than 90mmHg it is classified as hypertension [21-23]. It can lead to small vessel diseases, infarcts, changes in white matter (WM), hemorrhages and cerebral hypoperfusion [21-23]. It changes the function and structure of brain vessels and therefore causes disruptions of CBF [24]. As discussed before, proper CBF is crucial for cerebrovascular health. Reduced cerebral perfusion can exacerbate already existing AD symptoms and can lead to ischemic lesions which in turn cause improper cerebral circulation (Fig.3)[24].

Type of risk	Features
Stroke	Silent infarcts Stroke Transient ischemic attacks
Atherosclerosis	Carotid arteries Aortic arch Circle of Willis
Blood pressure	Hypertension systolic BP; >130 mm Hg; diastolic >95 mm Hg
Heart disease	Coronary artery disease Congestive heart failure Cardiac arrhythmia Atrial fibrillation
Diabetes	Type I Type II
Dyslipidemia	High cholesterol High triglycerides
Peripheral risk factors	Smoking Alcoholism Drug abuse Traumatic brain injury Diet Obesity Exercise Lower education Depression Sleep
Non-modifiable	Age Sex Genetic factors Menopause

Figure 2. Vascular risk factors. (adapted from 4, 11, 14-21)

Furthermore, chronic elevated BP leads vessel walls thickening and therewith decrease in the diameter of the micro vessels. This thickening in larger cerebral arteries also causes reduced diameter alongside with aggregated plaques. The plaques can cause rupture which leads to infarcts and complete occlusion of arteries resulting in stroke. [23]

c. Stroke and dementia

Thus, an important risk factor then stands out from hypertension, VaD, and AD: stroke. Risk factors for stroke can be categorized in three: modifiable (hypertension, diabetes mellitus, atrial fibrillation, hyperlipidemia, obesity, smoking etc.), potentially modifiable (alcohol/drug abuse, infections etc.) and non-modifiable (age, sex, genetics etc.) [25, 26]. So, there is a significant overlap between risk factors for VaD, AD, and stroke [25, 26]. While stroke is a risk factor for

VaD and AD, they in return increase the risk of stroke [27, 28]. According to the World Health Organization, ischemic heart diseases and stroke were accountable for 15.2 million deaths combined, which makes them the leading cause of death in 2016 [29]. Furthermore, dementias were the 5th cause of death in 2016 with approximately 2 million deaths [29].

Stroke can be divided into two classes: hemorrhagic or ischemic, but 80% of the cases are ischemic [30]. 2/3 of stroke patients later develop cognitive impairment while 1/3 develop post stroke dementia (PSD) (VaD, AD or mixed dementia) [31]. Ischemic strokes arise from thromboembolic occlusion of the cerebral arteries which leads to irreversible tissue injury and infarcts [32]. Global or focal ischemia has significant effects on cerebral circulation. Cerebral circulation is reduced significantly at the ischemic core in focal cerebral ischemia and is totally disturbed in global ischemia [24]. Clinical research shows stroke exacerbate the dementia symptoms [11]. Furthermore, brain damage due to stroke leads to increased mortality [33].

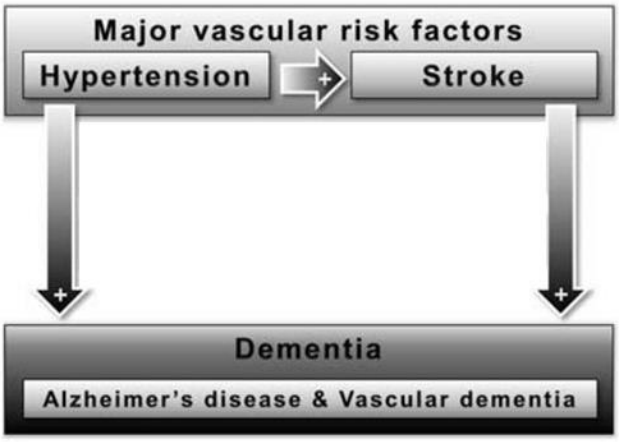

Figure 3. The connection between risk factors and dementia. (adapted from [25])

d. Sex differences in AD and stroke

Men and women show differences in AD development and progression. Studies suggest that the prevalence of AD is higher among women than men, but men have more rapid progression of the disease. [34]

Sex hormones play a significant role. In women, primary sex steroid hormone is estrogen. Overall exposure to estrogen is an important factor. Estrogen has neuroprotective factors (e.g. anti-inflammatory, protective against oxidative stress, Aβ clearance, and degradation). Research shows that reduced estrogen levels in adulthood is linked with increased risk of AD. [34, 35]

Especially, menopause is a critical time window. Menopause happens around the age of 50 and it is the cessation of the menstrual cycle that is accompanied with hormonal and endocrinal changes [34]. Studies show surgical menopause before the natural menopause significantly increases dementia risk [34-36]. Nevertheless, this interaction between low estrogen levels in adulthood and increased AD risk present in women and not men. This indicates increased vulnerability of women to AD [34]. In men, lower levels of testosterone during the normal course of aging is associated with increased risk of AD [34]. Yet again, decreased testosterone is a risk factor for men and not for women. Although different from women, men go through andropause which is a gradual decrease of testosterone that takes several decades [34]. So, the reproductive aging is different between sexes but in both normal depletion of sex hormones results in decreased neuroprotective effects and increased risk of AD. [34-36] Additionally, there are innate differences in brain function and structure between sexes that arise from sexual differentiation during early brain development [34]. Although there is a lack of evidence for the effect of chromosomal differences contribution to the prognosis of AD, there is no reason to assume that there is no interaction between the organizational and activational effect of sex hormones [34-36].

Research shows that there is a difference between the sexes in stroke prevalence. Risk of stroke increases with age, especially among women [37]. Although women are protected against stroke by estrogen before the menopause, this condition changes post menopause. Again, like in AD, menopause is a critical time window for stroke due to changes in the hormones, increased body weight, and higher BP [37]. So, at a later age, stroke risk changes; females being more at risk than men. Increased rates of stroke fatality and mortality at older aged women are higher and this may be attributable to higher life expectancy compared to men [37]. Another important factor is anatomical vasculature. Men have bigger arteries and hearts to due bigger body size which is a risk factor for stroke [37].

In conclusion, emerging evidence shows that stroke increases the risk of developing AD and in return, AD is a risk factor for stroke, but exact mechanisms behind this correlation are unknown and remain to be investigated. The main objective of this study is to evaluate the underlying mechanisms of stroke on AD pathophysiology and sex-specific differences.

3. Methods

a. Study Design

In this double-blinded, randomized study, male and female $APP_{swe}/PS1_{\Delta E9}$ (APP/PS1) and C57BL/6 wildtype (WT) mice were randomly divided into the 8 experimental groups: WT sham (male/female), AD sham (male/female), WT stroke (male/female) and AD stroke (male/female). First, at 3- months of age general health parameters (body weight and BP) and motor function to assess locomotion, activity and strength (via pole test, grip test, and open field) were measured as baseline. Then, stroke was induced by transient Middle Cerebral Artery occlusion (tMCAo) and sham mice went through the operation without getting the stroke. To assess the acute effects of the surgery, baseline measurements were repeated two weeks after the operation. Next, to evaluate the effect of stroke on brain structure, imaging experiments were conducted (rsfMRI, DTI, FAIR-ASL). All experiments were repeated periodically for the next eight months, until the mice were 12- months old. Finally, mice were euthanized via transcardial perfusion. Brain and tissues were collected for post mortem analysis.

Due to the longitudinal approach, amount of time-consuming experiments, and high number of animals, the present study was divided into six cohorts and distributed evenly across two years (Fig. 4). In each cohort the study design was the same (Fig. 5)

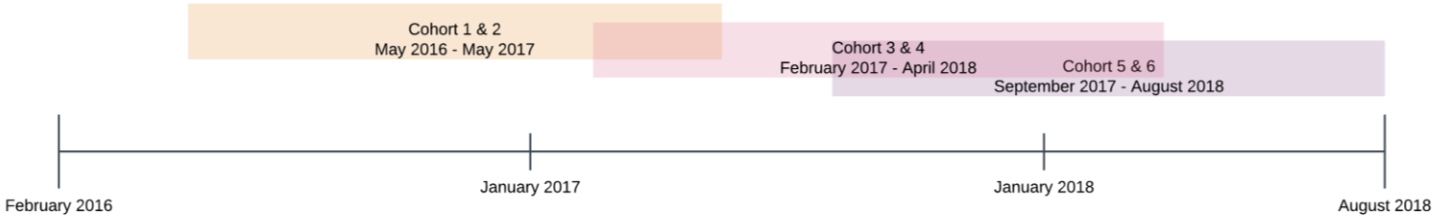

Figure 4. General outline of the study.

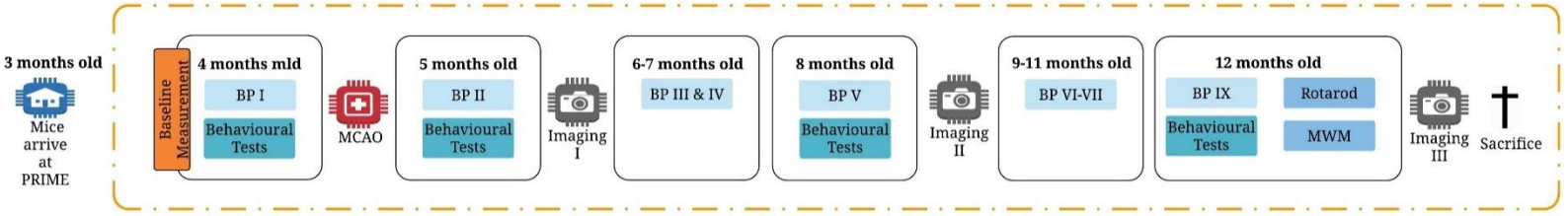

Figure 5. Study design.

b. Animals

$APP_{swe}/PS1_{\Delta E9}$ mouse model was selected for this study to be the AD model and C57BL/6 littermate mice were used as WT controls [38-40]. APP/PS1 mice was selected due to their increased expression of human amyloid precursor protein (APP). In this mice model Aβ deposition starts at six weeks of age at the neocortex and at 3-4 months of age at the hippocampus, thalamus and brainstem [38-40]. Cerebrospinal fluid (CSF) Aβ levels are inversely correlated with brain Aβ levels and decreases with aging. This mouse model presents 50% decrease in CSF Aβ level by the age of 6 months and 80% by the age of 18 months leading to increased cognitive impairments [38-40]. Furthermore, this model has shown to have increased systolic blood pressure (SBP) due to the reduction in regional CSF when subjected to stroke [38-40]. Mice models used in this study were created via the co-injection of mouse/human APP with the Swedish mutation (K595N and M596L) and human PS1 gene with the deletion of exon-9 [38-40]. APP/PS1 mouse was obtained from John Hopkins University Baltimore, MD, USA (D. Borchelt and J. Jankowsky, Dept. of Pathology). Then animals were bred at the Central Animal Facility at the Radboud University medical center (Nijmegen, The Netherlands) [40].

All animals were housed in digital ventilated cages (DVC) (Techniplast, Buguggiate, VA, Italy) with room temperature at 21°C, and artificial 12:12h light: dark cycle (lights on at 7 a.m.). Food and water were ad libitum and all mice were fed a standard diet (Ssniff rm/h V1534-000, Bio Services, Uden, The Netherlands) [33]. Before the stroke surgery, animals were grouped house with maximum of six mice/ cage and after the surgery mice were caged solitarily. All experiments were conducted at the preclinical imaging centre (PRIME) of the Radboudumc (Nijmegen, The Netherlands) between 7 a.m. and 18 p.m [33]. All experiments involving animal care and treatment were in accordance with guidelines of the Dutch federal regulations for animal protection. The experiments were approved by the Veterinary Authority of Radboud university medical center (Nijmegen, The Netherlands) and the Animal Experiment Committee (Dierenexperimentencomissie (DEC), (2012-248 & 2015-0079) of the Radboud University, (Nijmegen, The Netherlands). All performed animal experiments is in accordance with the ARRIVE guidelines [33].

All mice were divided into 8 experimental groups: wild type sham (male/female), AD sham (male, female), wild type stroke (male/ female) and AD stroke (male/female). Animal numbers per group were calculated with a power of 0.80 and $\alpha = 0.05$. Estimated mortality rates were taken into account in the calculation; WT sham (0%), APP/PS1 sham (10%), Wt stroke (35%)

and APP/PS1 stroke (60%). Total of 144 mice were used in the study and 92 mice completed all experiments (44 male, 48 female) (Fig. 6).

# of animals per group		Male	Female
WT	Sham	14	15
	Stroke	13	14
APP/PS1	Sham	9	10
	Stroke	8	9

Figure 6. Number of animals per experimental group.

c. Stroke induction

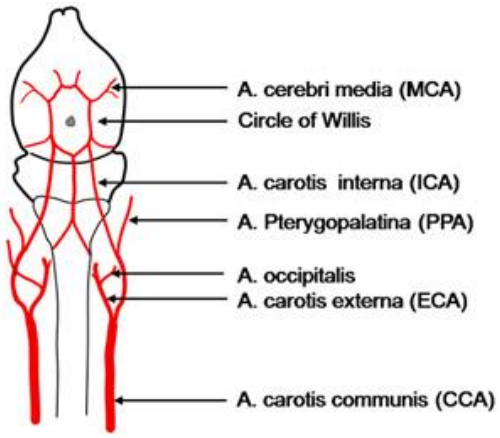

Figure 7. Cerebrovascular structure of a mouse.

MCA is the most common artery to be affected from ischemic strokes and occlusion [30], therefore all mice went through tMCAo at 3- months of age to mimic ischemic stroke. Before the surgery, Rimadyl (5mg/kg) was injected subcutaneously to prevent inflammation and pain after the surgery. During the surgery animals were anesthetized with 2-3% isoflurane (Abbott Animal Health, Abbot Park, IL, USA) in a 2:1 air and oxygen mix and placed on a heating pad to keep body temperature at constant levels [33]. A filament (70SPRePK5, Doccol Corp., Sharon, MA, USA) was inserted through the common carotid artery (CCA) and pushed forward until the tip of the filament occluded MCA for 30 minutes for the stroke mice. For the sham mice filament was introduced briefly but did not reached and occluded MCA (Fig. 7) [33]. After 30 min. the filament was retracted to allow reperfusion. Doppler probe (moorVMS-LDF2, Moor Instruments, UK) was attached to the skull prior to the surgery to monitor and validate reduction in CBF; 80 % decrease in regional CBF considered a successful stroke induction [33]. For the next 7 consecutive days after the surgery, mice were checked daily for signs of discomfort and ensure proper healing.

d. Tests

I. General health parameters

i. BW

Initially body weight was measured before and for seven consecutive days after the operation. After that, body weight has been measured periodically every month with a digital scale.

ii. SBP

SBP was measured with a heated tail-cuff plethysmography device (IITC Life Scientific Instruments, Woodland Hills, CA) [40]. At 3- months of age, all mice got habituated to the procedure in two consecutive days. In short, mice were placed in a heated plexiglass restrainer and stabilized with a head gate. Their tail was placed into the cuffs with pulse sensors that allows detection of the blood pressure. Mice were acclimatized for at least five minutes before each measurement. Every BP measurement consisted of 10 trials, each lasting 30 seconds. The trials were recorded with BPMonitor software (IITC Life Science Instruments, Woodland Hills, CA, USA) [33]. The recordings were manually analyzed, and first 4 trials of each measurement were considered as habituation and therefore excluded from the statistical analysis. This experiment was conducted periodically every month.

II. Behavioral tests

i. Pole test

The pole test was done to evaluate the motor coordination of the mice [33]. The mice were placed on ~7 cm from top of a rough surfaced pole (diameter 2.5 cm; height 60 cm). They were expected to turn downwards and descend to the base of the pole. Each mouse was tested five times, but the first trial was considered as habituation and therefore excluded from the statistical analysis. The velocity climb down was automatically assed by EthoVision XT10.1 (Noldus, Wageningen, The Netherlands) while rotation time was manually timed [33]. Pole test was conducted at 4-, 8- and 12- months of age (2 weeks, 4 months and 8 months after the surgery respectively).

ii. Grip test

Grip strength test was performed to evaluate forelimb and total limb (fore- and hindlimb) strength with a grip strength meter (Grip-Strength Meter, 47200, Ugo Basile, Italy) [33]. Mice were hold by their tails and then they were lowered close to the trapeze with forepaws or the grid with both fore- and hind paws. Once they grasped the trapeze/grid, mice were pulled back gently until they

released their grip and the grip strength meter measured the muscle strength (in gf). Experiment was repeated five times for both for the trapeze and the grid. Trials in which the animal did not use their two paws for the trapeze or four paws for the grid were considered invalid and excluded from the statistical analysis, then the muscle strength was determined by averaging the valid trials [33]. Grip test was conducted at 4-, 8- and 12- months of age (2 weeks, 4 months and 8 months after the surgery respectively).

iii. Open field

The open field was conducted to evaluate exploratory behavior, locomotion, motor function and anxiety related behaviors [40-42]. Mice were placed in a square shaped arena with transparent plexiglass walls that are high enough to prevent mice from escaping. Mice were given 10 minutes to explore the arena and were tracked with EthoVision XT10.1 (Noldus, Wageningen, The Netherlands). The floor of the arena was divided into different zones: center, periphery, and corners. The walking speed, walking distance, the frequency and the time spent in zones were automatically scored by the software. Additional behavioral measures (walking, sitting, wall leaning, jumping, rearing, grooming) were scored manually [40-42]. Open field was conducted at 4-, 8- and 12- months of age (2 weeks, 4 months and 8 months after the surgery respectively).

iv. Rotarod

Rotarod was conducted as a measure of motor coordination and balance [42]. Mice were placed on a rotating rod (IITC Inc., Woodland Hills, CA, USA) with a fixed diameter (3.18cm) that accelerates in speed over time (4-40 rounds per minute) for max. 300 seconds/ trial. The latency to fall was recorded manually. Rotarod was conducted at 12-months of age (8 months after the surgery) [42].

v. Morris water maze

Morris water maze (MWM) was used to assess spatial learning and memory [40, 42]. Mice were placed in a circular pool (diameter:104 cm) that was filled with opaque water (22°C water mixed with milk powder) and expected to use the visual cues that were placed on the walls around the maze to find the hidden platform that was in the North-East (NE) quadrant of the pool [40, 42]. Acquisition was done over four consecutive days. Each mouse was placed in the pool four times per day, starting from a different entry point each time (south, north, west, and east). Mice were given 120 seconds to find the hidden platform and upon finding, mice were expected to stay on

it for 30 seconds. Inter-trial time between starting points was set to 1 hour minimum. On the last day, the hidden platform was removed from the pool and mice were allowed to swim for 120 second. Latency to find the platform was manually timed in the acquisition phase, while velocity, distance, frequency and time spent in zones were automatically measured by EthoVision, XT10.1 (Noldus, Wageningen, The Netherlands) [40]. MWM was performed at 12-months of age (8 months after the surgery).

e. Magnetic Resonance Imaging

MRI measurements were done to assess and visualize the effect of stroke on brain structure. MRI was performed on a 11.7 T BioSpec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with an actively shielded gradient set of 600 mT/m and operating on Paravision 5.1 software platform (Bruker, Karlsruhe, Germany) [40]. Circular polarized volume resonator was used for signal transmission (Bruker BioSpin) along with an actively decoupled mouse brain quadrature surface coil [40]. All mice were anesthetized (3.5% for induction) in a 2:1 oxygen and N2O mix before the scanning sessions. They were placed in a stereotactic holder to minimize movement [40]. A rectal probe along with a heated airflow was used to monitor and maintain the body temperature of the mice at 37˚C. A pneumatic cushion respiratory monitoring system (Small Animal Instruments Inc, NY, USA) was used to monitor respiration during the scanning session [40]. During an imaging session the resting state fMRI (rsfMRI), diffusion tensor imaging (DT1) and flow-sensitive alternating inversion recovery (FAIR) – arterial spin labelling (ASL) were performed as in described previously [40]. FAIR-ASL was done to evaluate regional CBF for the selected regions of interest (ROI) that were in two different bregmas (according to the Paxinos and Franklin mouse brain atlas). The cerebral cortex, hippocampus, thalamus were located at bregma -1.82 to -1.94 and somatosensory cortex, caudate putamen, corpus callosum were located at bregma 0.38 to 0.50 [40].

f. Post mortem analysis

After the last scanning session, mice were sacrificed by transcardial perfusion. Post mortem tissue (ear, eyes, brain, CSF, heart, aorta, common carotid artery (CCA), and blood plasma) were collected. Brains were fixated with 4% paraformaldehyde for ~14h in 4°C and afterwards stored in in 0.1M PBS with 0.01% sodium azide at 4°C for immunohistochemical staining. Other organs and tissue were saved frozen in liquid nitrogen initially and afterwards stored in -80°C [40]. Furthermore, vaginal smears from female mice were collected for further investigation.

g. Statistics

All data were analyzed using IBM SPSS 24 software (IBM Corporation, New York, NY, USA). Repeated measures ANOVA and multivariate ANOVA with Bonferroni correction was used to analyze all data except for rotarod, ASL and MWM probe (univariate ANOVA) with a group dependent combination of between-subject-factors (genotype and surgery). All general health parameters and behavioral tests were split for sex. All data are expressed as mean ± SD. Statistical significance was set at $p \leq 0.05$; #, $0.05 < p < 0.08$ (tendency); *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

4. Results

All results are summarized in Appendix A.

a. Body Weight

i. Acute effects

Male mice showed a statistically significant time x genotype ($F(1,38) = 7.293$, $p = 0.01$) and time x surgery interactions ($F(1,38) = 4.886$, $p = 0.033$). Male WT mice were lighter two weeks after surgery compared to pre-surgery (Fig. 7A: $F(1,25) = 13.393$, $p = 0.001$). Also, male stroke mice were lighter two weeks after surgery compared to pre-surgery ($F(1,19) = 9.489$, $p = 0.006$; data not shown).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, were heavier two weeks after surgery compared to pre-surgery (Fig 7B: $F(1,44) = 14.535$, $p \leq 0.001$). Furthermore, all female stroke mice were heavier both at pre-surgery and two weeks after the surgery ($F(1,44) = 8.364$, $p = 0.006$; data not shown).

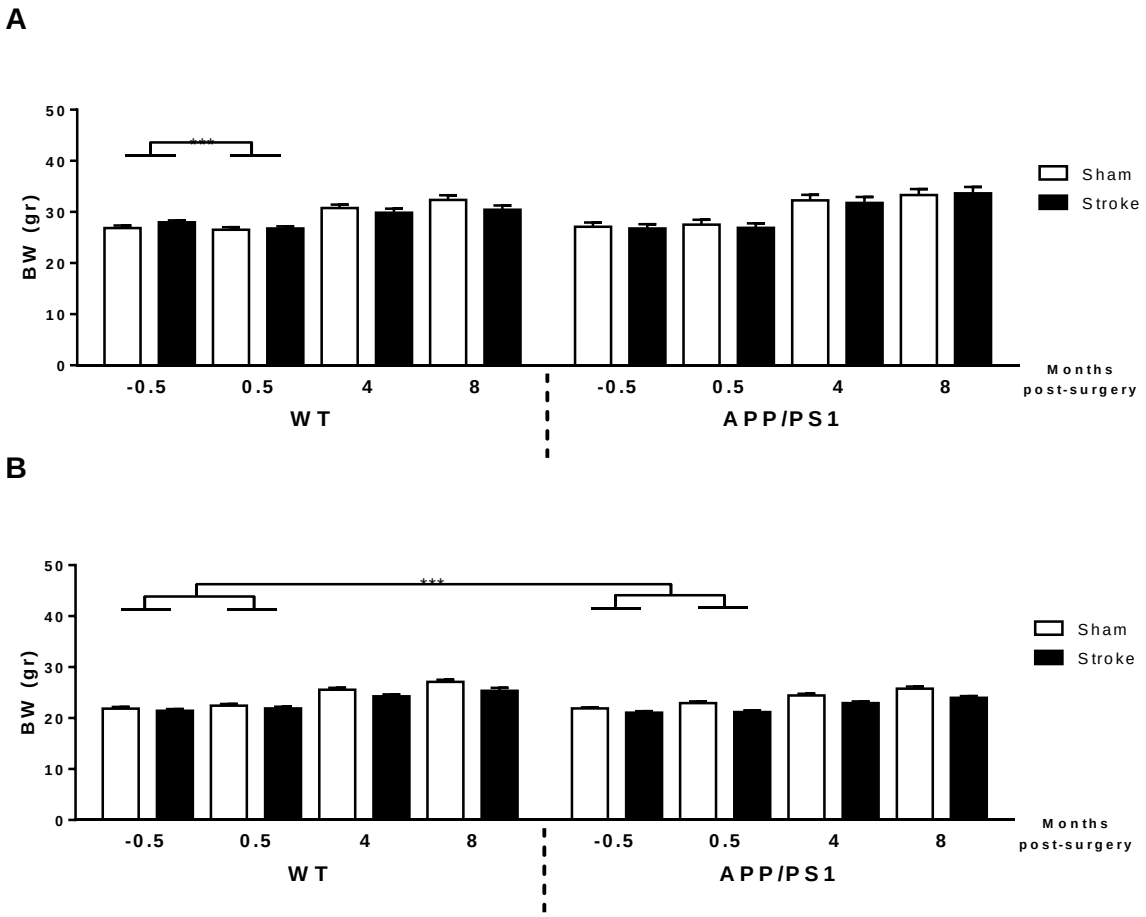

Figure 7. Acute effects on the body weight A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference). (A) Male WT and male stroke mice were lighter two weeks after surgery compared to two weeks before the surgery ($p = 0.001$). (B) All female mice (WT, APP/PS1, sham and stroke), were heavier two weeks after surgery compared to two weeks before the surgery ($p \leq 0.001$). Stroke mice were heavier than sham mice both two weeks before and two weeks after the surgery ($p = 0.006$).

ii. Chronic effects

Male mice showed a statistically significant time x genotype interaction ($F(1,38) = 7.086$, $p = 0.011$). In detail, both WT (Fig. 8A: $F(1,15) = 50.356$, $p \leq 0.001$) and APP/PS1 mice (Fig. 8A: $F(1,18) = 68.527$, $p \leq 0.001$) were heavier 4 months after surgery compared to pre-surgery. Furthermore, a significant time x surgery interaction was found ($F(1,38) = 5.056$, $p = 0.03$). In detail, both sham ($F(1,21) = 122.431$, $p \leq 0.001$; data not shown) and stroke mice ($F(1,19) = 24.349$, $p \leq 0.001$; data not shown) were heavier 4 months after surgery compared to pre-surgery. Female mice showed a statistically significant time x genotype interaction ($F(1,44) = 11.785$, $p = 0.001$). In detail, both WT (Fig 7B: $F(1,28) = 252.312$, $p \leq 0.001$) and APP/PS1 mice (Fig 7B: $F(1,18) = 81.041$, $p \leq 0.001$;) were heavier 4 months after surgery compared to pre-surgery. Furthermore, a significant time x surgery interaction was found ($F(1,44) = 6.236$, $p = 0.016$). In detail, both sham ($F(1,24) = 191.329$, $p \leq 0.001$; data not shown) and stroke mice ($F(1,22) = 108.523$, $p \leq 0.001$; data not shown) were heavier 4 months after surgery compared to pre-surgery. Additionally, female mice exhibited an overall genotype (Fig. 8B: $F(1,44) = 4.684$, $p = 0.036$) and surgery (Fig. 8B: $F(1,44) = 9.657$, $p = 0.003$) effect; APP/PS1 mice were lighter than WT mice and stroke mice were lighter than sham mice both at pre-surgery and 4 months after the surgery.

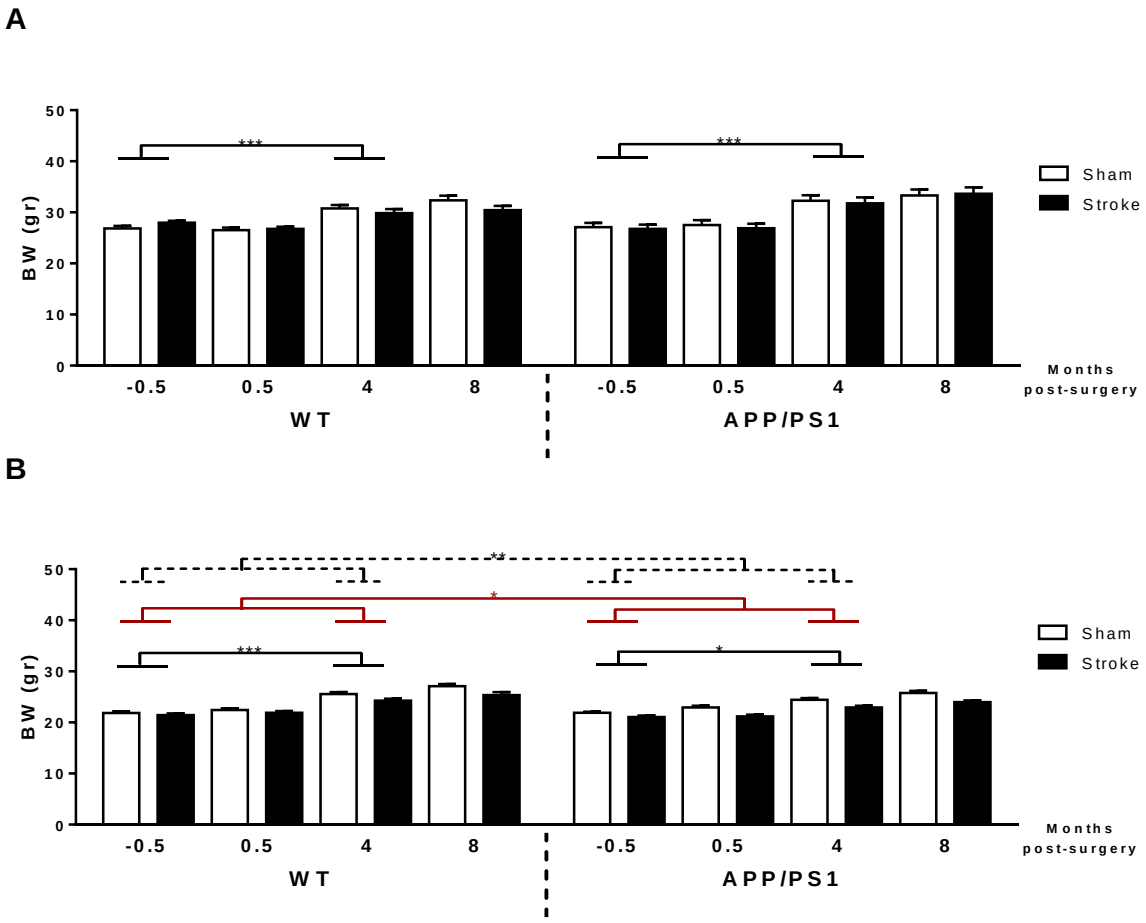

Figure 8. Chronic effects on the body weight A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference). (A) All male mice (WT, APP/PS1, sham and stroke) were heavier 4 months after surgery compared to two weeks before surgery ($p = 0.011$). (B) All female mice (WT, APP/PS1, sham and stroke) were heavier 4 months after surgery compared to two weeks before surgery ($p = 0.011$). APP/PS1 mice were lighter than WT mice ($p = 0.036$) and stroke mice were lighter than sham mice both at pre-surgery and 4 months after the surgery ($p = 0.003$).

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a statistically significant time x genotype x surgery interaction ($F(2,76) = 4.546$, $p = 0.014$). Specifically, WT mice demonstrated a time x surgery interaction (Fig. 9A: $F(2,48) = 9.026$, $p \leq 0.001$). In detail, both WT stroke ($F(2,48) = 9.026$, $p \leq 0.001$; data not shown) and WT sham mice ($F(2,24) = 41.486$, $p \leq 0.001$; data not shown) showed a time effect; both groups showed a gradual increase in body weight after the surgery. Additionally, APP/PS1 mice exhibited an overall time effect; body weight increased after the surgery (Fig. 9A: $F(2,28) = 96.951$, $p \leq 0.001$). Furthermore, a time x genotype interaction was found in stroke mice ($F(2,36) = 7.227$, $p \leq 0.01$). In detail, both WT stroke ($F(2,24) = 41.486$, $p \leq 0.001$; data not shown) and APP/PS1 stroke mice ($F(2, 12) = 69.803$, $p \leq 0.001$; data not shown) showed a gradual increase in body weight after the surgery. Additionally, sham mice exhibited an overall time effect; body weight increased at every time point after the surgery ($F(2,40) = 149.165$, $p \leq 0.001$; data not shown). Moreover, male mice revealed a significant overall surgery effect; stroke mice were lighter than sham mice at every time point after the surgery (Fig. 9A: $F(1,38) = 4.140$, $p = 0.049$). Female mice showed a statistically significant time x genotype interaction ($F(2,86) = 8.314$ $p \leq 0.001$). In detail, both WT (Fig. 9B: $F(2,54) = 146.203$, $p \leq 0.001$) and APP/PS1(Fig. 9B: $F(2,36) = 88.771$, $p \leq 0.001$) mice had a gradual increase in body weight after the surgery. Additionally, there female mice exhibited an overall genotype (Fig. 9B: $F(1,43) = 5.968$, $p = 0.019$) and surgery (Fig. 9B: $F(1,43) = 15.180$, $p \leq 0.001$) effect; APP/PS1 mice were lighter than WT mice and stroke mice were lighter than sham mice at every time point after the surgery.

