

Elsevier Editorial System(tm) for Brain,  
Behavior, and Immunity

Manuscript Draft

Manuscript Number: BBI-D-17-00762R2

Title: Inflammation, insulin signaling and cognitive function in aged  
APP/PS1 mice

Article Type: Full Length Article

Keywords: Alzheimer's disease; aging; neuroinflammation; insulin  
signaling; cognitive function; learning; memory; insulin sensitivity;  
cytokines

Corresponding Author: Dr. Paul A Denver, PhD

Corresponding Author's Institution: UCLA

First Author: Paul A Denver, PhD

Order of Authors: Paul A Denver, PhD; Andrew English; Paula L McClean,  
PhD

Abstract: Cognitive dysfunction and neuroinflammation are typical in Alzheimer's disease (AD), but are also associated with normal aging, albeit less severely. Insulin resistance in the brain has been demonstrated in AD patients and is thought to be involved in AD pathophysiology. Using 15-18 month-old APP/PS1 mice, this study measured peripheral and central insulin signaling and sensitivity, inflammatory markers in brain and plasma and oxidative stress and synapse density in the brain. Novel object recognition, Morris water maze and reversal water maze tasks were performed to assess cognitive function in aged APP/PS1 mice and wild type littermates. Glucose tolerance and insulin sensitivity were similar in APP/PS1 mice and wild type controls, however IRS-1 pSer616 was increased in cortex and dentate gyrus of APP/PS1 mice. Recognition and spatial memory was impaired in both APP/PS1 and wild type mice, however learning impairments were apparent in APP/PS1 mice. Expression of GLP-1 receptor, ERK2, IKK $\beta$ , mTOR, PKC $\theta$ , NF- $\kappa$ B1 and TLR4 was similar between aged APP/PS1 mice and age-matched wild types. Compared to age-matched wild type mice, IFN $\gamma$  and IL-4 were increased in brains of APP/PS1 mice. These results suggest that normal aging may be associated with enhanced neuroinflammation, oxidative stress, and cognitive decline, however distinctions are apparent in the brain of APP/PS1 mice in terms of inflammation and insulin signaling and in certain cognitive domains. Demarcation of pathological events that distinguish AD from normal aging will allow for improvements in diagnostic tools and the development of more effective therapeutics.

University of California, Los Angeles  
Department of Neurology  
710 Westwood Plaza, Los Angeles, CA, 90095  
West Los Angeles VA Healthcare Center  
11301 Wilshire Boulevard, Los Angeles, CA 90073  
Tel: +1 (310) 478-3711 ext. 42171  
Email: [pdenver@mednet.ucla.edu](mailto:pdenver@mednet.ucla.edu)

Professor Carmine M. Pariante, Editor-in-Chief, Brain, Behavior, and Immunity  
Professor of Biological Psychiatry and NIHR Senior Investigator Award,  
Stress, Psychiatry and Immunology Lab,  
Institute of Psychiatry, Psychology and Neuroscience,  
King's College London,  
The Maurice Wohl Clinical Neuroscience Institute,  
Cutcombe Road, London SE5 9RT

17<sup>th</sup> March 2018

Dear Professor Pariante,

On behalf of the authors, we are grateful for the thorough evaluation of our manuscript and the additional comments raised by the Reviewers and Editors. These have been very helpful and constructive. We have further revised our manuscript according to the points raised and we believe it has been strengthened as a result. Please see our responses to Reviewer's comments in a point-by-point fashion below.

We very much hope that you will consider our revised manuscript acceptable for publication in *Brain, Behavior and Immunity*.

Yours sincerely,

Dr Paul Denver

*Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice. (BBI-D-17-00762)*

## Highlights

- Peripheral insulin sensitivity and glucose tolerance in aged APP/PS1 mice is comparable to wild-type.
- Recognition and spatial memory is impaired in aged wild-type and APP/PS1 mice.
- Spatial learning is impaired in aged APP/PS1 mice, compared to age-matched controls.
- IRS-1 pSer<sup>616</sup> and astrocytes are elevated in brains of aged APP/PS1 mice compared to controls
- IFN $\gamma$ , IL-1 $\beta$  and IL-4 are elevated in brains of APP/PS1 mice compared to age-matched controls

*Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice. (BBI-D-17-00762R1)*

### **Reviewer #2**

The explanations offered by the authors are adequate.

**Thank you to the reviewer for acknowledging our previous response to reviewers' comments as adequate.**

### **Reviewer #3**

While the reviewers generally recognize the new and interesting contributions to knowledge provided by this paper several specific deficiencies are identified. In response the authors have added sections to the paper with additional references to address these deficiencies. In particular reviewers have indicated that young cohorts of wild type and transgenic mice should have been included in the study. The authors acknowledge this but cite previously published work to provide support for their speculative assertions that synaptophysin staining may be generally reduced in brains of aged wild type mice in their experiments.

Where it was possible to do so the authors have responded well to comments made by the reviewers by including additional data, figures, text and citations. This includes additional details of data analysis in the statistics section and the addition of Figure 7. The authors acknowledge that their future studies should include the younger age cohorts, more discrete studies including regional brain areas and examining changes in microglia.

As with most large studies that employ multiple sensitive techniques it will always be possible to identify interesting additions that might have been included if time and funds were available however the reality is that these are often limited.

**Thank you to the reviewer for their comments and for their appreciation for the limitations that were acknowledged in our previous response to reviewers' comments and in the manuscript itself. As noted by this reviewer, we added data, analysis, text and citations that we hope helps support our conclusions.**

### **Reviewer #4**

In the revision, the authors have addressed several of the issues raised with the initial reviews. However, one major issue that remains is the absence of young cohort of WT and APP/PS1 mice. This absence is very significant and, apparently, can not be addressed by the authors.

Because there is some value in the aged WT vs APP/PS1 comparison, the entire manuscript should be completely restructured, streamlined, and focused solely on this comparison. The extremely limited data of young WT mice is a distraction, not an addition to the aged mice data. Data interpretation and discussion should also be limited to the contributions of the APP/PS1 genotype to differences between the aged WT and APP/PS1 animals.

**Thank you to the reviewer for these helpful comments. The authors agree that our limited young data distracts from the aged data and detracts from the overall story of the manuscript, however, we feel that this young data is nevertheless of value here. We suggest that removing the young data from the**

main figures and condensing it into one supplementary figure may adequately address these concerns, while allowing the young data to be included in the article as supportive to the aged data, rather than as part of the main narrative. To this end, we removed the young data from figures 5 and 6, we added one supplementary figure consisting of 4 panels and made adjustments to the text in the following sections of the manuscript to reflect these changes:

- Abstract
- Results; 3.1.4 Peripheral insulin sensitivity and glucose tolerance and inflammatory and insulin signaling gene expression in brains of aged APP/PS1 mice
- Results; 3.1.5 Cytokine levels in brains of aged APP/PS1 mice
- Discussion

The authors feel that these changes have improved the manuscript and reiterate our thanks to the reviewer for their insight. However, should the reviewer and/or the editor feel that our response was inadequate and would prefer if the young data was removed entirely, then we would be happy to oblige and remove it.

# Inflammation, insulin signaling and cognitive function in aged

## APP/PS1 mice

Paul Denver<sup>a,1</sup>, Andrew English<sup>b</sup> and Paula L McClean<sup>b</sup>

<sup>a,1</sup> *Centre for Molecular Biosciences, University of Ulster, Coleraine, Northern Ireland*

<sup>b</sup> *Northern Ireland Centre for Stratified Medicine, Clinical, Translational and Research Innovation Centre (CTRIC), University of Ulster, Derry~Londonderry, Northern Ireland*

Abstract: 243 words

Main text: 4,491 words

Figures: 7

Supplementary Figures: 1

Corresponding author: Paul Denver,

West Los Angeles VA Healthcare Center,

11301 Wilshire Boulevard, Los Angeles, CA 90073

Email address: [pdenver@mednet.ucla.edu](mailto:pdenver@mednet.ucla.edu)

Telephone: 1 (310) 478-3711 ext. 42171

<sup>1</sup> *Present affiliations: Greater Los Angeles Veterans Affairs Healthcare System; West Los Angeles Medical Center and Dept. of Neurology, University of California, Los Angeles, CA, USA*

1  
2  
3  
4  
5  
6  
7  
8  
9 **Abstract**

10  
11 Cognitive dysfunction and neuroinflammation are typical in Alzheimer's disease (AD), but  
12 are also associated with normal aging, albeit less severely. Insulin resistance in the brain has  
13 been demonstrated in AD patients and is thought to be involved in AD pathophysiology.  
14 Using 15-18 month-old APP/PS1 mice, this study measured peripheral and central insulin  
15 signaling and sensitivity, inflammatory markers in brain and plasma and oxidative stress and  
16 synapse density in the brain. Novel object recognition, Morris water maze and reversal water  
17 maze tasks were performed to assess cognitive function in aged APP/PS1 mice and wild type  
18 littermates. Glucose tolerance and insulin sensitivity were similar in APP/PS1 mice and wild  
19 type controls, however IRS-1 pSer<sup>616</sup> was increased in cortex and dentate gyrus of APP/PS1  
20 mice. Recognition and spatial memory was impaired in both APP/PS1 and wild type mice,  
21 however learning impairments were apparent in APP/PS1 mice. Expression of GLP-1  
22 receptor, ERK2, IKK $\beta$ , mTOR, PKC $\theta$ , NF- $\kappa$ B1 and TLR4 was similar between aged  
23 APP/PS1 mice and age-matched wild types. Compared to age-matched wild type mice, IFN $\gamma$   
24 and IL-4 were increased in brains of APP/PS1 mice. These results suggest that normal aging  
25 may be associated with enhanced neuroinflammation, oxidative stress, and cognitive decline,  
26 however distinctions are apparent in the brain of APP/PS1 mice in terms of inflammation and  
27 insulin signaling and in certain cognitive domains. Demarcation of pathological events that  
28 distinguish AD from normal aging will allow for improvements in diagnostic tools and the  
29 development of more effective therapeutics.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



**Keywords: Alzheimer's disease; aging; neuroinflammation; insulin signalling; cognitive function; learning; memory; insulin sensitivity; cytokines**

**Abbreviations:** **A $\beta$**  Amyloid- $\beta$  **AD** Alzheimer's disease **ERK2** extracellular signal-regulated kinase 2 **IKK $\beta$**  Inhibitor of NF- $\kappa$ B kinase  $\beta$  **IRS-1** **pSer<sup>616</sup>** Insulin receptor substrate-1 phosphorylated at serine residue 616 **MAPK** mitogen-activated protein kinase **mTOR** mechanistic target of rapamycin **MWM** Morris water maze **ORT** Novel object recognition task **PKC** Protein kinase C **RI** Recognition index **RWM** Reversal water maze **TLR4** Toll-like receptor 4

## **1 Introduction**

As healthcare improves around the world, life expectancy continues to rise (1). Accordingly, the past 25 years have seen a dramatic increase in disorders associated with aging, including neurological diseases such as Alzheimer's disease (AD) (1), for which advancing age is the principal risk factor (2).

Many clinical and neuropathological features of AD parallel the normal progression of aging, making differentiation between normal brain aging and early-stage AD difficult. Generally, it can be said that healthy aging is associated with moderate decline in some cognitive abilities, whilst AD is characterized by severe deterioration of the same cognitive domains, with additional progressive decline of further cognitive functions, such that the patient's daily life is adversely affected to a severe degree (3). In AD, amyloid- $\beta$  (A $\beta$ ) accumulates into progressively larger fibrils, which become deposited as insoluble plaques in the brain parenchyma (4). Accumulating evidence suggests that the presence of A $\beta$  fibrils and plaques is not uncommon in the brains of non-demented, cognitively healthy older people (5, 6). Several studies have also shown that A $\beta$  deposition does not correlate with

1 cognitive impairment in elderly cohorts (6), highlighting the variability of age-related  
2 cognitive decline and suggesting that A $\beta$  *per se* does not directly influence cognitive  
3 function.  
4  
5

6  
7 Profound inflammation is evident in AD brain (7), primarily mediated by microglia  
8 and astrocytes (8, 9). Activated microglia and astrocytes phagocytose A $\beta$  oligomers and  
9 fibrils, degrade A $\beta$  plaques and reduce amyloid burden (10, 11). However, sustained  
10 microglial activation and unresolved inflammation in the brain is harmful to neurons and  
11 synapses and promotes chronic dysregulation of glial cells and subsequent deterioration of  
12 brain structure and function (12, 13). Inflammation in the brain increases with age (14) and  
13 several studies have shown elevated levels of inflammatory cytokines in the brains of aged  
14 rodents (15, 16). In the context of AD, primed microglia respond more readily to A $\beta$ ,  
15 producing increased levels of cytokines that exert direct toxic effects on neurons and at  
16 synapses (17).  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

31  
32 Insulin resistance has been demonstrated in postmortem brain tissue from AD patients  
33 and those with mild cognitive impairment, in the absence of diabetes and irrespective of  
34 ApoE- $\epsilon$ 4 status (18). Furthermore, IRS-1 pSer<sup>616</sup> was identified as a putative biomarker of  
35 brain insulin resistance in AD and was found to correlate positively with A $\beta$  oligomer levels  
36 and negatively with cognitive function (18). Additionally, Bomfim *et al.* (19) demonstrated  
37 increased levels of IRS-1 pSer<sup>616</sup> in the hippocampus of 13 month-old APP/PS1 mice. Other  
38 studies have also demonstrated impaired neuronal insulin signaling in AD brain and in  
39 response to A $\beta$  oligomer challenge (20, 21).  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51  
52 This study sought to determine differences in learning and memory, oxidative stress,  
53 glucose tolerance, central and peripheral insulin sensitivity between 15-18 month old wild  
54 type and age-matched APP/PS1 mice. Using novel object recognition and Morris water maze  
55 tasks, cognitive function was measured in aged wild type and APP/PS1 mice. Systemic  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 insulin sensitivity and glucose tolerance were compared between groups. Brain levels of A $\beta$ ,  
2 GFAP, 8-oxoguanine, IRS-1 pSer<sup>616</sup> and synaptophysin were measured by  
3 immunohistochemistry. Additionally inflammatory and insulin signalling associated genes,  
4  
5 GLP-1R, IKK $\beta$ , ERK2, mTOR, NF- $\kappa$ B1, PKC $\theta$ , and TLR4 and inflammatory cytokines  
6  
7 (IFN $\gamma$ , IL-10, IL-1 $\beta$ , IL-12p70, IL-2, IL-4, IL-5, IL-6 and KC/GRO (CXCL1)) were assessed  
8  
9 in brain tissue from aged APP/PS1 and wild type mice to delineate pathological changes from  
10  
11 those associated with ‘normal’ aging.  
12  
13  
14  
15  
16

## 17 **2 Materials and Methods**

### 18 *2.1.1 Animals*

19  
20 Male APP<sub>swe</sub>/PS1 $\Delta$ e9 (APP/PS1) mice with a C57Bl/6J background were bred with wild type  
21  
22 C57Bl/6J females at the Biomedical and Behavioural Research Unit at Ulster University in  
23  
24 Coleraine. Offspring were ear punched and positivity for the APP<sub>swe</sub>/PS1 $\Delta$ e9 transgene, or  
25  
26 lack thereof was confirmed by polymerase chain reaction, using primers specific for the APP  
27  
28 sequence of the APP/PS1 construct (Forward “GAATTCCGACATGACTCAGG”, Reverse:  
29  
30 “GTTCTGCTGCATCTTGGACA”). Offspring males heterozygous for the APP<sub>swe</sub>/PS1 $\Delta$ e9  
31  
32 transgenic construct were then age-matched with wild type littermates, not expressing the  
33  
34 transgene, which were used as controls. Both groups of mice were caged individually and  
35  
36 allowed access to food and water *ad libitum*. Animals were maintained on a 12:12 light-dark  
37  
38 cycle (lights on at 08:00, lights off at 20:00), within a temperature-controlled room (T:  
39  
40 21.5°C  $\pm$  1°C). All tests were performed during the light cycle. All experiments were  
41  
42 designed, analyzed and reported in accordance with ARRIVE guidelines. Experiments were  
43  
44 licensed according to UK Home Office regulations (UK Animals Scientific Procedures Act  
45  
46 1986) and associated guidelines (EU Directive 2010/63/EU). C57Bl/6 mice were derived  
47  
48 from a colony maintained in the Biomedical and Behavioural Research Unit at Ulster  
49  
50 University in Coleraine.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 *2.1.2 Glucose tolerance and insulin sensitivity tests*  
3

4 After an overnight fasting period, APP/PS1 mice and age-matched wild types received an i.p.  
5 injection of glucose (18 mmol/kg bw) in 0.9% NaCl or insulin (0.25  $\mu$ M/g). Blood glucose  
6 was measured at 0, 15, 30 and 60 minutes following glucose or insulin injection using a  
7 hand-held Ascencia Contour blood glucose meter (Bayer Health Care).  
8  
9  
10  
11  
12  
13  
14  
15  
16

17 *2.1.3 Behavioural Assessment*  
18

19 Mice were assessed in the ORT, as described previously (22). Briefly, mice were subjected to  
20 a 10 minute acquisition period, with two identical objects, followed by a 3 hour retention  
21 period and a 10 minute test phase, which involved replacing one of the objects with a novel  
22 object. A recognition index (RI) was calculated for each object, defined as amount of time  
23 spent exploring object A or B over the total time spent exploring both objects x 100 ( $t_A$  or  
24  $t_B / (t_A + t_B) \times 100$ ).  
25  
26  
27  
28  
29  
30  
31  
32  
33