Restoration:

Male mice showed a statistically significant time x genotype x surgery interaction ($F(1,38) = 4.530$, $p = 0.04$). Specifically, WT mice demonstrated a time x surgery interaction ($F(1,24) = 15.469$, $p = 0.001$). In detail, both WT sham ($F(1,12) = 142.664$, $p \leq 0.001$; data not shown) and WT stroke mice ($F(1,12) = 12.253$, $p = 0.004$; data not shown) were heavier 8 months after surgery compared to pre-surgery. Additionally, APP/PS1 mice were heavier 8 months after surgery compared to pre-surgery (Fig. 9A: $F(1,14) = 66.229$, $p \leq 0.001$). Furthermore, a time x genotype interaction was found in stroke mice ($F(1,18) = 9.393$, $p = 0.007$). In detail, both WT stroke ($F(1,12) = 12.253$, $p = 0.004$; data not shown) and APP/PS1 stroke ($F(1,6) = 33.128$, $p = 0.001$; data not shown) were heavier 8 months after the surgery compared to pre-surgery. Additionally, sham mice exhibited an overall time effect; mice were heavier 8 months after surgery compared to pre-surgery ($F(1,20) = 131.766$, $p \leq 0.001$; data not shown).

Female mice showed a statistically significant time x genotype interaction ($F(1,43) = 8.458$, $p \leq 0.001$). In detail, both WT (Fig. 9B: $F(1,27) = 243.811$, $p \leq 0.01$) and APP/PS1(Fig. 9B: $F(1,18) = 160.700$, $p \leq 0.001$) mice were heavier 8 months after surgery compared to pre-surgery. Furthermore, a statistically significant time x surgery interaction was found ($F(1,43) = 8.583$, $p \leq 0.01$). In detail, both sham ($F(1,24) = 295.058$, $p \leq 0.001$; data not shown) and stroke ($F(1,21) = 119.769$, $p \leq 0.001$; data not shown) mice were heavier 8 months after surgery compared to pre-surgery. Moreover, female mice revealed a significant overall genotype (Fig. 9B: $F(1,38) = 4.513$, $p = 0.039$) and surgery (Fig. 9B: $F(1,43) = 10.002$, $p = 0.003$) effect; APP/PS1 mice weighed less than WT mice, and stroke mice weighed less than sham mice at 8 months after surgery compared to pre-surgery respectively.

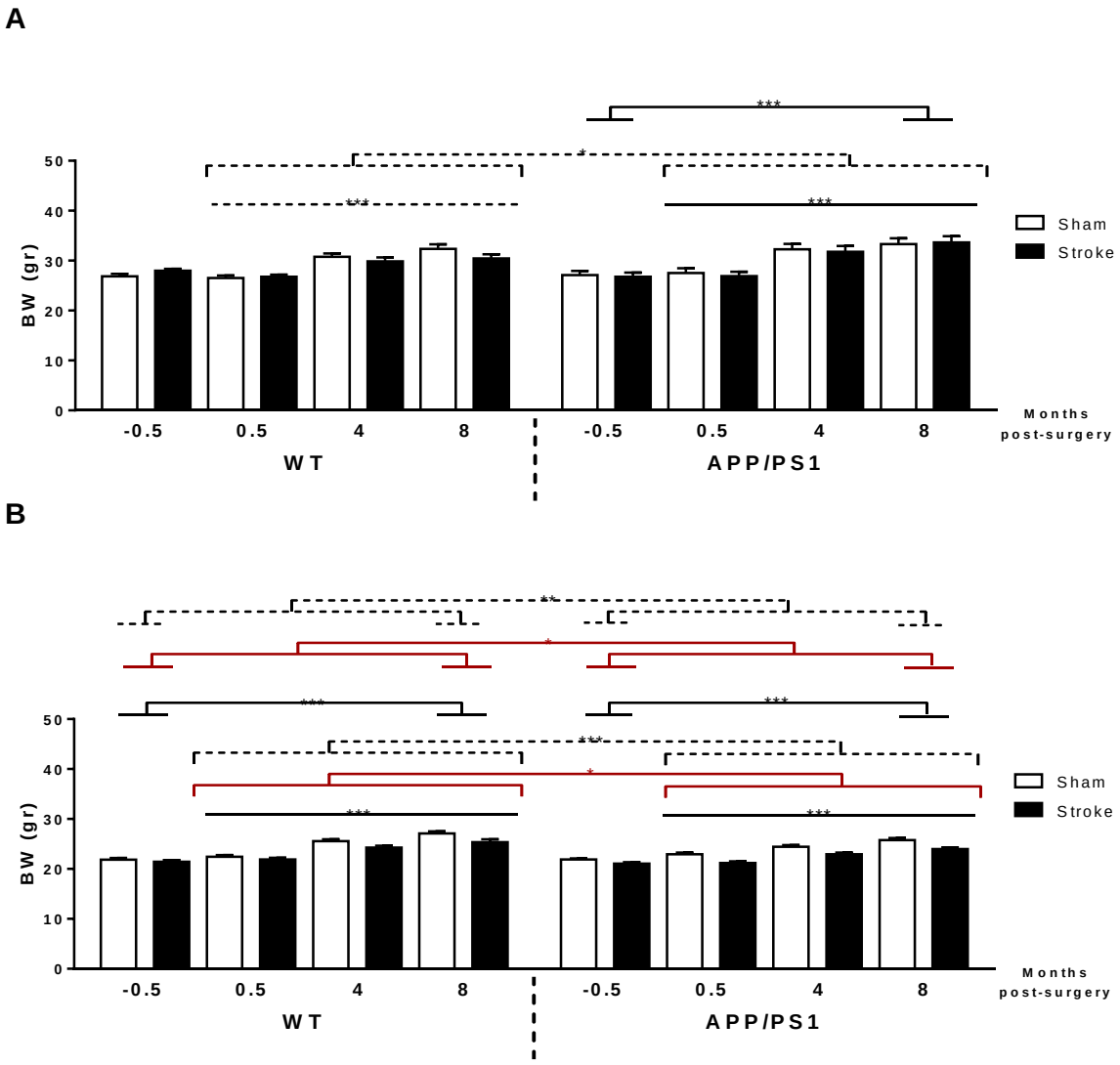

Figure 9. Revalidation and restoration effects on the body weight A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference). (A) Revalidation: All male groups (WT, APP/PS1, sham and stroke) showed a gradual increase in body weight after the surgery ($p = 0.04$). APP/PS1 had increased BW after the surgery ($p \leq 0.001$). Stroke mice were lighter than sham mice at every time point after the surgery ($p = 0.049$). Restoration: All male groups (WT, APP/PS1, sham and stroke) were heavier 8 months after surgery compared to two weeks before the surgery. (B) Revalidation: All female groups (WT, APP/PS1, sham and stroke) showed a gradual increase in body weight after the surgery ($p \leq 0.001$). Female APP/PS1 mice were lighter than WT mice ($p = 0.019$) and stroke mice were lighter than sham mice ($p \leq 0.001$) at every time point after the surgery. Restoration: All female groups (WT, APP/PS1, sham and stroke) were heavier 8 months after surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice weighed less than WT mice ($p = 0.039$), and stroke mice weighed less than sham mice ($p = 0.003$) at 8 months after surgery compared to two weeks before the surgery respectively.

b. Blood Pressure

i. Acute effects

Male mice showed a statistically significant genotype x surgery interaction ($F(1,37) = 7.114$, $p = 0.011$). Specifically, sham mice demonstrated a genotype effect ($F(1,37) = 7.114$, $p = 0.011$). In detail, APP/PS1 sham mice had an elevated SBP compared to WT sham mice ($F(1,18) = 12.787$, $p = 0.002$; data not shown). Furthermore, WT mice showed a surgery effect (Fig. 10A: $F(1,25) = 6.559$, $p = 0.017$). In detail, WT stroke mice had elevated SBP compared to WT sham mice. Moreover, male revealed a significant overall genotype effect; APP/PS1 mice had increased SBP compared to WT mice, regardless of their surgery, both at pre-surgery and two weeks after surgery (Fig. 10A: $F(1,37) = 4.767$, $p = 0.035$).

There were no significant effects observed in female mice (Fig.10B).

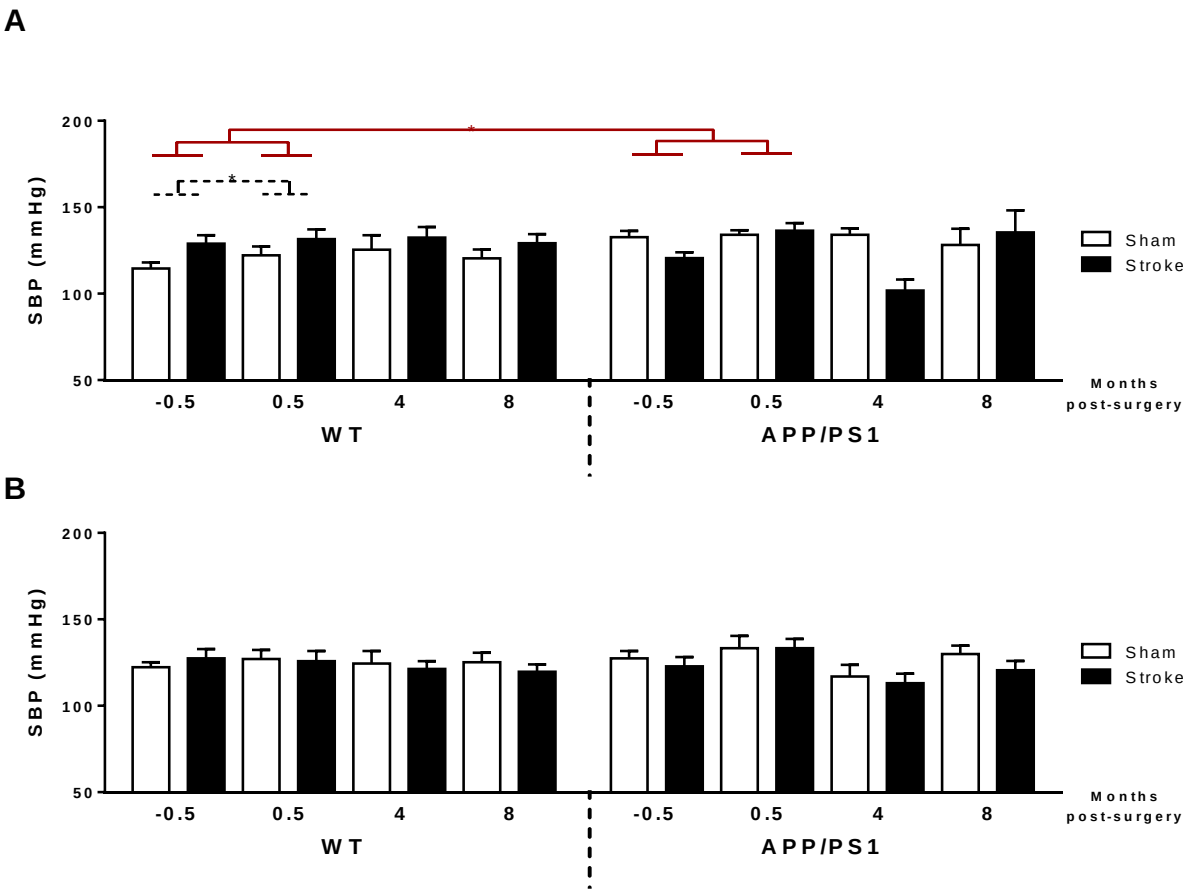

Figure 10. Acute effects on the systolic blood pressure A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference). (A) WT stroke mice had elevated SBP compared to WT sham mice ($p = 0.017$). APP/PS1 mice had increased SBP compared to WT mice, regardless of their surgery, both at two weeks before the surgery and two weeks after surgery ($p = 0.035$). (B) Female mice showed no significant interaction.

ii. Chronic effects

Male mice showed a statistically significant genotype x surgery interaction ($F(1,36) = 11.800$, $p = 0.002$). Specifically, both sham ($F(1,20) = 4.865$, $p = 0.039$; data not shown) and stroke (: $F(1,16) = 7.207$, $p = 0.016$; data not shown) mice demonstrated a genotype effect. In detail, while APP/PS1 sham mice had an elevated SBP compared to WT sham, APP/PS1 stroke mice had a decreased SBP compared to WT stroke mice both at pre-surgery and 4 months after the surgery. Additionally, APP/PS1 stroke mice had a decreased SBP compared to APP/PS1 sham mice both at pre-surgery and 4 months after the surgery (Fig. 11A: $F(1,12) = 6.042$, $p = 0.005$).

There were no significant effects observed in female mice (Fig. 11B)

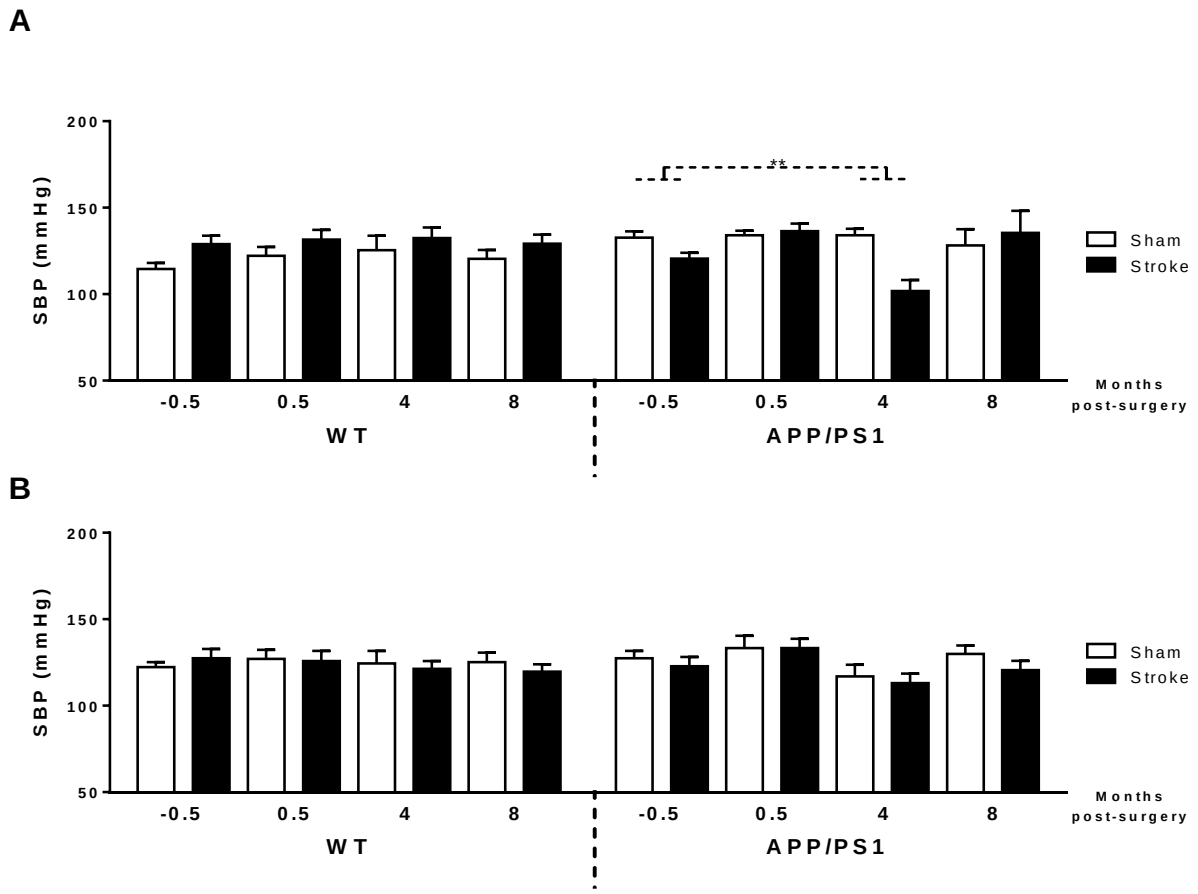

Figure 11. Chronic effects on the systolic blood pressure A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference). (A) APP/PS1 sham mice had an elevated SBP compared to WT sham ($p = 0.039$), APP/PS1 stroke mice had a decreased SBP compared to WT stroke mice ($p = 0.016$) both at two weeks before the surgery and 4 months after the surgery. APP/PS1 stroke mice had a decreased SBP compared to APP/PS1 sham mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.005$). (B) Female mice showed no significant interaction.

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a statistically significant genotype x surgery interaction ($F(1,25) = 4.398$, $p = 0.046$). In detail, APP/PS1 stroke mice had a decreased SBP compared to APP/PS1 sham mice (Fig. 12A: $F(1,5) = 7.014$, $p = 0.046$).

Female mice showed a slight trend in time; SBP decreased over time in all female groups (Fig. 12B: $F(1,70) = 2.939$, $p = 0.06$).

Restoration:

Male mice showed a slight trend in time; SBP decreased over time in all male groups (Fig. 12A: $F(1,29) = 3.554$, $p = 0.069$).

There were no significant effects observed in female mice (Fig. 12B)

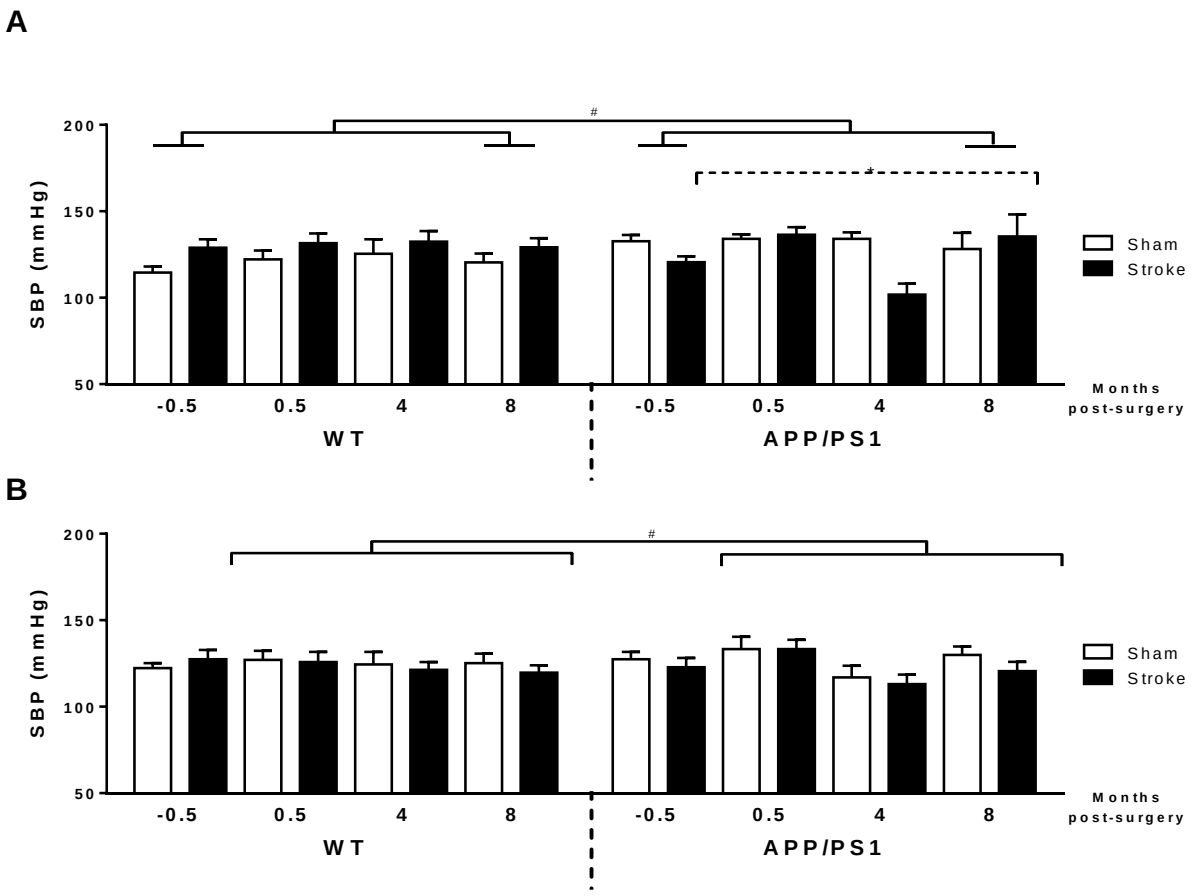

Figure 12. Revalidation and restoration effects on the systolic blood pressure A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Revalidation: APP/PS1 stroke mice had a decreased SBP compared to APP/PS1 sham mice after the surgery ($p = 0.046$). Restoration: All male groups (WT, APP/PS1, sham and stroke) had decreased SBP both at two weeks before the surgery and 8 months after the surgery ($p = 0.069$). (B) Revalidation: All female groups (WT, APP/PS1, sham and stroke) had decreased SBP after the surgery ($p = 0.06$). Restoration: Female mice showed no significant interaction.

c. Behavioral Tests

I. Pole test

I.I Velocity

i. Acute effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were slower to climb down the pole two weeks after surgery compared to pre-surgery (Fig. 13A: $F(1,40) = 23.530$, $p \leq 0.001$). Furthermore, an overall genotype effect was found; APP/PS1 mice were faster than WT mice to climb down the pole both at pre-surgery and two weeks after the surgery (Fig. 13A: $F(1,40) = 6.913$, $p = 0.012$). Specifically, two weeks before the surgery there was a trend in the velocity to climb down the pole, where APP/PS1 mice were faster than WT mice ($F(1,42) = 3.450$, $p = 0.070$; data not shown). APP/PS1 were again faster than WT in velocity two weeks after the surgery ($F(1,42) = 5.325$, $p = 0.026$; data not shown). Moreover, male mice revealed an overall surgery effect; stroke mice were faster than sham mice to climb down the pole (Fig. 13A: $F(1,40) = 8.491$, $p = 0.006$). Specifically, two weeks after the surgery stroke mice were significantly faster than sham mice ($F(1,42) = 7.442$, $p = 0.009$; data not shown).

Female mice showed an overall time effect; all female mice regardless of their genotype and surgery were slower to climb down the pole two weeks after surgery compared to pre-surgery (Fig. 13B: $F(1,44) = 21.711$, $p \leq 0.001$). Furthermore, an overall surgery effect was found; stroke mice were faster than sham mice to climb down the pole (Fig. 13B: $F(1,44) = 5.804$, $p = 0.020$; data not shown). Specifically, two weeks after the surgery stroke mice were significantly faster than sham mice ($F(1,46) = 4.041$, $p = 0.05$; data not shown).

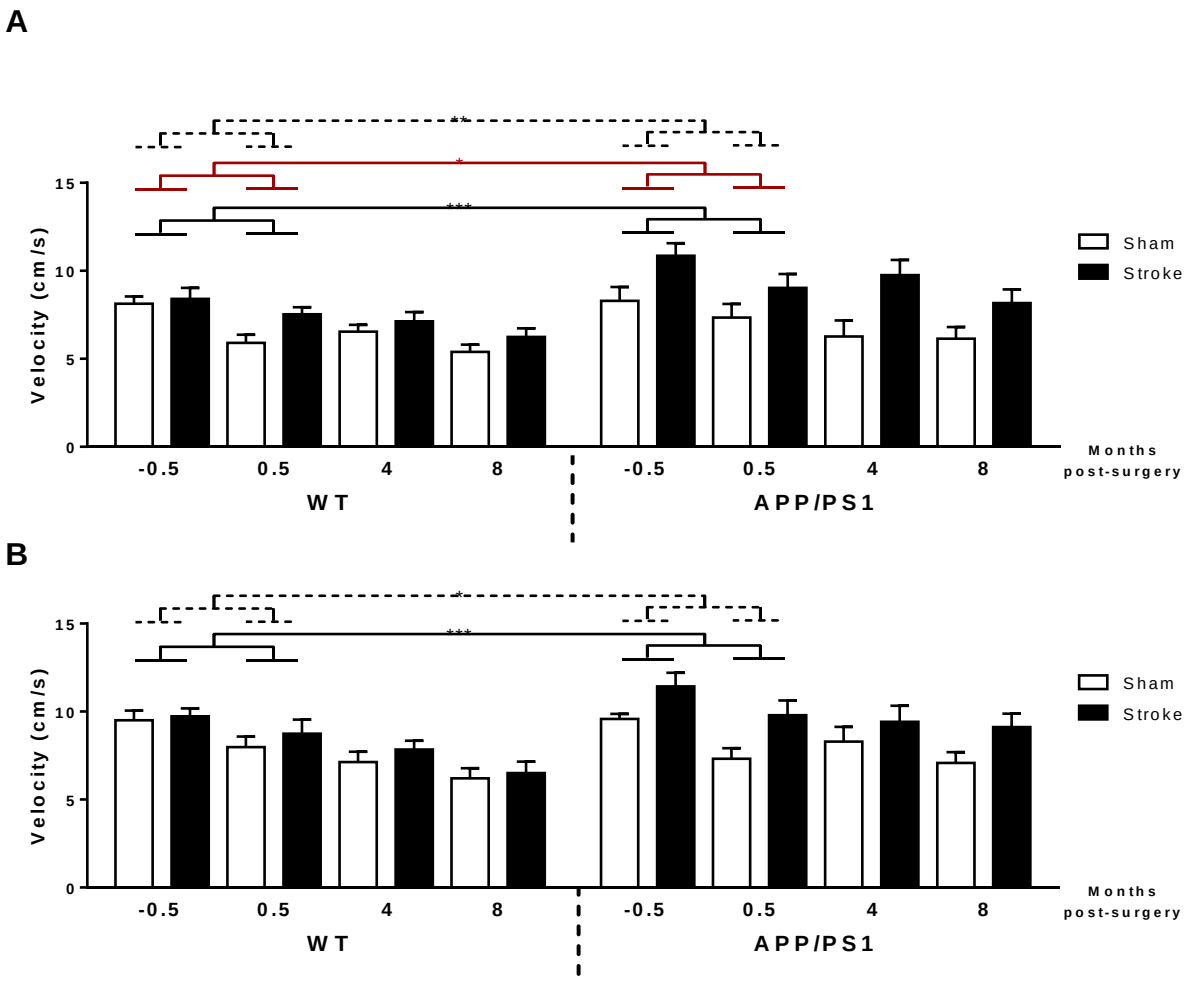

Figure 13. Acute effects on the velocity to climb down the pole A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) were slower to climb down the pole two weeks after surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were faster than WT mice ($p = 0.012$) and stroke mice were faster than sham mice ($p = 0.006$) to climb down the pole both at two weeks before the surgery and two weeks after the surgery. (B) All female groups (WT, APP/PS1, sham and stroke) were slower to climb down the pole two weeks after surgery compared to two weeks before the surgery ($p \leq 0.001$). Stroke mice were faster than sham mice to climb down the pole both at two weeks before the surgery and two weeks after the surgery ($p = 0.020$).

i. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were slower to climb down the pole 4 months after surgery compared to pre-surgery (Fig. 14A: $F(1,37) = 11.725$, $p = 0.002$). Furthermore, an overall genotype effect was found; APP/PS1 mice were faster than WT mice to climb down the pole both at pre-surgery and 4 months after the surgery (Fig. 14A: $F(1,37) = 5.195$, $p = 0.029$). Specifically, two weeks before the surgery there was a trend in the velocity to climb down the pole, where APP/PS1 mice were faster than WT mice ($F(1,42) = 3.450$, $p = 0.070$; data not shown). Moreover, male mice revealed an overall surgery effect; stroke mice were faster than sham mice to climb down the pole both at pre-surgery and 4 months after the surgery (Fig. 12A: $F(1,37) = 15.460$, $p \leq 0.001$). Specifically, 4 months after the surgery stroke mice were significantly faster than sham mice ($F(1,42) = 3.085$, $p = 0.009$; data not shown). In addition, male mice exhibited a significant genotype x surgery interaction ($F(1,37) = 4.792$, $p = 0.035$). Specifically, both WT (Fig. 14A: $F(1,23) = 3.747$, $p = 0.065$) and APP/PS1 (Fig. 14A: $F(1,23) = 8.584$, $p = 0.011$) mice showed a surgery effect. In detail, both WT and APP/PS1 stroke mice were faster than WT sham and APP/PS1 sham respectively. Furthermore, a genotype interaction was found for stroke mice; APP/PS1 stroke were faster than WT stroke mice ($F(1,18) = 12.084$, $p = 0.003$; data not shown).

Female mice showed an overall time effect; all female mice were regardless of their genotype and surgery, were slower to climb down the pole 4 months after the surgery compared to pre-surgery (Fig. 14B: $F(1,41) = 22.044$, $p \leq 0.001$). Furthermore, an overall surgery effect was found; stroke mice were faster than sham mice to climb down the pole (Fig. 14B: $F(1,41) = 4.516$, $p = 0.040$). Specifically, at 4 months after the surgery stroke mice were significantly faster than sham mice ($F(1,46) = 4.041$, $p = 0.05$; data not shown).

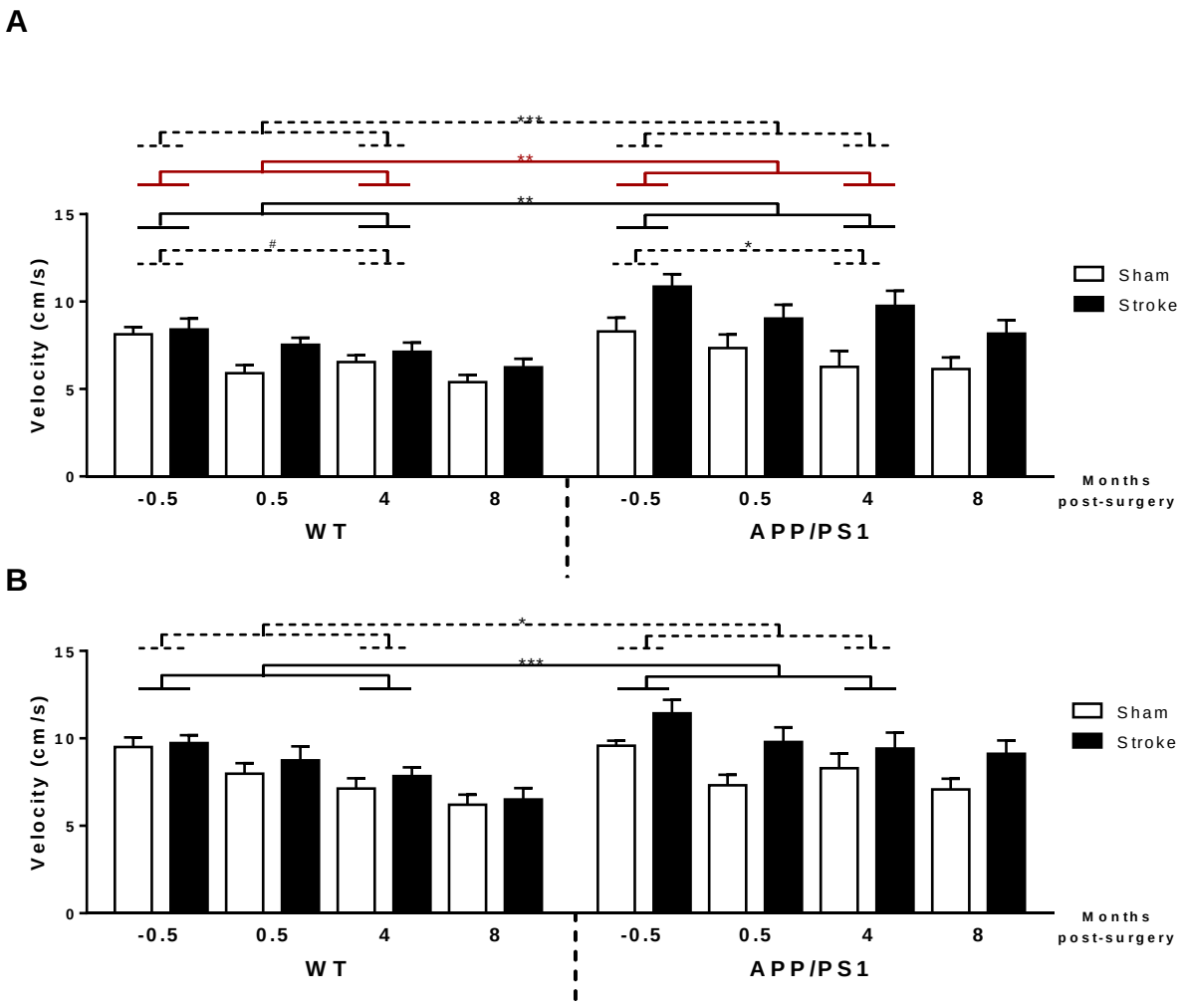

Figure 14. Chronic effects on the velocity to climb down the pole A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) were slower to climb down the pole 4 months after surgery compared to two weeks before the surgery ($p = 0.002$). APP/PS1 mice were faster than WT mice ($p = 0.029$) and stroke mice were faster than sham mice ($p \leq 0.001$) to climb down the pole both at two weeks before the surgery and 4 months after the surgery. WT stroke ($p = 0.065$) and APP/PS1 stroke ($p = 0.011$) were faster than WT sham and APP/PS1 sham respectively. APP/PS1 stroke were faster than WT stroke mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.003$). (B) All female groups (WT, APP/PS1, sham and stroke) slower to climb down the pole 4 months after the surgery compared to pre-surgery ($p \leq 0.001$). Stroke mice were faster than sham mice to climb down the pole both at two weeks before the surgery and 4 months after the surgery ($p = 0.040$).

ii. Revalidation and restoration effects

Revalidation:

Male mice showed a statistically significant time x genotype x surgery interaction ($F(1,72) = 4.917$, $p = 0.01$). In detail, both WT (Fig.14A; $F(2,44) = 3.907$, $p = 0.027$) and APP/PS1 (Fig. 15A: $F(2,28) = 3.446$, $p = 0.046$) showed a significant time effect; both groups showed a gradual decrease in the velocity to climb down the pole after the surgery. Stroke mice also showed a time effect; stroke mice showed a gradual decrease in the velocity to climb down the pole after the surgery ($F(2,34) = 6.307$, $p = 0.05$; data not shown). Furthermore, male mice showed a general genotype effect; APP/PS1 mice were faster than WT mice at every time point after the surgery (Fig. 15A; $F(1,36) = 6.570$, $p = 0.015$). Specifically, at two weeks after the surgery ($F(1,41) = 5.165$, $p = 0.028$; data not shown) and 8 months after the surgery ($F(1,41) = 4.842$, $p = 0.033$; data not shown) APP/PS1 mice were significantly faster than WT mice. Moreover, male mice showed a general surgery effect; stroke mice were faster than sham mice at every time point after the surgery (Fig. 15A: $F(1,36) = 11.328$, $p = 0.002$). Specifically, at two weeks after the surgery ($F(1,41) = 7.714$, $p = 0.008$; data not shown), 4 months after the surgery ($F(1,41) = 5.664$, $p = 0.022$; data not shown) and at 8 months after the surgery ($F(1,41) = 5.200$, $p = 0.028$; data not shown) stroke mice were faster to climb down the pole than sham mice.

Female mice showed a significant time x genotype interaction ($F(2,80) = 3.644$, $p = 0.031$). In detail, WT mice showed a gradual decrease in the velocity to climb down the pole after the surgery (Fig. 15B: $F(2,54) = 16.937$, $p \leq 0.001$). Furthermore, female mice showed an overall surgery effect; stroke mice were faster to climb down the pole than sham mice (Fig. 15B: $F(1,40) = 4.269$, $p = 0.045$). Specifically, two weeks after the surgery stroke mice are significantly faster than sham mice to climb down the pole ($F(1,45) = 3.846$, $p = 0.056$; data not shown).

Restoration:

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were slower to climb down the pole 8 months after surgery compared pre-surgery (Fig. 15A: $F(1,36) = 38.157$, $p \leq 0.001$). Furthermore, a general genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and 8 months after the surgery (Fig. 15A: $F(1,36) = 4.860$, $p = 0.034$). Specifically, at pre-surgery ($F(1,41) = 3.309$, $p = 0.076$; data not shown) and 8 months after the surgery (Fig.14A: $F(1,41) = 4.842$, $p = 0.033$; data not shown) APP/PS1 mice were significantly faster than WT. Moreover, male mice revealed a general surgery effect; stroke mice were faster than sham mice at pre-surgery and 8 months after the surgery (Fig. 15A: $F(1,36) = 10.862$, $p = 0.002$). Specifically, at pre-surgery ($F(1,41) = 3.251$, $p = 0.079$; data not shown) and 8 months after the surgery ($F(1,41) = 5.200$, $p = 0.028$; data not shown) stroke mice were faster to climb down the pole than sham.

Female mice showed an overall time effect; all female mice were regardless of their genotype and surgery, were slower to climb down the pole 8 months after the surgery compared to pre-surgery (Fig.14B: $F(1,40) = 67.101$, $p \leq 0.001$). Furthermore, an overall trend in genotype was found; APP/PS1 mice were faster to climb down the pole than WT mice both at pre-surgery and 8 months after the surgery (Fig.14B: $F(1,40) = 3.691$, $p = 0.062$). Specifically, 8 months after the surgery APP/PS1 mice were significantly faster than WT mice ($F(1,45) = 6.389$, $p = 0.015$; data not shown). Moreover, female mice revealed an overall surgery effect; stroke mice were faster to climb down the pole than sham mice at pre-surgery and 8 months after the surgery (Fig. 15B: $F(1,40) = 5.459$, $p = 0.025$).

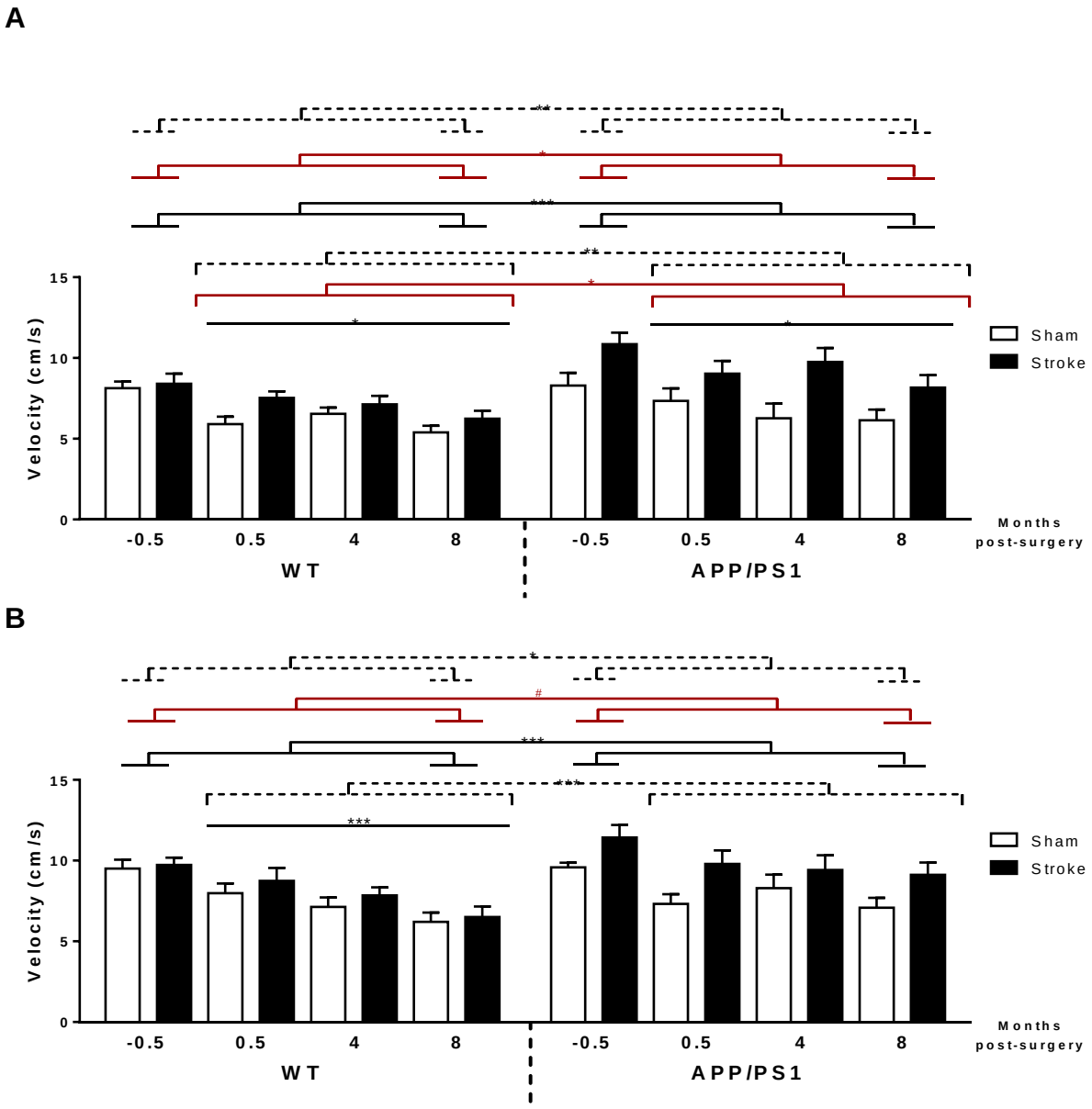

Figure 15. Revalidation and restoration effects on the velocity to climb down the pole A) males B) females (——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) Revalidation: Male WT and APP/PS1 mice showed a gradual decrease in the velocity to climb down the pole after the surgery ($p = 0.01$). Stroke mice showed a gradual decrease in the velocity to climb down the pole after the surgery ($p = 0.05$). APP/PS1 mice were faster than WT mice at every time point after the surgery ($p = 0.015$). Stroke mice were faster than sham mice at every time point after the surgery ($p = 0.002$). Restoration: All male groups (WT, APP/PS1, sham and stroke) were slower to climb down the pole 8 months after surgery compared two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.034$). Stroke mice were faster than sham mice at two weeks before the surgery and 8 months after the surgery ($p = 0.002$). (B) Revalidation: Female WT mice showed a gradual decrease in the velocity to climb down the pole after the surgery ($p \leq 0.001$). Stroke mice were faster to climb down the pole than sham mice at every time point after the surgery ($p = 0.045$). Restoration: All female groups (WT, APP/PS1, sham and stroke) were slower to climb down the pole 8 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were faster to climb down the pole than WT mice both at two weeks before the surgery and 8 months after the surgery (, $p = 0.062$). Stroke mice were faster to climb down the pole than sham mice at two weeks before the surgery and 8 months after the surgery ($p = 0.025$).

I.II Rotation time

i. Acute effects

Male mice showed a general surgery effect; stroke mice needed more time to turn on the pole than sham mice both at pre-surgery and two weeks after the surgery (Fig. 16A: $F(1,34) = 3.489$, $p = 0.07$). Specifically, stroke mice needed significantly more time to turn on the pole than sham mice two weeks after the surgery ($F(1,36) = 4.961$, $p = 0.032$; data not shown).

Female showed a trend in time x surgery interaction ($F(1,40) = 5.050$, $p = 0.030$). In detail, stroke mice needed more time to turn on the pole two weeks after the surgery compared to pre-surgery (Fig. 16B: $F(1,20) = 5.824$, $p = 0.013$). Moreover, two weeks after the surgery stroke mice needed significantly more time to turn on the pole than sham mice ($F(1,42) = 8.311$, $p = 0.006$; data not shown)

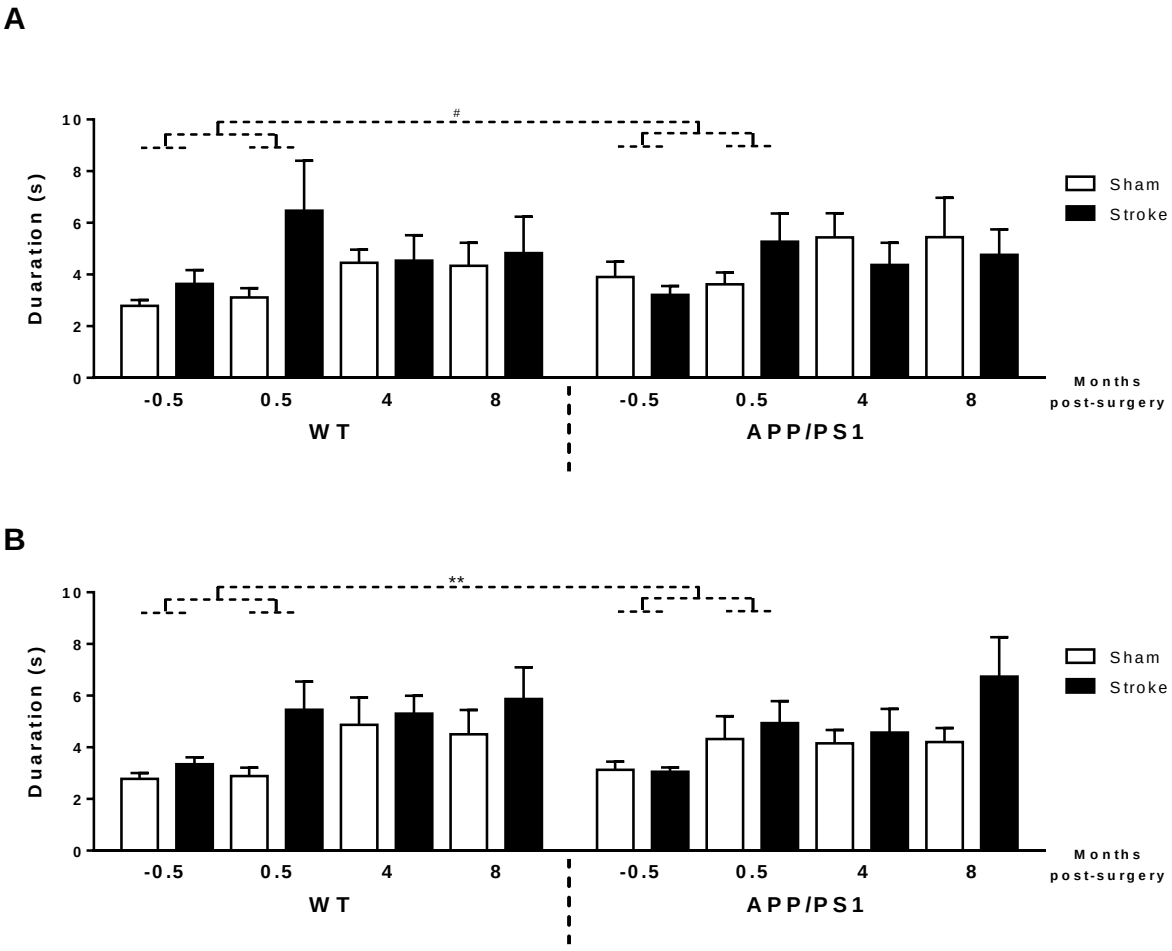

Figure 16. Acute effects on the rotation time on the pole A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Stroke mice needed more time to turn on the pole than sham mice at two weeks before and two weeks after the surgery ($p = 0.07$). (B) Stroke mice needed more time to turn on the pole two weeks after the surgery compared to two weeks before the surgery ($p = 0.013$).

ii. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, needed more time to turn on the pole 4 months after the surgery compared pre-surgery (Fig. 17A: $F(1,31) = 8.842$, $p = 0.006$).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, needed more time on the pole 4 months after the surgery compared pre-surgery (Fig. 17B: $F(1,40) = 11.123$, $p = 0.002$).

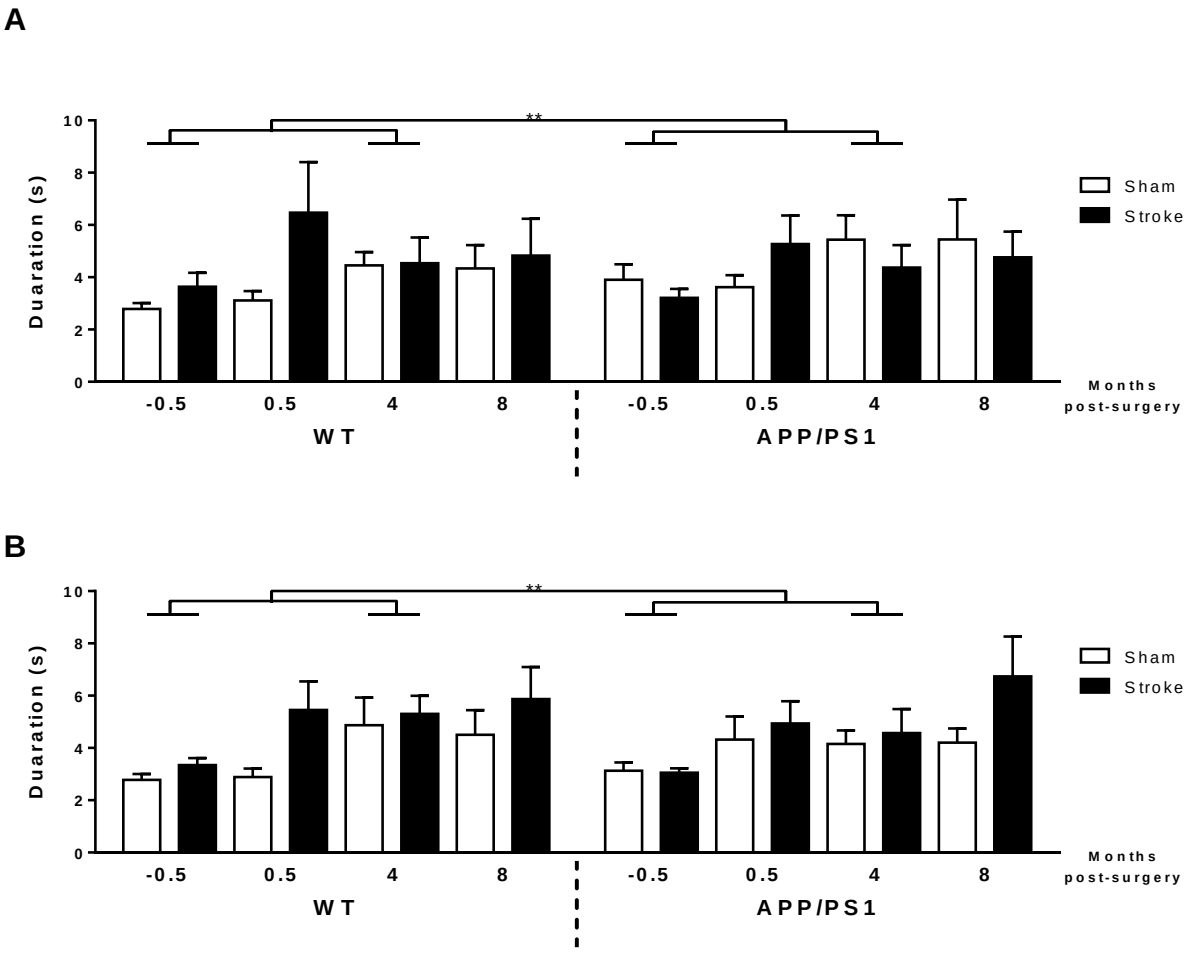

Figure 17. Chronic effects on the rotation time on the pole A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) needed more time to turn 4 months after surgery compared pre-surgery ($p = 0.006$). (B) All female groups (WT, APP/PS1, sham and stroke) needed more time turn 4 months after surgery compared pre-surgery ($p = 0.002$).

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a statistically significant time x surgery interaction. ($F(2,64) = 3.452$, $p = 0.038$). Specifically, stroke mice needed significantly more time to turn on the pole than sham mice 4 months after the surgery ($F(1,34) = 5.440$, $p = 0.026$; data not shown).

Female mice showed a trend in overall surgery effect; stroke mice needed more time to turn on the pole than sham mice at every time point after the surgery (Fig. 18B: $F(1,36) = 4.042$, $p = 0.052$). Specifically, stroke mice needed slightly more time to turn on the pole than sham mice 8 months after the surgery ($F(1,38) = 3.916$, $p = 0.055$; data not shown).

Restoration:

Male mice showed a trend in overall time effect; all male mice, regardless of their genotype and surgery, needed more time to turn pole 8 months after the surgery compared pre-surgery (Fig. 18A: $F(1,33) = 4.092$, $p = 0.051$).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, needed more time turn on the pole 8 months after the surgery compared pre-surgery (Fig. 18B: $(1,39) = 16.439$, $p \leq 0.001$). Moreover, 8 months after the surgery stroke mice needed slightly more time to turn than sham mice ($F(1,38) = 3.916$, $p = 0.055$; data not shown)

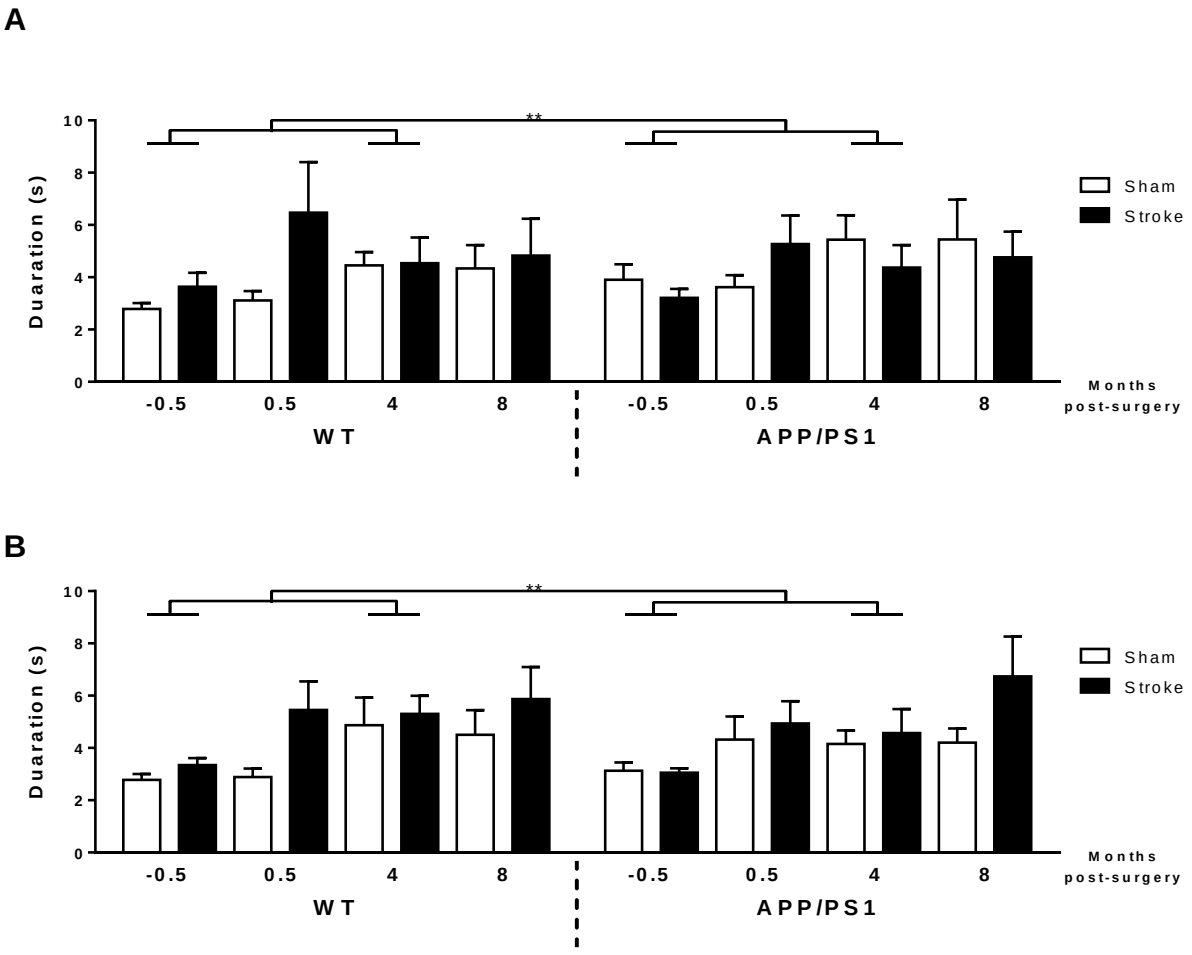

Figure 18. Revalidation and restoration effects on the rotation time on the pole A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Revalidation: stroke mice needed significantly more time to turn on the pole than sham mice 4 months after the surgery ($p = 0.026$). Restoration: all male groups (WT, APP/PS1, sham and stroke) needed more time to turn on the pole 8 months after surgery compared pre-surgery ($p = 0.051$). (B) Revalidation: stroke mice needed more time to turn on the pole than sham mice at every time point after the surgery ($p = 0.052$). Restoration: all female groups (WT, APP/PS1, sham and stroke) needed more time to turn on the pole 8 months after surgery compared pre-surgery ($p \leq 0.001$).

II. Grip Test

II.I Grid Average

i. Acute effects

Male mice showed a statistically significant time x surgery interaction ($F(1,40) = 5.637$, $p = 0.022$). In detail, WT mice showed a surgery effect; WT stroke mice were weaker than WT sham mice (Fig. 19A: $F(1,25) = 5.268$, $p = 0.03$). Furthermore, a genotype effect was found in stroke mice; APP/PS1 stroke mice were stronger than WT stroke mice ($F(1,19) = 10.971$, $p = 0.004$; data not shown). Moreover, APP/PS1 mice were stronger than WT mice two weeks after the surgery ($F(1,42) = 4.805$, $p = 0.034$; data not shown).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, were stronger two weeks after the surgery compared pre-surgery (Fig. 19B: $F(1,44) = 4.981$, $p = 0.031$). Female mice demonstrated a trend in overall genotype effect; APP/PS1 mice were stronger than sham WT mice both at pre-surgery and two weeks after the surgery. Specifically, APP/PS1 mice were significantly stronger than WT mice two weeks after the surgery ($F(1,45) = 4.247$, $p = 0.045$; data not shown).

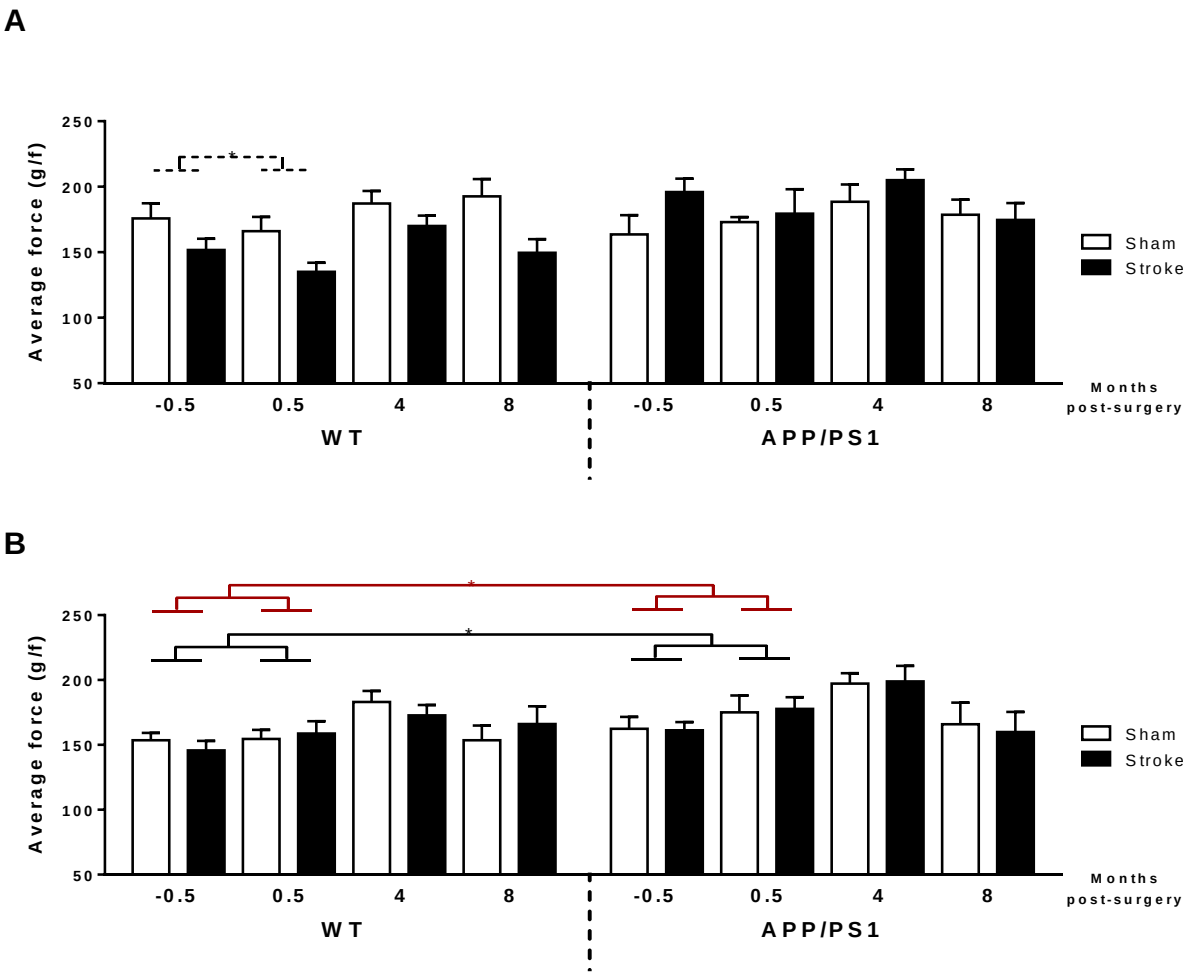

Figure 19. Acute effects on the average total muscle strength A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) WT stroke mice were weaker than WT sham mice ($p = 0.03$). APP/PS1 stroke mice were stronger than WT stroke mice ($p = 0.004$). APP/PS1 mice were stronger than WT mice two weeks after the surgery ($p = 0.034$). (B) All female groups (WT, APP/PS1, sham and stroke) were stronger two weeks after the surgery compared two weeks before the surgery ($p = 0.031$). APP/PS1 mice were stronger than sham WT mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.045$).

ii. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were stronger 4 months after the surgery compared pre-surgery (Fig. 20A: $F(1,39) = 9.338$, $p = 0.004$). Furthermore, statistically significant genotype x surgery interaction was found ($F(1,39) = 4.918$, $p = 0.032$). In detail, APP/PS1 stroke mice were stronger than WT stroke mice both at pre-surgery and 4 months after the surgery ($F(1,18) = 11.7007$, $p = 0.003$; data not shown).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, were stronger 4 months after the surgery compared pre-surgery (Fig. 20B: $F(1,44) = 51.739$, $p \leq 0.001$). Female mice exhibited an overall genotype effect; APP/PS1 mice were stronger than sham WT mice both at pre-surgery and 4 months after the surgery (Fig. 20B: $F(1,44) = 5.331$, $p = 0.026$). Specifically, APP/PS1 mice were significantly stronger than WT mice at 4 months after the surgery ($F(1,46) = 4.870$, $p = 0.032$; data not shown).

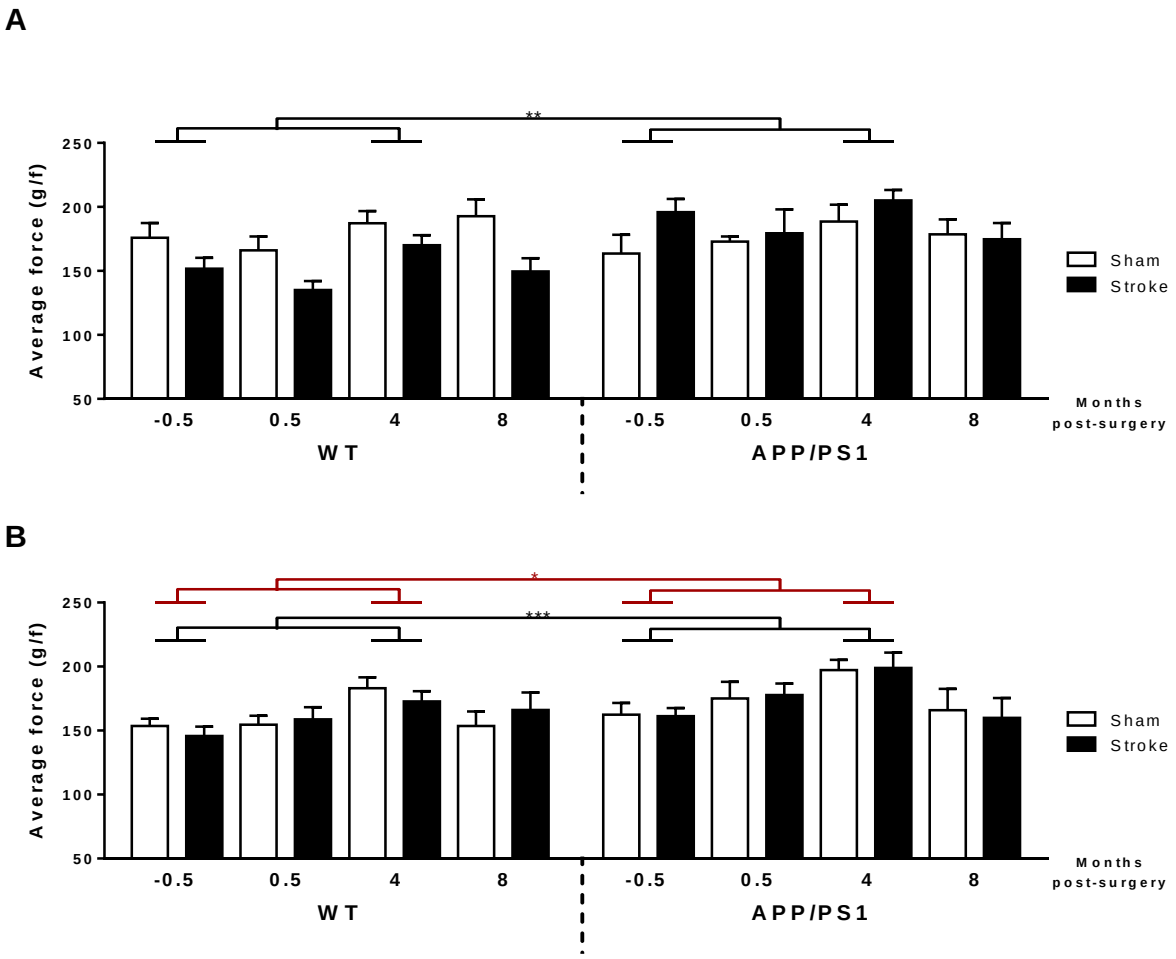

Figure 20. Chronic effects on the average total muscle strength A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) were stronger 4 months after the surgery compared two weeks before the surgery ($p = 0.004$). APP/PS1 stroke mice were stronger than WT stroke mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.003$). (B) All female groups (WT, APP/PS1, sham and stroke) were stronger 4 months after the surgery compared two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were stronger than sham WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.026$).

iii. Revalidation and restoration effects

Revalidation:

Male mice, regardless of their genotype and surgery, showed an overall time effect (Fig. 21A: $F(2,78) = 8.322$, $p = 0.001$).