34 Following ORT, mice were assessed in the Morris water maze (MWM) (22). The  
35 acquisition training phase consisted of 4 x 90 second trials per day, for 4 consecutive days,  
36 followed by a probe trial on the fifth day. The day after the probe trial, mice were subjected  
37 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
38 to northwest quadrant. There were 4 trials per day, for 4 consecutive days, followed by a  
39 reversal probe trial on day 5.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51 *2.1.4 Immunohistochemistry*  
52

53 Following sacrifice, animals were perfused with PBS and brains excised. One hemisphere  
54 was fixed in 4% paraformaldehyde and the other was frozen in liquid nitrogen. Hemi-brains  
55 for histology were then transferred to 30% sucrose and 40  $\mu$ m coronal sections were cut  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 using a cryostat (Leica Microsystems). One section in every 6 was collected sequentially and  
2 stored at -20°C. Staining was performed for A $\beta$ , GFAP, 8-oxoguanine, IRS-1 pSer<sup>616</sup> and  
3  
4 synaptophysin. All sections were incubated in H<sub>2</sub>O<sub>2</sub> and permeabilized using Triton X. For 8-  
5  
6 oxoguanine, sections were incubated at 37°C for 30 minutes with 2 M hydrochloric acid,  
7  
8 followed by 0.1 M borax (Sigma Aldrich) for 10 minutes. Blocking with 1.5%-10% normal  
9  
10 serum was performed prior to incubation with anti-A $\beta$  (1:200; Invitrogen; 71-5800) anti-  
11  
12 GFAP (1:250; Merck Millipore; MAB3402), anti-8-oxoguanine (1:250; Merck Millipore;  
13  
14 MAB3560), anti-IRS-1 pSer<sup>616</sup> (1:200; Invitrogen; 44-550G) or anti-synaptophysin (1:200;  
15  
16 Abcam; ab7837) antibodies overnight at 4°C. Sections were then incubated with secondary  
17  
18 antibodies and visualized using Vectastain Elite and SG substrate (Vector Laboratories).  
19  
20 Percentage area stained in each image was quantified using a multi threshold plug-in within  
21  
22 Image J (NIH, Bethesda, USA) in a blinded manner.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36

### 37 *2.1.5 Quantitative polymerase chain reaction (qPCR)*

38  
39 RNA was extracted from brain tissue using RNeasy Lipid Tissue Mini Kit (Qiagen)  
40  
41 according to manufacturer's instructions. For cDNA synthesis, transcript First Strand  
42  
43 cDNA synthesis kit (Roche Diagnostics) was used using 500 ng of RNA per sample. Real-  
44  
45 time PCR reactions were composed of; 5  $\mu$ l of PCR MasterMix (Roche Diagnostics), 1  $\mu$ l (10  
46  
47 pM/ $\mu$ l) gene-specific probes, 3  $\mu$ l of RNase free water and 1  $\mu$ l (25ng) of template cDNA.  
48  
49 Gene-specific probes (Roche Diagnostics) were as follows: GLP-1R (*Glp1r*), IKK $\beta$  (*Ikkkb*),  
50  
51 ERK2 (*Mapk1*), mTOR (*Mtor*), NF- $\kappa$ B1 (*Nfkb1*), PKC $\theta$  (*Prkcq*) and TLR4 (*Tlr4*).  
52  
53  
54  
55  
56  
57 Quantitative PCR was performed on Lightcycler 480 system (Roche Diagnostics), and  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

quantified on accompanying software package (Roche, Lightcycler 480 software, v1.5). Gene expression changes were calculated using Delta Delta CT mathematical model (23).

#### 2.1.6 Meso Scale Discovery multi-array

Whole hemi-brains were homogenized under liquid nitrogen, followed by addition of 10 ml/g of lysis buffer (1 mM EDTA in PBS supplemented with protease inhibitor cocktail). Samples were centrifuged at 14,000 G for 20 min at 4°C and supernatant was removed and added to Meso Scale Discovery (MSD<sup>®</sup>) plate. Bradford protein assay was performed to measure protein content and data were normalized to the total amount of protein present in each sample. Levels of IFN $\gamma$ , IL1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, TNF- $\alpha$  and KC/GRO (CXCL1) were quantified in brain and plasma using MSD<sup>®</sup> Multi-spot Assay Pro-inflammatory panel 1 kit (Rockville, MD, USA) according to manufacturer's instructions.

#### 2.1.7 Statistical Analysis

Data were analyzed using Graphpad Prism (v6.0h). Differences were deemed to be significant if  $p \leq 0.05$ . Data are expressed as means  $\pm$  SEM. Tests included one-way or two-way ANOVA and unpaired Student's  $t$  tests. Data heterogeneity was tested and, where variance was significant, appropriate non-parametric tests were used. Corrections for multiple comparisons were performed using appropriate *post-hoc* tests. Linear relationships between two variables were measured by Pearson's correlation analysis.

### 3 Results

#### 3.1.1 Spatial learning is impaired in aged APP/PS1 mice

During the acquisition phase of the MWM, escape latency significantly decreased over time ( $p < 0.0001$ ), as expected, and was also significantly greater overall in APP/PS1 mice (Fig.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1A;  $p=0.0264$ ). However, *post-hoc* analysis indicated that average escape latency was not significantly different between aged wild type and APP/PS1 mice on any of the training days (Fig. 1A). In the probe trial, time spent in each quadrant by wild type mice was not significantly different (Fig. 1D). Similarly, APP/PS1 mice spent a similar amount of time swimming in all 4 quadrants in the probe trial and although significant variation in the time spent in each quadrant was detected ( $p=0.0174$ ), *post-hoc* analysis showed that time spent in the target quadrant by APP/PS1 mice was not significantly different from any other quadrant (Fig. 1G). In the acquisition phase of the RWM, escape latency decreased over time (Fig. 1B;  $p=0.0009$ ) and was also significantly affected by genotype (Fig. 1B;  $p=0.0020$ ). *Post-hoc* analysis revealed that average escape latency was significantly greater in APP/PS1 mice, compared to wild types on days 2 ( $p<0.05$ ), 3 ( $p<0.01$ ) and 4 ( $p<0.05$ ; Fig. 1B).

In the reversal probe trial, time spent in each of the quadrants by wild types (Fig. 1E) or APP/PS1 (Fig. 1H) mice did not differ significantly

### 3.1.2 Recognition memory is impaired in aged APP/PS1 and wild type mice

In the acquisition phase of the ORT, recognition indices for the identical objects were not significantly different in 15-18 month old APP/PS1 or wild type mice (Fig. 1C). In the test phase, recognition index for the novel object was not significantly different from the familiar in the aged APP/PS1 mice or the age-matched control group (Fig. 1F).

### 3.1.3 Immunohistochemistry

#### 3.1.3.1 $A\beta$ deposition is ubiquitous in brains of aged APP/PS1 mice

1 Representative micrographs from wild type mice show that A $\beta$  immunopositivity was almost  
2 completely absent from the cortex (0.0054%  $\pm$  0.0012) and dentate gyrus (0.0178  $\pm$  0.0136)  
3 (Fig. 2A and B). However, widespread A $\beta$  deposition was apparent in the cerebral cortex and  
4 dentate gyrus of APP/PS1 mice (Fig. 2E and F). Quantification confirmed that A $\beta$   
5 immunopositivity was significantly higher in the cortex (Fig. 2D;  $p$ <0.0001) and dentate  
6 gyrus (Fig. 2H;  $p$ <0.0001) of APP/PS1 mice compared to wild type controls.  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16

### 17 *3.1.3.2 IRS-1 pSer<sup>616</sup> is elevated in brains of aged APP/PS1 mice*

18 Representative micrographs illustrate increased levels of IRS-1 pSer<sup>616</sup> in the cerebral cortex  
19 (Fig. 2M) and dentate gyrus (Fig. 2N) of APP/PS1 mice, compared to age-matched wild  
20 types (Fig. 2I and J). Although distribution of IRS-1 pSer<sup>616</sup> staining was similar between  
21 groups in both brain regions, staining intensity was greater in APP/PS1 mice (Fig. 2O)  
22 compared to wild types (Fig. 2K). As such, quantification showed that IRS-1 pSer<sup>616</sup> was  
23 significantly greater in the cortex (Fig. 2L;  $p$ =0.0303) and dentate gyrus (Fig. 2P;  $p$ =0.0429)  
24 of aged APP/PS1 mice compared to wild type controls. Pearson's correlation analysis  
25 identified negative correlations between recognition index for the novel object in ORT and  
26 IRS-1 pSer<sup>616</sup> immunopositivity in the cortex (Fig. 7A) dentate gyrus (Fig. 7B) of wild type  
27 and APP/PS1 mice. Although the negative correlation between cortical IRS-1 pSer<sup>616</sup> staining  
28 and ORT recognition index in APP/PS1 approached significance (Fig. 7A;  $r$ =-0.7744,  
29  $p$ =0.0706) the negative trends between IRS-1 pSer<sup>616</sup> staining and ORT recognition index  
30 remained insignificant in both brain regions of both genotypes.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53

### 54 *3.1.3.3 Oxidative stress is comparable in brains of aged APP/PS1 and wild type mice*

55 Representative micrographs shown in Fig. 3A-C and E-G illustrate the similarity in oxidative  
56 stress levels between the brains of aged APP/PS1 mice and wild type controls. Quantitative  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 analysis demonstrated that 8-oxoguanine immunopositivity was not significantly different in  
2 the cortex (Fig. 3D) or the dentate gyrus (Fig. 3H) of APP/PS1 mice compared to age-  
3 matched wild type controls.  
4  
5  
6

#### 7 8 9 10 *3.1.3.4 Astrocytes are elevated in brains of aged APP/PS1 mice*

11 Representative micrographs illustrate increased levels of GFAP-positive astrocytes in the  
12 cerebral cortex (Fig. 3I and J) and dentate gyrus (Fig. 3M and N) of aged APP/PS1 mice  
13 compared to wild type controls. Quantitative analysis revealed a significant increase in GFAP  
14 immunopositivity in the cortex (Fig. 3L;  $p=0.0010$ ) and dentate gyrus (Fig. 3P;  $p=0.0007$ ),  
15 compared to age-matched wild type mice.  
16  
17  
18  
19  
20  
21  
22  
23

#### 24 25 26 27 *3.1.3.5 Synaptophysin is reduced in the polymorphic layer of the dentate gyrus of aged* 28 *APP/PS1 mice*

29 Representative images illustrate reduced synaptophysin in the hippocampal polymorphic  
30 layer of 15-18 month old APP/PS1 mice (Fig. 4D) compared to wild types (Fig. 4A;  
31  $p=0.0338$ ). Synaptophysin staining was similar in all other layers of the hippocampus  
32 between wild type and APP/PS1 mice and was not significantly different in the granule cell  
33 (Fig. 4D), molecular layer (Fig. 4D), strata radiatum (Fig. 4E), pyramidale (Fig. 4E) or oriens  
34 (Fig. 4E) of APP/PS1 mice, compared to wild type controls (Fig. 4A and B). Furthermore,  
35 synaptophysin optical density did not differ significantly in the inner or outer (Fig. 4F)  
36 cortical layers of APP/PS1 mice, compared to age-matched wild types (Fig. 4C).  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
Quantification confirmed that synaptophysin staining was reduced in the polymorphic layer  
of APP/PS1 mice, but was comparable with wild types in all other layers of the hippocampus  
and cortex (Fig. 4G).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

### 3.1.4 Peripheral insulin sensitivity and glucose tolerance and inflammatory and insulin signaling gene expression in brains of aged APP/PS1 mice

Expression of GLP-1R, IKK $\beta$ , ERK2, mTOR, NF- $\kappa$ B1, PKC $\theta$  and TLR4 was comparable in brains of aged APP/PS1 and wild type mice and genotype did not have a significant effect on gene expression (Fig. 5A). Additional analysis comparing aged APP/PS1 mice with younger C57Bl/6 mice (17-22 weeks old) identified a significant effect of genotype on gene expression (Supplementary Fig. 1A;  $p < 0.0001$ ) and *post-hoc* analysis showed that expression of IKK $\beta$  ( $p < 0.01$ ), ERK2 ( $p < 0.05$ ) and mTOR ( $p < 0.01$ ) was significantly down-regulated and TLR4 ( $p < 0.05$ ) was up-regulated in brains of aged APP/PS1 mice, compared to young C57Bl/6 controls. As illustrated in Fig. 5C, in response to an insulin sensitivity test, a significant decrease in blood glucose over time was detected ( $p < 0.0001$ ), however genotype had no significant effect on blood glucose levels. Similarly, glucose tolerance was comparable in both groups and although time significantly affected blood glucose levels ( $p < 0.0001$ ), genotype was not associated with a change in peripheral glucose tolerance (Fig. 5D).

### 3.1.5 Cytokine levels in brains of aged APP/PS1 mice

Brain levels of IFN $\gamma$  (Fig. 6A;  $p = 0.0046$ ) and IL-4 (Fig. 6F;  $p = 0.0013$ ) were significantly elevated in brains of 15-18 month-old APP/PS1 mice compared to age-matched wild type mice (Fig. 6A). A trend towards elevated IL-1 $\beta$  was detected in the brains of APP/PS1 mice compared to age-matched wild types, however this failed to reach significance (Fig. 6C;  $p = 0.0965$ ). Additional analysis indicated that IL-1 $\beta$  was significantly elevated in the brains of 15-18 month-old wild type ( $p < 0.01$ ) and APP/PS1 ( $p < 0.0001$ ) mice, compared to young wild types (Supplementary Fig. 1B). A significant increase in IL-4 (Supplementary Fig. 1C;  $p < 0.001$ ) and IFN $\gamma$  (Supplementary Fig. 1D;  $p < 0.01$ ) was also detected in the brains of aged

1 APP/PS1 mice, compared to young wild type mice. In addition, Pearson's correlation  
2 analysis identified a significant negative correlation between levels of IFN $\gamma$  and novel object  
3 recognition index in APP/PS1 mice (Fig. 7C;  $r=-0.8362$ ,  $p=0.0381$ ), suggesting that higher  
4 levels of IFN $\gamma$  in the brain were associated with worse ORT performance in APP/PS1 mice.  
5  
6  
7  
8  
9  
10 No significant correlations were identified between IFN $\gamma$  and IRS-1 pSer<sup>616</sup> immunopositivity  
11  
12 in the cortex or dentate gyrus (Fig. 7D and E).  
13  
14  
15  
16

#### 17 **4. Discussion**

18  
19 This study showed that peripheral glucose tolerance and insulin sensitivity were comparable  
20 between aged APP/PS1 and wild type mice, conflicting with a number of other studies (24,  
21  
22 25). It has been suggested that 5/6 hours fasting is optimal for glucose and insulin tolerance  
23  
24 tests, as this was sufficient for normalization of glucose levels and phosphorylation of insulin  
25  
26 signaling proteins (26, 27). The current study performed glucose tolerance and insulin  
27  
28 sensitivity tests following an overnight fasting period, so it is possible that results presented  
29  
30 here reflect an exaggerated suppression of basal glucose levels in mice as a result of  
31  
32 prolonged fasting. This suggestion is supported by Jimenez-Palomares *et al.* (28) who also  
33  
34 found that glucose tolerance and insulin sensitivity were not significantly different in 8  
35  
36 month-old APP/PS1 mice, compared to wild types following overnight fasting periods.  
37  
38 Future studies should avoid overnight fasting prior to glucose and insulin tolerance tests in  
39  
40 order to achieve optimal normalization of metabolic parameters and to avert potentially  
41  
42 dangerous hypoglycemic effects of insulin. Other reports suggest that insulin insensitivity  
43  
44 and glucose intolerance also exists in aged animals (29-31), including C57Bl/6 mice (32-34);  
45  
46 a possible explanation for the similarity between APP/PS1 mice and controls in the present  
47  
48 study. To better understand the impact of central insulin resistance on global insulin  
49  
50 utilisation in the APP/PS1 model, future studies should assess the impact of hypothalamic  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

insulin administration alone and in combination with insulin sensitising drugs, such as metformin, in hyperinsulinemic euglycemic clamp models.

Recognition memory was impaired in APP/PS1 mice here, consistent with several other studies (35-37). However, since wild type controls also exhibited impaired recognition memory, the deficits may be related to advanced age, rather than the APP/PS1 genotype; a suggestion supported by other studies reporting recognition memory deficits in aged C57Bl/6 mice (38, 39). Another study found several indications of cognitive dysfunction in 18-20 month-old C57Bl/6 mice, including impaired novel location memory, but not object recognition memory (40). Spatial learning was impaired in aged APP/PS1 mice, in agreement with other studies (41, 42). Spatial memory recall was impaired in APP/PS1 mice and wild type mice, similar to Barreto *et al.* (43), who showed that spatial learning and memory were impaired in 18 month-old C57Bl/6 mice. Other reports have highlighted age-related decline in learning and memory in C57Bl/6 mice (44, 45) consistent with the findings of the present study, providing further evidence that there exists age-related deterioration of cognitive function in C57Bl/6 mice. Learning in the reversal water maze task was impaired in aged APP/PS1 mice, compared to controls, while both APP/PS1 mice and wild types failed to recognize the reversal target quadrant. Some (46), but not others (47) have shown that reversal learning and memory are impaired in APP/PS1 mice. Results presented here, suggest that reversal learning is a cognitive domain that is especially vulnerable to the effects of AD pathology in aged mice.