Female mice, regardless of their genotype and surgery, showed an overall time effect (Fig. 21B: $F(2,88) = 15.279$, $p \leq 0.001$). Moreover, APP/PS1 mice were stronger than WT mice both at 4 weeks after the surgery ($F(1,46) = 4.247$, $p = 0.045$; data not shown) and 8 months after the surgery ($F(1,46) = 4.870$, $p = 0.032$; data not shown).

Restoration:

Male mice showed a statistically significant genotype x surgery interaction ($F(1,40) = 5.693$, $p = 0.022$). In detail, WT mice showed a surgery effect; WT stroke mice were weaker than WT sham mice both at pre-surgery and 8 months after the surgery (Fig. 21B: $F(1,40) = 5.693$, $p = 0.022$). Specifically, stroke mice were weaker than sham mice 8 months after the surgery ($F(1, 42) = 5.082$, $p = 0.029$; data not shown). Furthermore, a genotype effect was found in stroke mice; APP/PS1 stroke mice were stronger than WT stroke mice both at pre-surgery and 8 months after the surgery ($F(1,19) = 8.103$, $p = 0.01$; data not shown).

There were no significant effects observed in female mice (Fig. 21B).

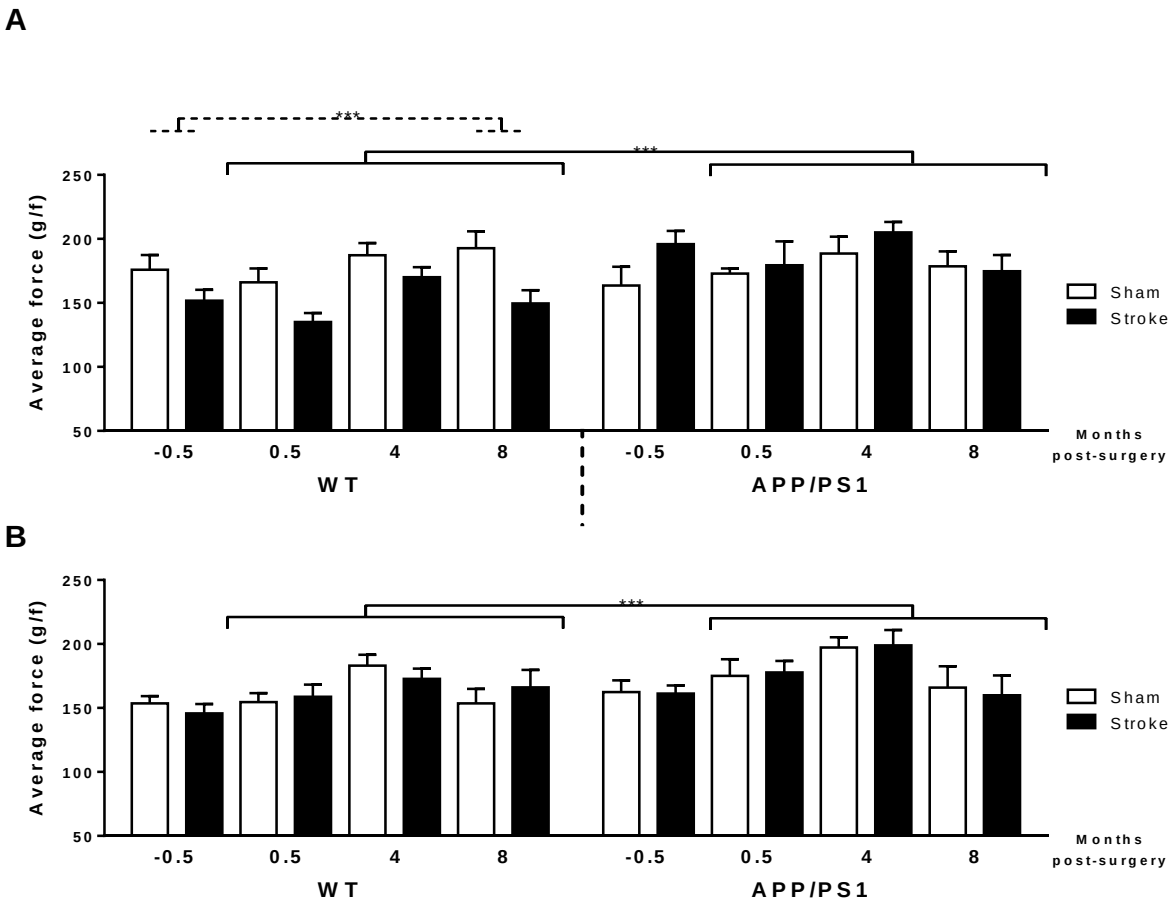

Figure 21. Revalidation and restoration effects on the average total muscle strength A) males B) females (——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) Revalidation: all male groups (WT, APP/PS1, sham and stroke) showed an overall time effect ($p = 0.001$). Restoration: WT stroke mice were weaker than WT sham mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.01$). (B) Revalidation: all female groups (WT, APP/PS1, sham and stroke) showed an overall time effect ($p \leq 0.001$). APP/PS1 mice were stronger than WT mice both at two weeks before and 4 weeks after the surgery ($p = 0.045$). Restoration: female mice showed no significant effects.

I.II. Trapeze Average

i. Acute effects

Male mice showed a statistically significant time x genotype interaction ($F(1,36) = 4.864$, $p = 0.034$). In detail, WT mice showed a time effect; WT stroke mice were weaker two weeks after the surgery compared to pre-surgery (Fig. 22A: $F(1,23) = 18.721$, $p \leq 0.001$). Furthermore, a genotype effect was found; APP/PS1 mice were stronger than WT both at pre-surgery and two weeks after the surgery (Fig. 22A: $F(1,36) = 8.806$, $p = 0.005$). Specifically, APP/PS1 mice were significantly stronger than WT mice two weeks after the surgery ($F(1,38) = 10.868$, $p = 0.002$; data not shown).

Female mice showed a significant genotype effect; APP/PS1 mice were stronger than WT both at pre-surgery and two weeks after the surgery (Fig. 22B: $F(1,43) = 6.380$, $p = 0.015$). Specifically, APP/PS1 mice were significantly stronger than WT mice two weeks after the surgery ($F(1,45) = 9.974$, $p = 0.003$; data not shown). Furthermore, a trend in surgery effect was found; stroke mice were stronger than sham mice both at pre-surgery and two weeks after the surgery (Fig. 22B: $F(1,43) = 3.401$, $p = 0.072$).

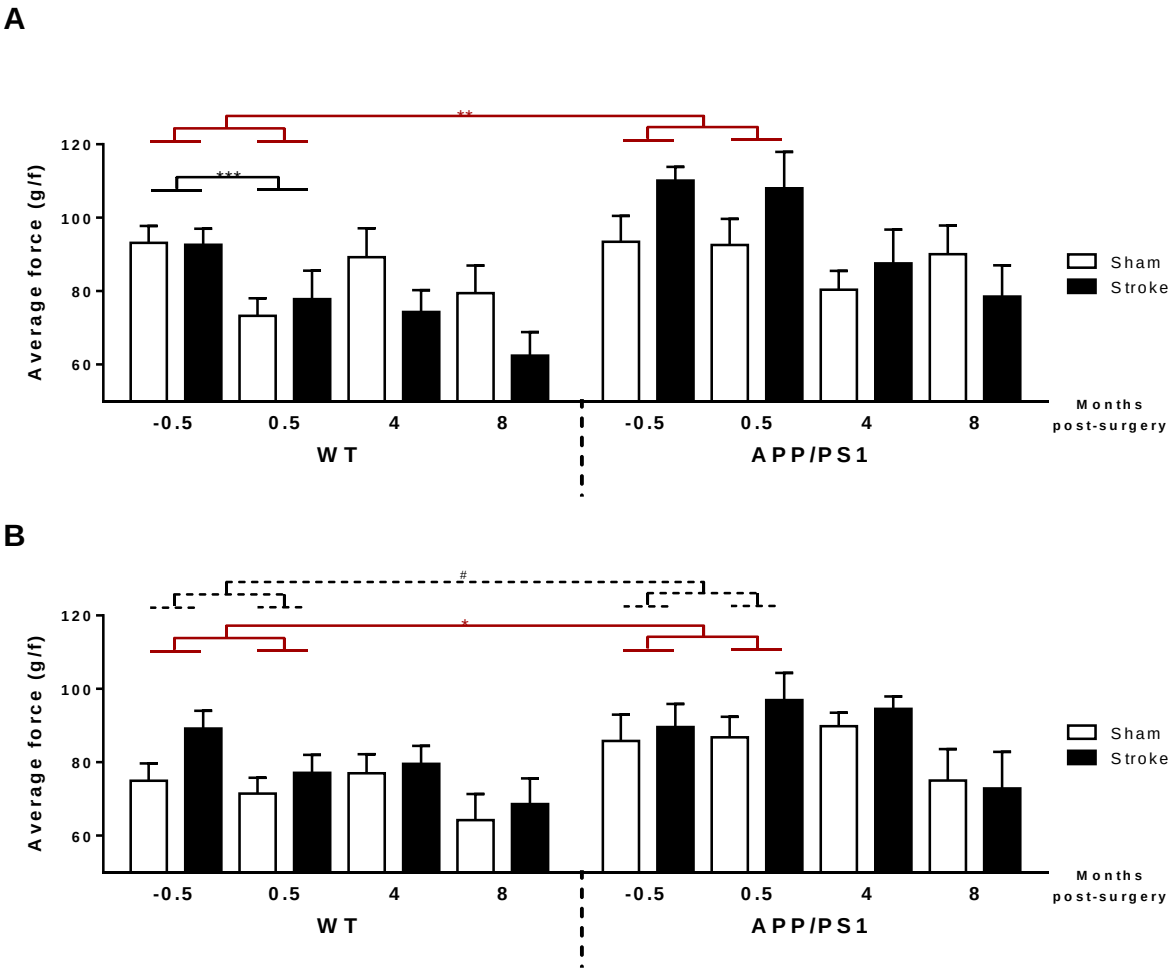

Figure 22. Acute effects on the average forelimb muscle strength A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) WT stroke mice were weaker two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were stronger than WT both at two weeks before the surgery and two weeks after the surgery ($p = 0.005$). (B) APP/PS1 mice were stronger than WT both at two weeks before the surgery and two weeks after the surgery ($p = 0.015$). Stroke mice were stronger than sham mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.072$).

ii. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were weaker 4 months after the surgery compared pre-surgery (Fig. 23A: $F(1,36) = 10.772$, $p = 0.002$). Additionally, APP/PS1 mice were stronger than WT mice at pre-surgery ($F(1,38) = 8.681$, $p = 0.005$; data not shown).

Female mice showed a significant genotype effect; APP/PS1 mice were stronger than WT both at pre-surgery and 4 months after the surgery (Fig. 23B: $F(1,43) = 3.159$, $p = 0.01$). Specifically, APP/PS1 mice were significantly stronger than WT mice 4 months after the surgery ($F(1,45) = 8.457$, $p = 0.006$; data not shown).

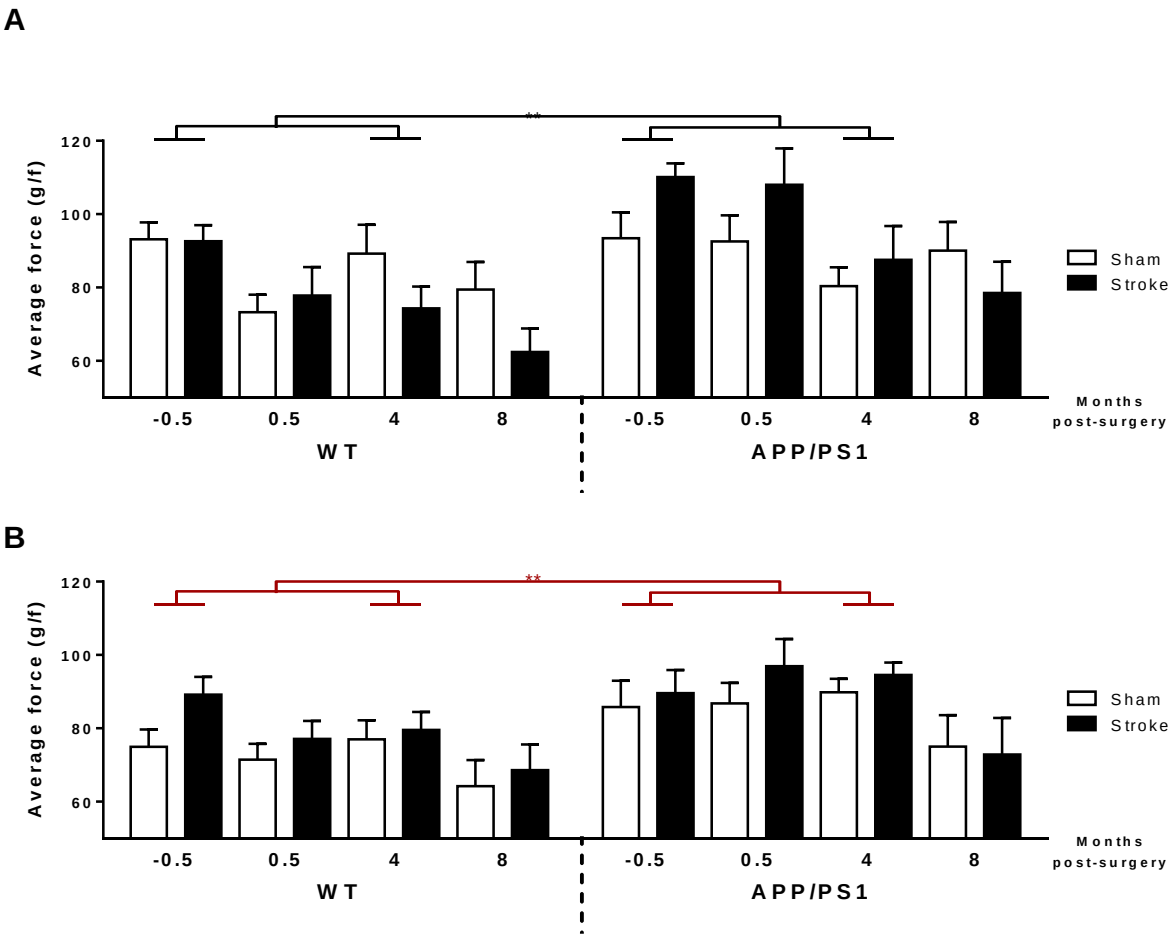

Figure 23. Chronic effects on the average forelimb muscle strength A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) were weaker 4 months after the surgery compared two weeks before the surgery ($p = 0.002$). APP/PS1 mice were stronger than WT mice at two weeks before the surgery ($p = 0.005$). (B) APP/PS1 mice were stronger than WT both at two weeks before the surgery and 4 months after the surgery ($p = 0.01$).

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a genotype effect; APP/PS1 mice were stronger than WT after the surgery (Fig. 24A: $F(1,25) = 7.444$, $p = 0.01$). Specifically, APP/PS1 mice were significantly stronger than WT mice two weeks after the surgery ($F(1,37) = 11.824$, $p = 0.001$; data not shown).

Female mice, regardless of their genotype and surgery, showed an overall time effect (Fig. 24B: $F(2,64) = 5.257$, $p = 0.007$). Female mice exhibited a significant genotype effect; APP/PS1 mice were stronger than WT mice at every time point after the surgery (Fig. 24B: $F(1,42) = 16.303$, $p = 0.01$). Specifically, APP/PS1 mice were stronger than WT mice at two weeks after the surgery ($F(1,44) = 9.149$, $p = 0.004$; data not shown) and 4 months after the surgery ($F(1,44) = 10.036$, $p = 0.003$; data not shown).

Restoration:

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, were weaker 8 months after the surgery compared pre-surgery (Fig. 24A: $F(1,38) = 14.497$, $p \leq 0.001$). Male mice exhibited a significant genotype effect; APP/PS1 mice were stronger than WT mice both at pre-surgery and 8 months after the surgery (Fig. 24A: $F(1,38) = 5.226$, $p = 0.028$).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, were weaker 8 months after the surgery compared pre-surgery (Fig. 24B: $F(1,44) = 6.702$, $p = 0.013$).

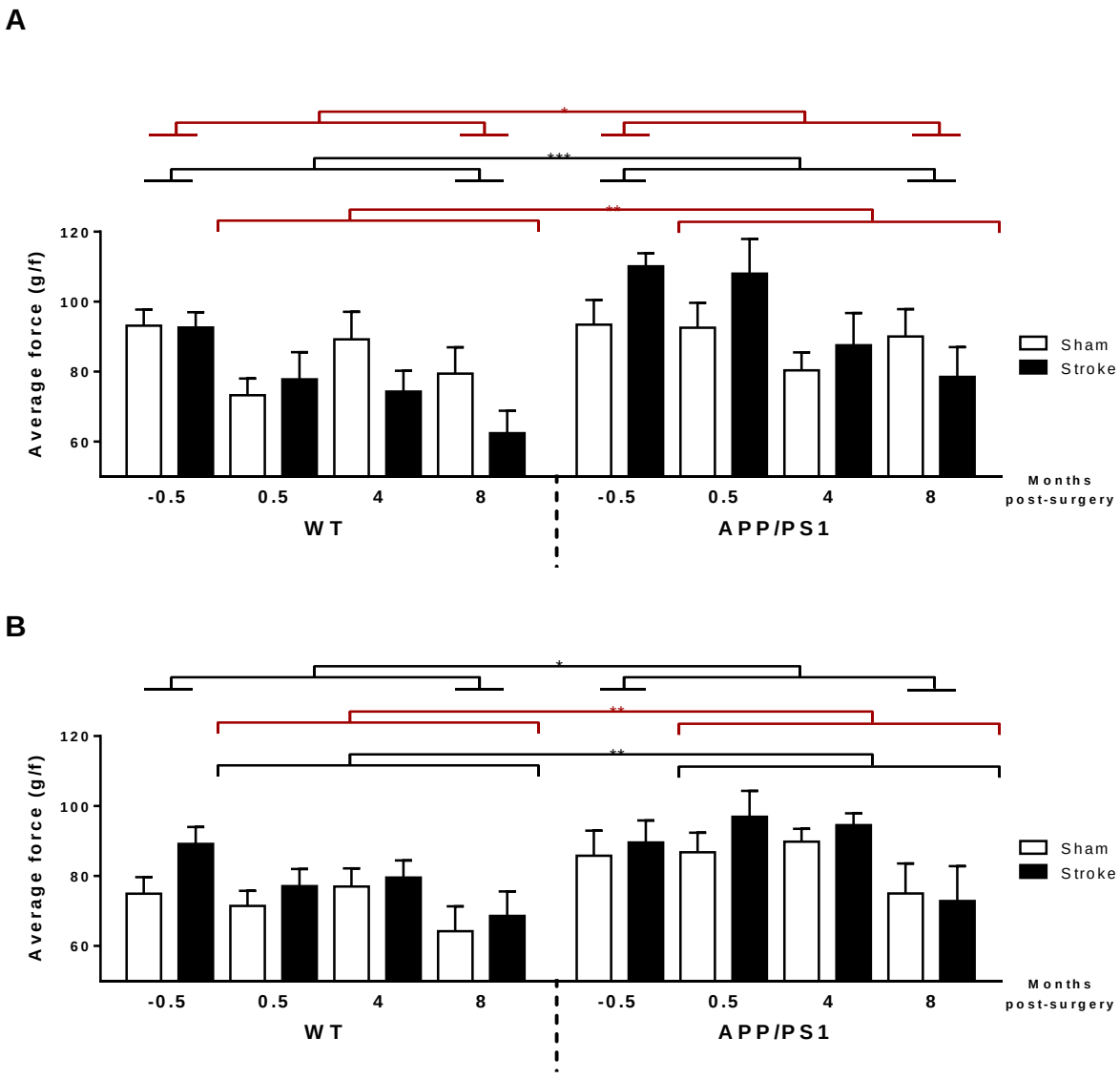

Figure 24. Revalidation and restoration effects on the average forelimb muscle strength A) males B) females (——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) Revalidation: APP/PS1 mice were stronger than WT after the surgery ($p = 0.01$). Restoration All male groups (WT, APP/PS1, sham and stroke) were weaker 8 months after the surgery compared two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were stronger than WT mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.028$). (B) Revalidation: all female groups (WT, APP/PS1, sham and stroke) showed an overall time effect ($p = 0.007$). APP/PS1 mice were stronger than WT mice at every time point after the surgery ($p = 0.01$). Restoration: all female groups (WT, APP/PS1, sham and stroke) were weaker 8 months after the surgery compared two weeks before the surgery ($p = 0.013$).

III. Open Field

III. I Velocity

i. **Acute effects**

Male mice showed a trend in time x genotype interaction ($F(1,28) = 4.195$, $p = 0.05$). In detail, both WT (Fig. 25A: $F(1,17) = 12.186$, $p = 0.003$) and (Fig. 25A: $F(1,13) = 15.413$, $p = 0.002$) APP/PS1 mice were slower two weeks after the surgery compared to pre-surgery. Male mice a significant surgery effect was found; stroke mice were faster than sham mice both at pre-surgery and two weeks after the surgery (Fig. 25A: $F(1, 28) = 4.214$, $p = 0.05$). Specifically, stroke mice were faster than sham mice two weeks after the surgery ($F(1,30) = 18.346$, $p \leq 0.001$; data not shown). Moreover, APP/PS1 mice were faster than WT mice at pre-surgery ($F(1,30) = 4.912$, $p = 0.034$; data not shown).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, were slower two weeks after the surgery compared to pre-surgery (Fig. 25B: $F(1,36) = 34.719$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and two weeks after the surgery (Fig. 25B: $F(1,36) = 10.927$, $p = 0.002$). Specifically, APP/PS1 mice were significantly faster than WT mice two weeks before the surgery ($F(1,38) = 6.505$, $p = 0.015$; data not shown) and two weeks after the surgery ($F(1,38) = 5.866$, $p = 0.02$; data not shown). Additionally, female mice exhibited a trend in surgery effect; stroke mice were faster than sham mice both at pre-surgery and two weeks after the surgery (Fig. 25B: $(1,36) = 3.563$, $p = 0.067$).

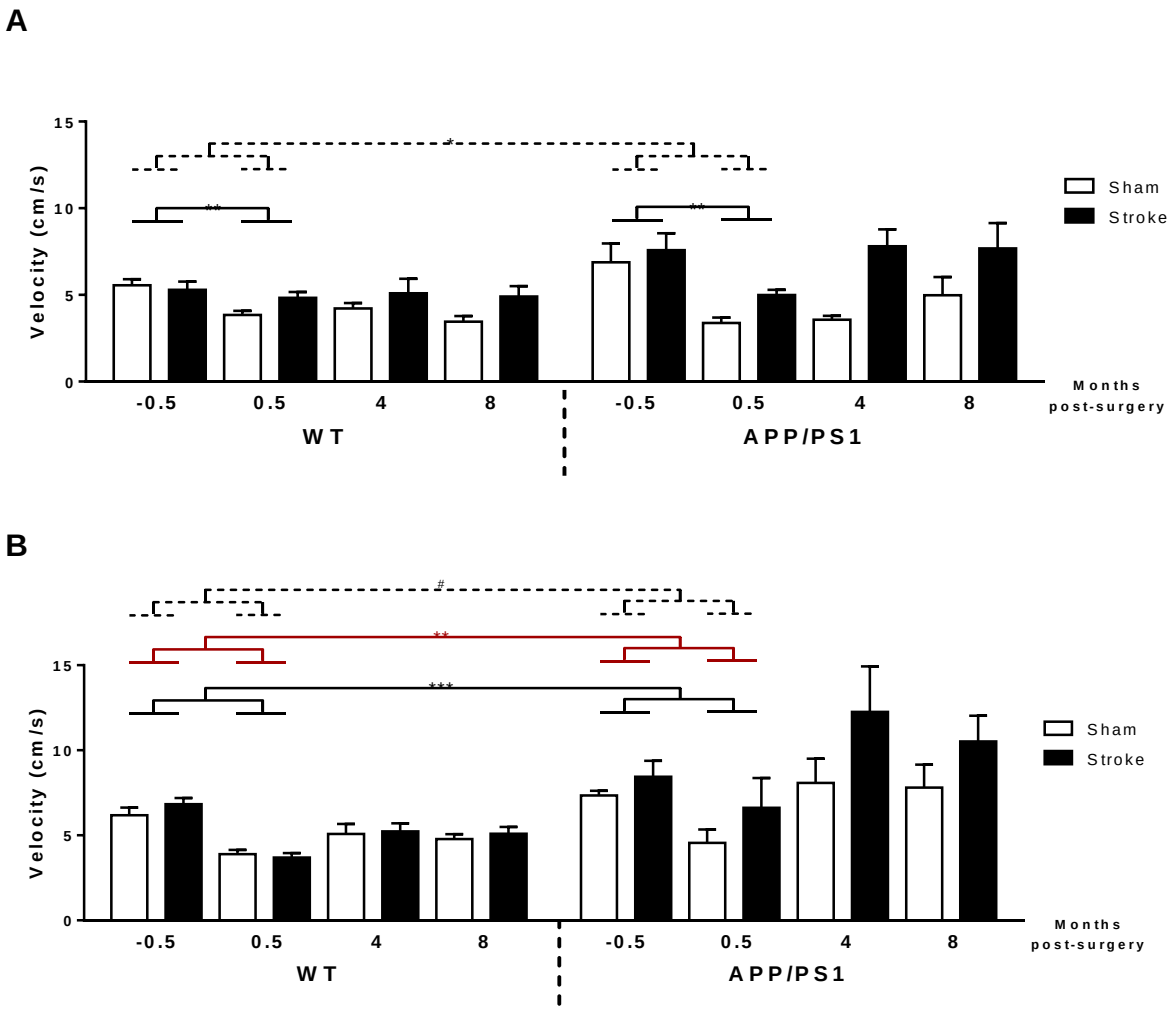

Figure 25. Acute effects on the walking velocity A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) WT and APP/PS1 mice were slower two weeks after the surgery compared to two weeks before the surgery ($p = 0.05$). Stroke mice were faster than sham mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.05$). (B) All female groups (WT, APP/PS1, sham and stroke) were slower two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.002$). Stroke mice were faster than sham mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.067$).

ii. Chronic effects

Male mice showed a significant time x surgery interaction ($F(1,32) = 8.900$, $p = 0.005$). In detail, sham mice were slower 4 months after the surgery compared to pre-surgery (Fig. 26A: $F(1,16) = 14.223$, $p = 0.02$). Furthermore, a significant genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and 4 months after the surgery (Fig. 26A: $F(1,32) = 5.762$, $p = 0.022$). Specifically, APP/PS1 mice were faster than WT mice two weeks before the surgery ($F(1,34) = 7.767$, $p = 0.009$; data not shown). Additionally, male mice exhibited a significant surgery effect; stroke mice were faster than sham mice both at pre-surgery and 4 months after the surgery (Fig. 26A: $F(1,32) = 5.527$, $p = 0.025$). Specifically, stroke mice were faster than sham mice 4 months before the surgery ($F(1,34) = 8.513$, $p = 0.006$; data not shown). Female mice showed a significant time x surgery interaction ($F(1,37) = 9.367$, $p = 0.04$). In detail, WT mice were slower 4 months after the surgery compared to pre-surgery (Fig. 26B: $F(1,26) = 13.358$, $p = 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and 4 months after the surgery (Fig. 26B: $F(1,37) = 20.457$, $p \leq 0.001$). Specifically, APP/PS1 were faster than WT mice two weeks before the surgery ($F(1,39) = 6.793$, $p = 0.013$; data not shown) and 4 months after the surgery ($F(1,39) = 16.575$, $p \leq 0.001$; data not shown). Additionally, female mice exhibited a significant surgery effect; stroke mice were faster than sham mice both at pre-surgery and 4 months after the surgery (Fig. 26B: $F(1,37) = 4.640$, $p = 0.038$).

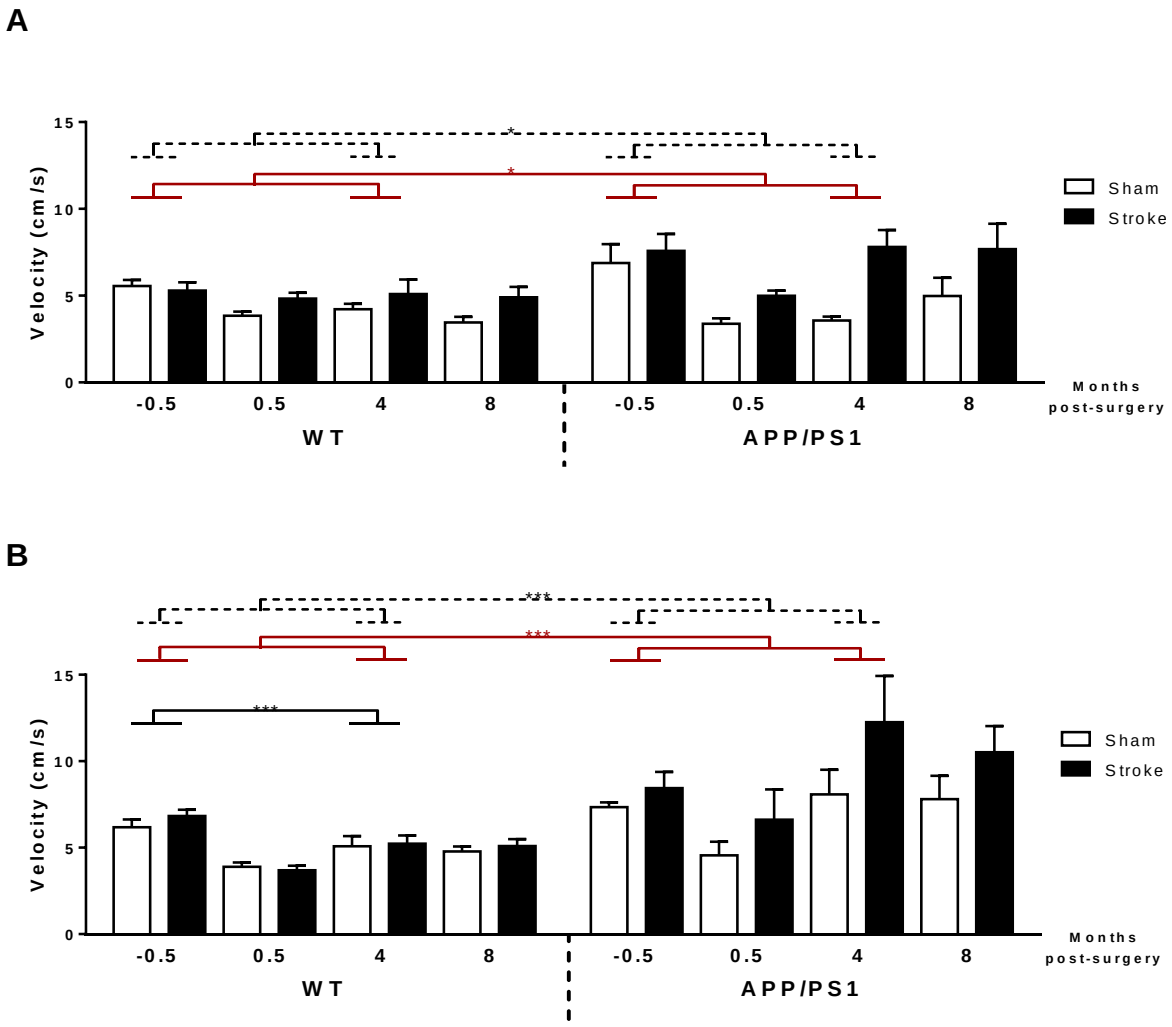

Figure 26. Chronic effects on the walking velocity A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Sham mice were slower 4 months after the surgery compared to two weeks before the surgery ($p = 0.02$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.022$). Stroke mice were faster than sham mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.025$). (B) WT mice were slower 4 months after the surgery compared to two weeks before the surgery ($p = 0.001$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and 4 months after the surgery ($p \leq 0.001$). Stroke mice were faster than sham mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.038$).

iii. Revalidation and restoration effects

Revalidation:

Male mice, regardless of their genotype and surgery, showed a trend in overall time effect (Fig. 26A: $F(2,56) = 3.008$, $p = 0.057$). Furthermore, a trend in overall genotype effect was found; APP/PS1 mice were faster than WT mice at every time point after the surgery (Fig. 27A: $F(1,28) = 3.882$, $p = 0.059$). Specifically, APP/PS1 mice were significantly faster than WT at 8 months after the surgery ($F(1,30) = 4.538$, $p = 0.027$; data not shown). Additionally, male mice exhibited a significant surgery effect; stroke mice were faster than sham mice at every time point after the surgery (Fig. 27A: $F(1,28) = 24.229$, $p \leq 0.001$). Specifically, sham mice were faster than stroke mice two weeks after the surgery ($F(1,30) = 18.346$, $p \leq 0.001$; data not shown), 4 months after the surgery ($F(1,30) = 13.684$, $p = 0.01$; data not shown) and 8 months after the surgery ($F(1,30) = 5.912$, $p = 0.021$; data not shown).