Amyloid- $\beta$  (A $\beta$ ) deposits were detected throughout the brains of 15-18 month-old APP/PS1 mice, while A $\beta$  was undetectable in wild type controls. APP/PS1 mice develop plaque deposition by 6 months of age, which progressively worsens, leading to abundant and widespread A $\beta$  plaque pathology by the age of 14 months (48, 49). The finding that A $\beta$

1 deposition was significant in APP/PS1 brains and absent from wild types suggests that the  
2 spatial memory deficits in both groups were not directly related to A $\beta$  burden.  
3

4  
5 Oxidative stress levels were similar between APP/PS1 and wild type mice in the  
6 cortex and dentate gyrus. This was unexpected given previous reports showing elevated  
7 oxidative damage in brains of aged APP/PS1 mice (50-52). However, since aging is  
8 associated with accumulation of oxidative stress in the brain (53, 54), results presented here  
9 may reflect age-related accumulation of oxidative DNA damage in both APP/PS1 and wild  
10 type mice.  
11  
12  
13  
14  
15  
16  
17

18  
19 IRS-1 pSer<sup>616</sup> was increased in brains of APP/PS1 mice, as has also been observed in  
20 AD patients (18, 19, 55) and in experimental models (55, 56). The findings of the present  
21 study corroborate those of Talbot *et al.* (18) that demonstrated elevated IRS-1 pSer<sup>616</sup> in the  
22 hippocampus of APP/PS1 mice. IRS-1 pSer<sup>616</sup> has been shown to robustly correlate with  
23 cognitive impairment and brain insulin resistance associated with AD (18) and is likely  
24 related to the cognitive impairment in APP/PS1 mice here. It is interesting to note that the  
25 increased brain insulin resistance in aged APP/PS1 mice was apparent in the absence of any  
26 significant indications of peripheral insulin insensitivity or glucose intolerance.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38

39 Astrocytes were increased in the cortex and dentate gyrus of APP/PS1 mice,  
40 consistent with previous reports (50, 57). Neuroinflammation and glial cell proliferation,  
41 recruitment and activation is a commonly associated with AD pathology (13). The fact that  
42 A $\beta$  deposition remained substantial in the brains of APP/PS1 mice suggests that clearance of  
43 A $\beta$  was minimal, providing support for the proposal that astrocyte function is defective in  
44 AD (58). Expression of inflammatory and insulin signaling genes was similar in brains of  
45 aged APP/PS1 mice and age-matched wild type controls. It has been shown previously that  
46 expression of TLR4 is up-regulated in brains of APP/PS1 and wild type mice in an age-  
47 related manner (59) and the present report provides further evidence that TLR4 expression in  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 brain is increased with normal aging, to levels comparable with APP/PS1 mice. Th1 cytokine  
2 IFN $\gamma$  was elevated in brains of APP/PS1 mice compared to wild types, in agreement with  
3  
4 another study that showed age-related enhancement of IFN $\gamma$  in brains of AD mice from 3 to  
5  
6 19 months of age (60). It has also been shown that IFN $\gamma$  has opposing functions in AD brain,  
7  
8 whereby overexpression of IFN $\gamma$  in the hippocampus augments neuroinflammation and  
9  
10 worsens A $\beta$  burden, but abrogates tau pathology and enhances synaptic markers and  
11  
12 neurogenesis (61). Further experimentation should determine whether the increased IFN $\gamma$  in  
13  
14 brains of APP/PS1 mice here represents a component of pathogenic neuroinflammation or an  
15  
16 up-regulation of protective processes. A significant negative correlation was identified here  
17  
18 between IFN $\gamma$  levels and novel object recognition memory in APP/PS1 mice, in line with a  
19  
20 recent study demonstrating improved hippocampal synaptic plasticity and cognitive  
21  
22 performance in mice deficient for IFN $\gamma$  (62), suggesting that increased IFN $\gamma$  in the brain may  
23  
24 impair cognitive function in aged APP/PS1 mice. The increase in IFN $\gamma$  is mirrored by a  
25  
26 comparable increase in anti-inflammatory IL-4 in brains of APP/PS1 mice, which likely  
27  
28 reflects an attempt to suppress the Th1 response. Interestingly IFN $\gamma$  has also been implicated  
29  
30 in attenuation of insulin signaling (63) and may be similarly associated with the brain insulin  
31  
32 resistance in the present study. Although we failed to detect significant correlations between  
33  
34 brain levels of IFN $\gamma$  and IRS-1 pSer<sup>616</sup>, this potential mechanism certainly warrants further  
35  
36 exploration.  
37

38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48 Previous studies have detected increased IL-1 $\beta$  in the brains of APP/PS1 mice (64,  
49  
50 65). The present report, however, detected a non-significant trend towards an increase in IL-  
51  
52 1 $\beta$  in the brains of APP/PS1 mice, possibly due to a parallel, age-related elevation of IL-1 $\beta$  in  
53  
54 wild-type mice. This suggests that IL-1 $\beta$  is involved with neuroinflammation that  
55  
56 accompanies normal aging, while IFN $\gamma$  and IL-4 are not part of the normal process of aging,  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 but are components of the neuroinflammatory processes associated with AD, since these were  
2 elevated in aged APP/PS1 mice, compared to both young and old wild types.  
3

4  
5 Expression of mTOR and ERK2 was comparable in brains of aged APP/PS1 mice,  
6 compared to wild types. Extracellular signal-regulated kinase 2 (ERK2) signaling facilitates  
7 learning and memory (68, 69), suggesting that impaired cognitive function in aged mice may  
8 be due, in part to reduced expression of ERK2 in the brain. Dineley *et al.* (70) showed that  
9 A $\beta$  reduces ERK2 activity and that ERK2 expression is down-regulated in brains of 20  
10 month-old AD mice. Similarly, dysregulation of signaling downstream of mTOR has been  
11 demonstrated in post-mortem brain tissue from AD patients (71). Signaling through mTOR  
12 contributes to synaptic plasticity, learning and memory (72, 73). It has also been shown that  
13 insulin promotes neurogenesis, dendrite and synapse formation by signaling through IRS-  
14 mediated activation of mTOR (74, 75). Amyloid- $\beta$  (A $\beta$ ) perturbs mTOR signaling in neurons  
15 (76) and mTOR inhibition impairs hippocampal LTP in an AD mouse model (77). Our results  
16 suggest that expression of insulin signaling components is comparable in aged APP/PS1 and  
17 wild-type mice. Disruption of insulin signaling in the brain may be involved in the  
18 pathophysiology of AD and may contribute to cognitive impairments associated with aging, a  
19 proposition that should be further probed in future studies.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40

41 Synaptophysin staining was reduced in the polymorphic layer of the dentate gyrus of  
42 APP/PS1 mice. Several previous studies have shown that synapse density is decreased in the  
43 brains of APP/PS1 mice (78-80). These studies did not consider the discrete cellular layers of  
44 the hippocampus and may have overlooked subtle variation in synapse density between  
45 subregions (78-80). However, one report showed that synaptophysin levels in the  
46 hippocampus of 7 and 17 month-old APP/PS1 mice were similar to age-matched wild types  
47 (81), which more closely aligns with the findings of the present study. It has also been shown  
48 in Tg2576 mice, that synaptophysin levels were no different from controls at 3, 9, 14 and 19  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 months of age (82), while Xu *et al.* (45) reported age-related decline in hippocampal synaptic  
2 spine density in C57Bl/6 mice. Results of the present report reflect a similar pattern, with  
3 synapse density being comparable to wild types in 15-18 month-old APP/PS1 mice. Since  
4 synapse density in the polymorphic layer was reduced in aged transgenic mice, it is  
5 reasonable to suggest that this subregion was selectively susceptible to synaptotoxicity  
6 associated with AD neuropathology.  
7  
8  
9  
10  
11  
12  
13

14 A limitation of the present study is the absence of young cohorts of wild type and  
15 APP/PS1 mice. Based on evidence from the literature, it is likely that peripheral insulin  
16 sensitivity, cognitive function and synapse density were influenced by aging. Future studies  
17 should include groups of young wild type and transgenic mice in order to more robustly  
18 characterize the differences between AD pathology and changes associated with normal  
19 aging, throughout the lifecourse. Another limitation of the present report is that cytokines and  
20 mRNA were measured in whole hemi-brains, while analysis of immunohistochemistry was  
21 performed on brain sections allowing for quantification within discrete brain regions. This  
22 means that comparing the results of our biochemical assays with our immunohistochemical  
23 data is difficult. Future studies will analyse mRNA and associated proteins and activation  
24 states in discrete brain regions to allow for more accurate demarcation of differences between  
25 APP/PS1 mice and wild types with regard to insulin signaling dysregulation and  
26 inflammation the brain.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45

46 Nevertheless, this study has demonstrated memory deficits and neuroinflammation in  
47 aged APP/PS1 and wild type mice. Astrocyte accumulation, IL-1 $\beta$  and IRS-1 pSer<sup>616</sup> levels  
48 were increased in the brain of APP/PS1 mice in the absence of systemic insulin insensitivity  
49 or glucose intolerance. Pharmacological agents targeting impaired insulin signaling and  
50 inflammation in the brain may prove efficacious in treating AD, a suggestion requiring  
51 further investigation.  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1  
2 **Acknowledgements**  
3

4 Some of the data has been submitted, in abstract form, to the 2017 Southern California  
5 Alzheimer's Disease Centers Research Symposium at the University of California, Beckman  
6  
7 Center of the National Academies of Sciences & Engineering, 100 Academy Way, Irvine,  
8  
9 CA, USA 92617  
10  
11  
12  
13  
14  
15

16 **Funding**  
17

18 This work was supported by the Department of Education and Learning, Northern Ireland.  
19  
20  
21  
22  
23

24 **Declaration / Conflict of Interest**  
25

26 All authors declare that there is no duality of interest associated with their contribution to this  
27  
28 manuscript.  
29  
30  
31  
32  
33

34 **Author Contribution Statement**  
35

36 PMcC conceived the study, participated in the analysis and interpretation of data, drafted the  
37  
38 manuscript and revised it critically for intellectual content. PD participated in data  
39  
40 generation, analysis and interpretation and drafted the manuscript and revised it critically for  
41  
42 intellectual content. AE participated in data generation and analysis. All authors approved the  
43  
44 final version of the manuscript. PD is the guarantor of this work and, as such, had full access  
45  
46 to all the data in the study and takes responsibility for the integrity of the data and the  
47  
48 accuracy of the data analysis.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## References

1. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* (London, England). 2016;388(10053):1459-544.
2. Querfurth HW, LaFerla FM. Alzheimer's disease. *The New England journal of medicine*. 2010;362(4):329-44.
3. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 2011;7(3):263-9.
4. De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. *Subcellular biochemistry*. 2012;65:329-52.
5. Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. *The New England journal of medicine*. 2009;360(22):2302-9.
6. Malek-Ahmadi M, Perez SE, Chen K, Mufson EJ. Neuritic and Diffuse Plaque Associations with Memory in Non-Cognitively Impaired Elderly. *Journal of Alzheimer's disease : JAD*. 2016;53(4):1641-52.
7. Meraz-Rios MA, Toral-Rios D, Franco-Bocanegra D, Villeda-Hernandez J, Campos-Pena V. Inflammatory process in Alzheimer's Disease. *Front Integr Neurosci*. 2013;7:59.
8. Rezai-Zadeh K, Gate D, Town T. CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease? *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*. 2009;4(4):462-75.
9. Schilling M, Besselmann M, Leonhard C, Mueller M, Ringelstein EB, Kiefer R. Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. *Experimental neurology*. 2003;183(1):25-33.
10. Fu Y, Hsiao JT, Paxinos G, Halliday GM, Kim WS. ABCA7 Mediates Phagocytic Clearance of Amyloid-beta in the Brain. *Journal of Alzheimer's disease : JAD*. 2016;54(2):569-84.
11. El-Shimy IA, Heikal OA, Hamdi N. Minocycline attenuates Abeta oligomers-induced pro-inflammatory phenotype in primary microglia while enhancing Abeta fibrils phagocytosis. *Neuroscience letters*. 2015;609:36-41.
12. Leszek J, Barreto GE, Gasiorowski K, Koutsouraki E, Avila-Rodrigues M, Aliev G. Inflammatory Mechanisms and Oxidative Stress as Key Factors Responsible for Progression of Neurodegeneration: Role of Brain Innate Immune System. *CNS & neurological disorders drug targets*. 2016;15(3):329-36.
13. Steardo L, Jr., Bronzuoli MR, Iacomino A, Esposito G, Steardo L, Scuderi C. Does neuroinflammation turn on the flame in Alzheimer's disease? Focus on astrocytes. *Frontiers in neuroscience*. 2015;9:259.
14. Gabuzda D, Yankner BA. Physiology: Inflammation links ageing to the brain. *Nature*. 2013;497(7448):197-8.
15. Maher FO, Martin DS, Lynch MA. Increased IL-1beta in cortex of aged rats is accompanied by downregulation of ERK and PI-3 kinase. *Neurobiology of aging*. 2004;25(6):795-806.

16. Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2005;19(10):1329-31.
17. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science (New York, NY)*. 2016;352(6286):712-6.
18. Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *The Journal of clinical investigation*. 2012;122(4):1316-38.
19. Bomfim TR, Fornyy-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, et al. An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease-associated Abeta oligomers. *The Journal of clinical investigation*. 2012;122(4):1339-53.
20. Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiology of aging*. 2010;31(2):224-43.
21. Liu Y, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. *The Journal of pathology*. 2011;225(1):54-62.
22. McClean PL, Parthasarathy V, Faivre E, Holscher C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011;31(17):6587-94.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif)*. 2001;25(4):402-8.
24. Clarke JR, Lyra ESNM, Figueiredo CP, Frozza RL, Ledo JH, Beckman D, et al. Alzheimer-associated Abeta oligomers impact the central nervous system to induce peripheral metabolic deregulation. *EMBO molecular medicine*. 2015;7(2):190-210.
25. Pedros I, Petrov D, Allgaier M, Sureda F, Barroso E, Beas-Zarate C, et al. Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. *Biochimica et biophysica acta*. 2014;1842(9):1556-66.
26. Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *American journal of physiology Endocrinology and metabolism*. 2008;295(6):E1323-32.
27. Agouni A, Owen C, Czopek A, Mody N, Delibegovic M. In vivo differential effects of fasting, re-feeding, insulin and insulin stimulation time course on insulin signaling pathway components in peripheral tissues. *Biochemical and biophysical research communications*. 2010;401(1):104-11.
28. Jimenez-Palomares M, Ramos-Rodriguez JJ, Lopez-Acosta JF, Pacheco-Herrero M, Lechuga-Sancho AM, Perdomo G, et al. Increased Abeta production prompts the onset of glucose intolerance and insulin resistance. *American journal of physiology Endocrinology and metabolism*. 2012;302(11):E1373-80.
29. Catalano KJ, Bergman RN, Ader M. Increased susceptibility to insulin resistance associated with abdominal obesity in aging rats. *Obesity research*. 2005;13(1):11-20.

- 1 30. Yamamoto M, Otsuki M. Effect of inhibition of alpha-glucosidase on age-related  
2 glucose intolerance and pancreatic atrophy in rats. *Metabolism: clinical and*  
3 *experimental*. 2006;55(4):533-40.
- 4 31. Romanatto T, Fiamoncini J, Wang B, Curi R, Kang JX. Elevated tissue omega-3  
5 fatty acid status prevents age-related glucose intolerance in fat-1 transgenic mice.  
6 *Biochimica et biophysica acta*. 2014;1842(2):186-91.
- 7 32. Houtkooper RH, Argmann C, Houten SM, Canto C, Jenning EH, Andreux PA, et al.  
8 The metabolic footprint of aging in mice. *Scientific reports*. 2011;1:134.
- 9 33. Lipina C, Vaanholt LM, Davidova A, Mitchell SE, Storey-Gordon E, Hambly C, et al.  
10 CB1 receptor blockade counters age-induced insulin resistance and metabolic  
11 dysfunction. *Aging cell*. 2016;15(2):325-35.
- 12 34. Park D, Lee EK, Jang EJ, Jeong HO, Kim BC, Ha YM, et al. Identification of the  
13 dichotomous role of age-related LCK in calorie restriction revealed by integrative  
14 analysis of cDNA microarray and interactome. *Age (Dordrecht, Netherlands)*.  
15 2013;35(4):1045-60.
- 16 35. Gu XH, Xu LJ, Liu ZQ, Wei B, Yang YJ, Xu GG, et al. The flavonoid baicalein rescues  
17 synaptic plasticity and memory deficits in a mouse model of Alzheimer's disease.  
18 *Behavioural brain research*. 2016;311:309-21.
- 19 36. Yang YJ, Zhao Y, Yu B, Xu GG, Wang W, Zhan JQ, et al. GluN2B-containing NMDA  
20 receptors contribute to the beneficial effects of hydrogen sulfide on cognitive and  
21 synaptic plasticity deficits in APP/PS1 transgenic mice. *Neuroscience*. 2016;335:170-83.
- 22 37. Zhou D, Liu H, Li C, Wang F, Shi Y, Liu L, et al. Atorvastatin ameliorates cognitive  
23 impairment, A $\beta$ 1-42 production and Tau hyperphosphorylation in APP/PS1  
24 transgenic mice. *Metabolic brain disease*. 2016;31(3):693-703.
- 25 38. Qiu J, Dunbar DR, Noble J, Cairns C, Carter R, Kelly V, et al. Decreased Npas4 and  
26 Arc mRNA Levels in the Hippocampus of Aged Memory-Impaired Wild-Type But Not  
27 Memory Preserved 11beta-HSD1 Deficient Mice. *Journal of neuroendocrinology*.  
28 2016;28(1).
- 29 39. Mehan AO, Wyss A, Rieger H, Mohajeri MH. A comparison of learning and  
30 memory characteristics of young and middle-aged wild-type mice in the IntelliCage.  
31 *Journal of neuroscience methods*. 2009;180(1):43-51.
- 32 40. Benice TS, Rizk A, Kohama S, Pfankuch T, Raber J. Sex-differences in age-related  
33 cognitive decline in C57BL/6J mice associated with increased brain microtubule-  
34 associated protein 2 and synaptophysin immunoreactivity. *Neuroscience*.  
35 2006;137(2):413-23.
- 36 41. Fol R, Braudeau J, Ludewig S, Abel T, Weyer SW, Roederer JP, et al. Viral gene  
37 transfer of APP $\alpha$  rescues synaptic failure in an Alzheimer's disease mouse model.  
38 *Acta neuropathologica*. 2016;131(2):247-66.
- 39 42. Xiao Q, Shi R, Yang W, Zou Y, Du Y, Zhang M, et al. Time-Dependent Increase of  
40 Chitinase1 in APP/PS1 Double Transgenic Mice. *Neurochemical research*.  
41 2016;41(7):1604-11.
- 42 43. Barreto G, Huang TT, Giffard RG. Age-related defects in sensorimotor activity,  
43 spatial learning, and memory in C57BL/6 mice. *Journal of neurosurgical anesthesiology*.  
44 2010;22(3):214-9.
- 45 44. Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in  
46 C57BL/6J mice from young adulthood to middle age. *Molecular brain*. 2016;9:11.
- 47 45. Xu B, Sun A, He Y, Qian F, Liu L, Chen Y, et al. Running-induced memory  
48 enhancement correlates with the preservation of thin spines in the hippocampal area  
49 CA1 of old C57BL/6 mice. *Neurobiology of aging*. 2017;52:106-16.
- 50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
46. Cheng D, Low JK, Logge W, Garner B, Karl T. Novel behavioural characteristics of female APPSwe/PS1DeltaE9 double transgenic mice. *Behavioural brain research*. 2014;260:111-8.
  47. O'Leary TP, Brown RE. Visuo-spatial learning and memory deficits on the Barnes maze in the 16-month-old APPSwe/PS1dE9 mouse model of Alzheimer's disease. *Behavioural brain research*. 2009;201(1):120-7.
  48. Gallagher JJ, Minogue AM, Lynch MA. Impaired performance of female APP/PS1 mice in the Morris water maze is coupled with increased Abeta accumulation and microglial activation. *Neuro-degenerative diseases*. 2013;11(1):33-41.
  49. McManus RM, Higgins SC, Mills KH, Lynch MA. Respiratory infection promotes T cell infiltration and amyloid-beta deposition in APP/PS1 mice. *Neurobiology of aging*. 2014;35(1):109-21.
  50. Duffy AM, Holscher C. The incretin analogue D-Ala2GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience*. 2013;228:294-300.
  51. Jin JL, Liou AK, Shi Y, Yin KL, Chen L, Li LL, et al. CART treatment improves memory and synaptic structure in APP/PS1 mice. *Scientific reports*. 2015;5:10224.
  52. Yun HM, Jin P, Park KR, Hwang J, Jeong HS, Kim EC, et al. Thiacecremonone Potentiates Anti-Oxidant Effects to Improve Memory Dysfunction in an APP/PS1 Transgenic Mice Model. *Molecular neurobiology*. 2016;53(4):2409-20.
  53. Chakrabarti S, Munshi S, Banerjee K, Thakurta IG, Sinha M, Bagh MB. Mitochondrial Dysfunction during Brain Aging: Role of Oxidative Stress and Modulation by Antioxidant Supplementation. *Aging and disease*. 2011;2(3):242-56.
  54. Manczak M, Jung Y, Park BS, Partovi D, Reddy PH. Time-course of mitochondrial gene expressions in mice brains: implications for mitochondrial dysfunction, oxidative damage, and cytochrome c in aging. *Journal of neurochemistry*. 2005;92(3):494-504.
  55. Yarchoan M, Toledo JB, Lee EB, Arvanitakis Z, Kazi H, Han LY, et al. Abnormal serine phosphorylation of insulin receptor substrate 1 is associated with tau pathology in Alzheimer's disease and tauopathies. *Acta neuropathologica*. 2014;128(5):679-89.
  56. Zhang B, Tang XC, Zhang HY. Alternations of central insulin-like growth factor-1 sensitivity in APP/PS1 transgenic mice and neuronal models. *Journal of neuroscience research*. 2013;91(5):717-25.
  57. Galea E, Morrison W, Hudry E, Arbel-Ornath M, Bacskai BJ, Gomez-Isla T, et al. Topological analyses in APP/PS1 mice reveal that astrocytes do not migrate to amyloid-beta plaques. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(51):15556-61.
  58. Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM. The effect of amyloid associated proteins on the expression of genes involved in amyloid-beta clearance by adult human astrocytes. *Experimental neurology*. 2012;233(1):373-9.
  59. Lopez-Gonzalez I, Schluter A, Aso E, Garcia-Esparcia P, Ansoleaga B, F LL, et al. Neuroinflammatory signals in Alzheimer disease and APP/PS1 transgenic mice: correlations with plaques, tangles, and oligomeric species. *Journal of neuropathology and experimental neurology*. 2015;74(4):319-44.
  60. Abbas N, Bednar I, Mix E, Marie S, Paterson D, Ljungberg A, et al. Up-regulation of the inflammatory cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP(SWE) transgenic mice. *Journal of neuroimmunology*. 2002;126(1-2):50-7.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
61. Mastrangelo MA, Sudol KL, Narrow WC, Bowers WJ. Interferon- $\gamma$  differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. *The American journal of pathology*. 2009;175(5):2076-88.
  62. Monteiro S, Ferreira FM, Pinto V, Roque S, Morais M, de Sa-Calcada D, et al. Absence of IFN $\gamma$  promotes hippocampal plasticity and enhances cognitive performance. *Translational psychiatry*. 2016;6:e707.
  63. McGillicuddy FC, Chiquoine EH, Hinkle CC, Kim RJ, Shah R, Roche HM, et al. Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. *The Journal of biological chemistry*. 2009;284(46):31936-44.
  64. Xuan AG, Pan XB, Wei P, Ji WD, Zhang WJ, Liu JH, et al. Valproic acid alleviates memory deficits and attenuates amyloid-beta deposition in transgenic mouse model of Alzheimer's disease. *Molecular neurobiology*. 2015;51(1):300-12.
  65. Guo HB, Cheng YF, Wu JG, Wang CM, Wang HT, Zhang C, et al. Donepezil improves learning and memory deficits in APP/PS1 mice by inhibition of microglial activation. *Neuroscience*. 2015;290:530-42.
  66. Salminen A, Ojala J, Suuronen T, Kaarniranta K, Kauppinen A. Amyloid-beta oligomers set fire to inflammasomes and induce Alzheimer's pathology. *Journal of cellular and molecular medicine*. 2008;12(6a):2255-62.
  67. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493(7434):674-8.
  68. Thomas GM, Haganir RL. MAPK cascade signalling and synaptic plasticity. *Nature reviews Neuroscience*. 2004;5(3):173-83.
  69. Satoh Y, Endo S, Ikeda T, Yamada K, Ito M, Kuroki M, et al. Extracellular signal-regulated kinase 2 (ERK2) knockdown mice show deficits in long-term memory; ERK2 has a specific function in learning and memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007;27(40):10765-76.
  70. Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD. Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal  $\alpha 7$  nicotinic acetylcholine receptors: In vitro and in vivo mechanisms related to Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21(12):4125-33.
  71. Yates SC, Zafar A, Hubbard P, Nagy S, Durant S, Bicknell R, et al. Dysfunction of the mTOR pathway is a risk factor for Alzheimer's disease. *Acta neuropathologica communications*. 2013;1:3.
  72. Sosanya NM, Cacheaux LP, Workman ER, Niere F, Perrone-Bizzozero NI, Raab-Graham KF. Mammalian Target of Rapamycin (mTOR) Tagging Promotes Dendritic Branch Variability through the Capture of Ca<sup>2+</sup>/Calmodulin-dependent Protein Kinase II  $\alpha$  (CaMKII $\alpha$ ) mRNAs by the RNA-binding Protein HuD. *The Journal of biological chemistry*. 2015;290(26):16357-71.
  73. Garza-Lombo C, Gonsebatt ME. Mammalian Target of Rapamycin: Its Role in Early Neural Development and in Adult and Aged Brain Function. *Frontiers in cellular neuroscience*. 2016;10:157.
  74. Lee CC, Huang CC, Hsu KS. Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 signaling pathways. *Neuropharmacology*. 2011;61(4):867-79.

- 1 75. Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, et al. Ezh2 regulates adult hippocampal  
2 neurogenesis and memory. *The Journal of neuroscience : the official journal of the*  
3 *Society for Neuroscience*. 2014;34(15):5184-99.
- 4 76. Chen TJ, Wang DC, Chen SS. Amyloid-beta interrupts the PI3K-Akt-mTOR  
5 signaling pathway that could be involved in brain-derived neurotrophic factor-induced  
6 Arc expression in rat cortical neurons. *Journal of neuroscience research*.  
7 2009;87(10):2297-307.
- 8 77. Ma T, Hoeffler CA, Capetillo-Zarate E, Yu F, Wong H, Lin MT, et al. Dysregulation of  
9 the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of  
10 Alzheimer's disease. *PloS one*. 2010;5(9).
- 11 78. Liu SJ, Yang C, Zhang Y, Su RY, Chen JL, Jiao MM, et al. Neuroprotective effect of  
12 beta-asarone against Alzheimer's disease: regulation of synaptic plasticity by increased  
13 expression of SYP and GluR1. *Drug design, development and therapy*. 2016;10:1461-9.
- 14 79. Ostapchenko VG, Chen M, Guzman MS, Xie YF, Lavine N, Fan J, et al. The Transient  
15 Receptor Potential Melastatin 2 (TRPM2) Channel Contributes to beta-Amyloid  
16 Oligomer-Related Neurotoxicity and Memory Impairment. *The Journal of neuroscience :*  
17 *the official journal of the Society for Neuroscience*. 2015;35(45):15157-69.
- 18 80. Zhang Y, Huang LJ, Shi S, Xu SF, Wang XL, Peng Y. L-3-n-butylphthalide Rescues  
19 Hippocampal Synaptic Failure and Attenuates Neuropathology in Aged APP/PS1 Mouse  
20 Model of Alzheimer's Disease. *CNS neuroscience & therapeutics*. 2016.
- 21 81. Minkeviciene R, Ihalainen J, Malm T, Matilainen O, Keksa-Goldsteine V,  
22 Goldsteins G, et al. Age-related decrease in stimulated glutamate release and vesicular  
23 glutamate transporters in APP/PS1 transgenic and wild-type mice. *Journal of*  
24 *neurochemistry*. 2008;105(3):584-94.
- 25 82. King DL, Arendash GW. Maintained synaptophysin immunoreactivity in Tg2576  
26 transgenic mice during aging: correlations with cognitive impairment. *Brain research*.  
27 2002;926(1-2):58-68.
- 28  
29  
30  
31  
32  
33  
34  
35

### 36 **Figure Legends**

37  
38 **Figure 1. Learning and memory in aged APP/PS1 and wild type mice.** The acquisition  
39 training phase of the Morris water maze (MWM) involved four training sessions per day  
40 over four consecutive days, followed by a probe trial on the fifth day, 24 hours following the  
41 final training session. Escape latency during the training phase is shown (A), as is the  
42 proportion of time spent in each quadrant during the probe trial by 15-18 month-old wild  
43 type (solid line with circles; D) and APP/PS1 (dotted line with squares; G) mice. Reversal  
44 water maze acquisition training began 24 hours following the MWM probe trial and  
45 consisted of four consecutive days with four training sessions per day, followed by a reversal  
46 probe trial on the fifth day. Illustrated are training phase escape latency (B) and time spent in  
47 each quadrant during the reversal probe trial by wild type (E) and APP/PS1 (H) mice. For  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 the novel object recognition task, recognition index, a measure of the percentage of time  
2 spent exploring either object, is illustrated in the acquisition phase (C) during exposure to  
3 two identical objects, and the test phase (F), in the presence of one familiar (black bars) and  
4 one novel (white bars) object. \* $p < 0.05$ , \*\* $p < 0.01$  APP/PS1 vs. wild type; two-way repeated  
5 measures ANOVA with Bonferroni's *post-hoc* test (A, B), ordinary one-way ANOVA with  
6 Dunnett's *post-hoc* test (D, E, G, H), multiple *t* tests with Holm-Šidák's *post-hoc* test (C, F).  
7 Data represent mean  $\pm$  SEM for 13-15 mice per group.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18

19 **Figure 2. A $\beta$  deposition and IRS-1 pSer<sup>616</sup> in the cerebral cortex and dentate gyrus of**  
20 **aged APP/PS1 and wild type mice.** Representative images (10x magnification) are shown  
21 that depict A $\beta$  staining in the cerebral cortex (A) and dentate gyrus (B) of 15-18 month old  
22 wild type mice and the cerebral cortex (E) and dentate gyrus (F) of age-matched APP/PS1  
23 mice. Also shown is an exemplary magnified image (20x magnification) of A $\beta$  staining in  
24 brains of wild type (C) and APP/PS1 (G) mice. Quantification of A $\beta$  immunopositivity in  
25 the cortex (D) and dentate gyrus (H) of 15-18 month old APP/PS1 and wild type mice is also  
26 shown. Representative images (20x magnification) are also shown that depict IRS-1 pSer<sup>616</sup>  
27 staining in the cerebral cortex (I) and dentate gyrus (J) of 15-18 month old wild type mice  
28 and the cerebral cortex (M) and dentate gyrus (N) of age-matched APP/PS1 mice. Also  
29 shown are exemplary magnified images (40x magnification) from wild type (K) and  
30 APP/PS1 (O) mice. Quantification of IRS-1 pSer<sup>616</sup> immunopositivity in cortex (L) and  
31 dentate gyrus (P) of 15-18 month old APP/PS1 and wild type mice is also illustrated.  
32 \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ , Student's *t* test. Data represent mean  $\pm$  SEM for 6 per group.  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

56 **Figure 3. Oxidative stress and astrocytes in the cerebral cortex and dentate gyrus of**  
57 **aged APP/PS1 and wild type mice.** Representative images (20x magnification) are shown  
58  
59  
60  
61  
62  
63  
64  
65



1 that depict the 8-oxoguanine staining in cerebral cortex (A) and dentate gyrus (B) of 15-18  
2 month old wild type mice and cerebral cortex (E) and dentate gyrus (F) of age-matched  
3 APP/PS1 mice. Also shown are exemplary magnified images (40x magnification) from wild  
4 type (C) and APP/PS1 (G) mice. Quantification of 8-oxoguanine immunopositivity in cortex  
5 (D) and dentate gyrus (H) of 15-18 month old APP/PS1 and wild type mice is also  
6 illustrated. Representative images (20x magnification) are also shown that depict GFAP  
7 staining in the cerebral cortex (I) and dentate gyrus (J) of 15-18 month-old wild type mice  
8 and the cerebral cortex (M) and dentate gyrus (N) of age-matched APP/PS1 mice. Also  
9 shown are exemplary magnified images (100x magnification) from wild type (K) and  
10 APP/PS1 (O) mice. Quantification of GFAP immunopositivity in cortex (L) and dentate  
11 gyrus (P) of 15-18 month old APP/PS1 and wild type mice is also shown. \*\*\* $p < 0.001$ ;  
12 Student's *t* test. Data represent mean  $\pm$  SEM for 6 per group.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

31 **Figure 4. Synapse density is decreased in the polymorphic layer of the dentate gyrus in**  
32 **APP/PS1 mice.** Illustrated are representative images depicting synaptophysin staining of  
33 brain sections from 15-18 month-old wild type (A, B, C) and APP/PS1 (D, E, F) mice. A  
34 and D show the polymorphic layer (PL), granule cell layer (GCL) and molecular layer (ML)  
35 of the dentate gyrus. C and D show the stratum radiatum (SR), stratum pyramidale (SP) and  
36 stratum oriens (SO) of the hippocampus, while B and E show the inner (IC) and outer (OC)  
37 cerebral cortex. Also illustrated is quantification of synaptophysin optical density values for  
38 the polymorphic layer, granule cell layer and molecular layer of the dentate gyrus and the  
39 stratum radiatum, stratum pyramidale and stratum oriens of the hippocampus, inner and outer  
40 cortex of 15-18 month-old APP/PS1 and wild type mice (G). \* $p < 0.05$ ; Student's *t* tests. Data  
41 represent mean  $\pm$  SEM for 6 per group.  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Figure 5. Peripheral insulin sensitivity, glucose tolerance and expression of inflammatory and insulin signaling genes in brains of aged APP/PS1 mice.** Illustrated is quantification of the expression of genes associated with inflammatory pathways and insulin signaling in brains of 15-18 month-old APP/PS1 mice (black bars), compared with age-matched wild type controls (white bars) (A). Also shown are blood glucose levels following insulin injection (B) and following glucose injection (C). Wild type (solid line with circles) and APP/PS1 mice (dotted line with squares) aged 15-18 months were administered insulin or glucose via i.p. injection and blood glucose levels were measured at 15, 30 and 60 minutes post-injection. \* $p < 0.05$ , \*\* $p < 0.01$ ; ordinary two-way ANOVA with Holm-Šidák's *post-hoc* test (A) or 13-15 per group, two-way repeated measures ANOVA with Holm-Šidák's *post-hoc* test (B, C). Data represent mean  $\pm$  SEM for 5 per group.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Figure 6. Cytokine levels in the brains of aged APP/PS1 and wild type mice.** MSD multiplex analysis of 8 cytokines was performed on supernatant extracted from brain tissue. Protein levels of IFN $\gamma$  (A), IL-10 (B), IL-1 $\beta$  (C), IL-12p70 (D), IL-2 (E), IL-4 (F), IL-5 (E), IL-6 (G) and KC/GRO (CXCL1) (H) were measured and compared between 15-18 month-old APP/PS1 mice (black bars) and age-matched wild types (white bars).\*\* $p < 0.01$ ; Student's *t* tests. Data represent mean  $\pm$  SEM for 6 per group..