Female mice showed a significant time x genotype interaction ($F(2,72) = 6.609$, $p = 0.002$). In detail, both WT (Fig. 27B: $F(2,50) = 11.938$, $p \leq 0.001$) and APP/PS1 (Fig. 27B: $F(2,26) = 9.509$, $p = 0.001$) mice showed a change in their walking velocity over time after the surgery. Furthermore, a significant overall genotype effect was found; APP/PS1 mice were faster than WT mice at every time point after the surgery (Fig. 27B: $F(1,36) = 22.126$, $p \leq 0.001$). Specifically, APP/PS1 mice were significantly faster than WT 4 months after the surgery ($F(1,38) = 15.671$, $p \leq 0.001$; data not shown) and 8 months after the surgery ($F(1,38) = 26.026$, $p \leq 0.001$; data not shown). Additionally, female mice exhibited a trend in overall surgery effect; stroke mice were faster than sham mice at every time point after the surgery (Fig. 27B: $F(1,36) = 3.825$, $p = 0.058$).

Restoration:

Male mice showed a trend in overall time effect; all male mice, regardless of their genotype and surgery, were slower 8 months after the surgery compared pre-surgery (Fig. 27A: $F(1,32) = 3.412$, $p = 0.074$). Furthermore, a trend in overall genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and 8 months after the surgery (Fig. 27A: $F(1, 32) = 14.304$, $p = 0.001$). Specifically, APP/PS1 mice were significantly faster than WT at pre-surgery ($F(1,34) = 7.67$, $p = 0.009$; data not shown) at 8 months after the surgery ($F(1,34) = 6.633$, $p = 0.015$; data not shown). Additionally, male mice exhibited a significant surgery effect; stroke mice were faster than sham mice both at pre-surgery and 8 months after the surgery (Fig. 27A: $F(1, 32) = 4.843$, $p = 0.035$). Specifically, stroke mice were significantly faster than sham 8 months after the surgery ($F(1,34) = 5.312$, $p = 0.027$; data not shown).

Female mice showed a significant time x genotype interaction ($F(1,37) = 10.700$, $p = 0.002$). In detail, WT mice were slower 8 months after the surgery compared to pre-surgery (Fig. 27B: $F(1,26) = 25.121$, $p \leq 0.001$). Furthermore, an overall genotype effect was found; APP/PS1 mice were faster than WT mice both at pre-surgery and 8 months after the surgery (Fig. 27B: $F(1,37) = 29.686$, $p \leq 0.001$). Specifically, APP/PS1 mice were significantly faster than WT mice two weeks before the surgery ($F(1,39) = 6.793$, $p = 0.013$; data not shown) and 8 months after the surgery ($F(1,39) = 26.903$, $p \leq 0.001$; data not shown). Additionally, female mice exhibited a significant surgery effect; stroke mice were faster than sham mice both at pre-surgery and 8 months after the surgery (Fig. 27B: $F(1,37) = 5.832$, $p = 0.026$).

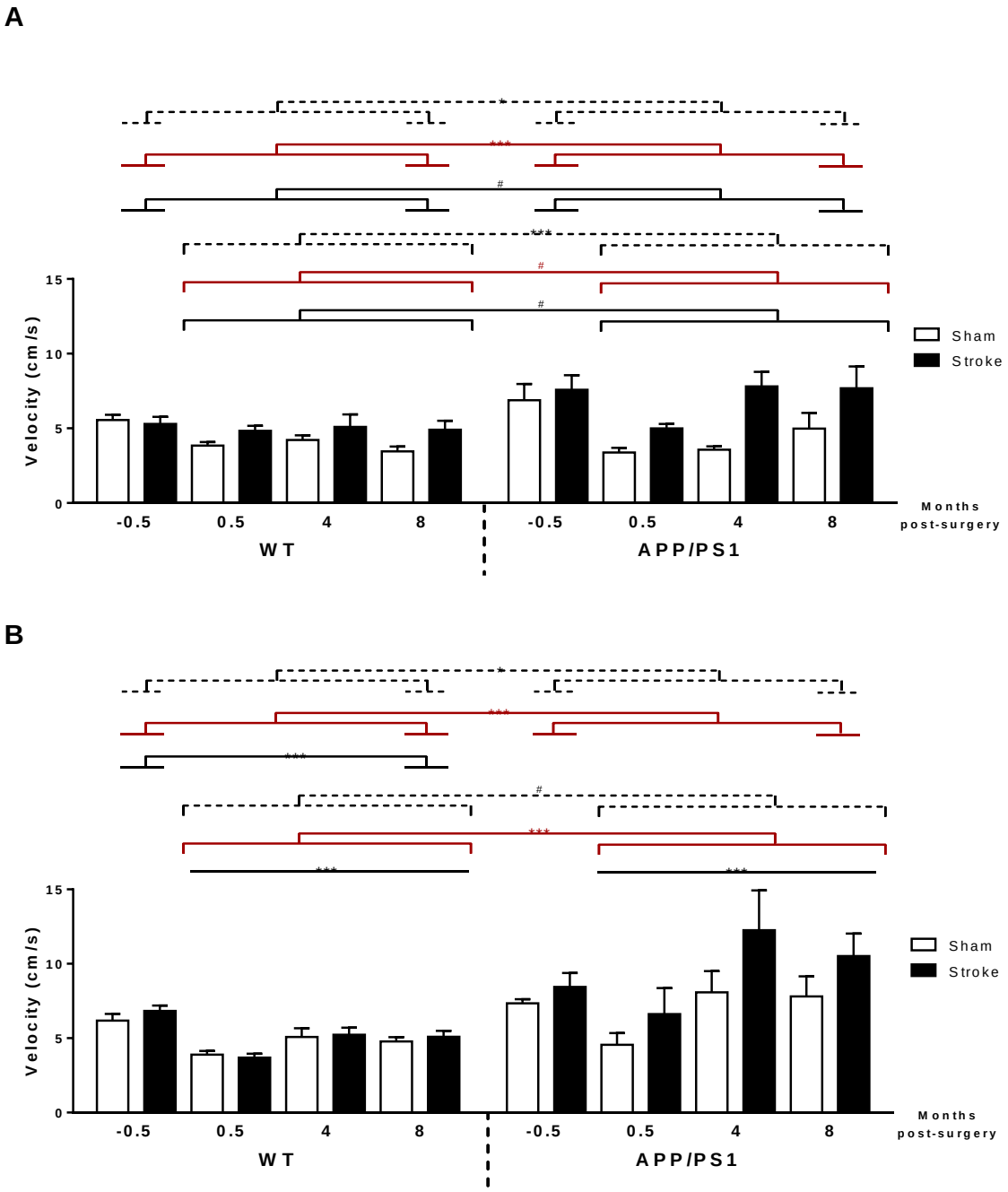

Figure 27. Revalidation and restoration effects on the walking velocity A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Revalidation: all male groups (WT, APP/PS1, sham and stroke) showed a trend in overall time effect ($p = 0.057$). APP/PS1 mice were faster than WT mice at every time point after the surgery ($p = 0.059$). Stroke mice were faster than sham mice at every time point after the surgery ($p \leq 0.001$). Restoration: all male groups (WT, APP/PS1, sham and stroke) were slower 8 months after the surgery compared two weeks before the surgery ($p = 0.074$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.001$). Stroke mice were faster than sham mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.035$). (B) Revalidation: Both WT and APP/PS1 mice showed a change in their walking velocity after the surgery ($p = 0.002$). APP/PS1 mice were faster than WT mice at every time point after the surgery ($p \leq 0.001$). Stroke mice were faster than sham mice at every time point after the surgery ($p = 0.058$). Restoration: WT mice were slower 8 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice were faster than WT mice both at two weeks before the surgery and 8 months after the surgery ($p \leq 0.001$). Stroke mice were faster than sham mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.026$).

III.II Distance

i. Acute effects

Male mice showed a significant time x genotype interaction ($F(1,28) = 4.181$, $p = 0.05$). In detail, both WT (Fig. 28A: $F(1,17) = 19.137$, $p \leq 0.001$) and APP/PS1 (Fig. 28A: $F(1,13) = 520.680$, $p = 0.001$) mice walked less two weeks after the surgery compared to pre-surgery. Moreover, APP/PS1 mice walked more than WT mice at pre-surgery ($F(1,30) = 5.199$, $p = 0.030$; data not shown). Additionally, a significant surgery effect was found; stroke mice walked more than sham mice both at pre-surgery and two weeks after the surgery (Fig 27A: $F(1,28) = 5.013$, $p = 0.033$). Specifically, APP/PS1 mice walked significantly more than sham mice two weeks after the surgery ($F(1,30) = 10.355$, $p = 0.003$; data not shown).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, mice walked less two weeks after the surgery compared to pre-surgery (Fig. 28B: $F(1,36) = 57.086$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice walked more than WT mice both at pre-surgery and two weeks after the surgery (Fig. 28B: $F(1,36) = 7.901$, $p = 0.008$). Specifically, APP/PS1 mice walked more than WT mice at pre-surgery ($F(1,38) = 56.751$, $p = 0.013$; data not shown). Additionally, female mice exhibited a significant surgery effect; stroke mice walked more than sham mice both at pre-surgery and two weeks after the surgery (Fig. 28B: $F(1,36) = 7.589$, $p = 0.009$).

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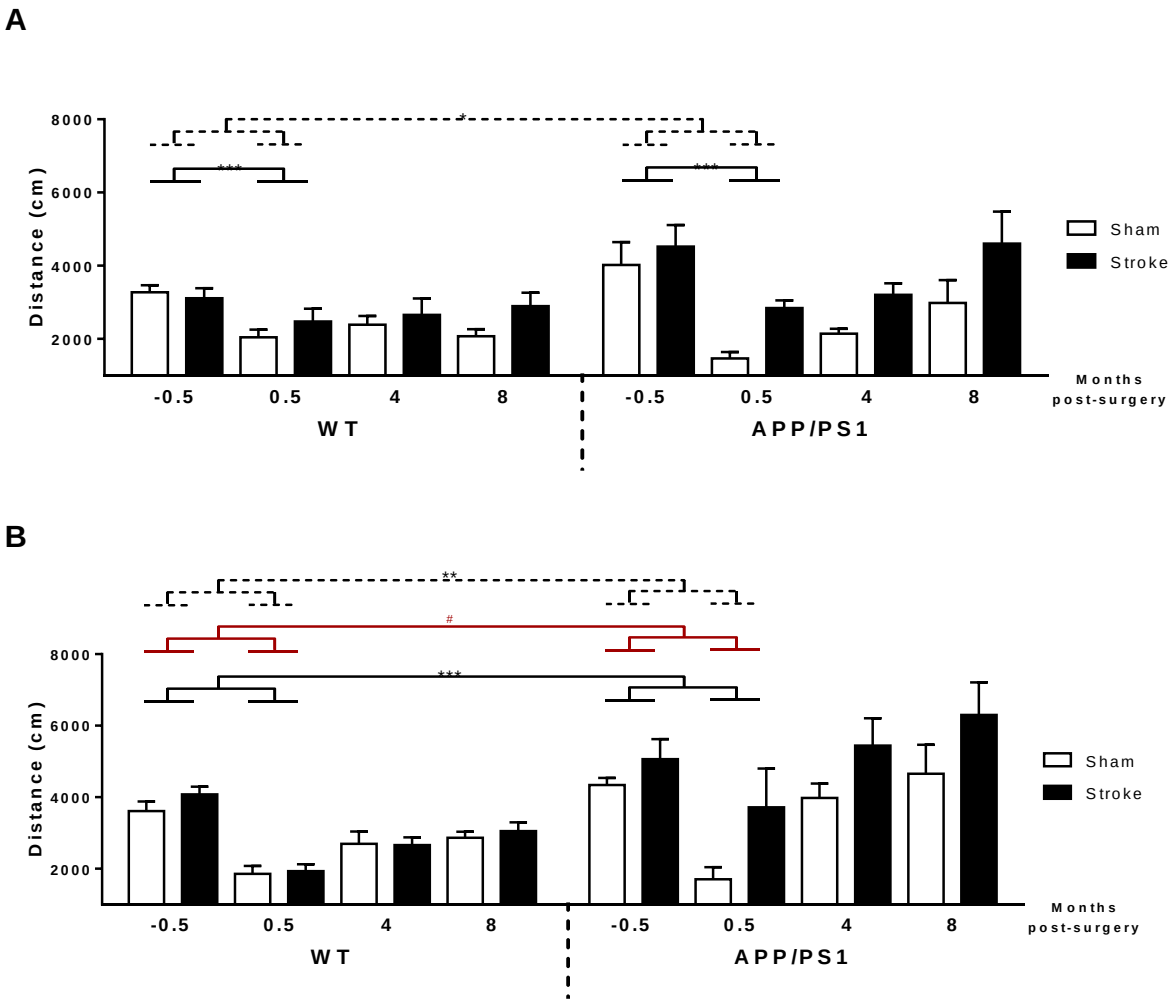

Figure 28. Acute effects on the walking distance A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Both WT and APP/PS1 mice walked less two weeks after the surgery compared to two weeks before the surgery ($p = 0.05$). Stroke mice walked more than sham mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.033$). (B) All female groups (WT, APP/PS1, sham and stroke) mice walked less two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice walked more than WT mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.008$). Stroke mice walked more than sham mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.009$).

ii. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, mice walked less 4 months after the surgery compared to pre-surgery (Fig. 29A: $F(1,32) = 17.788$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice walked more than WT mice both at pre-surgery and 4 months after the surgery (Fig. 29A: $F(1,32) = 5.360$, $p = 0.027$). Specifically, APP/PS1 mice walked more than WT mice two weeks before the surgery ($F(1,34) = 8.194$, $p = 0.007$; data not shown).

Female mice showed a significant time x genotype interaction ($F(1,37) = 6.2752$, $p = 0.017$). In detail, WT mice walked less 4 months after the surgery compared to pre-surgery (Fig. 29B: $F(1,19) = 69.425$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice walked more than WT mice both at pre-surgery and 4 months after the surgery (Fig. 29B: $F(1,37) = 24.656$, $p \leq 0.001$). Specifically, APP/PS1 mice walked more than WT two weeks before the surgery ($F(1,39) = 7.012$, $p = 0.012$; data not shown) and 4 months after the surgery ($F(1,39) = 22.050$, $p \leq 0.001$; data not shown). Additionally, female mice exhibited a significant surgery effect; stroke mice walked more than sham mice both at pre-surgery 4 months after the surgery (Fig. 29B: $F(1,37) = 5.065$, $p = 0.03$).

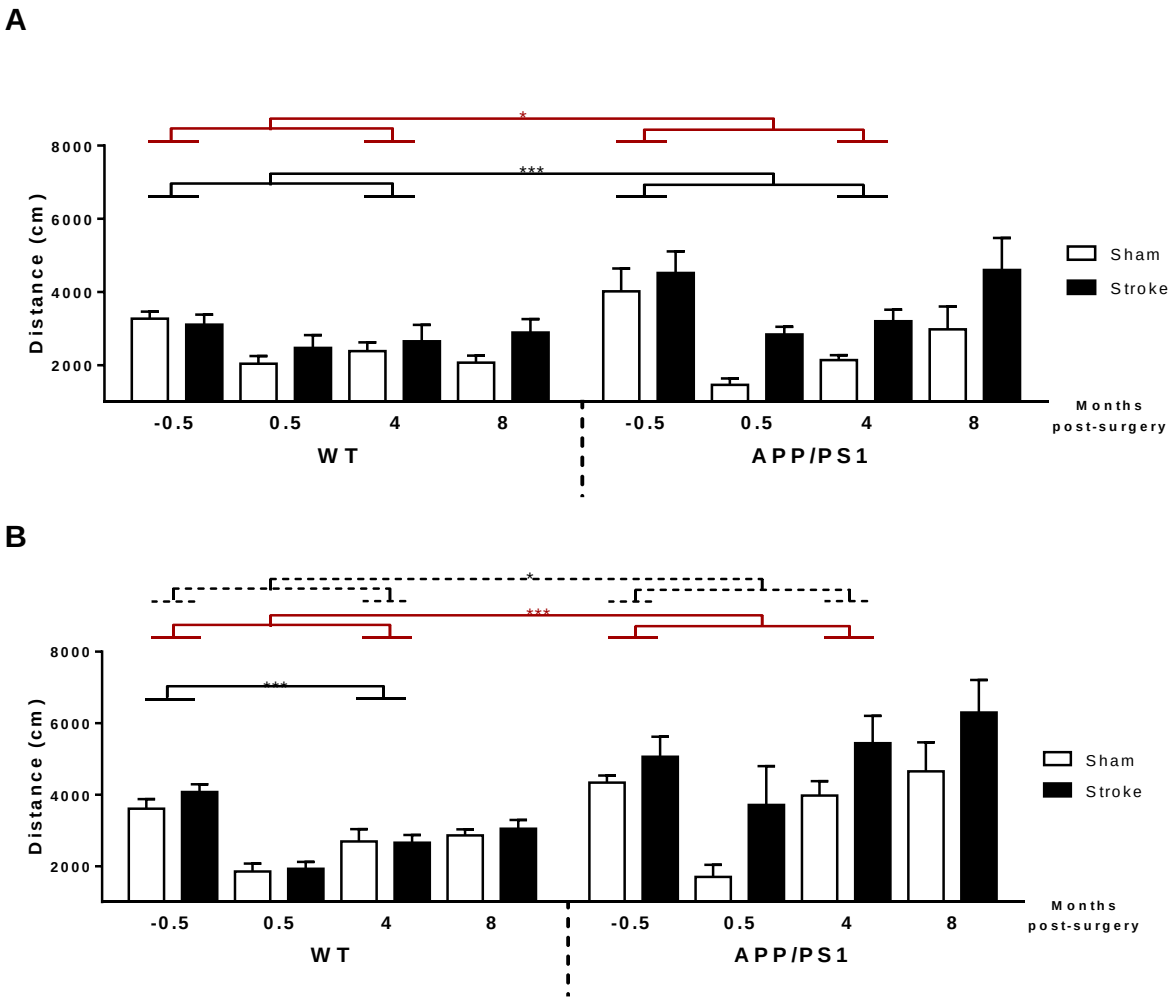

Figure 29. Chronic effects on the walking distance A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) mice walked less 4 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice walked more than WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.027$). (B) WT mice walked less 4 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice walked more than WT mice both at two weeks before the surgery and 4 months after the surgery ($p \leq 0.001$). Stroke mice walked more than sham mice both at two weeks before the surgery 4 months after the surgery ($p = 0.03$).

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a significant time x genotype interaction ($F(2,56) = 3.559$, $p = 0.035$). In detail, APP/PS1 mice walked gradually more after the surgery (Fig. 30A: $F(1,17) = 5.200$, $p = 0.013$). Moreover, APP/PS1 mice walked more than WT mice 8 months after the surgery ($F(1,30) = 5.652$, $p \leq 0.05$; data not shown). Furthermore, a significant surgery effect was found; stroke mice walked more than sham mice at every time point after the surgery (Fig. 30A: $F(1,28) = 13.972$, $p = 0.001$). Specifically, stroke mice walked more than sham mice at two weeks ($F(1,30) = 10.355$, $p \leq 0.01$; data not shown), 4 months ($F(1,30) = 4.937$, $p \leq 0.05$; data not shown) and 8 months after the surgery ($F(1,30) = 5.580$, $p \leq 0.05$; data not shown).

Female mice showed a significant time x genotype interaction ($F(2,72) = 7.998$, $p = 0.001$). In detail; both WT mice (Fig. 30B: $F(2,50) = 15.674$, $p \leq 0.001$) and APP/PS1 mice (Fig. 30B: $F(2,26) = 18.908$, $p \leq 0.001$) walked gradually more after the surgery. Moreover, APP/PS1 mice walked more than WT mice 4 months ($F(1,38) = 20.947$, $p \leq 0.001$; data not shown) and 8 months after the surgery ($F(1,38) = 25.769$, $p \leq 0.001$; data not shown). Furthermore, female mice a significant genotype x surgery interaction was found ($F(1,36) = 4.689$, $p = 0.037$). In detail, APP/PS1 sham mice walked more than WT sham mice ($F(1,18) = 5.333$, $p = 0.033$; data not shown) and APP/PS1 stroke mice walked more than WT stroke mice at every time point after the surgery ($F(1,18) = 17.234$, $p = 0.001$; data not shown).

Restoration:

Male mice showed a significant genotype effect; APP/PS1 mice walked more than WT mice at pre-surgery and 8 months after the surgery (Fig. 30A: $F(1,32) = 14.705$, $p = 0.001$). Specifically, APP/PS1 mice walked more than WT mice two weeks before ($F(1,34) = 8.194$, $p = 0.07$; data not shown) and 8 months after the surgery ($F(1,34) = 6.845$, $p = 0.013$; data not shown). Furthermore, a significant surgery effect was found; stroke mice walked more than sham mice at pre-surgery and 8 months after the surgery (Fig. 30A: $F(1,32) = 5.915$, $p = 0.032$). Specifically, stroke mice walked more than sham mice 8 months after the surgery ($F(1,34) = 5.036$, $p = 0.031$; data not shown).

Female mice showed a significant time x genotype interaction ($F(1,37) = 10.286$, $p = 0.003$). In detail, WT mice walked less 8 months after the surgery compared to pre-surgery (Fig. 30B: $F(1,26) = 21.878$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice walked more than WT mice at 8 months after the surgery compared to pre-surgery (Fig. 30B: $F(1,37) = 29.586$, $p \leq 0.001$). Moreover, female mice exhibited a significant surgery effect; stroke mice walked more than sham mice at 8 months after the surgery compared to pre-surgery (Fig. 30B: $F(1,37) = 5.915$, $p = 0.020$).

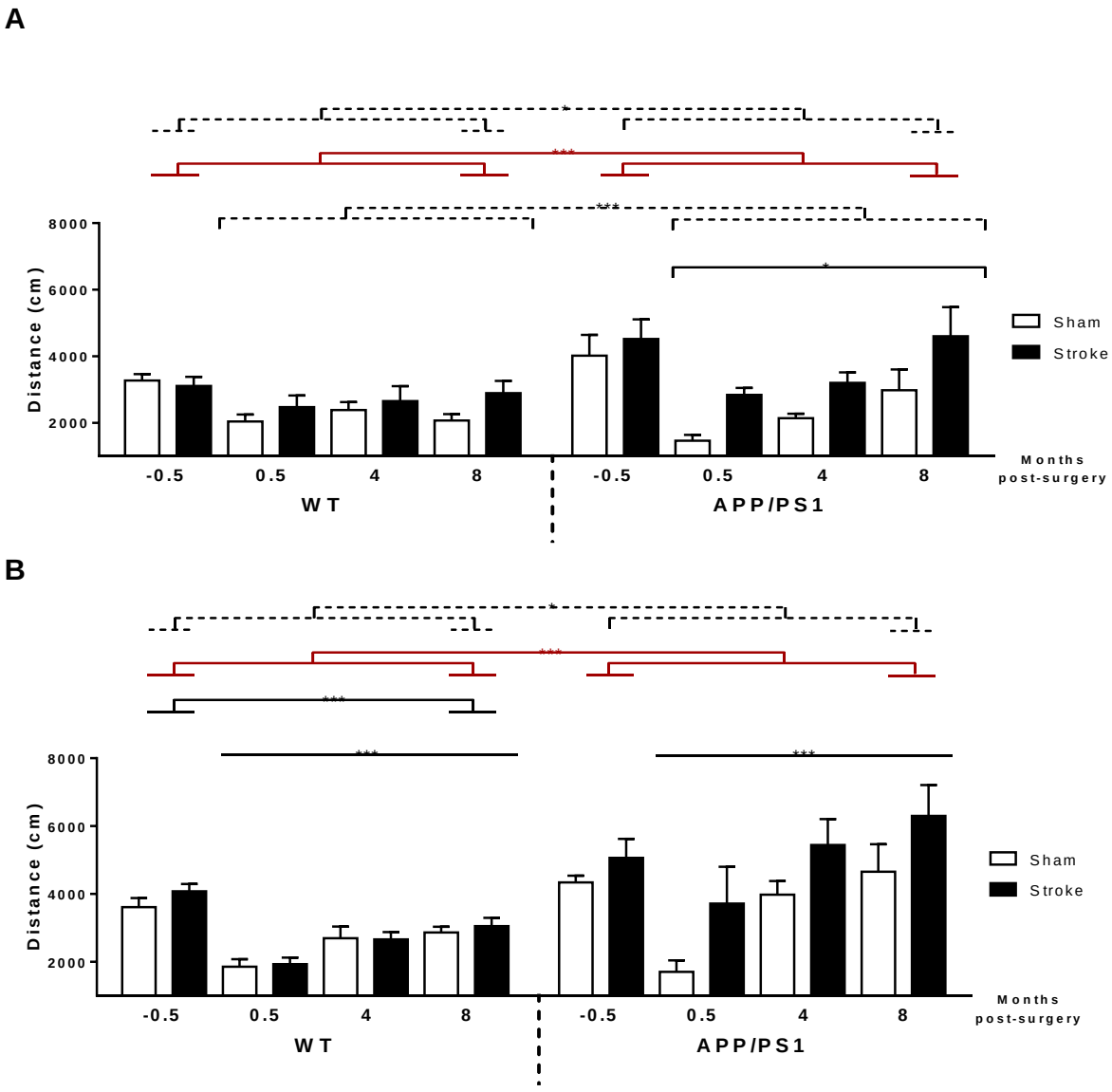

Figure 30. Revalidation and restoration effects on the walking distance A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Revalidation: APP/PS1 mice walked gradually more after the surgery ($p = 0.013$). Stroke mice walked more than sham mice at every time point after the surgery ($p = 0.001$). Restoration: APP/PS1 mice walked more than WT mice at two weeks before the surgery and 8 months after the surgery ($p = 0.001$). Stroke mice walked more than sham mice at every time point after the surgery ($p = 0.032$). (B) Revalidation: WT and APP/PS1 mice walked gradually more after the surgery ($p = 0.001$). APP/PS1 sham mice walked more than WT sham mice ($p = 0.033$) and APP/PS1 stroke mice walked more than WT stroke mice at every time point after the surgery ($p = 0.001$). Restoration: WT mice walked less 8 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice walked more than WT mice at 8 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). Stroke mice walked more than sham mice at 8 months after the surgery compared to two weeks before the surgery ($p = 0.020$).

III.III Walking duration

i. Acute effects

Male mice showed a significant time x genotype interaction ($F(1,28) = 8.417$, $p = 0.007$). In detail, APP/PS1 mice spent less time walking two weeks after the surgery compared to pre-surgery (Fig 30A: $F(1,13) = 21.280$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice spent more time walking than WT mice both at pre-surgery and two weeks after the surgery (Fig. 31A: $F(1,28) = 4.225$, $p = 0.049$). Specifically, APP/PS1 mice spent more time walking than WT mice two weeks before the surgery ($F(1,30) = 7.061$, $p = 0.013$; data not shown).

Female mice an overall time effect; all female mice, regardless of their genotype and surgery, spent less time walking two weeks after the surgery compared to pre-surgery (Fig. 31B: $F(1,36) = 30.214$, $p \leq 0.001$).

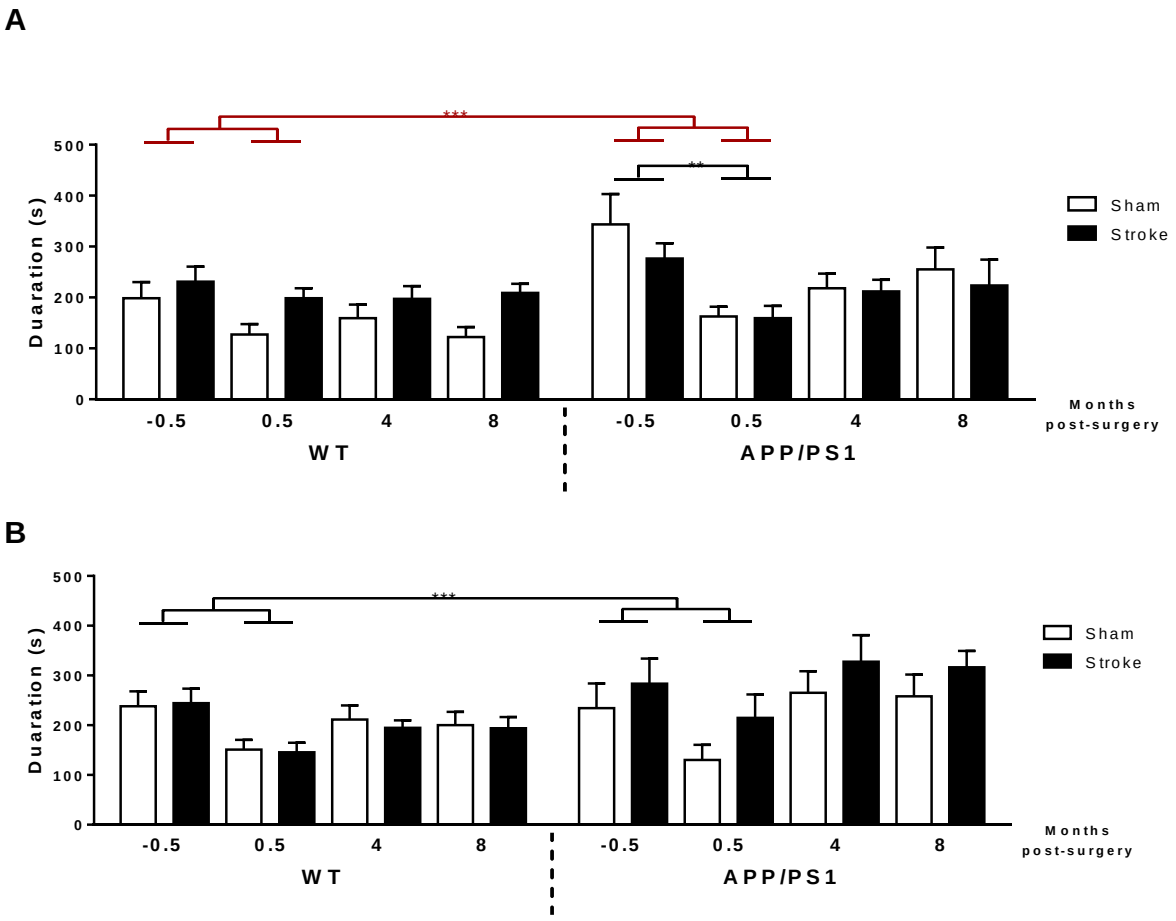

Figure 31. Acute effects on the walking time in the open field A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) APP/PS1 mice spent less time walking two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice spent more time walking than WT mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.049$). (B) All female groups (WT, APP/PS1, sham and stroke) spent less time walking two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$).

ii. Chronic effects

All male mice, regardless of their genotype and surgery, spent less time walking 4 months after the surgery compared to pre-surgery (Fig. 32A: $F(1,32) = 10.678$, $p = 0.03$). Furthermore, male mice a significant genotype effect was found; APP/PS1 mice spent more time walking than WT mice both at pre-surgery and 4 months after the surgery (Fig. 32A: $F(1,32) = 6.736$, $p = 0.028$). Specifically, APP/PS1 mice spent more time walking than WT mice two weeks before the surgery ($F(1,34) = 6.138$, $p = 0.018$; data not shown).

Female mice showed a significant time x genotype interaction (Fig. 32B: $F(1,37) = 5.217$, $p = 0.028$). In detail, WT mice spent less time walking 4 months after the surgery compared to pre-surgery (Fig. 32B: $F(1,26) = 4.485$, $p = 0.044$). Moreover, APP/PS1 mice spent more time walking than WT mice at 4 months after surgery ($F(1,39) = 7.820$, $p = 0.008$; data not shown).

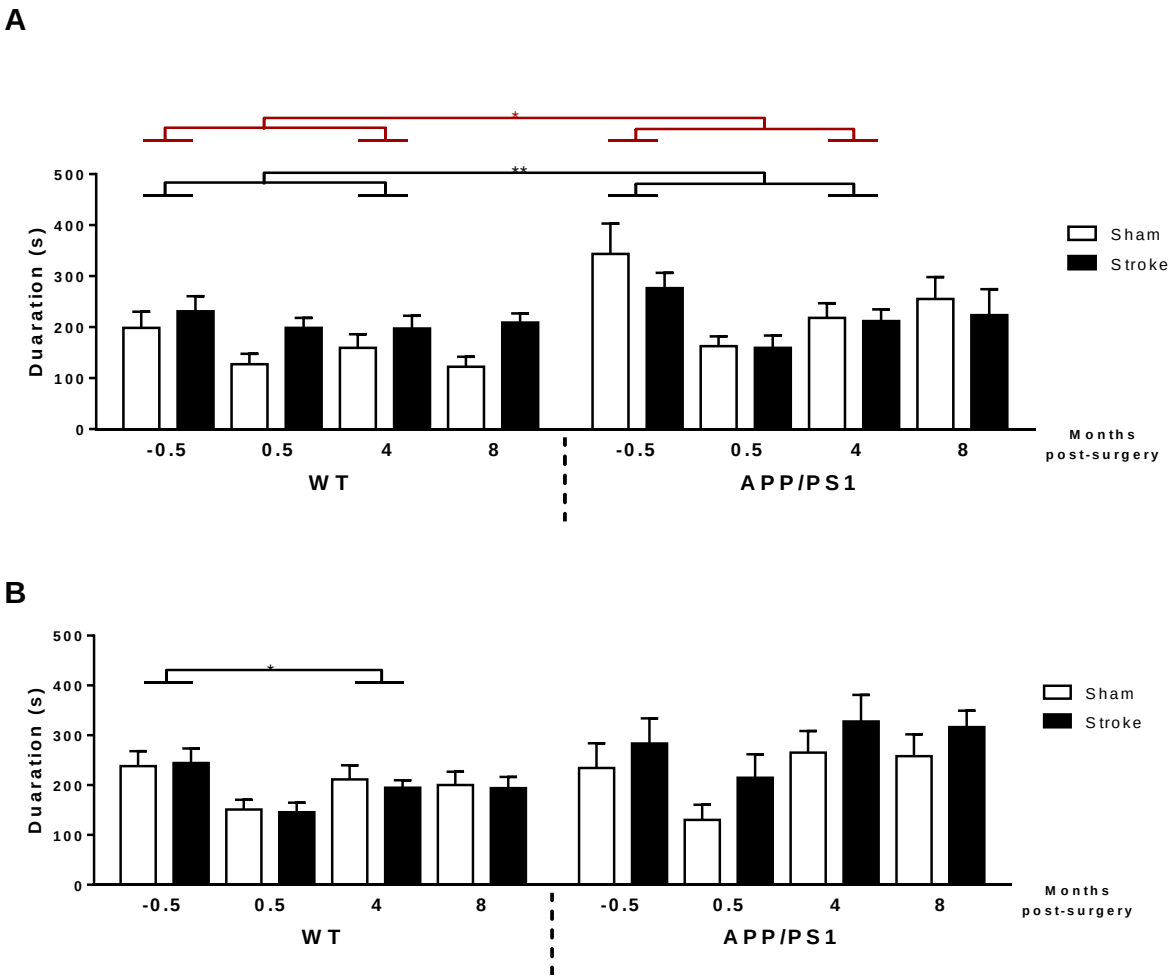

Figure 32. Chronic effects on the walking time in the open field A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)

(A) All male groups (WT, APP/PS1, sham and stroke) spent less time walking 4 months after the surgery compared to two weeks before the surgery ($p = 0.03$). APP/PS1 mice spent more time walking than WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.028$). (B) WT mice spent less time walking 4 months after the surgery compared to two weeks before the surgery ($p = 0.044$).

iii. Revalidation and restoration effects

Revalidation:

Male mice showed a significant time x genotype interaction ($F(2,56) = 3.359$, $p = 0.042$). In detail, APP/PS1 mice showed a change in their walking duration after the surgery (Fig. 33A: $F(2,26) = 3.621$, $p = 0.041$). Furthermore, a significant genotype effect was found; APP/PS1 mice spent more time walking than WT mice at every time point after the surgery (Fig. 33A: $F(1,28) = 4.194$, $p = 0.05$). Specifically, APP/PS1 mice spent more time walking than WT mice at 4 months after the surgery ($F(1,30) = 6.511$, $p = 0.016$; data not shown).

Female mice showed a significant time x genotype interaction ($F(2,72) = 5.036$, $p = 0.009$). In detail; both WT (Fig.33B: $F(2,50) = 12.773$, $p \leq 0.001$) and APP/PS1(Fig. 33B: $F(2,26) = 12.950$, $p \leq 0.001$) mice spent more time walking at every time point after the surgery. Furthermore, a significant genotype effect was found; APP/PS1 mice spent more time walking than WT mice at every time point after the surgery (Fig. 33B: $F(1,36) = 6.830$, $p = 0.013$). Specifically, APP/PS1 mice spent more time walking than WT mice at 4 months ($F(1,38) = 7.633$, $p = 0.009$; data not shown) and 8 months after the surgery ($F(1,38) = 9.648$, $p = 0.004$; data not shown).

Restoration:

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, spent less time walking 8 months after the surgery compared to pre-surgery (Fig. 33A: $F(1,32) = 7.721$, $p = 0.009$). Furthermore, a significant genotype effect was found; APP/PS1 mice spent more time walking than WT mice both at pre-surgery and 8 months after the surgery (Fig. 33B: $F(1,32) = 9.817$, $p = 0.004$). Specifically, APP/PS1 mice spent more time walking than WT mice at two weeks before ($F(1,34) = 6.138$, $p = 0.018$; data not shown) and 8 months after the surgery ($F(1,34) = 4.616$, $p = 0.039$; data not shown).

Female mice showed a significant time x genotype interaction ($F(1,37) = 5.436$, $p = 0.025$). In detail; WT mice spent less time walking 8 months after the surgery compared to pre-surgery (Fig. 33B: $F(1,26) = 8.161$, $p = 0.008$). Moreover, APP/PS1 mice spent more time walking than WT mice at 8 months after the surgery ($F(1,39) = 8.428$, $p = 0.006$; data not shown).

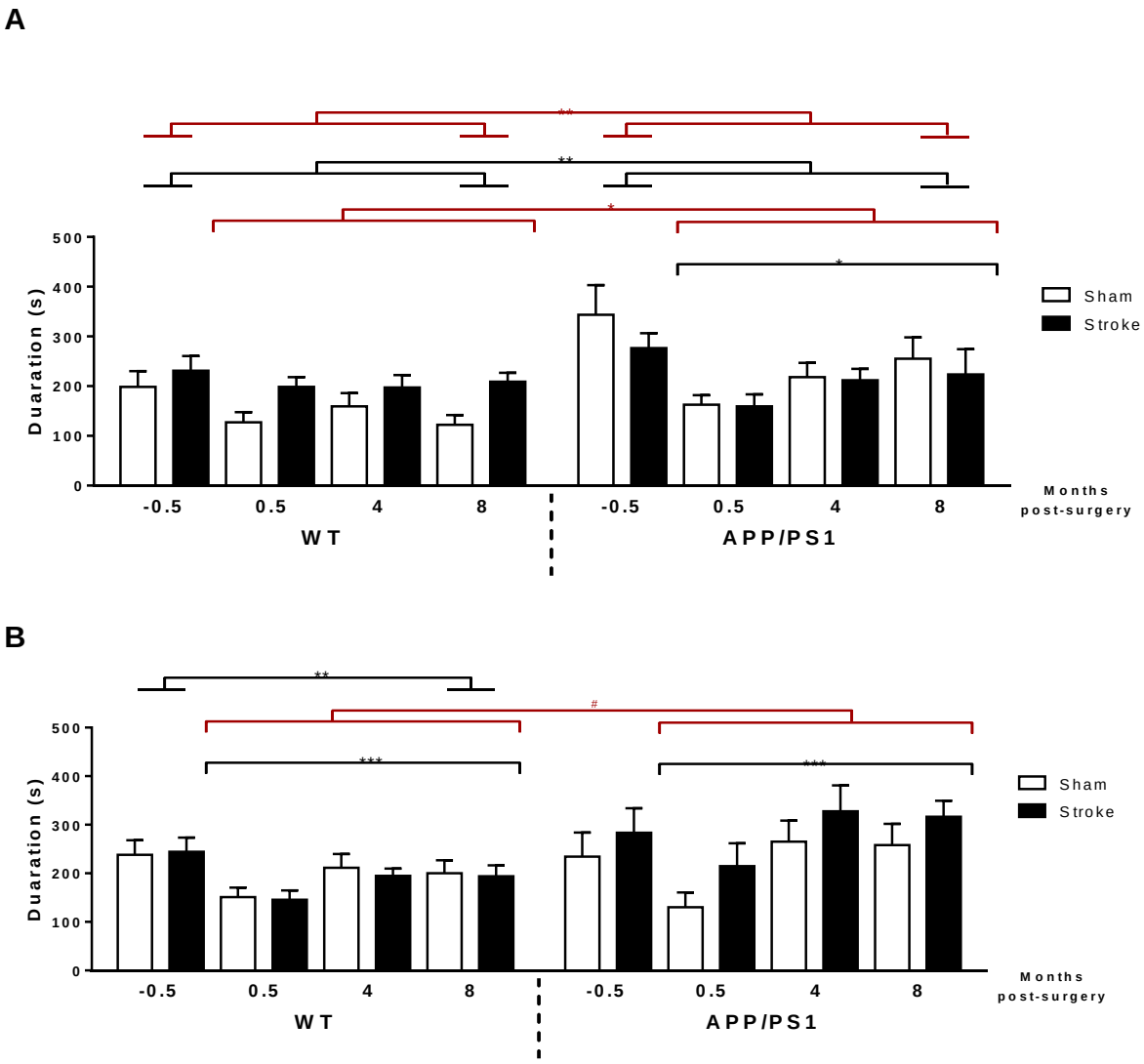

Figure 33. Revalidation and restoration effects on the walking time in the open field A) males B) females (——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) Revalidation: APP/PS1 mice showed a change in their walking duration after the surgery ($p = 0.041$). APP/PS1 mice spent more time walking than WT mice at every time point after the surgery ($p = 0.05$). Restoration: All male groups (WT, APP/PS1, sham and stroke) spent less time walking 8 months after the surgery compared to two weeks before the surgery ($p = 0.009$). APP/PS1 mice spent more time walking than WT mice both at two weeks before the surgery and 8 months after the surgery ($p = 0.004$). (B) Revalidation: WT and APP/PS1mice spent more time walking at every time point after the surgery ($p = 0.009$). APP/PS1 mice spent more time walking than WT mice at every time point after the surgery ($p = 0.013$). Restoration: WT mice spent less time walking 8 months after the surgery compared to two weeks before the surgery ($p = 0.008$).

III. IV Sitting duration

i. Acute effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, spent more time sitting two weeks after the surgery compared to pre-surgery (Fig. 34A: $F(1,28) = 39.860$, $p \leq 0.001$). Furthermore, APP/PS1 mice spent less time sitting than WT mice two weeks before the surgery (Fig. 34A: $F(1,30) = 4.026$, $p = 0.054$)

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, spent more time sitting two weeks after the surgery compared to pre-surgery (Fig. 34B: $F(1,36) = 49.824$, $p \leq 0.001$). Furthermore, a trend in overall genotype effect was found; APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and two weeks after the surgery (Fig. 34B: $F(1,36) = 3.437$, $p = 0.072$). Specifically, APP/PS1 mice spent less time sitting than WT two weeks after the surgery ($F(1,38) = 4.104$, $p = 0.05$; data not shown).

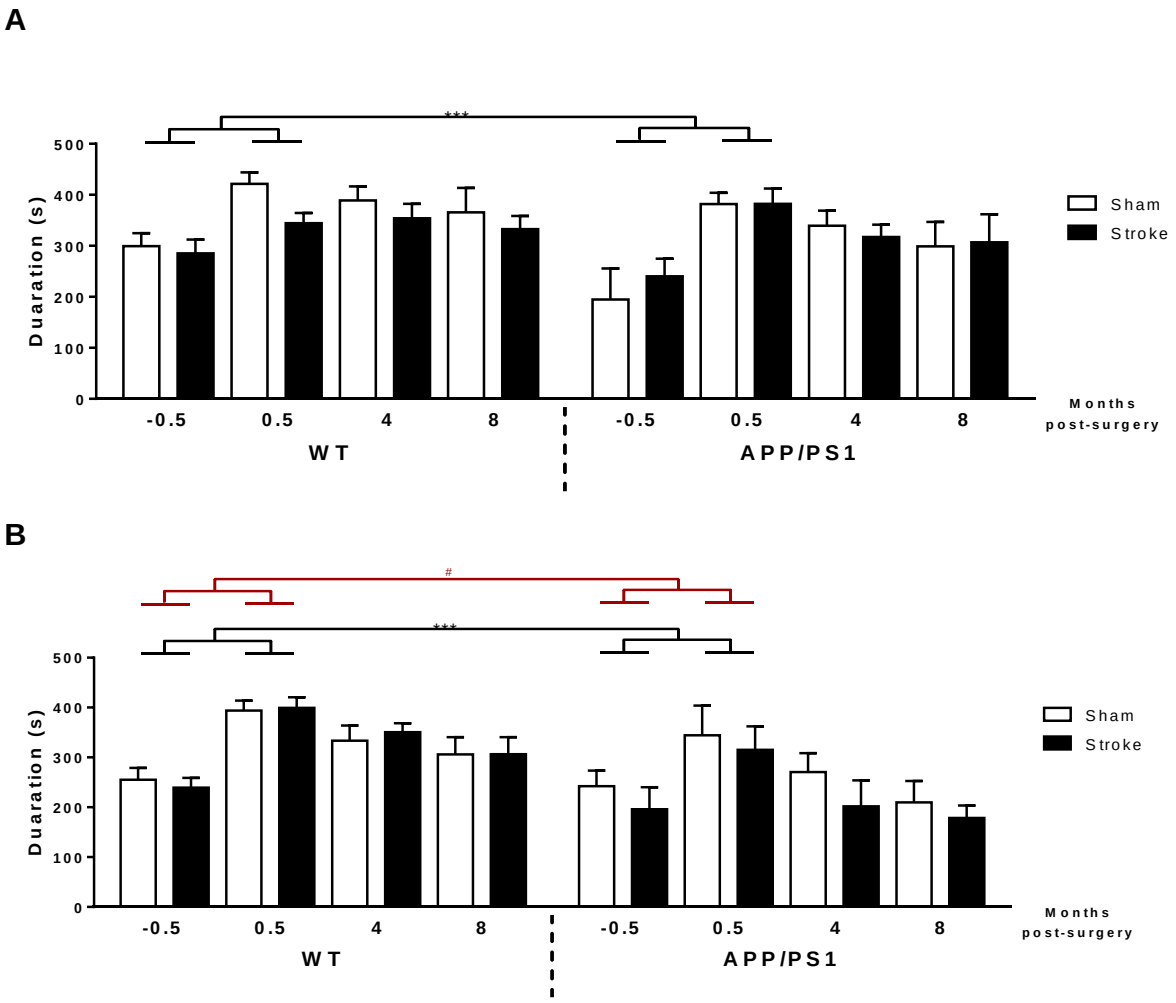

Figure 34. Acute effects on the sitting time in the open field A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) spent more time sitting two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice two weeks before the surgery ($p = 0.054$). (B) All female groups (WT, APP/PS1, sham and stroke) spent more time sitting two weeks after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice both at two weeks before the surgery and two weeks after the surgery ($p = 0.072$).

ii. Chronic effects

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, spent more time sitting 4 months after the surgery compared to pre-surgery (Fig. 35A: $F(1,32) = 21.597$, $p \leq 0.001$). Furthermore, a trend in overall genotype effect was found; APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 4 months after the surgery (Fig. 35A: $F(1,32) = 5.629$, $p = 0.024$). Specifically, APP/PS1 mice spent less time sitting than WT two weeks after the surgery ($F(1,34) = 4.437$, $p = 0.043$; data not shown).

Female mice showed a significant time x genotype interaction ($F(1,37) = 6.796$, $p = 0.013$). In detail, WT mice spent more time sitting 4 months after the surgery compared to pre-surgery. (Fig. 35B: $F(1,26) = 31.428$, $p \leq 0.001$). Specifically, APP/PS1 mice spent less time sitting than WT 4 months after the surgery ($F(1,39) = 9.639$, $p = 0.004$; data not shown). Furthermore, a trend in overall genotype effect was found; APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 4 months after the surgery (Fig. 35B: $F(1,37) = 5.781$, $p = 0.021$).

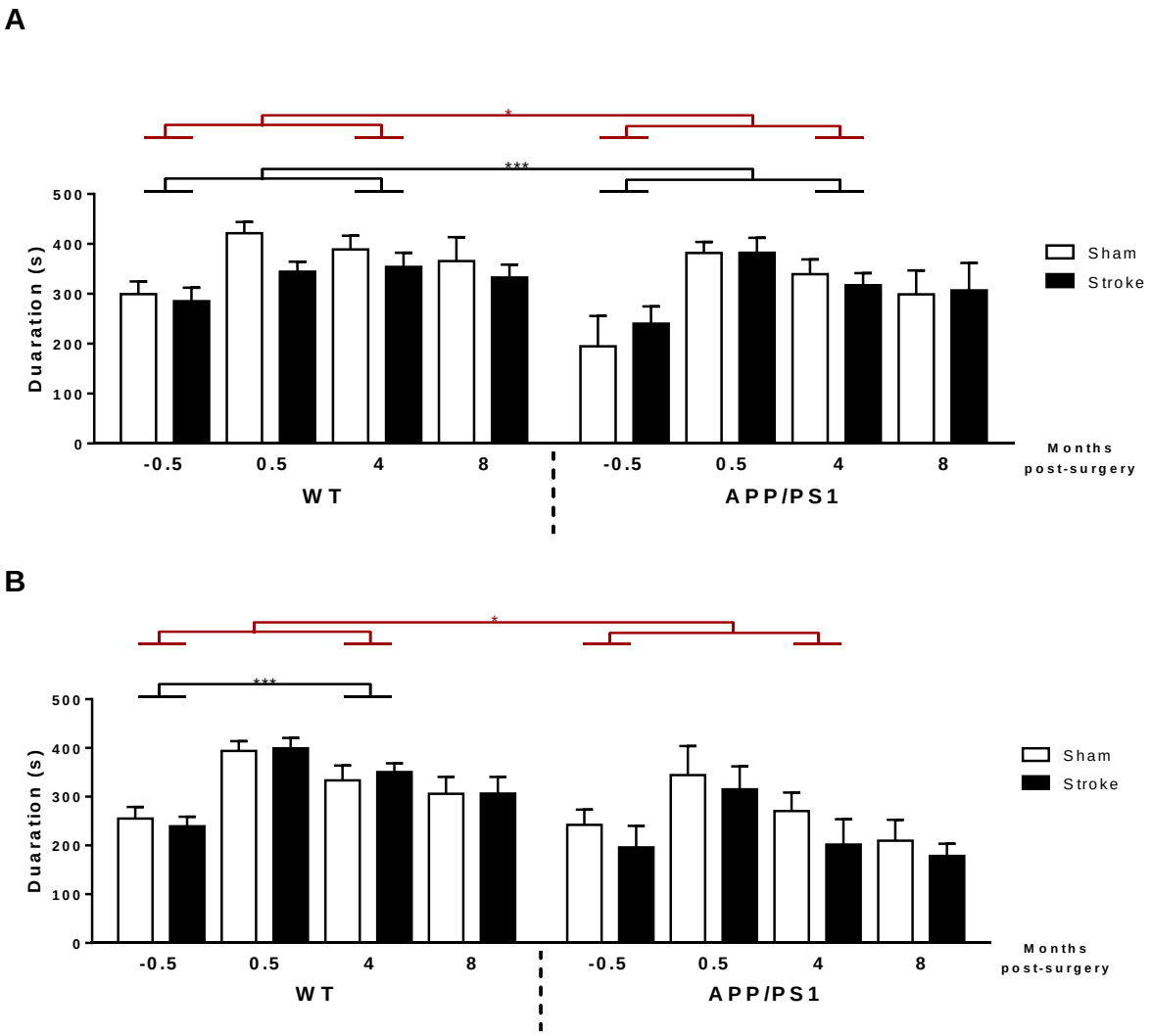

Figure 35. Chronic effects on the sitting time in the open field A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) All male groups (WT, APP/PS1, sham and stroke) spent more time sitting 4 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.024$). (B) WT mice spent more time sitting 4 months after the surgery compared to two weeks before the surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice both at two weeks before the surgery and 4 months after the surgery ($p = 0.021$).

iii. Revalidation and restoration effects

Revalidation:

There were no significant effects observed in male mice (Fig. 36A).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, showed a gradual decrease in time spent sitting at every time point after the surgery. (Fig. 36B: $F(2,72) = 14.544$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice spent less time sitting than WT mice at every time point after the surgery (Fig. 36B: $F(1,36) = 13.338$, $p = 0.001$). Specifically, APP/PS1 mice spent less time sitting than WT mice at two weeks after ($F(1,38) = 4.104$, $p = 0.05$; data not shown), 4 months after ($F(1,38) = 9.349$, $p = 0.004$; data not shown) and 8 months after the surgery ($F(1,38) = 9.493$, $p = 0.004$; data not shown).

Restoration:

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, spent more time sitting 8 months after the surgery compared to pre-surgery (Fig. 36A: $F(1,32) = 21.597$, $p \leq 0.001$). Furthermore, an overall genotype effect was found; APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 8 months after the surgery (Fig. 36A: $F(1,32) = 4.594$, $p = 0.040$). Specifically, APP/PS1 mice spent less time sitting than WT mice two weeks before the surgery ($F(1,34) = 4.437$, $p = 0.043$; data not shown).

Female mice showed a significant genotype effect; APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 8 months after the surgery (Fig. 36B: $F(1,37) = 8.300$, $p = 0.07$). Specifically, APP/PS1 mice spent less time sitting than WT mice 8 months after the surgery ($F(1,39) = 8.982$, $p = 0.005$; data not shown).

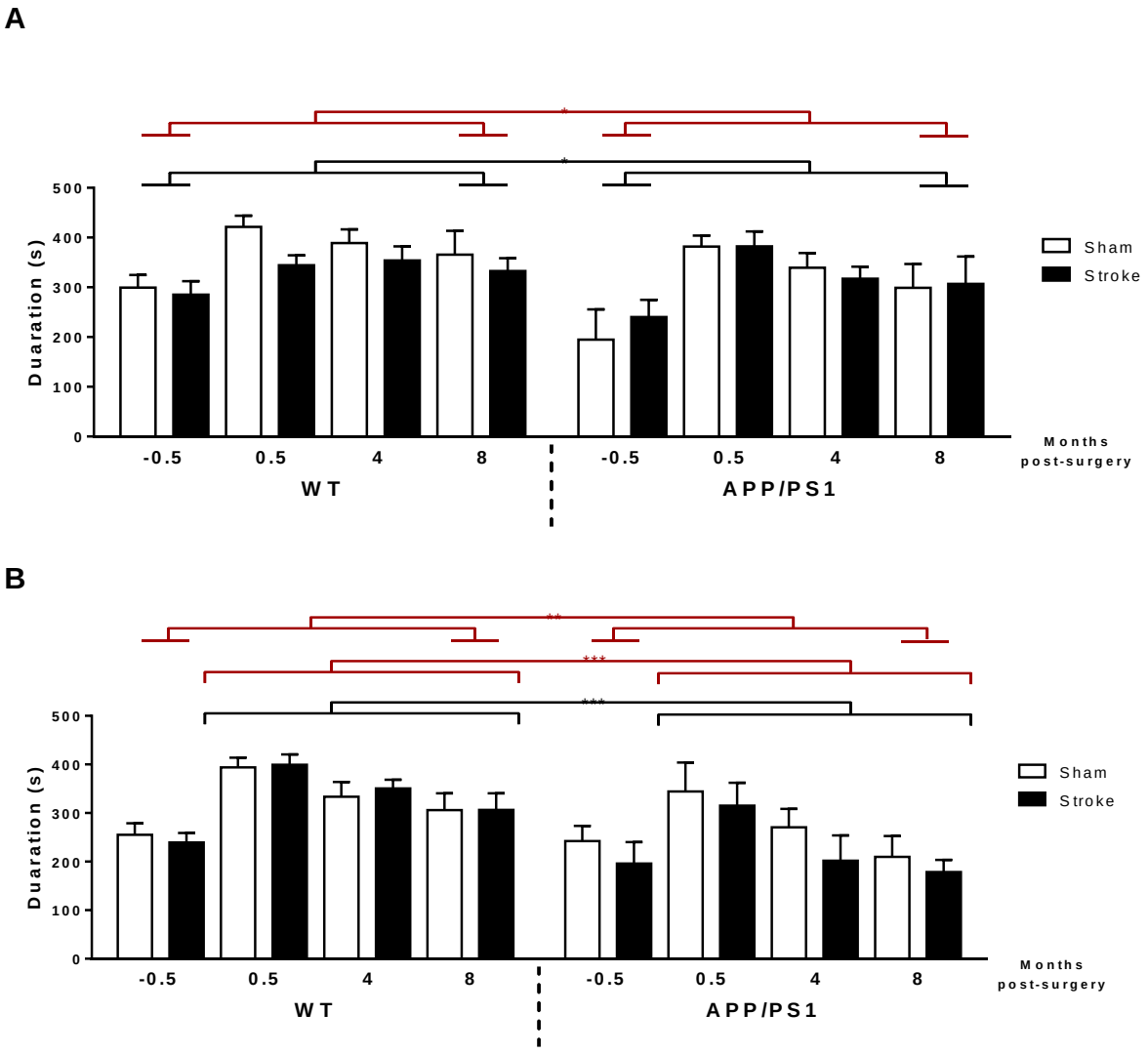

Figure 36. Revalidation and restoration effects on the sitting time in the open field A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Revalidation: Male mice showed no significant interaction. Restoration: All male groups (WT, APP/PS1, sham and stroke) spent more time sitting 8 months after the surgery compared to pre-surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 8 months after the surgery ($p = 0.040$). (B) Revalidation: all female groups (WT, APP/PS1, sham and stroke) showed a gradual decrease in time spent sitting at every time point after the surgery ($p \leq 0.001$). APP/PS1 mice spent less time sitting than WT mice at every time point after the surgery ($p = 0.001$). Restoration: APP/PS1 mice spent less time sitting than WT mice both at pre-surgery and 8 months after the surgery ($p = 0.07$).

IV. Rotarod

Male mice showed a trend in genotype effect; APP/PS1 mice demonstrated decreased latency to fall from the rotating rod than WT mice (Fig. 37: F (1,42) = 3.563, p = 0.067).

Female mice showed a trend in surgery effect; stroke mice demonstrated decreased latency to fall from the rotating rod than sham mice (Fig. 37: F (1,46) = 3.871, p = 0.056).

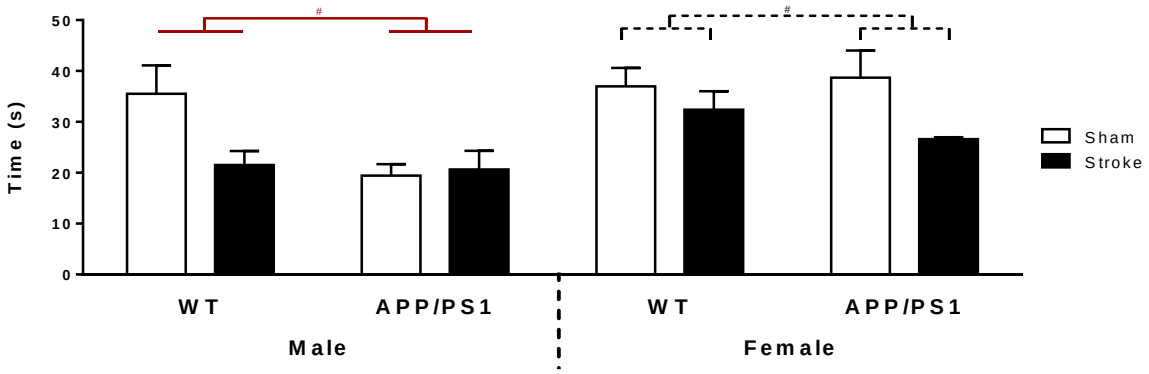

Figure 37. Latency to fall in rotarod.
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
Male APP/PS1 mice demonstrated decreased latency to fall than WT mice (p = 0.067). Female stroke mice demonstrated decreased latency to fall than sham mice (p = 0.056).

V. Morris Water Maze

V.I Acquisition

i. Latency

Male mice found the hidden platform faster on the fourth day compared to the first day (Fig. 38A: $F(3,108) = 14.429$, $p \leq 0.001$).

Female mice found the hidden platform faster on the fourth day compared to the first day (Fig. 38B: $F(3,105) = 18.108$, $p \leq 0.001$). Furthermore, APP/PS1 mice were slower to find the platform than WT mice on every day of the acquisition (Fig. 38B: $F(1,35) = 17.027$, $p \leq 0.001$).

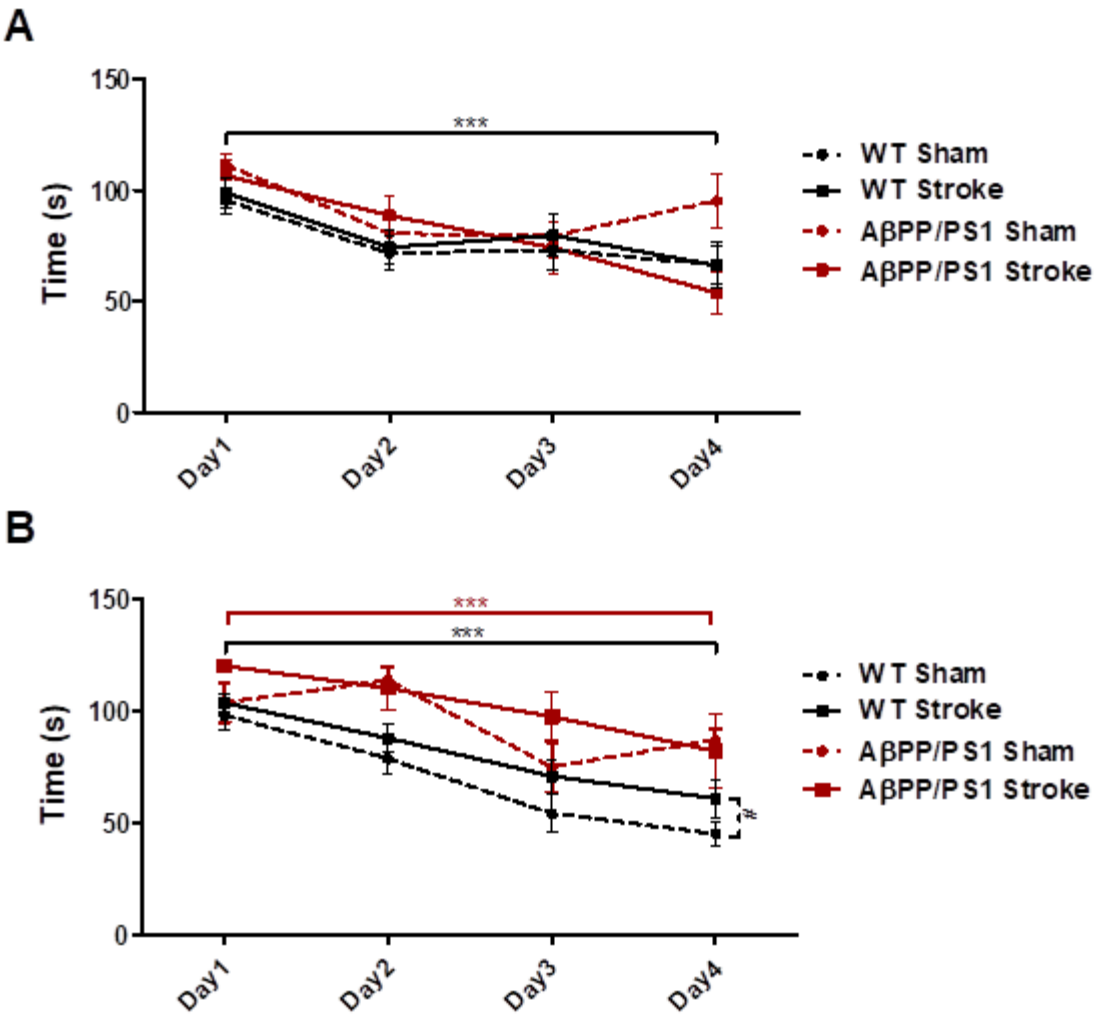

Figure 38. Latency to find the hidden platform in MWM A) males B) females
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Male mice reached the platform faster on the fourth day compared to the first day ($p \leq 0.001$). (B) Female mice reached the platform faster on the fourth day compared to the first day ($p \leq 0.001$). APP/PS1 mice were slower to find the platform than WT mice ($p \leq 0.001$).

ii. Velocity

Male mice showed a significant time x genotype interaction ($F(3,108) = 3.736$, $p = 0.013$). In detail, both WT mice (Fig. 39A: $F(3,78) = 24.074$, $p \leq 0.001$) and APP/PS1 mice (Fig. 39A: $F(3,36) = 2.958$, $p = 0.045$) swam slower on the fourth day compared to the first day. Furthermore, a significant genotype effect was found; APP/PS1 swam faster than WT mice (Fig. 39A: $F(1,36) = 6.751$, $p = 0.013$).