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Figure 7. Correlations between IFN $\gamma$ , IRS-1 pSer<sup>616</sup> and novel object recognition memory in aged APP/PS1 and wild type mice.** Pearson's correlation analysis was performed between IRS-1 pSer<sup>616</sup> immunopositivity and novel object recognition index (A, B), between IFN $\gamma$  and novel object recognition index (C) and between IFN $\gamma$  and IRS-1 pSer<sup>616</sup> immunopositivity (D, E) in wild type (open circles and dotted best fit line) and APP/PS1 (black squares and solid best fit line) mice. Lines of best fit, *r* and *p* values were

1 also added to the graphs. Each data point represents an *XY* pair for a total of 6 *XY* pairs per  
2 genotype on each graph. Significance of correlation was determined using two-tailed *t* tests.  
3  
4  
5  
6

7 **Supplementary Figure 1. Cytokines and gene expression in brains of young wild type**  
8 **mice.** Quantification of expression of inflammatory and insulin signaling genes in the brains  
9 of young wild type mice (17-22 weeks old) is shown (black bars), compared to aged wild  
10 types (white bars) and APP/PS1 mice (dark grey bars) (A). Also illustrated are brain levels of  
11 IFN $\gamma$  (B), IL-1 $\beta$  (C) and IL-4 (D) in young wild type mice, compared with aged wild types  
12 and APP/PS1 mice. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, \*\*\*\* $p$ <0.0001; ordinary one-way  
13 ANOVA with Holm-Šídák's *post-hoc* test (A) and Student's *t* test (B-D). Data represent  
14 mean  $\pm$  SEM for 5 (A) or 6 (B-D) per group.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Figure 1

Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice

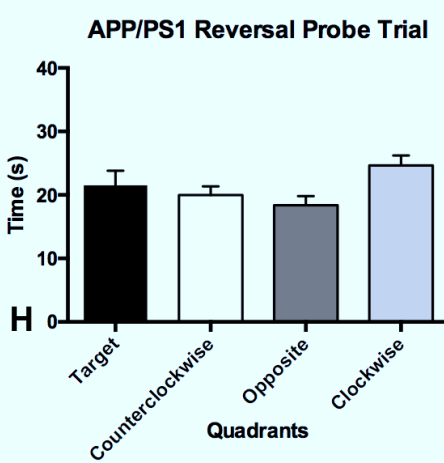
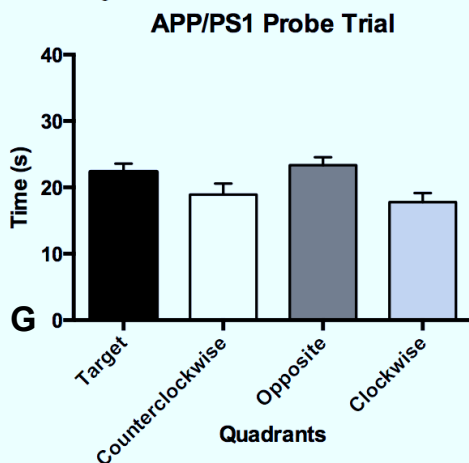
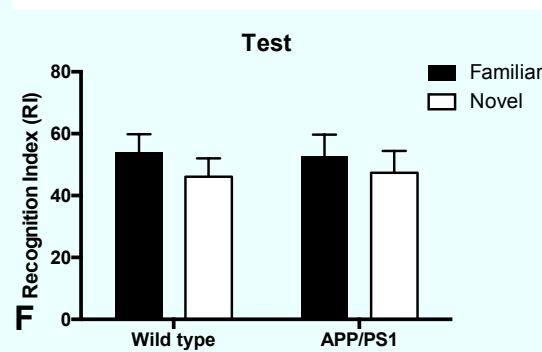
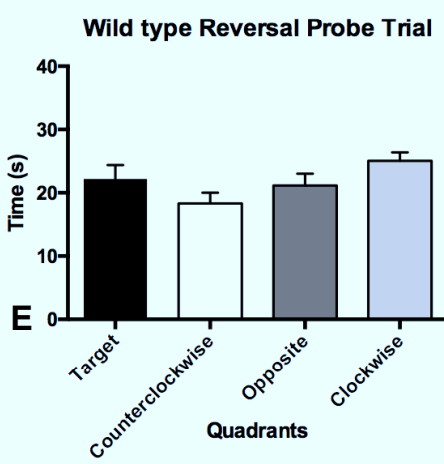
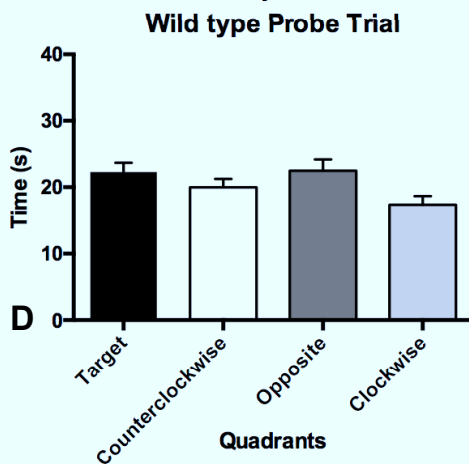
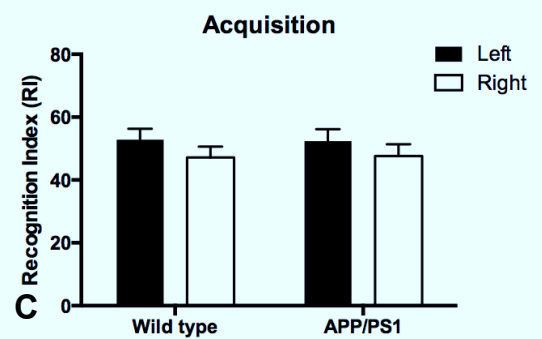
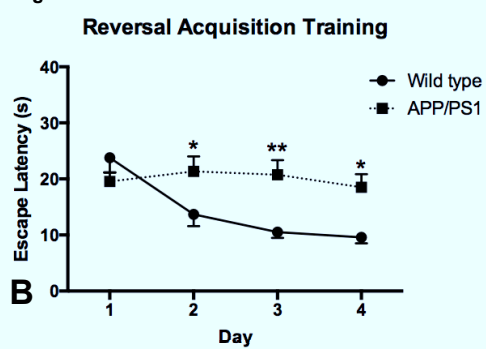
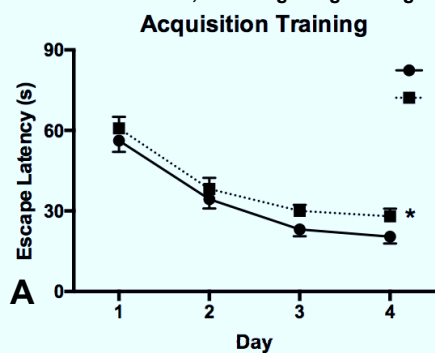


Figure 2  
[Click here to download high resolution image](#)

Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice

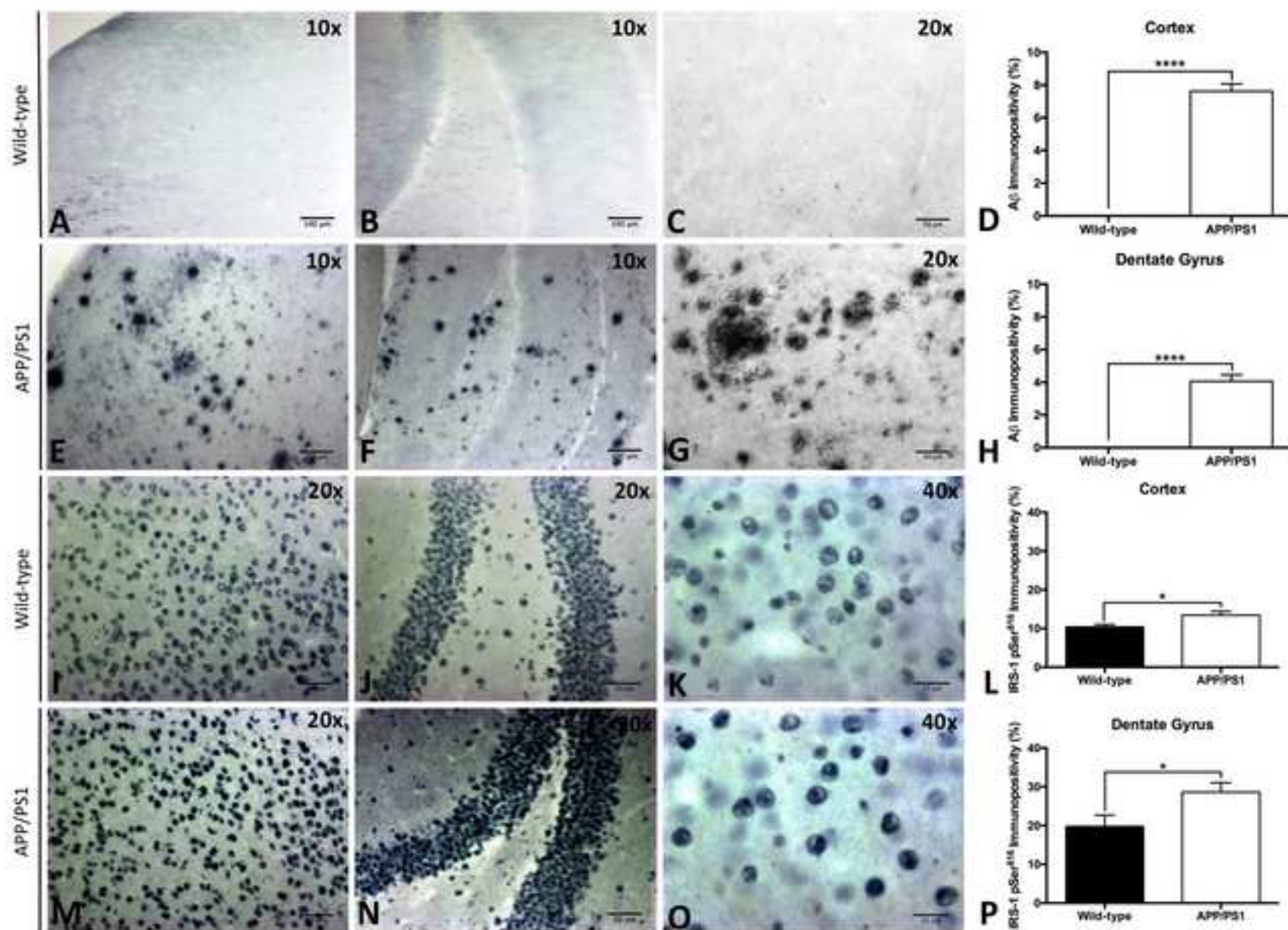


Figure 3  
[Click here to download high resolution image](#)

Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice

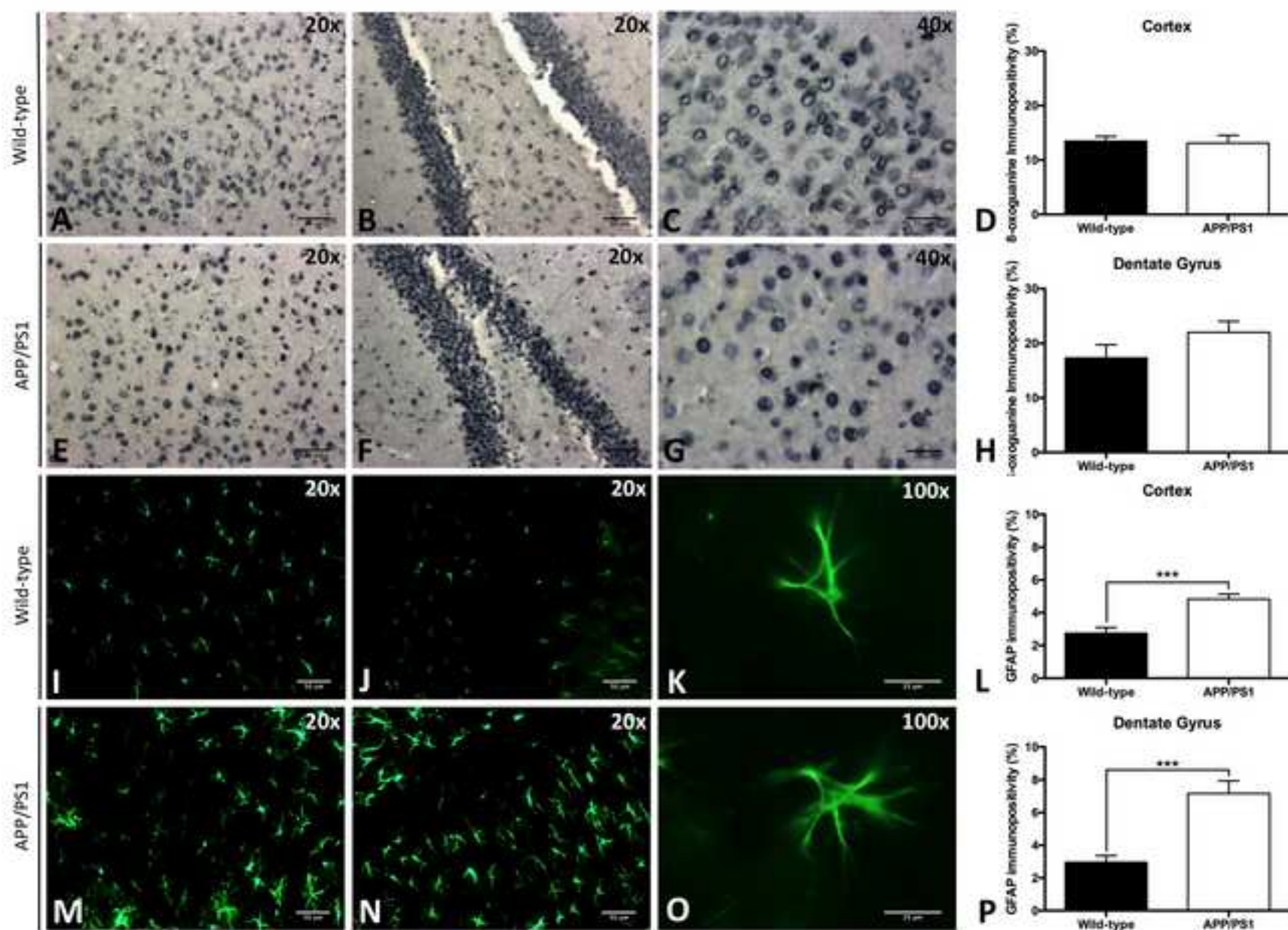
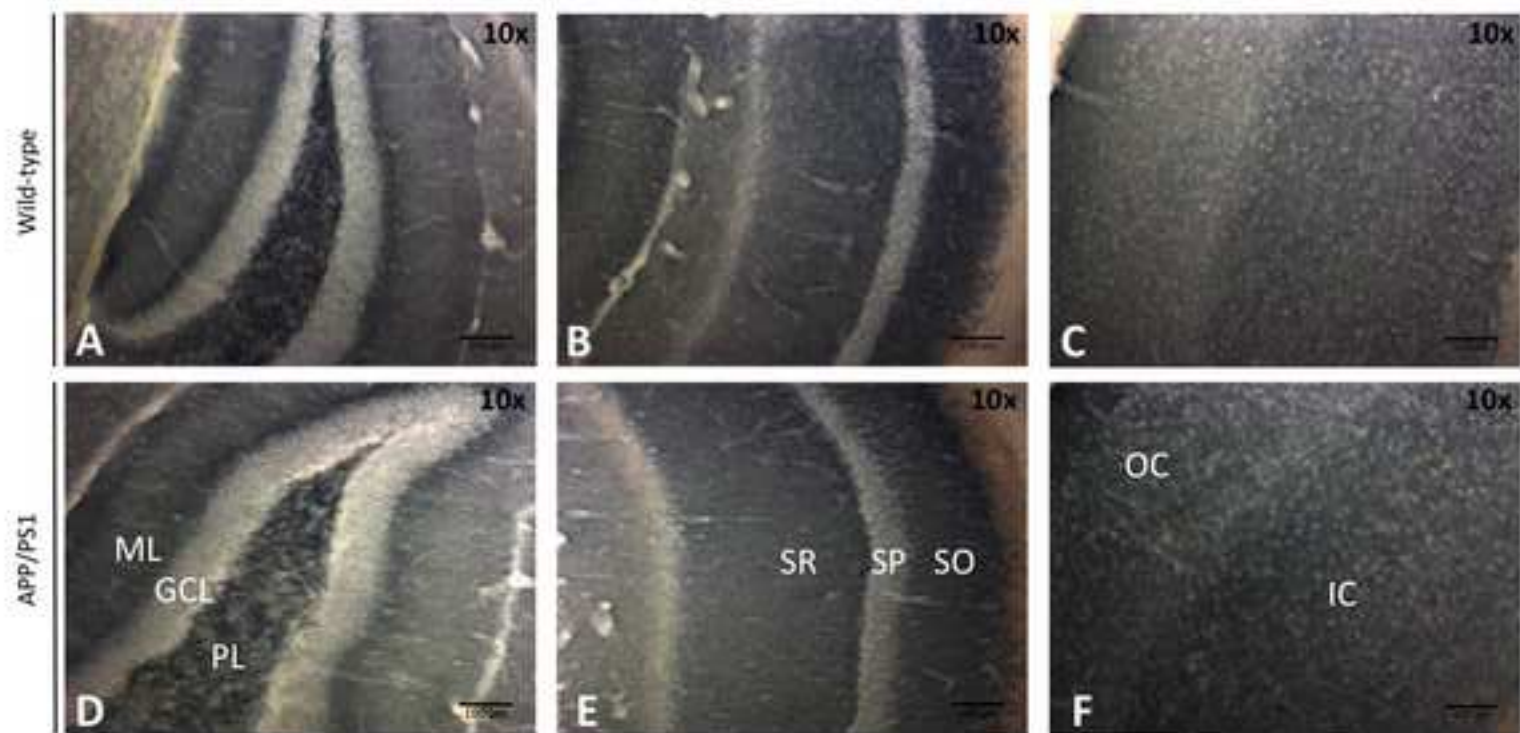


Figure 4  
[Click here to download high resolution image](#)

Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice



Synaptophysin

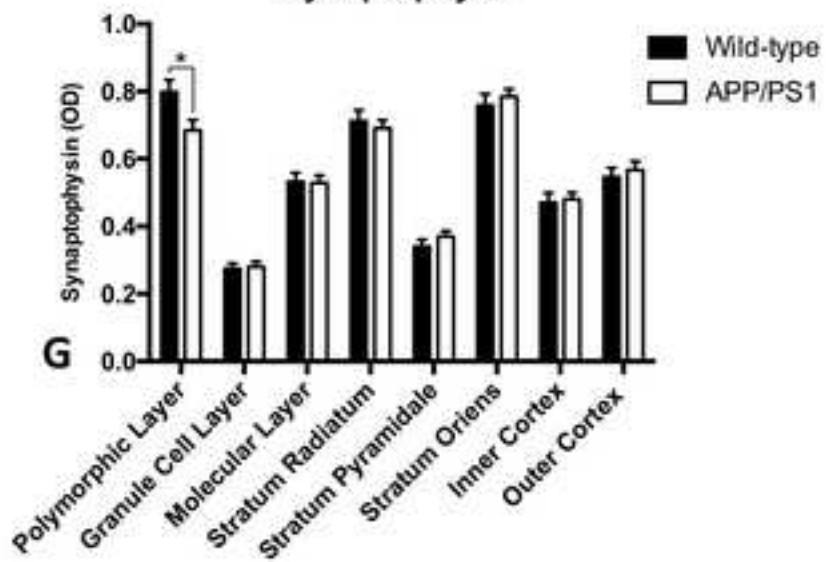


Figure 5 (revised)

Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice

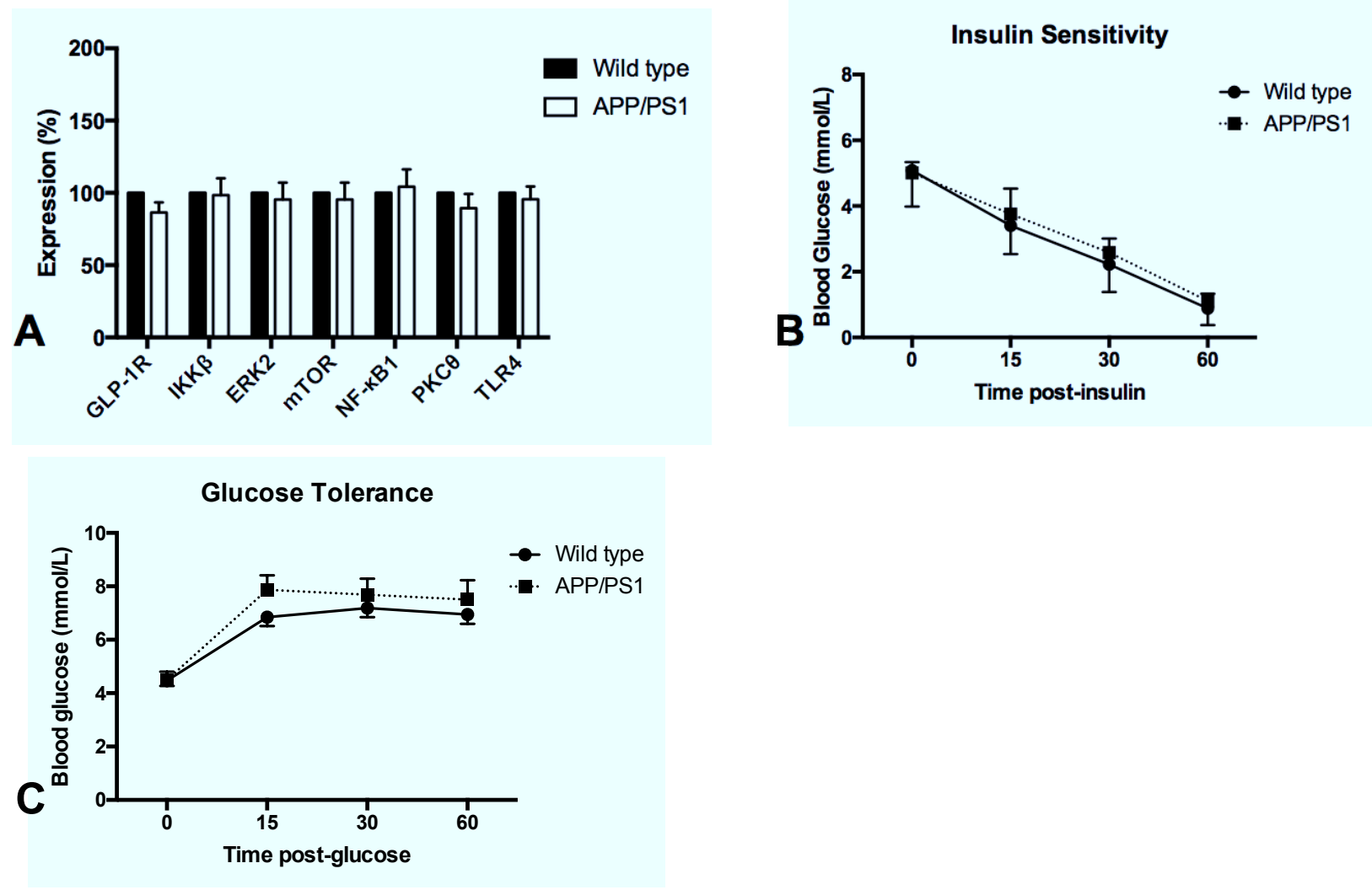
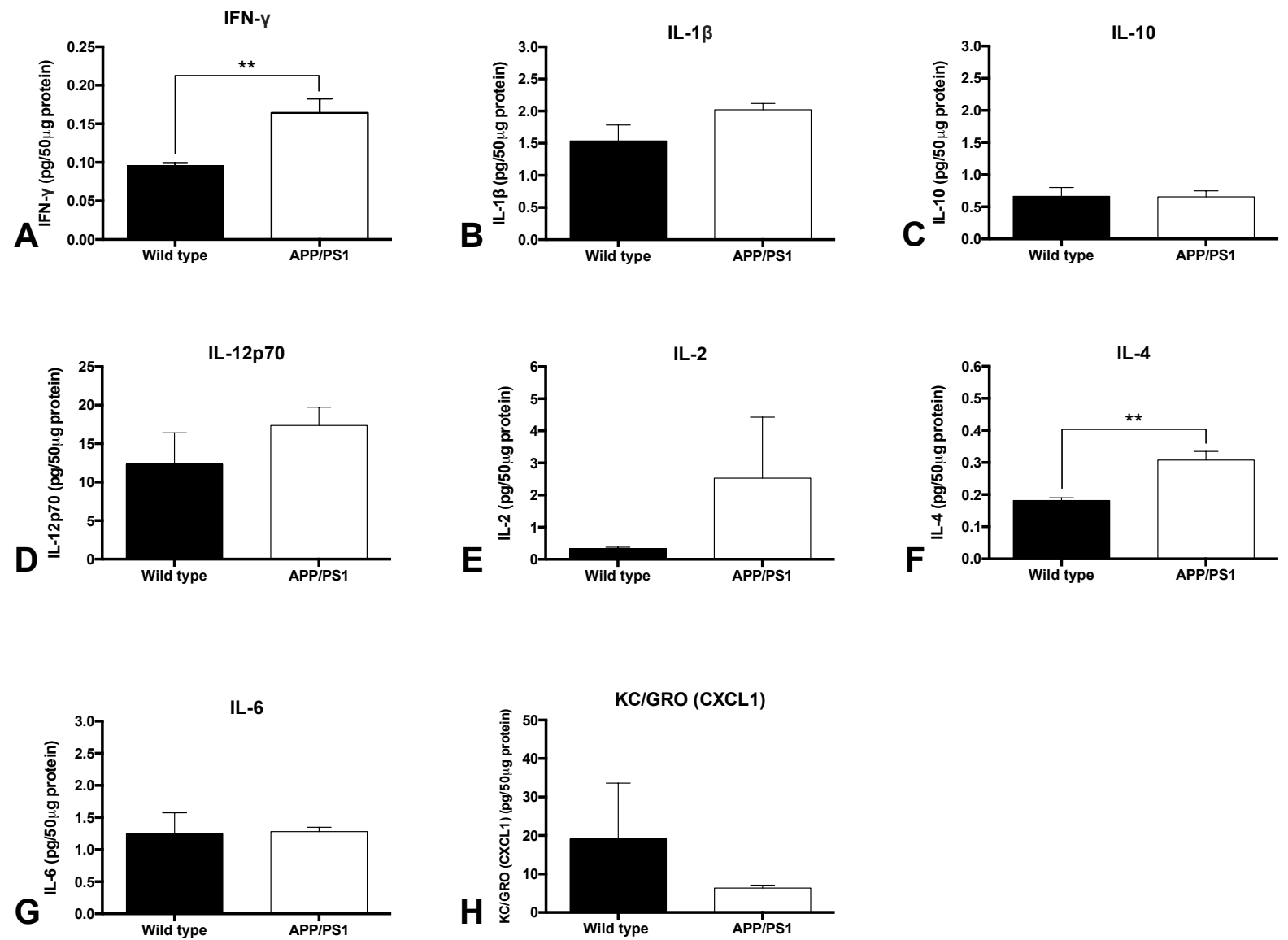


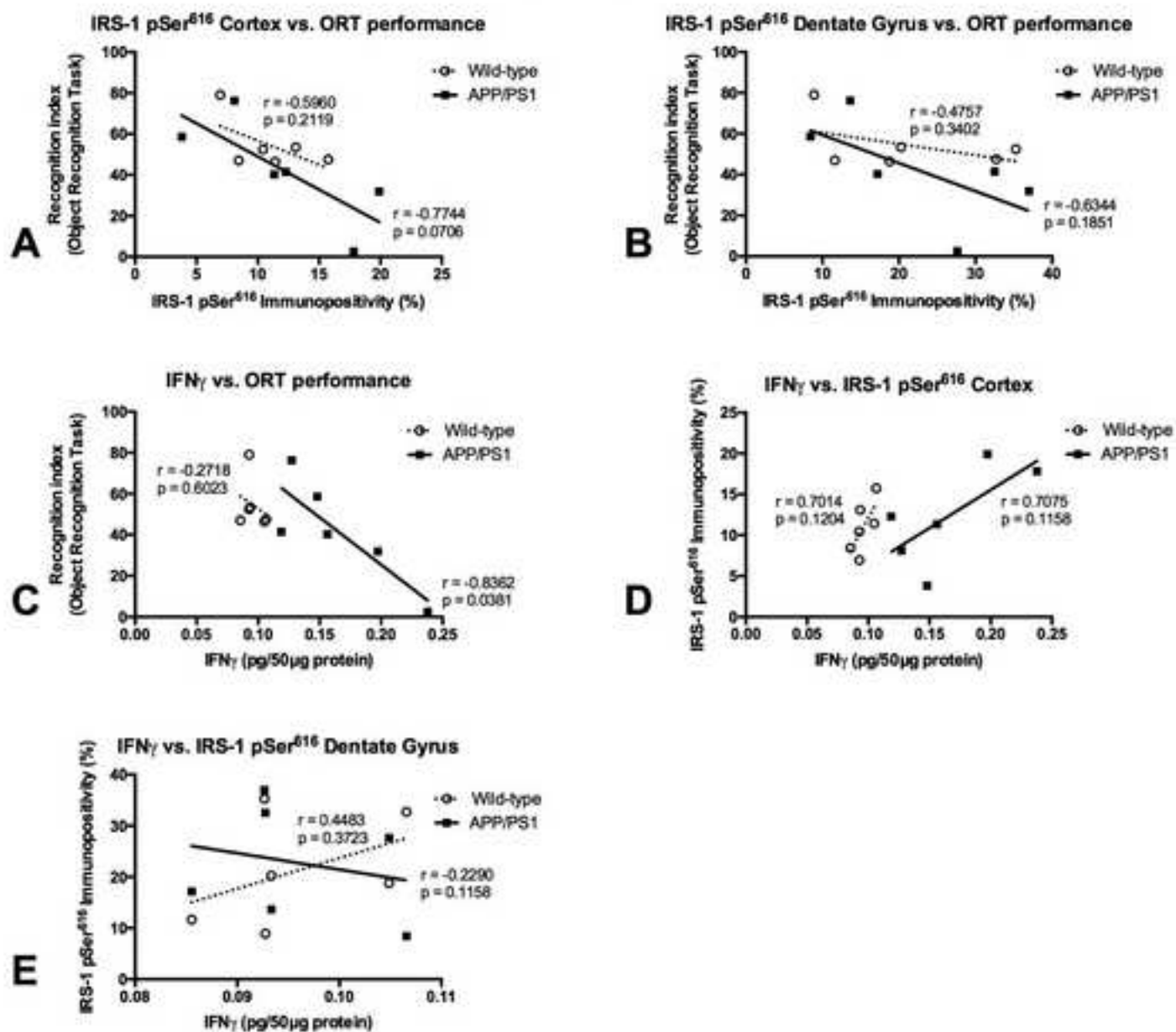


Figure 6 (revised)

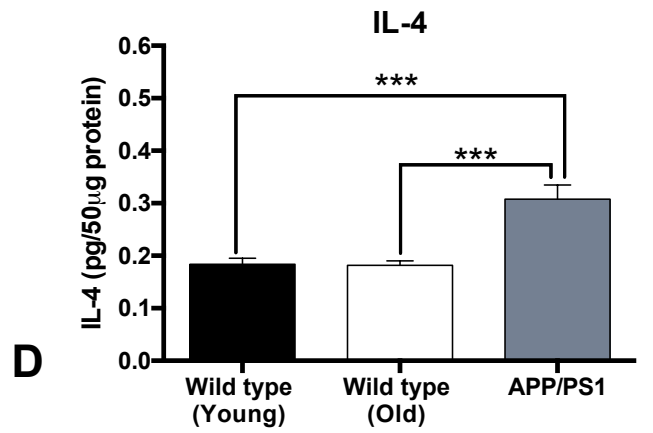
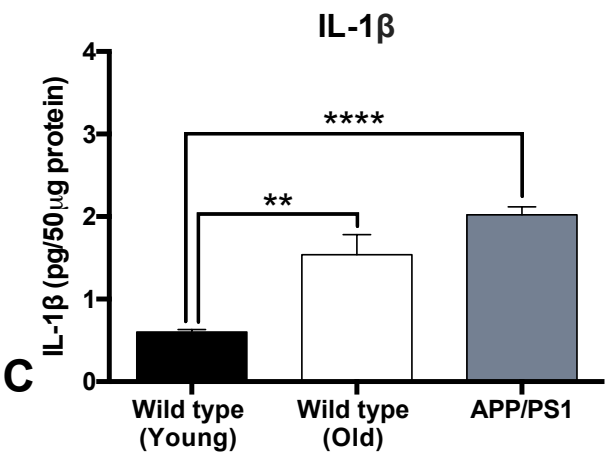
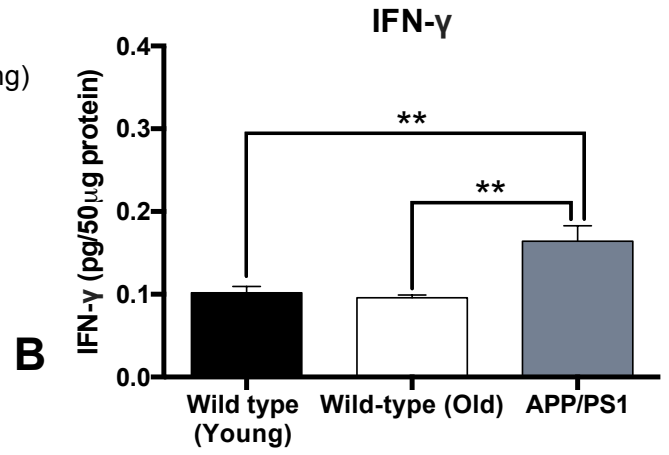
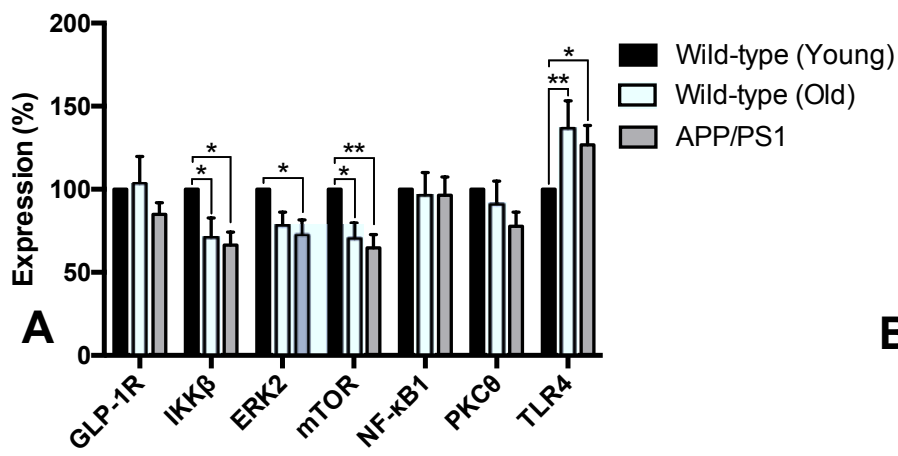
Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice



## Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice



Denver et al. Inflammation, insulin signaling and cognitive function in aged APP/PS1 mice



1  
2 **Inflammation, insulin signaling and cognitive function in aged**  
3  
4  
5 **APP/PS1 mice**  
6

7  
8  
9 Paul Denver<sup>a,1</sup>, Andrew English<sup>b</sup> and Paula L McClean<sup>b</sup>  
10

11  
12 *<sup>a,1</sup>Centre for Molecular Biosciences, University of Ulster, Coleraine, Northern Ireland*

13  
14 *<sup>b</sup>Northern Ireland Centre for Stratified Medicine, Clinical, Translational and Research Innovation Centre (C-*  
15  
16 *TRIC), University of Ulster, Derry~Londonderry, Northern Ireland*  
17

18  
19  
20 Abstract: 287 words

21  
22 Main text: 4,532 words

23  
24  
25 Figures: 7  
26

27  
28  
29 Corresponding author: Paul Denver,

30  
31 West Los Angeles VA Healthcare Center,

32  
33 11301 Wilshire Boulevard, Los Angeles, CA 90073

34  
35 Email address: pdenver@mednet.ucla.edu

36  
37 Telephone: 1 (310) 478-3711 ext. 42171  
38

39  
40 <sup>1</sup>*Present affiliations: Greater Los Angeles Veterans Affairs Healthcare System; West Los Angeles Medical*  
41 *Center and Dept. of Neurology, University of California, Los Angeles, CA, USA*  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Abstract

Cognitive dysfunction and neuroinflammation are typical in Alzheimer's disease (AD), but are also associated with normal aging, albeit less severely. Insulin resistance in the brain has been demonstrated in AD patients and is thought to be involved in AD pathophysiology. Using 15-18 month-old APP/PS1 mice, this study measured peripheral and central insulin signaling and sensitivity, inflammatory markers in brain and plasma and oxidative stress and synapse density in the brain. Novel object recognition, Morris water maze and reversal water maze tasks were performed to assess cognitive function in aged APP/PS1 mice and wild type littermates. Glucose tolerance and insulin sensitivity were similar in APP/PS1 mice and wild type controls, however IRS-1 pSer<sup>616</sup> was increased in cortex and dentate gyrus of APP/PS1 mice. Recognition and spatial memory was impaired in both APP/PS1 and wild type mice, however learning impairments were apparent in APP/PS1 mice. Expression of GLP-1 receptor, ERK2, IKK $\beta$ , mTOR, PKC $\theta$ , NF- $\kappa$ B1 and TLR4 was similar between aged APP/PS1 mice and age-matched wild types, ~~however, compared to young wild types (7-9 month old), IKK $\beta$ , mTOR and ERK2 were decreased in brains of APP/PS1 mice, while TLR4 expression was increased.~~ Compared to age-matched ~~and younger~~ wild type mice, IFN $\gamma$  and IL-4 were increased in brains of APP/PS1 mice, ~~whereas IL-1 $\beta$  was increased in the brains of aged wild type and APP/PS1 mice, compared to young controls.~~ These results suggest that normal aging may be associated with enhanced neuroinflammation, oxidative stress, and cognitive decline, however distinctions are apparent in the brain of APP/PS1 mice in terms of inflammation and insulin signaling and in certain cognitive domains. Demarcation

1  
2 of pathological events that distinguish AD from normal aging will allow for improvements in  
3  
4 diagnostic tools and the development of more effective therapeutics.  
5  
6

7  
8 **Keywords: Alzheimer’s disease; aging; neuroinflammation; insulin signaling; cognitive**  
9 **function; learning; memory; insulin sensitivity; cytokines**  
10

11  
12  
13 **Abbreviations: A $\beta$**  Amyloid- $\beta$  **AD** Alzheimer’s disease **ERK2** extracellular signal-regulated  
14 kinase 2 **IKK $\beta$**  Inhibitor of NF- $\kappa$ B kinase  $\beta$  **IRS-1 pSer<sup>616</sup>** Insulin receptor substrate-1  
15 phosphorylated at serine residue 616 **MAPK** mitogen-activated protein kinase **mTOR**  
16 mechanistic target of rapamycin **MWM** Morris water maze **ORT** Novel object recognition  
17 task **PKC** Protein kinase C **RI** Recognition index **RWM** Reversal water maze **TLR4** Toll-  
18 like receptor 4  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28

## 29 **1 Introduction**

30

31 As healthcare improves around the world, life expectancy continues to rise (1). Accordingly,  
32 the past 25 years have seen a dramatic increase in disorders associated with aging, including  
33 neurological diseases such as Alzheimer’s disease (AD) (1), for which advancing age is the  
34 principal risk factor (2).  
35  
36  
37  
38  
39

40 Many clinical and neuropathological features of AD parallel the normal progression  
41 of aging, making differentiation between normal brain aging and early-stage AD difficult.  
42 Generally, it can be said that healthy aging is associated with moderate decline in some  
43 cognitive abilities, whilst AD is characterized by severe deterioration of the same cognitive  
44 domains, with additional progressive decline of further cognitive functions, such that the  
45 patient’s daily life is adversely affected to a severe degree (3). In AD, amyloid- $\beta$  (A $\beta$ )  
46 accumulates into progressively larger fibrils, which become deposited as insoluble plaques in  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 the brain parenchyma (4). Accumulating evidence suggests that the presence of A $\beta$  fibrils  
3  
4 and plaques is not uncommon in the brains of non-demented, cognitively healthy older  
5  
6 people (5, 6). Several studies have also shown that A $\beta$  deposition does not correlate with  
7  
8 cognitive impairment in elderly cohorts (6), highlighting the variability of age-related  
9  
10 cognitive decline and suggesting that A $\beta$  *per se* does not directly influence cognitive  
11  
12 function.  
13

14  
15 Profound inflammation is evident in AD brain (7), primarily mediated by microglia  
16  
17 and astrocytes (8, 9). Activated microglia and astrocytes phagocytose A $\beta$  oligomers and  
18  
19 fibrils, degrade A $\beta$  plaques and reduce amyloid burden (10, 11). However, sustained  
20  
21 microglial activation and unresolved inflammation in the brain is harmful to neurons and  
22  
23 synapses and promotes chronic dysregulation of glial cells and subsequent deterioration of  
24  
25 brain structure and function (12, 13). Inflammation in the brain increases with age (14) and  
26  
27 several studies have shown elevated levels of inflammatory cytokines in the brains of aged  
28  
29 rodents (15, 16). In the context of AD, primed microglia respond more readily to A $\beta$ ,  
30  
31 producing increased levels of cytokines that exert direct toxic effects on neurons and at  
32  
33 synapses (17).  
34  
35

36  
37 Insulin resistance has been demonstrated in postmortem brain tissue from AD patients  
38  
39 and those with mild cognitive impairment, in the absence of diabetes and irrespective of  
40  
41 ApoE- $\epsilon$ 4 status (18). Furthermore, IRS-1 pSer<sup>616</sup> was identified as a putative biomarker of  
42  
43 brain insulin resistance in AD and was found to correlate positively with A $\beta$  oligomer levels  
44  
45 and negatively with cognitive function (18). Additionally, Bomfim *et al.* (19) demonstrated  
46  
47 increased levels of IRS-1 pSer<sup>616</sup> in the hippocampus of 13 month-old APP/PS1 mice. Other  
48  
49 studies have also demonstrated impaired neuronal insulin signaling in AD brain and in  
50  
51 response to A $\beta$  oligomer challenge (20, 21).  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 This study sought to determine differences in learning and memory, oxidative stress,  
3  
4 glucose tolerance, central and peripheral insulin sensitivity between 15-18 month old wild  
5  
6 type and age-matched APP/PS1 mice. Using novel object recognition and Morris water maze  
7  
8 tasks, cognitive function was measured in aged wild type and APP/PS1 mice. Systemic  
9  
10 insulin sensitivity and glucose tolerance were compared between groups. Brain levels of A $\beta$ ,  
11  
12 GFAP, 8-oxoguanine, IRS-1 pSer<sup>616</sup> and synaptophysin were measured by  
13  
14 immunohistochemistry. Additionally inflammatory and insulin signaling associated genes,  
15  
16 GLP-1R, IKK $\beta$ , ERK2, mTOR, NF- $\kappa$ B1, PKC $\theta$ , and TLR4 and inflammatory cytokines  
17  
18 (IFN $\gamma$ , IL-10, IL-1 $\beta$ , IL-12p70, IL-2, IL-4, IL-5, IL-6 and KC/GRO (CXCL1)) were assessed  
19  
20 in brain tissue from aged APP/PS1 and wild type mice to delineate pathological changes from  
21  
22 those associated with ‘normal’ aging.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35

## 36 **2 Materials and Methods**

### 37 *2.1.1 Animals*

38  
39 Male APP<sub>swe</sub>/PS1 $\Delta$ e9 (APP/PS1) mice with a C57Bl/6J background were bred with wild type  
40  
41 C57Bl/6J females at the Biomedical and Behavioural Research Unit at Ulster University in  
42  
43 Coleraine. Offspring were ear punched and positivity for the APP<sub>swe</sub>/PS1 $\Delta$ e9 transgene, or  
44  
45 lack thereof was confirmed by polymerase chain reaction, using primers specific for the APP  
46  
47 sequence of the APP/PS1 construct (Forward “GAATTCGACATGACTCAGG”, Reverse:  
48  
49 “GTTCTGCTGCATCTTGGACA”). Offspring males heterozygous for the APP<sub>swe</sub>/PS1 $\Delta$ e9  
50  
51 transgenic construct were then age-matched with wild type littermates, not expressing the  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1  
2 transgene, which were used as controls. Both groups of mice were caged individually and  
3  
4 allowed access to food and water *ad libitum*. Animals were maintained on a 12:12 light-dark  
5  
6 cycle (lights on at 08:00, lights off at 20:00), within a temperature-controlled room (T:  
7  
8 21.5°C ± 1°C). All tests were performed during the light cycle. All experiments were  
9  
10 designed, analyzed and reported in accordance with ARRIVE guidelines. Experiments were  
11  
12 licensed according to UK Home Office regulations (UK Animals Scientific Procedures Act  
13  
14 1986) and associated guidelines (EU Directive 2010/63/EU). C57Bl/6 mice were derived  
15  
16 from a colony maintained in the Biomedical and Behavioural Research Unit at Ulster  
17  
18 University in Coleraine.  
19  
20  
21  
22

### 23 *2.1.2 Glucose tolerance and insulin sensitivity tests*

24  
25 After an overnight fasting period, APP/PS1 mice and age-matched wild types received an i.p.  
26  
27 injection of glucose (18 mmol/kg bw) in 0.9% NaCl or insulin (0.25 µM/g). Blood glucose  
28  
29 was measured at 0, 15, 30 and 60 minutes following glucose or insulin injection using a  
30  
31 hand-held Ascencia Contour blood glucose meter (Bayer Health Care).  
32  
33  
34

### 35 *2.1.3 Behavioural Assessment*

36  
37 Mice were assessed in the ORT, as described previously (22). Briefly, mice were subjected to  
38  
39 a 10 minute acquisition period, with two identical objects, followed by a 3 hour retention  
40  
41 period and a 10 minute test phase, which involved replacing one of the objects with a novel  
42  
43 object. A recognition index (RI) was calculated for each object, defined as amount of time  
44  
45 spent exploring object A or B over the total time spent exploring both objects x 100 ( $t_A$  or  
46  
47  $t_B / (t_A + t_B) \times 100$ ).  
48  
49

50 Following ORT, mice were assessed in the Morris water maze (MWM) (22). The  
51  
52 acquisition training phase consisted of 4 x 90 second trials per day, for 4 consecutive days,  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 followed by a probe trial on the fifth day. The day after the probe trial, mice were subjected  
2 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
3 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
4 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
5 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
6 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
7 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
8 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
9 to reversal water maze (RWM), wherein the escape platform was moved from the southwest  
10 to reversal water maze (RWM), wherein the escape platform was moved from the southwest

#### 11 12 *2.1.4 Immunohistochemistry*

13 Following sacrifice, animals were perfused with PBS and brains excised. One hemisphere  
14 was fixed in 4% paraformaldehyde and the other was frozen in liquid nitrogen. Hemi-brains  
15 was fixed in 4% paraformaldehyde and the other was frozen in liquid nitrogen. Hemi-brains  
16 for histology were then transferred to 30% sucrose and 40  $\mu\text{m}$  coronal sections were cut  
17 using a cryostat (Leica Microsystems). One section in every 6 was collected sequentially and  
18 stored at  $-20^{\circ}\text{C}$ . Staining was performed for  $\text{A}\beta$ , GFAP, 8-oxoguanine, IRS-1 pSer<sup>616</sup> and  
19 synaptophysin. All sections were incubated in  $\text{H}_2\text{O}_2$  and permeabilized using Triton X. For 8-  
20 oxoguanine, sections were incubated at  $37^{\circ}\text{C}$  for 30 minutes with 2 M hydrochloric acid,  
21 followed by 0.1 M borax (Sigma Aldrich) for 10 minutes. Blocking with 1.5%-10% normal  
22 serum was performed prior to incubation with anti- $\text{A}\beta$  (1:200; Invitrogen; 71-5800) anti-  
23 GFAP (1:250; Merck Millipore; MAB3402), anti-8-oxoguanine (1:250; Merck Millipore;  
24 MAB3560), anti-IRS-1 pSer<sup>616</sup> (1:200; Invitrogen; 44-550G) or anti-synaptophysin (1:200;  
25 Abcam; ab7837) antibodies overnight at  $4^{\circ}\text{C}$ . Sections were then incubated with secondary  
26 antibodies and visualized using Vectastain Elite and SG substrate (Vector Laboratories).  
27 Percentage area stained in each image was quantified using a multi threshold plug-in within  
28 Image J (NIH, Bethesda, USA) in a blinded manner.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

#### 50 *2.1.5 Quantitative polymerase chain reaction (qPCR)*

51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 RNA was extracted from brain tissue using RNeasy Lipid Tissue Mini Kit (Qiagen)  
3 according to manufacturer's instructions. For cDNA synthesis, transcriptor First Strand  
4 cDNA synthesis kit (Roche Diagnostics) was used using 500 ng of RNA per sample. Real-  
5 time PCR reactions were composed of; 5  $\mu$ l of PCR MasterMix (Roche Diagnostics), 1  $\mu$ l (10  
6 pM/ $\mu$ l) gene-specific probes, 3  $\mu$ l of RNase free water and 1  $\mu$ l (25ng) of template cDNA.  
7  
8 Gene-specific probes (Roche Diagnostics) were as follows: GLP-1R (*Glp1r*), IKK $\beta$  (*Ikkbb*),  
9  
10 ERK2 (*Mapk1*), mTOR (*Mtor*), NF- $\kappa$ B1 (*Nfkb1*), PKC $\theta$  (*Prkcq*) and TLR4 (*Tlr4*).  
11  
12 Quantitative PCR was performed on Lightcycler 480 system (Roche Diagnostics), and  
13  
14 quantified on accompanying software package (Roche, Lightcycler 480 software, v1.5). Gene  
15  
16 expression changes were calculated using Delta Delta CT mathematical model (23).  
17  
18  
19  
20  
21  
22  
23  
24

#### 25 *2.1.6 Meso Scale Discovery multi-array*

26  
27 Whole hemi-brains were homogenized under liquid nitrogen, followed by addition of 10 ml/g  
28  
29 of lysis buffer (1 mM EDTA in PBS supplemented with protease inhibitor cocktail). Samples  
30  
31 were centrifuged at 14,000 G for 20 min at 4°C and supernatant was removed and added to  
32  
33 Meso Scale Discovery (MSD<sup>®</sup>) plate. Bradford protein assay was performed to measure  
34  
35 protein content and data were normalized to the total amount of protein present in each  
36  
37 sample. Levels of IFN $\gamma$ , IL1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, TNF- $\alpha$  and KC/GRO  
38  
39 (CXCL1) were quantified in brain and plasma using MSD<sup>®</sup> Multi-spot Assay Pro-  
40  
41 inflammatory panel 1 kit (Rockville, MD, USA) according to manufacturer's instructions.  
42  
43  
44  
45

#### 46 *2.1.7 Statistical Analysis*

47  
48 Data were analyzed using Graphpad Prism (v6.0h). Differences were deemed to be  
49  
50 significant if  $p \leq 0.05$ . Data are expressed as means  $\pm$  SEM. Tests included one-way or two-  
51  
52 way ANOVA and unpaired Student's *t* tests. Data heterogeneity was tested and, where  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 variance was significant, appropriate non-parametric tests were used. Corrections for multiple  
3  
4 comparisons were performed using appropriate *post-hoc* tests. Linear relationships between  
5  
6 two variables were measured by Pearson's correlation analysis.  
7  
8  
9