Female mice an overall time effect; all female mice, regardless of their genotype and surgery, swam slower on the fourth day compared to the first day (Fig. 39B: $F(3, 99) = 24.271$, $p \leq 0.001$). Furthermore, a significant surgery effect was found; stroke mice swam slower than sham mice (Fig. 39B: $F(1,33) = 6,527$, $p = 0.015$).

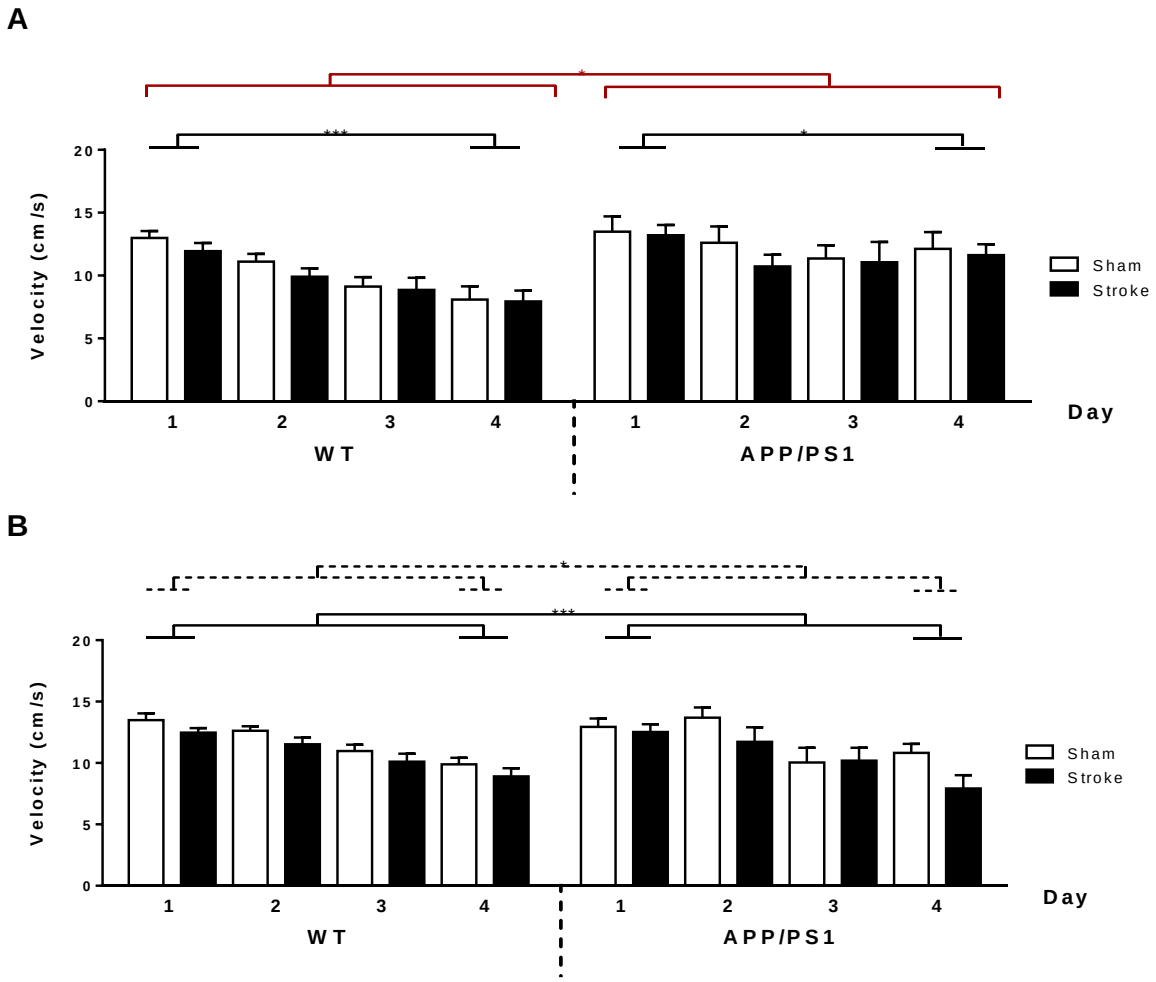

Figure 39. Swim velocity in MWM A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) WT and APP/PS1 mice swam slower on the fourth day compared to the first day ($p = 0.013$). APP/PS1 swam faster than WT mice ($p = 0.013$). (B) All female groups (WT, APP/PS1, sham and stroke) swam slower on the fourth day compared to the first day ($p \leq 0.001$). Stroke mice swam slower than sham mice ($p = 0.015$).

iii. Distance

Male mice showed an overall time effect; all male mice, regardless of their genotype and surgery, swam a shorter distance on day four compared to the first day (Fig. 40A: $F(3,108) = 22.359$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 swam longer distances than WT mice (Fig. 40A: $F(1,36) = 10.167$, $p = 0.003$).

Female mice showed an overall time effect; all female mice, regardless of their genotype and surgery, swam a shorter distance on day four compared to the first day (Fig. 40B: $F(3, 99) = 27.043$, $p \leq 0.001$). Furthermore, a significant genotype effect was found; APP/PS1 mice swam longer distances than WT mice (Fig. 40B: $F(1,33) = 7.600$, $p = 0.009$).

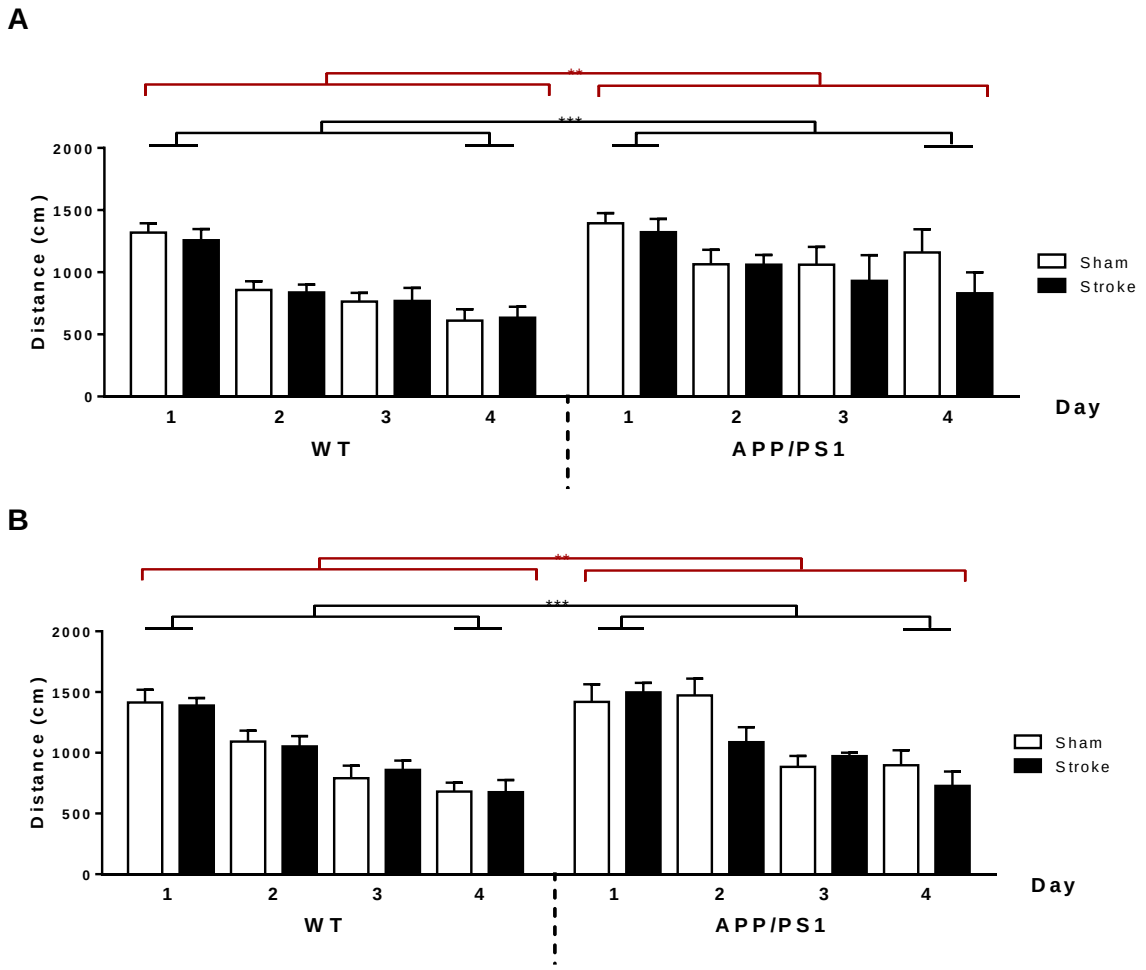

Figure 40. Swim distance in MWM A) males B) females

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference) (A) All male groups (WT, APP/PS1, sham and stroke) swam a shorter distance on day four compared to the first day ($p \leq 0.001$). APP/PS1 swam longer distances than WT mice ($p = 0.003$). (B) All female groups (WT, APP/PS1, sham and stroke) swam a shorter distance on day four compared to the first day ($p \leq 0.001$). APP/PS1 swam longer distances than WT mice ($p = 0.009$).

V.II. Probe

i. Velocity

There were no significant effects observed in male mice (Fig. 41A).

Female mice demonstrated a significant surgery effect; stroke mice swam slower than sham mice (Fig.40A: $F(3,38) = 9.543$, $p = 0.004$).

ii. Distance

There were no significant effects observed in male mice (Fig. 41B).

Female mice demonstrated a significant surgery effect; stroke mice swam shorter distances than sham mice (Fig.40B $F(3,38) = 5.989$, $p = 0.020$).

iii. Duration in platform zone

There were no significant effects observed in male and female mice (Fig. 41C).

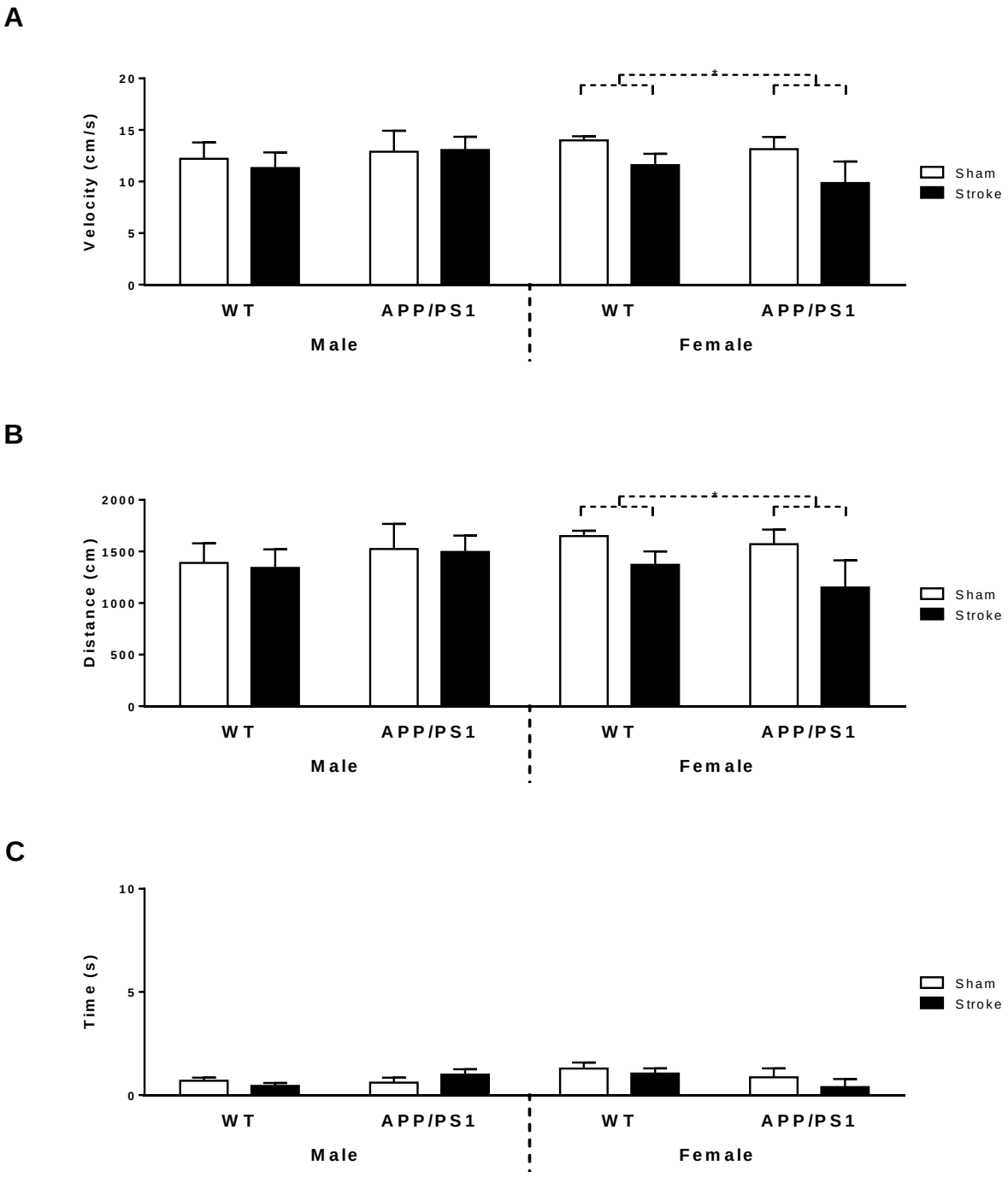

Figure 41. Swim velocity, distance and time spent in platform zone in MWM probe trial. A) velocity B) distance C) time spent in platform zone.

(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
(A) Male mice showed no significant interaction. Female stroke mice swam slower than female sham mice ($p = 0.004$). (B) Male mice showed no significant interaction. Female stroke mice swam shorter distances than female sham mice ($p = 0.004$). (C) No significant effects were found in male and female mice.

d. Imaging

I. ASL

I. I. Cortex

i. Left and right cortex CBF level differences

Two weeks after the surgery CBF in right cortex (Fig: 42/45: WT sham: $F(1,22) = 29.360$, $p \leq 0.001$, WT stroke: $F(1,16) = 19.103$, $p \leq 0.001$, APP/PS1 sham: $F(1,12) = 11.823$, $p = 0.005$ and APP/PS1 stroke: $F(1,6) = 8.175$, $p = 0.029$) was lower than corresponding left ROI for WT sham, WT stroke, APP/PS1 sham and APP/PS1 stroke.

4 months after the surgery CBF in right cortex (Fig: 42/45: WT sham: $F(1,22) = 16.203$, $p = 0.001$, APP/PS1 sham: $F(1,12) = 4.445$, $p = 0.055$ and APP/PS1 stroke: $F(1,6) = 8.207$, $p = 0.029$) was lower than corresponding left ROI for WT sham, APP/PS1 sham and APP/PS1 stroke.

8 months after the surgery CBF in right cortex (Fig: 42/45: WT sham: $F(1,22) = 4.681$, $p = 0.043$, APP/PS1 sham $F(1,12) = 3.972$, $p = 0.072$) was lower than corresponding left ROI for WT sham and APP/PS1 sham.

ii. Genotype effects

No genotype effect on the CBF levels in the cortex was observed (Fig. 42/45).

iii. Surgery effects

No surgery effect on the CBF levels in the cortex was observed (Fig. 42/45)

I.II Hippocampus

i. Left and right hippocampus CBF level differences:

Two weeks after the surgery CBF in right hippocampus (Fig: 43/45: WT sham: $F(1,22) = 47.822$, $p \leq 0.001$, WT stroke: $F(1,16) = 5.964$, $p = 0.027$, APP/PS1 sham $F(1,12) = 27.368$, $p \leq 0.001$ and APP/PS1 stroke: $F(1,6) = 9.965$, $p = 0.020$) was lower than corresponding left ROI for WT sham, WT stroke, APP/PS1 sham and APP/PS1 stroke.

4 months after the surgery CBF in right hippocampus (Fig: 43/45: WT sham: $F(1,22) = 33.111$, $p \leq 0.001$, APP/PS1 sham $F(1,12) = 27.575$, $p \leq 0.001$, and APP/PS1 stroke: $F(1,6) = 8.028$, $p = 0.030$) was lower than corresponding left ROI for WT sham, APP/PS1 sham and APP/PS1 stroke.

8 months after the surgery CBF in right hippocampus (Fig: 43/45: WT sham: $F(1,22) = 98.995$, $p \leq 0.001$, APP/PS1 sham $F(1,12) = 8.539$, $p = 0.014$, and APP/PS1 stroke: $F(1,6) = 14.430$, $p = 0.009$) was lower than corresponding left ROI for WT sham, APP/PS1 sham and APP/PS1 stroke.

ii. Genotype effects

Two weeks after the surgery, CBF in the right hippocampus tended to be lower in APP/PS1 stroke mice than WT stroke mice (Fig: 43/45: $F(1,23) = 27.883$, $p = 0.052$).

4 months after the surgery, CBF in the right hippocampus was lower in APP/PS1 stroke mice than WT stroke mice (Fig: 43/45: $F(1,23) = 38.646$, $p = 0.036$).

8 months after the surgery, CBF in the right and left hippocampus was lower in APP/PS1 sham mice than WT sham mice (Fig: 43/45: right HPC: $F(1,32) = 15.247$, $p = 0.017$, left HPC: $F(1,32) = 15.247$, $p = 0.017$). Additionally, CBF in the right hippocampus tended to be lower in APP/PS1 stroke mice than WT stroke mice (Fig: 43/45: $F(1,23) = 39.928$, $p = 0.066$).

iii. Surgery effects

Two weeks after the surgery, CBF in the right hippocampus was higher in WT stroke mice than WT sham mice (Fig: 43/45: $F(1,39) = 17.249$, $p = 0.003$).

4 months after the surgery, CBF in the right hippocampus was higher in WT stroke mice than WT sham mice (Fig: 43/45: $F(1,39) = 24.750$, $p \leq 0.001$). Additionally, CBF in the right and left hippocampus was higher in APP/PS1 stroke mice than APP/PS1 sham mice (Fig. 43B: right HPC: $F(1,19) = 19.611$, $p = 0.057$, left HPC: $F(1,19) = 18.990$, $p = 0.006$).

8 months after the surgery, CBF in the right hippocampus was higher in WT stroke mice than WT sham mice (Fig: 43/45: $F(1,39) = 24.335$, $p = 0.012$). Additionally, CBF in the left hippocampus tended to be higher in APP/PS1 stroke mice than APP/PS1 sham mice (Fig: 43/45: $F(1,19) = 20.553$, $p = 0.064$).

I.III Thalamus

i. Left and right cortex CBF level differences

4 months after the surgery CBF in right cortex (Fig: 44/45: WT sham: $F(1,22) = 16.203$, $p = 0.001$, APP/PS1 sham: $F(1,12) = 4.919$, $p = 0.045$) was lower than corresponding left ROI for WT sham and APP/PS1 sham. On the contrary, CBF in right cortex (Fig: 44/45: $F(1,16) = 4.857$, $p = 0.043$) was higher than corresponding left ROI for WT stroke.

8 months after the surgery CBF in right cortex (Fig: 44/45: F (1,16) = 18.740, p = 0.001) was higher than corresponding left ROI for WT stroke.

ii. Genotype effects

8 months after the surgery, CBF in the right thalamus was lower in APP/PS1 stroke mice than WT stroke mice (Fig: 44/45: F (1,23) = 11.852, p = 0.029).

iii. Surgery effects

Two weeks after the surgery, CBF in the right thalamus was higher in WT stroke mice than WT sham mice (Fig: 44/45: F (1,39) = 17.249, p = 0.003).

4 months after the surgery, CBF in the right thalamus was higher in WT stroke mice than WT sham mice (Fig: 44/45: F (1,39) = 24.750, $p \leq 0.001$). Additionally, CBF in the right and left thalamus was higher in APP/PS1 stroke mice than APP/PSS1 sham mice (Fig. 42: right HPC: F (1,19) = 19.611, p = 0.057, left HPC: F (1,19) = 18.990, p = 0.006).

8 months after the surgery, CBF in the right thalamus was higher in WT stroke mice than WT sham mice (Fig: 44/45: F (1,39) = 24.335, p = 0.012). Additionally, CBF in the left thalamus was higher in APP/PS1 stroke mice than APP/PS1 sham mice (Fig. 42: F (1,19) = 20.553, p = 0.064).

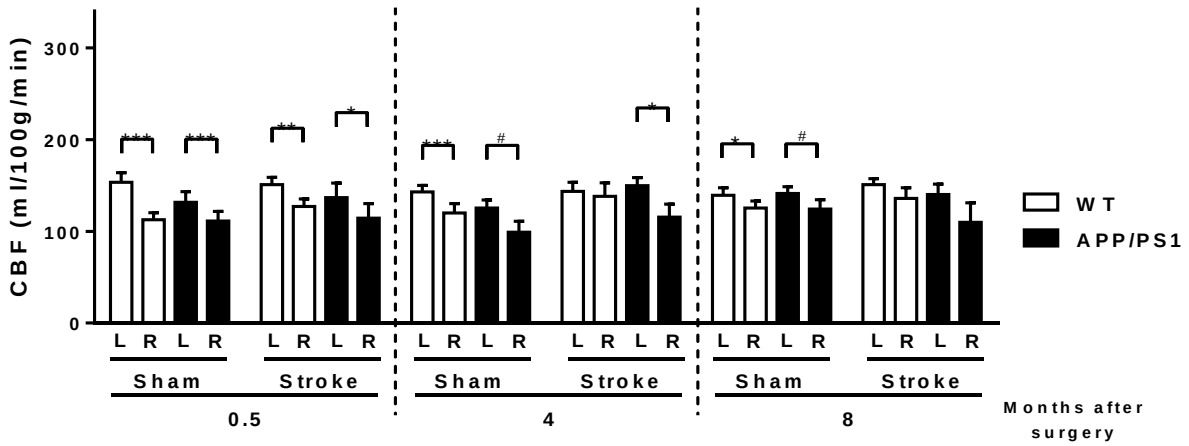

Figure 42. CBF in the in the left and right cortex 2 weeks, 4 months and 8 months after surgery.
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
Two weeks after the surgery: CBF in right cortex was lower than corresponding left ROI for WT sham ($p \leq 0.001$), WT stroke ($p \leq 0.001$), APP/PS1 sham ($p = 0.005$) and APP/PS1 stroke ($p = 0.029$). 4 months after the surgery: CBF in right cortex was lower than corresponding left ROI for WT sham ($p = 0.001$), APP/PS1 sham ($p = 0.055$) and APP/PS1 stroke ($p = 0.029$). 8 months after the surgery: CBF in right cortex was lower than corresponding left ROI for WT sham ($p = 0.043$) and APP/PS1 sham ($p = 0.072$).

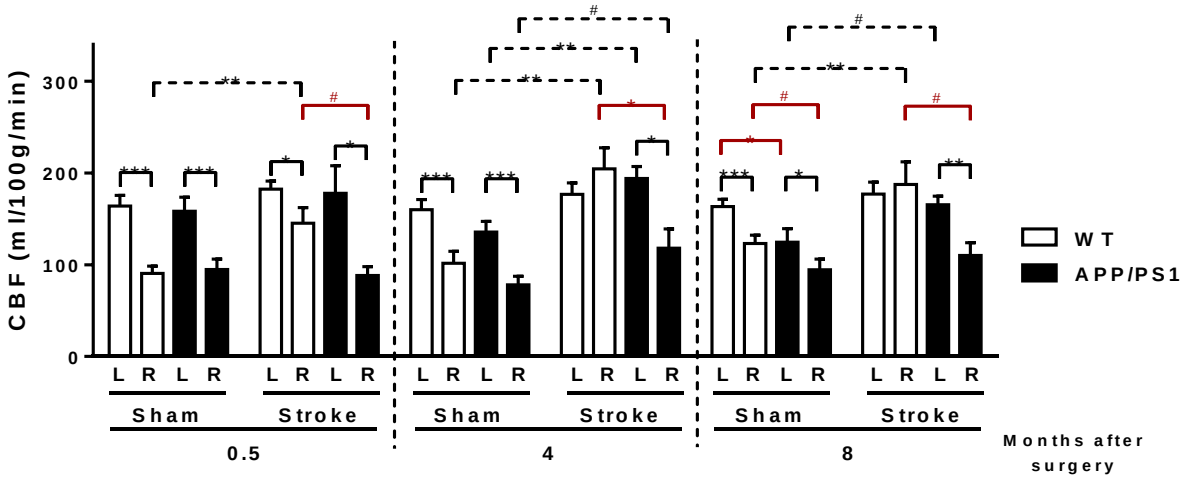

Figure 43. CBF in the in the left and right hippocampus 2 weeks, 4 months and 8 months after surgery.
(——— significant time difference; ——— significant genotype difference; ------- significant surgery difference)
Two weeks after the surgery: CBF in right hippocampus was lower than corresponding left ROI for WT sham ($p \leq 0.001$), WT stroke ($p = 0.027$), APP/PS1 sham ($p \leq 0.001$) and APP/PS1 stroke ($p = 0.020$). CBF in the right hippocampus was lower in APP/PS1 stroke mice than WT stroke mice ($p = 0.052$). CBF in the right hippocampus was higher in WT stroke mice than WT sham mice ($p = 0.003$). 4 months after the surgery: CBF in right hippocampus was lower than corresponding left ROI for WT sham ($p \leq 0.001$), APP/PS1 sham ($p \leq 0.001$) and APP/PS1 stroke ($p = 0.030$). CBF in the right hippocampus was lower in APP/PS1 stroke mice than WT stroke mice ($p = 0.036$). CBF in the right hippocampus was higher in WT stroke mice than WT sham mice ($p \leq 0.001$) and CBF in the right ($p = 0.057$) and left ($p = 0.006$) hippocampus was higher in APP/PS1 stroke mice than APP/PS1 sham mice. 8 months after the surgery: CBF in right hippocampus was lower than corresponding left ROI for WT sham ($p \leq 0.001$), APP/PS1 sham ($p = 0.014$) and APP/PS1 stroke ($p = 0.009$). CBF in the right ($p = 0.017$) and left ($p = 0.017$) hippocampus was lower in APP/PS1 sham mice than WT sham mice. CBF in the right hippocampus was higher in WT stroke mice than WT sham mice ($p = 0.012$) and CBF in the left hippocampus was higher in APP/PS1 stroke mice than APP /PS1 sham mice ($p = 0.064$).

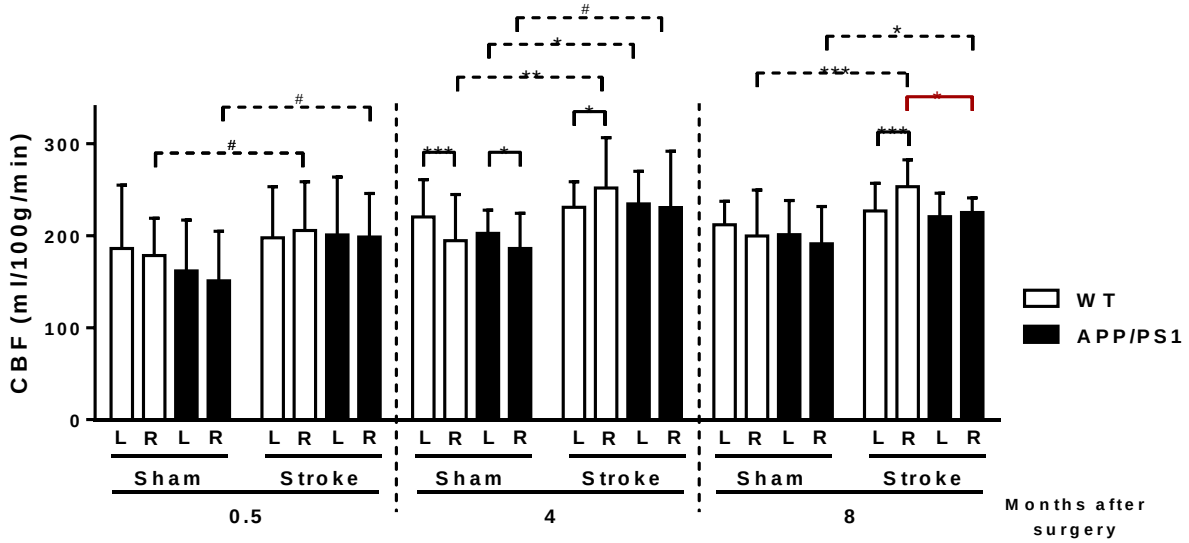

Figure 44. CBF in the in the left and right thalamus 2 weeks, 4 months and 8 months after surgery.
(——— significant time difference;——— significant genotype difference; ------- significant surgery difference)
Two weeks after the surgery: CBF in the right thalamus was higher in WT stroke mice than WT sham mice ($p = 0.003$).4 months after the surgery: CBF in right cortex was lower than corresponding left ROI for WT sham ($p = 0.001$) and APP/PS1 sham ($p = 0.045$). CBF in right cortex was higher than corresponding left ROI for WT stroke ($p = 0.043$). CBF in the right thalamus was higher in WT stroke mice than WT sham mice ($p \leq 0.001$). CBF in the right ($p = 0.057$) and left ($p = 0.006$) thalamus was higher in APP/PS1 stroke mice than APP/PS1 sham mice. 8 months after the surgery: CBF in right cortex was higher than corresponding left ROI for WT stroke ($p = 0.001$). CBF in the right thalamus was lower in APP/PS1 stroke mice than WT stroke mice ($p = 0.029$). CBF in the right thalamus was higher in WT stroke mice than WT sham mice ($p = 0.012$). CBF in the left thalamus was higher in APP/PS1stroke mice than APP/PS1 sham mice ($p = 0.064$).

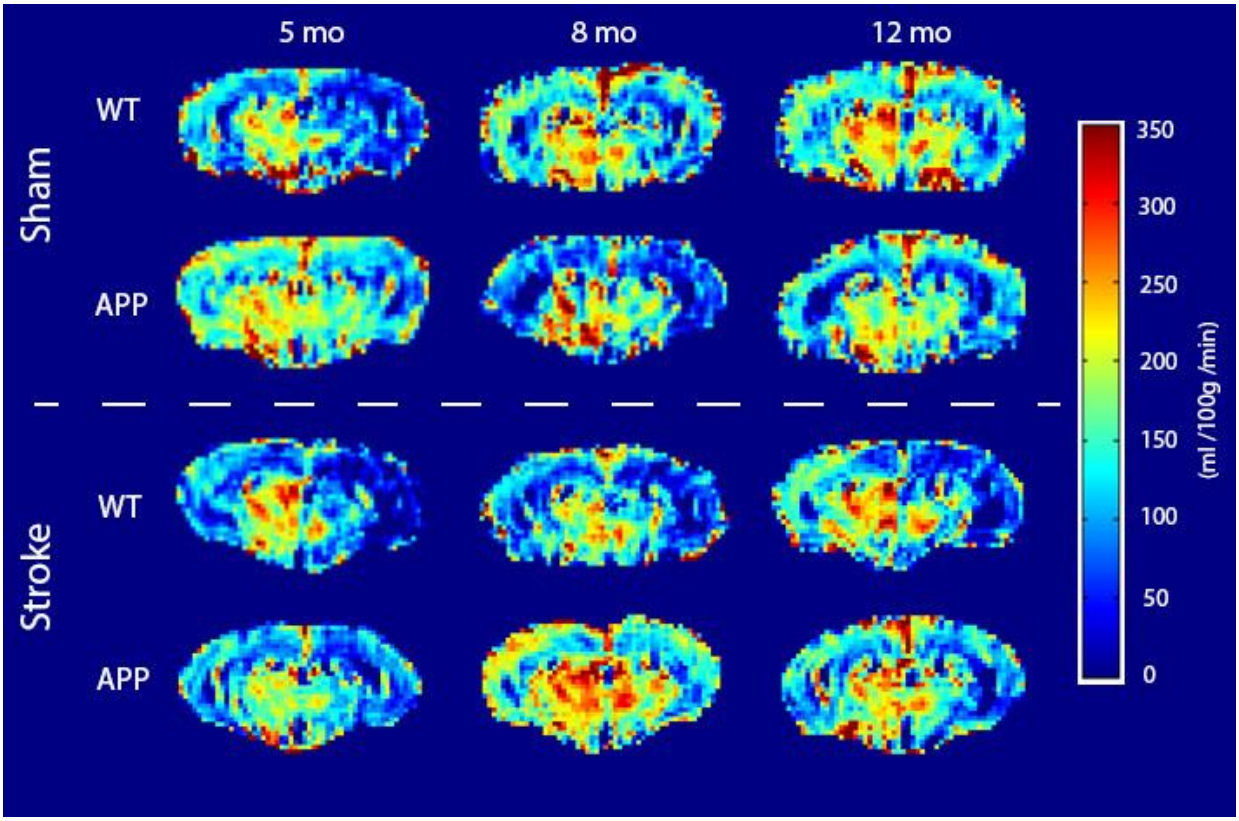

Figure 45. CBF was determined by FAIR-ASL at 5-, 8- and 12- months old age (2 weeks, 4 months and 8 months after the surgery, respectively) for the cortex, hippocampus and thalamus.

5. Discussion

In this present study, we assessed the interaction between stroke and AD. We evaluated acute and long-term effects of ischemic stroke on the health parameters, motor function, locomotion, and cognition in WT mice and APP/PS1 mice for both sexes. Our aim was to shed light on the underlying vascular mechanisms of stroke on AD pathophysiology and sex-specific differences.

a. Physiological parameters: body weight and blood pressure

Clinically, weight loss after stroke is common and occurs due to impaired feeding, inactivity and metabolic imbalance [43]. In the acute phase, we observed weight loss in male mice but interestingly, female mice demonstrated weight gain. Although both AD and stroke are more prevalent in women, disease course and progression are worse for men than women [34]. As mentioned above, it is important to note that there is a significant difference in sexual aging between men and women; women are protected by estrogen against AD and stroke [34]. Therefore, the effect we observed in female mice is attributable to the estrogen protectiveness. Weight loss observed in male mice can be a part of the regular course of the stroke and/or AD. Because clinically, both stroke and AD patients show decline in body weight over time [43, 44]. In the chronic and revalidation phases, we observed that both male and female mice gained weight showing that they were able to recover from acute body weight loss. Furthermore, again in both male and female mice, stroke mice were lighter than sham mice. Also, female APP/PS1 mice were lighter than WT mice. In line with previous research, a possible explanation of decreased weight of stroke and APP/PS1 mice compared to sham and WT mice arises from increased anxiety which was reflected to the mice behavior as increased walking and hyperactivity, leading to weight loss [45]. Post stroke agitation is observed in pre-clinical and clinical studies. In humans, 15- 35% of stroke patients demonstrate high agitation and aggression. Also, in mice, even the ones with motor problems present hyperactivity after stroke [45-47]. The reason behind this high locomotion is increased anxiety [45-47]. Furthermore, AD patients present increased hyperactivity (wandering, increased walking) [48]. Another reason behind the observed acute weight loss might be due to decreased feeding, but food intake was not monitored in this study. So, further studies should evaluate food/water intake at home cages to verify the reasons behind decreased body weight of stroke and APP/PS1 animals.

As mentioned above healthy blood pressure is crucial for cerebrovascular health. Particularly, hypertension is an important risk factor for stroke and AD [21-23]. It can cause infarcts, small vessel diseases, and causes disruptions in the CBF [21-23]. Moreover, hypertension causes changes in the cerebrovascular autoregulation which refers to maintaining constant blood flow despite the changes in the arterial pressure [49]. Two important outcomes should be mentioned: first that male APP/PS1 sham mice had a higher SBP than APP/PS1 stroke mice in all time points, and secondly male APP/PS1 stroke mice had an increased SBP compared to male WT stroke mice only in the acute and chronic phases. The reason behind APP/PS1 mice having increased SBP compared to its stroke littermates can be explained by autoregulation. Sham mice might have altered their arterial pressure to maintain regular levels of CBF which in turn, might have caused them to have hypertension. On the other hand, stroke mice, who already had higher BP, failed to do so resulting in lowered levels of SBP compared to sham mice. This explanation should be verified with vasoreactivity research in the future since vasoreactivity was not evaluated in the current study. Another explanation might come from human studies. Although three-quarter of the patients shows increased BP after an acute stroke, BP decreases spontaneously in two-thirds of the patients within the first seven days [50]. So, it might be that stroke mice also showed a drop in the BP after the stroke operation. Secondly, APP/PS1 is a mouse model that shows increased BP and higher Aβ accumulation already as discussed before. This leads to impaired autoregulation and stroke which are known to increase BP. Therefore, in the acute and chronic phase, combined genotype and surgery effect might led APP/PS1 mice to have a higher BP compared to WT stroke mice. Furthermore, when compared with pre-surgery, all male mice also showed decreased SBP at 8 months after the surgery. This also aligns with recent studies. Clinical research showed BP declines gradually after the onset of AD. The underlying reason is revealed to be the impaired autoregulation caused by increased Aβ and causing hypoperfusion and therefore decreased BP [49]. Although the link between BP and AD though studied extensively, underlying mechanisms are still unclear. Hypertension can be present in patients long before the onset of AD and AD can progress without patients showing overt symptoms for decades. So hypertension may precede or follow AD [49]. The explained results were only seen in males and not in females. Interestingly, female mice did not show any significant SBP effects over the course of the study. As mentioned before, women have the estrogen protectiveness against stroke and AD. Female mice are less prone to the outcomes of these diseases. However, an important distinction between mice and humans should get attention.

Both species undergo reproductive aging but while humans' sex hormones decrease with aging (menopause and andropause in humans), mice's sex hormones do not decrease with aging [51]. Therefore, we assume that female mice are protected from severe effects of stroke and AD. Further studies could include gonadectomy to study the effect of sex hormone depletion and reflect the human conditions more appropriately, therefore, increasing translation of the study to the clinical settings [51].

b. Motor function

Motor deficits are one of the most common impairments that are observed after stroke in patients, which include muscle weakness [52]. Majorly we observed genotype effects with the trapeze where forelimb strength was assessed and surgery effect with the grid where total muscle strength was evaluated. In the acute phase, we found that male WT stroke mice were weaker than sham littermates. Similar results were found in previous studies rat studies but also observed in the clinical settings. Grip strength of stroke patients may not result from the voluntary muscle movement but may be a result of involuntary flexing or as in explained in rodent studies, it may be due to failure to initiate movement and fail to relax the grip [53, 54]. In the chronic and the restoration phases, we also found that APP/PS1 stroke mice are stronger that WT stroke mice. This could be the effect of the failed relaxation of the muscles due to stroke combined with increased hyperactivity of APP/PS1 mice which might cause increased forepaw strength. Nevertheless, it demonstrated that the combined effect of AD and stroke has severe effects on the behavior. Again, these effects were solely presented in male mice and not in females. Proving the increased protection of females by estrogen. Although with the trapeze test, in the acute phase we observed decreased muscle strength in all mice groups. Interestingly, in all time points including the acute phase, APP/PS1 mice were stronger than WT mice both in male and females. AD related hyperactivity might be the underlying reason. An interesting distinction was made between the grid and the trapeze; there was an overall decrease in muscle strength measured in trapeze but an overall increase in muscle strength in the grid. While the trap measures the forelimbs, the grid measures both fore- and hindlimbs. Increase in total muscle strength in the grid can be explained by the remarkable compensation of mice after stroke, mice are capable of recover from it within the next 14-21 days [55]. Although hind limb strength is not extensively researched, hind limbs may be less affected and therefore show higher strength in the grid. Also, mice are using all limbs in the grid test compared to two possibly comprised forepaws due to

stroke in the trapeze test. Nevertheless, it was always challenging to differentiate compensation from a recovery in mice, especially in longer assessments [55, 56]. Not only due to their compensation mechanisms but animals inheritably try to hide their impairments in the wild and that might be a confounding effect [55, 56]. Also, in longer assessments animals do become habituated and adapt to the task, therefore, lose motivation [55, 56]. In this sense, trapeze might be more sensitive than grid to evaluate the motor function and muscle strength than the grid. Furthermore, since strength is related to body mass, weights of the mice at the time of the grip test should be taken as a covariate.

In addition to the grip test, pole test was used to assess motor function. Pole test has been widely used to evaluate differences between stroke and sham groups [45, 55]. In line with the previous studies, rotation time increased in all animal groups, but stroke mice needed more time to turn than sham mice [57]. Although this effect was only observed in the acute and the revalidation phase and was not present in the restoration phase. It is coherent with previous studies since pole test have been shown to be useful in detecting deficits up to a month after the surgery but was not sensitive enough to detect differences afterward [45, 55, 58]. Furthermore, in all groups velocity to descend the pole was decreased after the surgery. Also, in all time points stroke mice were faster than sham mice and APP/PS1 mice were faster than WT mice to descend the pole. The increased velocity of stroke and APP/PS1 mice can be explained by the increased hyperactivity due to anxiety as explained above. Interestingly, we only observed strong genotype x surgery interactions in the male mice, proving protection of estrogen in females. In male mice, APP/PS1 stroke mice were faster than APP/PS1 sham mice showing that stroke exacerbate the already existing AD symptoms. But also, WT stroke mice were faster than WT sham mice, indicating stroke can lead to AD like pathology in mice.

c. Locomotion

Open field was used not only to evaluate locomotor activity but also the explorative and anxiety related behaviors [45]. Two weeks after the surgery, velocity and distance decreased significantly in all animals. Nevertheless, APP/PS1 mice were faster than WT mice and stroke mice were faster than sham mice. This suggests, stroke not only influences the locomotion, it leads mice to be more hyperactive, anxious. We can conclude that stroke worsens the symptoms of AD. This finding also agrees with previous findings. Mice displayed decreased velocity and distance also because they spent less time walking and more time sitting two weeks after the surgery [59, 60].

When we compared the velocity and the distance 8 months after the surgery, we observed that most animal groups, except for male APP/PS1 sham and female APP/PS1 stroke, were able to restore back to the baseline measurements. Overall, both in female and male, APP/PS1 mice were faster, walked more and sat less than WT mice at all time points. As mentioned above, observed hyperactivity of APP/PS1 mice is due to increased anxiety that was previously observed both in pre-clinical and clinical settings [45-48]. But, male and female comparison was not part of the current statistical analysis and needs to be checked to evaluate sex differences more profoundly. Moreover, other behavior measures need to be analyzed further to confirm high anxiety. Nevertheless, it is important to mention that mice become habituated to the test and the environment is perceived not novel with repeated testing [45, 56]. Therefore, exploration which is a direct measure of locomotion, might decrease [45, 56].

Rotarod test was performed to assess locomotion, coordination, and balance [45]. In our study the rotarod test did not yield significant results. Male mice showed a trend in genotype; APP/PS1 demonstrated decreased latency to fall compared to WT. Female mice showed a trend in surgery; stroke demonstrated decreased latency to fall than sham mice. Although previous studies found a similar difference that was found in male mice. But differently from our present study, in previous studies mice were trained at the baseline [45, 55-57]. Furthermore, previous studies state that rotarod can detect differences between sham and stroke mice seven days after the surgery but was unreliable in the long term [45, 55-57]. Therefore, performing rotarod training at a later time point with no baseline training might be the reason behind insignificant results.

d. Cognition

In the acquisition phase of the MWM, mice need to find the platform with the aid of the visual cues. Mice needs to learn the location of the platform, consolidate and then retrieve the information following day to navigate to the platform [42]. All mice were able to learn and find the platform because latency was decreased on the fourth day compared to the first day. Yet, female APP/PS1 mice had increased latency to find the platform compared to WT mice indicating impaired learning. Increased latency of female mice resulted from their slow swim speed and long swim distances. Prolonged latencies and longer swim distances indicates that mice did not have spatial, goal directed (hippocampus dependent) search strategies but did demonstrated non-goal directed search (thigmotaxic, random search, scanning) [61]. Non- goal directed search is not based on spatial memory and thus hippocampus independent [61]. Although this explanation

should be verified with other MWM measures. Since increased latency was only observed in female mice, obtained vaginal smears needs to be analyzed for any further effects that might arise from disruptions of estrogen levels. Furthermore, male mice also displayed decreased speed, swim distances. But both in male and female mice APP/PS1 mice swam longer distances than WT mice. Again, indicating hyperactivity. Additionally, female stroke mice were slower than sham mice. These kinds of effects were also reported previously in other studies and clinically it was shown that AD patients also suffer from disruptions in orientation, navigation, and recall [42, 58]. In the probe phase, we did not observe any significant differences in any animal group. MWM was done once at 12 months of age but in further studies, could implement a cognitive test such as reverse MWM, radial arm test, or Barnes maze before the surgery as a baseline for cognitive parameters along with imaging. Overall, impaired learning we observed was predicted to arise from hippocampal dysfunction. But impairments in in the sensory and motor areas may be confounding. Although it is important to make a remark about the translational value because sole total hippocampal deficits are not very common among post stroke patients [62]. Impairments in sensory motor areas are involved in cognitive deficits [62]. But a recent study showed that hippocampal injury due to a stroke may become more pronounce in the patients with AD [63]. Additionally, there are differences between mice and human cognition; humans have more lateralized cognition than mice[58]. Nevertheless, to evaluate the cognitive impairments observed in MWM and to investigate the CBF, MRI was performed after the MWM.

To evaluate and visualize CBF we used ASL technique. ASL uses the arterial blood and magnetizes it by radiofrequencies [64]. As labelled flow flows through the brain arteries images are taken alongside with control images that were taken previously to the labeling [64]. The signal difference between the control and the labeled images than provides with CBF maps [64]. We looked at three ROI: cortex, hippocampus, and thalamus. Due to the high mortality rate of APP/PS1 and stroke mice, especially in male mice, the number of animals /groups was low. Therefore, we did not split the imaging data for sex.

Somatosensory cortex has a role in motor control function in humans and mice [65]. While CBF in the right cortex was lower than left ROI at 5- months of age for all animal groups, it was restored for WT stroke at 8 -months of age and for APP/PS1 stroke and WT stroke at 12- months of age. Which is interesting if we look back at the locomotion from open field data, where WT sham mice did not show any signs of motor impairments. Although it can explain persistent hyperactivity of APP/PS1 mice. Because APP/PS1 mice are already prone to high Aβ load and

combined with stroke, can cause low CBF. What is also interesting is while both WT and APP/PS1 stroke mice restored, sham littermates did not. Further investigation of the other imaging parameters and immunohistological analysis should be conducted. Furthermore, the hippocampus was investigated. Hippocampus has a crucial role in learning and memory formation both in humans and mice [66]. Two weeks after the surgery, CBF in the right hippocampus was lower than left ROI for all animal groups. Notably, at 8- and 12- months of age WT stroke animals show elevated CBF levels in the right side, indicating recovery after the stroke induction. But again, WT shams did not present a similar recovery, and further investigation is needed on this result. Moreover, APP/PS1 stroke mice showed decreased CBF in the right hippocampus compared to the left side in all time points, which was not present in WT stroke animals at 8- and 12- months of age. Additionally, APP/PS1 stroke mice had lowered CBF in the right hippocampus compared to WT stroke mice at all time points. This effect can account for increased latencies and impaired learning of APP/PS1 mice in MWM. Here again, we observed lowered CBF in the right hippocampus in WT sham mice compared to WT stroke mice in all time points. Although WT sham animals had decreased CBF in the right hippocampus compared to WT stroke, they did not show any cognitive impairments in MWM. Indicating, the drop in CBF in the right side might just be a relative decrease. During the stroke procedure, CCA is occluded by a suture and it stays occluded for the rest of the experiments. This might cause a drop in CBF levels but because sham mice did not have occluded MCA they might have compensated and did not show any cognitive deficits. Finally, we looked at the thalamus. Thalamus receives and sends information to the cortex and therefore thalamocortical circuits are essential for perception and action [67]. Interestingly, we did not observe decreased CBF in the right thalamus compared to left ROI at 5 -months of age. Thalamus is distal to the stroke site and therefore immediate effects might not be seen. But at 8- months of age CBF in the right thalamus was higher than left ROI for WT sham and APP/PS1 sham. Additionally, at all-time points, CBF in the right thalamus of sham mice was lowered than stroke mice. Leading to similar results we observed for cortex and hippocampus. In the mouse brain anterior choroidal artery (AchA), the lateral hypothalamic artery (LHA) and the ventral thalamic artery (VTA) supplies blood to the subcortical areas such as the thalamus and the hippocampus [67]. These arteries can branch out from the CCA and in our surgery method CCA stays occluded but CCA is proximal to the stroke area [67]. Therefore, a decline in the distal areas from the stroke area can result from the surgery method and/or also proximal occlusion of MCA can alone cause proximal damage. Thalamus

also is known to have a role in the circadian rhythm and the DVC data can be used verified observed connections [67]. Nevertheless, despite sham mice showing reduced CBF in the right side of all three ROI, they did not exhibit any cognitive deficits in the MWM. This could mean that the drop in CBF is relative and without any further damage is caused. Still, further investigation should be done with other imaging parameters and post mortem tissue analysis to reveal the differences in CBF between sham and stroke mice.

e. Surgery, tests and mouse model

In this study, we used tMCAo for 30 min. to induce an ischemic stroke in the right hemisphere. In this procedure, CCA remains occluded indefinitely. With ASL analysis, we found sham mice seemed to have lower levels of CBF for the chosen ROI. Previous studies showed that duration and the location of the stroke change the results excessively [45, 58]. Though other imaging parameters still need to be assessed in our study, alternative methods can be implemented for future studies. Sham operations could be done without the insertion of the filament, or another control group without the surgery can be used.

To investigate the effect of sex, we included female mice groups in our study. Female mice, as well as women, are protected by estrogen against many disorders, but menopause is a critical time window. But female mice do not undergo menopause as mentioned above. Also, male mice' sex hormones do not decline with age. To better represent the human characteristics, further studies could implement gonadectomy. Furthermore, while the female mice estrous cycle was studied, there is a lack of information on the differences of the estrous cycle between strains, such as WT and APP/PS1, that needs to be filled. Most importantly, sex bias and sex omission are still a present problem in neuroscience [51, 68-71]. This is especially crucial since most diseases such as AD and stroke are more prominent in women, but the preclinical and clinical tests are being conducted on male mice and men. Including both sexes will be more beneficial in the long run both for clinical drug trials by avoiding adverse events that can arise from sex differences [51, 68-71].

Some of the tests were unable or limited to assess long term functional behaviors. Longitudinal functional assessments are extremely important yet there are few tests that reached a consensus and that can evaluate the given parameters correctly. Since it is natively difficult to assess behavior because mice inherently want to hide their deficits and their compensation mechanism can mask it further leading false recovery signs. Even if not, not all deficits and impairments are

overtly observable. With that in mind, most tests are adapted from rat studies. Development of appropriate tests and further enhancements and corrections of the existing tests more important than ever. Furthermore, detailed information about the animal's inclusion and exclusion criteria as well as the negative results should be included in the preclinical stroke publications [30, 45, 55, 58, 72]. But the utmost importance is that there should be more animals with comorbidities, older animals and female animals included in research, especially in stroke and AD due to their heterogeneous and sporadic nature [30, 45, 55, 58, 72]. Additionally, confounding factors such as diet, sleep, social interaction and hormones should be evaluated [30, 45, 55, 58, 72]. This kind of advancement will not only lead to less failure in drug tests but also to the development of better and alternate therapeutic approaches which will close the gap between animal and clinical studies [30, 45, 55, 58, 72].

APP/PS1 is a valid, well studied model for AD. It has been transgenically modified to express high Aβ accumulation. But with increasing evidence fails to replicate the correlation between AD and Aβ. Since most patients with AD do not have Aβ depositions or people with high Aβ accumulations do not have AD [3, 11-13]. Therefore, current mice model of AD present some of the features of the human AD but the pathogenesis is not identical. Transgenic mice are forced to imitate the genetic components of the disease which in the case of AD, relates more to the familial form of the disease while the majority of the patients have the sporadic form. So additional measures are needed for better mouse models that imitate the human conditions better [73].

6. Conclusion

In the aging world, AD becomes more prevalent than ever. In this study, we showed that stroke exacerbates the symptoms of AD. Physiological parameters, motor function, locomotion, and cognition were affected by stroke induction. Overall, APP/PS1 and stroke mice were more hyperactive, more anxious, showed deficits in motor function, locomotion and cognitive impairments than their WT and sham littermates. Male mice showed greater surgery effects than female mice, but further comparisons are needed to validate. Also, greater importance should be made on the sex hormones and their effect on disease progression. While the correlation between vascular risk factors, AD and stroke have been made, the exact underlying mechanisms are yet to be investigated. Further studies should investigate a variety of vascular risk factors to establish which alters the AD prognosis and evaluate whether treatment of vascular risk factors can prevent AD or delay the onset of the disease. More extensive research should be conducted to establish better models and tests for preclinical studies to increase translation to the clinical area. There is no effective treatment or cure available for stroke and AD yet. But considering the variations we showed between sex, genotype, and surgery, development of preventative and therapeutic strategies for cerebrovascular neurodegenerative disease should be personalized and be tailor based.

7. References

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8. Appendix

A. Overview of the results

Male						
Experiments		**Difference**	**Acute**	**Long term**	**Revalidation**	**Restore**
Weight		**Time**				
		Genotype	WT ↓	WT↑ APP/PS1 ↑	WT stroke ↑ APP/PS1 stroke ↑ APP ↑	WT stroke ↑ APP/PS1 stroke ↑ APP/PS1 ↑
		Surgery	stroke ↓	sham ↑ stroke ↑	sham ↑ WT sham ↑ WT stroke ↑ stroke < sham	sham ↑ WT sham ↑ WT stroke ↑
SBP		**Time**				↓
		Genotype	APP/PS1 > WT APP/PS1 sham > WT sham	APP/PS1 sham > WT sham APP/PS1 stroke < WT stroke		
		Surgery	WT stroke > WT sham	APP/PS1 stroke < APP/PS1 sham	APP/PS1 stroke < APP/PS1 sham	
Grip test	**Trapeze**	**Time**		↓		↓
		Genotype	WT stroke ↓ APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery				
	Grid	**Time**		↑	↑	
		Genotype	APP/PS1 > WT			APP/PS1 stroke > WT stroke
		Surgery	WT stroke < WT sham	APP/PS1 stroke > WT stroke		WT stroke < WT sham

Pole test	**Velocity**	**Time**	↓	↓		↓
		Genotype	APP/PS1 > WT	APP/PS1 stroke > WT stroke APP/PS1 > WT	WT ↓ APP/PS1 ↓ APP/PS1 > WT	APP/PS1 > WT
		Surgery	stroke > sham	WT stroke > WT sham APP/PS1 stroke > APP/PS1 sham APP/PS1 stroke > WT stroke	stroke ↓ stroke > sham	stroke > sham
	Rotation time	**Time**		↑		↑
		Genotype				
		Surgery	stroke > sham		stroke > sham	
Open field	**Velocity**	**Time**			↓	↓
		Genotype	WT ↓ APP/PS1 ↓	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery	stroke > sham	sham ↓ stroke > sham	stroke > sham	stroke > sham
	Distance	**Time**		↓		
		Genotype	WT ↓ APP/PS1 ↓	APP/PS1 > WT	APP/PS1 ↑	APP/PS1 > WT
		Surgery	stroke > sham		stroke > sham	stroke > sham
	Center	**Time**	sham ↓	sham ↓		
		Genotype				APP/PS1 > WT
		Surgery				stroke > sham
	Periphery	**Time**	↓			↑
		Genotype				
		Surgery				
	Corners	**Time**		APP/PS1 stroke ↓		↓
		Genotype				
		Surgery				

Open field	**Walking: time**	**Time**	APP/PS1 ↓	↓	APP/PS1 ↑	↓
		Genotype	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery				
	Walking: frequency	**Time**	↓	↓		↓
		Genotype	APP/PS1 > WT	APP/PS1 > WT		APP/PS1 > WT
		Surgery			stroke > sham	
	Sitting: time	**Time**	↑	↑		↑
		Genotype	APP/PS1 < WT	APP/PS1 < WT		APP/PS1 < WT
		Surgery				
	Sitting: frequency	**Time**	↓			
		Genotype		APPPS1 stroke > WT stroke APP/PS1 sham < WT sham	APP/PS1 sham < WT sham	APP/PS1 sham < WT sham
		Surgery		APP/PS1 stroke > APP/PS1 sham	APP/PS1 stroke > APP/PS1 sham	APP/PS1 stroke > APP/PS1 sham
	Wall leaning: time	**Time**	↓	↓		
		Genotype				APP/PS1 stroke > WT stroke
		Surgery			stroke > sham	APP/PS1 stroke > APP/PS1 sham
	Wall leaning: frequency	**Time**	↓	↓		↓
		Genotype	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery			stroke > sham	
	Rearing: time	**Time**	↓	↓	↓	↓
		Genotype		APP/PS1 < WT	APP/PS1 < WT	APP/PS1 < WT
		Surgery				
	Rearing: frequency	**Time**	↓	↓	↓	↓
		Genotype	APP/PS1 < WT		APP/PS1 < WT	
		Surgery				

Open field	**Grooming: time**	**Time**				↓
		Genotype		APP/PS1 stroke < WT stroke		
		Surgery		APP/PS1 stroke > APP/PS1 sham		
	Grooming: frequency	**Time**				
		Genotype	stroke > sham	stroke > sham	APP/PS1 stroke < WT stroke	
		Surgery			APP/PS1 stroke > APP/PS1 sham	APP/PS1 stroke > APP/PS1 sham

Parameter			**Time**	**Genotype**	**Surgery**
Rotarod			N/A	APP/PS1< WT	
MWM	**Acquisition**	**Latency**	↓		
		Velocity	↓	APPPS1> WT	
		Distance	↓	APPPS1> WT	
	Probe (30s)	**Velocity**			
		Distance			
		Frequency in platfrom zone			
		Total time in in platfrom zone			
		Frequency in NE			
		Total time in NE		APP/PS1 < WT	
		Frequency in NW			
		Total time in NW			
		Frequency in SE			
		Total time in SE		APP/PS1 < WT	
		Frequency in SW			stroke < sham
		Total time in SW		APP/PS1 stroke > WT stroke	APP/PS1 stroke > WT stroke

MWM	**Probe (120s)**	**Velocity**			
		Distance			
		Frequency in platfrom zone			
		Total time in in platfrom zone			
		Frequency in NE			
		Total time in NE		APP/PS1 < WT	
		Frequency in NW		stroke > sham	
		Total time in NW			
		Frequency in SE			
		Total time in SE			
		Frequency in SW			
		Total time in SW			

Female						
Experiments		**Difference**	**Acute**	**Long term**	**Revalidation**	**Restore**
Weight		**Time**	↑			
		Genotype		WT ↑ APP/PS1 ↑ APP/PS1 < WT	WT ↑ APP/PS1 ↓ APP/S1 < WT	WT ↑ APP/PS1 ↑ APP/PS1 < WT
		Surgery	stroke > sham	sham ↑ stroke ↑ stroke < sham	stroke < sham	sham ↑ stroke↑ stroke < sham
SBP		**Time**			↓	
		Genotype				
		Surgery				
Grip test	**Trapeze**	**Time**			↓	↓
		Genotype	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT	
		Surgery	stroke > sham			
	Grid	**Time**	↑	↑	↓	
		Genotype	APP/PS1 > WT	APP/PS1 > WT		
		Surgery				
Pole test	**Velocity**	**Time**	↓	↓		↓
		Genotype			WT ↓	APP/PS1 > WT
		Surgery	stroke > sham	stroke > sham	stroke > sham	stroke > sham
	Rotation time	**Time**		↑		↑
		Genotype				
		Surgery	stroke ↑ stroke > sham		stroke > sham	

Open field	**Velocity**	**Time**	↓			
		Genotype	APP/PS1 > WT	WT ↓ APP/PS1 > WT	WT ↑ APP/PS1 ↑ APP/PS1 > WT	WT ↓ APP/PS1 > WT
		Surgery	stroke > sham	stroke > sham	stroke > sham	stroke > sham
	Distance	**Time**	↓			
		Genotype	APP/PS1 > WT	WT ↓ APP/PS1 > WT	APP/PS1 > WT	WT ↓ APP/PS1 > WT
		Surgery	stroke > sham	stroke > sham	stroke > sham	stroke > sham
	Center	**Time**	↓	sham ↓		WT ↓
		Genotype				APP/PS1 > WT
		Surgery		stroke > sham	stroke > sham	stroke > sham
	Periphery	**Time**	APP/PS1 ↓			
		Genotype				
		Surgery				
	Corners	**Time**	↓			↓
		Genotype				APP/PS1 > WT
		Surgery				
	Walking: time	**Time**	↓	WT ↓	↑	WT ↓
		Genotype		APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery				
	Walking: frequency	**Time**	↓	WT ↓	↑	↓
		Genotype		APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery				
	Sitting: time	**Time**	↑	WT ↑	↓	
		Genotype	APP/S1 < WT	APP/S1 < WT	APP/S1 < WT	APP/S1 < WT
		Surgery				

Open field	**Sitting: frequency**	**Time**	↓			
		Genotype				
		Surgery				
	Wall leaning: time	**Time**	↓	↓		↓
		Genotype	WT stroke < APP/PS1 stroke	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery	APP/S1 stroke > APP/PS1 sham			
	Wall leaning: frequency	**Time**	↓	↓	↓	↓
		Genotype	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT	APP/PS1 > WT
		Surgery				
	Rearing: time	**Time**	↓	↓	↓	↓
		Genotype				
		Surgery				
	Rearing: frequency	**Time**	↓	↓	↓	↓
		Genotype				
		Surgery				
	Grooming: time	**Time**		↓		
		Genotype				
		Surgery				
	Grooming: frequency	**Time**		↓	↓	↓
		Genotype				
		Surgery				

Parameter			Time	Genotype	Surgery
Rotarod			N/A	APP/PS1< WT	stroke < sham
MWM	**Acquisition**	**Latency**	↓		
		Velocity	↓		stroke < sham
		Distance	↓	APPPS1> WT	
	Probe (30s)	**Velocity**			stroke < sham
		Distance			stroke < sham
		Frequency in platfrom zone			
		Total time in in platfrom zone			
		Frequency in NE			
		Total time in NE		APP/PS1 < WT	
		Frequency in NW		APP/PS1 < WT	
		Total time in NW			
		Frequency in SE			
		Total time in SE			
		Frequency in SW			stroke < sham
		Total time in SW			stroke < sham

MWM	**Probe (120s)**	**Velocity**			stroke < sham
		Distance			stroke < sham
		Frequency in platfrom zone			
		Total time in in platfrom zone			
		Frequency in NE			
		Total time in NE			
		Frequency in NW		APP/PS1 < WT	
		Total time in NW			
		Frequency in SE		APP/PS1 stroke < WT stroke	stroke < sham APP/PS1 stroke < APP/PS1 sham
		Total time in SE			
		Frequency in SW		APP/PS1 stroke < WT stroke	APP/PS1 stroke < WT stroke
		Total time in SW			APP/PS1 stroke < APP/PS1 sham

B. List of abbreviations

AchA Anterior choroidal artery

AD Alzheimer´s disease

APP/PS1 $APP_{SWE}/PS1_{\Delta E9}$

ASL Arterial spin labeling

Aβ Amyloid-β

AβPP Amyloid-β precursor protein

BP Blood pressure

CAA Cerebral amyloid angiopathy

CBF Cerebral blood flow

CSF Cerebrospinal fluid

DBP Diastolic blood pressure

DTI Diffusion tensor imaging

DVC Digitally ventilated cages

LHA lateral hypothalamic artery

MCA Middle cerebral artery

MRI Magnetic Resonance Imaging

MWM Morris water maze

NE North-East

NW North-West

OF Open field

PSEN1 Presenilins1

PSEN2 Presenilins2

ROI Regions of interest

rsfMRI Resting state fMRI

SBP Systolic blood pressure

SE- South-East

SW South-West

tMCAo Transient occlusion of the middle cerebral artery

VaD Vascular dementia

VCI Vascular cognitive impairment

VTA Ventral thalamic artery

WT Wild-type