### 10 **3 Results**

#### 11 *3.1.1 Spatial learning is impaired in aged APP/PS1 mice*

12  
13 During the acquisition phase of the MWM, escape latency significantly decreased over time  
14  
15 ( $p<0.0001$ ), as expected, and was also significantly greater overall in APP/PS1 mice (Fig.  
16  
17 1A;  $p=0.0264$ ). However, *post-hoc* analysis indicated that average escape latency was not  
18  
19 significantly different between aged wild type and APP/PS1 mice on any of the training days  
20  
21 (Fig. 1A). In the probe trial, time spent in each quadrant by wild type mice was not  
22  
23 significantly different (Fig. 1D). Similarly, APP/PS1 mice spent a similar amount of time  
24  
25 swimming in all 4 quadrants in the probe trial and although significant variation in the time  
26  
27 spent in each quadrant was detected ( $p=0.0174$ ), *post-hoc* analysis showed that time spent in  
28  
29 the target quadrant by APP/PS1 mice was not significantly different from any other quadrant  
30  
31 (Fig. 1G). In the acquisition phase of the RWM, escape latency decreased over time (Fig. 1B;  
32  
33  $p=0.0009$ ) and was also significantly affected by genotype (Fig. 1B;  $p=0.0020$ ). *Post-hoc*  
34  
35 analysis revealed that average escape latency was significantly greater in APP/PS1 mice,  
36  
37 compared to wild types on days 2 ( $p<0.05$ ), 3 ( $p<0.01$ ) and 4 ( $p<0.05$ ; Fig. 1B).  
38  
39  
40  
41

42 In the reversal probe trial, time spent in each of the quadrants by wild types (Fig. 1E)  
43  
44 or APP/PS1 (Fig. 1H) mice did not differ significantly  
45  
46  
47

#### 48 *3.1.2 Recognition memory is impaired in aged APP/PS1 and wild type mice*

49  
50 In the acquisition phase of the ORT, recognition indices for the identical objects were not  
51  
52 significantly different in 15-18 month old APP/PS1 or wild type mice (Fig. 1C). In the test  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 phase, recognition index for the novel object was not significantly different from the familiar  
3  
4 in the aged APP/PS1 mice or the age-matched control group (Fig. 1F).  
5  
6  
7  
8  
9

### 10 11 12 *3.1.3 Immunohistochemistry*

#### 13 *3.1.3.1 A $\beta$ deposition is ubiquitous in brains of aged APP/PS1 mice*

14  
15 Representative micrographs from wild type mice show that A $\beta$  immunopositivity was almost  
16  
17 completely absent from the cortex ( $0.0054\% \pm 0.0012$ ) and dentate gyrus ( $0.0178 \pm 0.0136$ )  
18  
19 (Fig. 2A and B). However, widespread A $\beta$  deposition was apparent in the cerebral cortex and  
20  
21 dentate gyrus of APP/PS1 mice (Fig. 2E and F). Quantification confirmed that A $\beta$   
22  
23 immunopositivity was significantly higher in the cortex (Fig. 2D;  $p < 0.0001$ ) and dentate  
24  
25 gyrus (Fig. 2H;  $p < 0.0001$ ) of APP/PS1 mice compared to wild type controls.  
26  
27  
28  
29  
30

#### 31 *3.1.3.2 IRS-1 pSer<sup>616</sup> is elevated in brains of aged APP/PS1 mice*

32  
33 Representative micrographs illustrate increased levels of IRS-1 pSer<sup>616</sup> in the cerebral cortex  
34  
35 (Fig. 2M) and dentate gyrus (Fig. 2N) of APP/PS1 mice, compared to age-matched wild  
36  
37 types (Fig. 2I and J). Although distribution of IRS-1 pSer<sup>616</sup> staining was similar between  
38  
39 groups in both brain regions, staining intensity was greater in APP/PS1 mice (Fig. 2O)  
40  
41 compared to wild types (Fig. 2K). As such, quantification showed that IRS-1 pSer<sup>616</sup> was  
42  
43 significantly greater in the cortex (Fig. 2L;  $p = 0.0303$ ) and dentate gyrus (Fig. 2P;  $p = 0.0429$ )  
44  
45 of aged APP/PS1 mice compared to wild type controls. Pearson's correlation analysis  
46  
47 identified negative correlations between recognition index for the novel object in ORT and  
48  
49 IRS-1 pSer<sup>616</sup> immunopositivity in the cortex (Fig. 7A) dentate gyrus (Fig. 7B) of wild type  
50  
51 and APP/PS1 mice. Although the negative correlation between cortical IRS-1 pSer<sup>616</sup> staining  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 and ORT recognition index in APP/PS1 approached significance (Fig. 7A;  $r=-0.7744$ ,  
3  
4  $p=0.0706$ ) the negative trends between IRS-1 pSer<sup>616</sup> staining and ORT recognition index  
5  
6 remained insignificant in both brain regions of both genotypes.  
7  
8  
9

### 10 *3.1.3.3 Oxidative stress is comparable in brains of aged APP/PS1 and wild type mice*

11  
12 Representative micrographs shown in Fig. 3A-C and E-G illustrate the similarity in oxidative  
13  
14 stress levels between the brains of aged APP/PS1 mice and wild type controls. Quantitative  
15  
16 analysis demonstrated that 8-oxoguanine immunopositivity was not significantly different in  
17  
18 the cortex (Fig. 3D) or the dentate gyrus (Fig. 3H) of APP/PS1 mice compared to age-  
19  
20 matched wild type controls.  
21  
22  
23  
24

### 25 *3.1.3.4 Astrocytes are elevated in brains of aged APP/PS1 mice*

26  
27 Representative micrographs illustrate increased levels of GFAP-positive astrocytes in the  
28  
29 cerebral cortex (Fig. 3I and J) and dentate gyrus (Fig. 3M and N) of aged APP/PS1 mice  
30  
31 compared to wild type controls. Quantitative analysis revealed a significant increase in GFAP  
32  
33 immunopositivity in the cortex (Fig. 3L;  $p=0.0010$ ) and dentate gyrus (Fig. 3P;  $p=0.0007$ ),  
34  
35 compared to age-matched wild type mice.  
36  
37  
38  
39

### 40 *3.1.3.5 Synaptophysin is reduced in the polymorphic layer of the dentate gyrus of aged* 41 *APP/PS1 mice*

42  
43 Representative images illustrate reduced synaptophysin in the hippocampal polymorphic  
44  
45 layer of 15-18 month old APP/PS1 mice (Fig. 4D) compared to wild types (Fig. 4A;  
46  
47  $p=0.0338$ ). Synaptophysin staining was similar in all other layers of the hippocampus  
48  
49 between wild type and APP/PS1 mice and was not significantly different in the granule cell  
50  
51 (Fig. 4D), molecular layer (Fig. 4D), strata radiatum (Fig. 4E), pyramidale (Fig. 4E) or oriens  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 (Fig. 4E) of APP/PS1 mice, compared to wild type controls (Fig. 4A and B). Furthermore,  
3  
4 synaptophysin optical density did not differ significantly in the inner or outer (Fig. 4F)  
5  
6 cortical layers of APP/PS1 mice, compared to age-matched wild types (Fig. 4C).  
7  
8 Quantification confirmed that synaptophysin staining was reduced in the polymorphic layer  
9  
10 of APP/PS1 mice, but was comparable with wild types in all other layers of the hippocampus  
11  
12 and cortex (Fig. 4G).  
13

#### 14 15 16 *3.1.4 Peripheral insulin sensitivity and glucose tolerance and inflammatory and insulin* 17 18 *signaling gene expression in brains of aged APP/PS1 mice*

19  
20 Expression of GLP-1R, IKK $\beta$ , ERK2, mTOR, NF- $\kappa$ B1, PKC $\theta$  and TLR4 was comparable in  
21  
22 brains of aged APP/PS1 and wild type mice and genotype did not have a significant effect on  
23  
24 gene expression (Fig. 5A). Additional analysis comparing aged APP/PS1 mice with younger  
25  
26 C57Bl/6 mice (17-22 weeks old) identified a significant effect of genotype on gene  
27  
28 expression ([Supplementary Fig. 1A](#); ~~Fig. 5B~~;  $p < 0.0001$ ) and *post-hoc* analysis showed that  
29  
30 expression of IKK $\beta$  ( $p < 0.01$ ), ERK2 ( $p < 0.05$ ) and mTOR ( $p < 0.01$ ) was significantly down-  
31  
32 regulated and TLR4 ( $p < 0.05$ ) was up-regulated in brains of aged APP/PS1 mice, compared to  
33  
34 young C57Bl/6 controls. As illustrated in Fig. 5C, in response to an insulin sensitivity test, a  
35  
36 significant decrease in blood glucose over time was detected ( $p < 0.0001$ ), however genotype  
37  
38 had no significant effect on blood glucose levels. Similarly, glucose tolerance was  
39  
40 comparable in both groups and although time significantly affected blood glucose levels  
41  
42 ( $p < 0.0001$ ), genotype was not associated with a change in peripheral glucose tolerance (Fig.  
43  
44  
45 5D).  
46  
47  
48  
49

#### 50 *3.1.5 Cytokine levels in brains of aged APP/PS1 mice*

51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 Brain levels of IFN $\gamma$  ( $p=0.0015$ Fig. 6A;  $p=0.0046$ ), IL-1 $\beta$  ( $p<0.0001$ ) and IL-4 ( $p=0.0002$ Fig.  
3 ~~6F;  $p=0.0013$~~ ) were significantly impacted by genotype (Fig. 6A, C and F). *Post hoc* analysis  
4 ~~revealed that IFN $\gamma$  was significantly elevated~~ in brains of 15-18 month-old APP/PS1 mice  
5 ~~compared to age-matched ( $p<0.01$ ) and young ( $p<0.01$ ) wild type mice (Fig. 6A). A trend~~  
6 ~~towards elevated IL-1 $\beta$  was comparable detected~~ in the brains of APP/PS1 mice ~~and~~  
7 ~~compared to~~ age-matched wild types, however ~~a significant increase in IL-1 $\beta$  protein was~~  
8 ~~detected in the brains of APP/PS1 mice, compared to 17-22 week old wild type mice~~  
9 ~~this~~  
10 ~~failed to reach significance~~ (Fig. 6C;  $p=0.0965001$ ). Furthermore ~~Additional analysis~~  
11 ~~indicated that~~, IL-1 $\beta$  was significantly elevated in the brains of 15-18 month-old wild type  
12 ( $p<0.01$ ) and APP/PS1 ( $p<0.0001$ ) mice, compared to young wild types (Supplementary Fig.  
13 1B) (Fig. 6C;  $p<0.01$ ). A significant increase in IL-4 (Supplementary Fig. 1C;  $p<0.001$ ) and  
14 IFN $\gamma$  (Supplementary Fig. 1D;  $p<0.01$ ) was also detected in the brains of aged APP/PS1  
15 mice, compared to ~~age-matched ( $p<0.001$ ) and young ( $p<0.001$ ) wild type mice (Fig. 6F)~~. In  
16 addition, Pearson's correlation analysis identified a significant negative correlation between  
17 levels of IFN $\gamma$  and novel object recognition index in APP/PS1 mice (Fig. 7C;  $r=-0.8362$ ,  
18  $p=0.0381$ ), suggesting that higher levels of IFN $\gamma$  in the brain were associated with worse  
19 ORT performance in APP/PS1 mice. No significant correlations were identified between  
20 IFN $\gamma$  and IRS-1 pSer<sup>616</sup> immunopositivity in the cortex or dentate gyrus (Fig. 7D and E).

#### 4. Discussion

21 This study showed that peripheral glucose tolerance and insulin sensitivity were comparable  
22 between aged APP/PS1 and wild type mice, conflicting with a number of other studies (24,  
23 25). It has been suggested that 5/6 hours fasting is optimal for glucose and insulin tolerance  
24 tests, as this was sufficient for normalization of glucose levels and phosphorylation of insulin  
25 signaling proteins (26, 27). The current study performed glucose tolerance and insulin  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Formatted: Font: Not Italic

Formatted: Font: Italic

Formatted: Font: Not Italic

Formatted: Font: Italic



1  
2 sensitivity tests following an overnight fasting period, so it is possible that results presented  
3  
4 here reflect an exaggerated suppression of basal glucose levels in mice as a result of  
5  
6 prolonged fasting. This suggestion is supported by Jimenez-Palomares *et al.* (28) who also  
7  
8 found that glucose tolerance and insulin sensitivity were not significantly different in 8  
9  
10 month-old APP/PS1 mice, compared to wild types following overnight fasting periods.  
11  
12 Future studies should avoid overnight fasting prior to glucose and insulin tolerance tests in  
13  
14 order to achieve optimal normalization of metabolic parameters and to avert potentially  
15  
16 dangerous hypoglycemic effects of insulin. Other reports suggest that insulin insensitivity  
17  
18 and glucose intolerance also exists in aged animals (29-31), including C57Bl/6 mice (32-34);  
19  
20 a possible explanation for the similarity between APP/PS1 mice and controls in the present  
21  
22 study. To better understand the impact of central insulin resistance on global insulin  
23  
24 utilisation in the APP/PS1 model, future studies should assess the impact of hypothalamic  
25  
26 insulin administration alone and in combination with insulin sensitising drugs, such as  
27  
28 metformin, in hyperinsulinemic euglycemic clamp models.  
29  
30

31 Recognition memory was impaired in APP/PS1 mice here, consistent with several  
32  
33 other studies (35-37). However, since wild type controls also exhibited impaired recognition  
34  
35 memory, the deficits may be related to advanced age, rather than the APP/PS1 genotype; a  
36  
37 suggestion supported by other studies reporting recognition memory deficits in aged C57Bl/6  
38  
39 mice (38, 39). Another study found several indications of cognitive dysfunction in 18-20  
40  
41 month-old C57Bl/6 mice, including impaired novel location memory, but not object  
42  
43 recognition memory (40). Spatial learning was impaired in aged APP/PS1 mice, in agreement  
44  
45 with other studies (41, 42). Spatial memory recall was impaired in APP/PS1 mice and wild  
46  
47 type mice, similar to Barreto *et al.* (43), who showed that spatial learning and memory were  
48  
49 impaired in 18 month-old C57Bl/6 mice. Other reports have highlighted age-related decline  
50  
51 in learning and memory in C57Bl/6 mice (44, 45) consistent with the findings of the present  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 study, providing further evidence that there exists age-related deterioration of cognitive  
3 function in C57Bl/6 mice. Learning in the reversal water maze task was impaired in aged  
4 APP/PS1 mice, compared to controls, while both APP/PS1 mice and wild types failed to  
5 recognize the reversal target quadrant. Some (46), but not others (47) have shown that  
6 reversal learning and memory are impaired in APP/PS1 mice. Results presented here, suggest  
7 that reversal learning is a cognitive domain that is especially vulnerable to the effects of AD  
8 pathology in aged mice.  
9

10  
11 Amyloid- $\beta$  (A $\beta$ ) deposits were detected throughout the brains of 15-18 month-old  
12 APP/PS1 mice, while A $\beta$  was undetectable in wild type controls. APP/PS1 mice develop  
13 plaque deposition by 6 months of age, which progressively worsens, leading to abundant and  
14 widespread A $\beta$  plaque pathology by the age of 14 months (48, 49). The finding that A $\beta$   
15 deposition was significant in APP/PS1 brains and absent from wild types suggests that the  
16 spatial memory deficits in both groups were not directly related to A $\beta$  burden.  
17

18  
19 Oxidative stress levels were similar between APP/PS1 and wild type mice in the  
20 cortex and dentate gyrus. This was unexpected given previous reports showing elevated  
21 oxidative damage in brains of aged APP/PS1 mice (50-52). However, since aging is  
22 associated with accumulation of oxidative stress in the brain (53, 54), results presented here  
23 may reflect age-related accumulation of oxidative DNA damage in both APP/PS1 and wild  
24 type mice.  
25

26  
27 IRS-1 pSer<sup>616</sup> was increased in brains of APP/PS1 mice, as has also been observed in  
28 AD patients (18, 19, 55) and in experimental models (55, 56). The findings of the present  
29 study corroborate those of Talbot *et al.* (18) that demonstrated elevated IRS-1 pSer<sup>616</sup> in the  
30 hippocampus of APP/PS1 mice. IRS-1 pSer<sup>616</sup> has been shown to robustly correlate with  
31 cognitive impairment and brain insulin resistance associated with AD (18) and is likely  
32 related to the cognitive impairment in APP/PS1 mice here. It is interesting to note that the  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 increased brain insulin resistance in aged APP/PS1 mice was apparent in the absence of any  
3  
4 significant indications of peripheral insulin insensitivity or glucose intolerance.  
5

6 Astrocytes were increased in the cortex and dentate gyrus of APP/PS1 mice,  
7  
8 consistent with previous reports (50, 57). Neuroinflammation and glial cell proliferation,  
9  
10 recruitment and activation is a commonly associated with AD pathology (13). The fact that  
11  
12 A $\beta$  deposition remained substantial in the brains of APP/PS1 mice suggests that clearance of  
13  
14 A $\beta$  was minimal, providing support for the proposal that astrocyte function is defective in  
15  
16 AD (58). ~~E~~Although expression of ~~several~~ inflammatory and insulin signaling genes was  
17  
18 similar in brains of aged APP/PS1 mice and age-matched wild type controls, ~~comparison~~  
19  
20 ~~with younger control mice showed that IKK $\beta$ , ERK2 and mTOR were reduced, while TLR4~~  
21  
22 ~~was increased in brains of APP/PS1 mice compared to young wild types.~~ It has been shown  
23  
24 previously that expression of TLR4 is up-regulated in brains of APP/PS1 and wild type mice  
25  
26 in an age-related manner (59) and the present report provides further evidence that TLR4  
27  
28 expression in brain is increased with normal aging, to levels comparable with APP/PS1 mice.  
29  
30 Th1 cytokine IFN $\gamma$  was elevated in brains of APP/PS1 mice compared to ~~young and old~~ wild  
31  
32 types, in agreement with another study that showed age-related enhancement of IFN $\gamma$  in  
33  
34 brains of AD mice from 3 to 19 months of age (60). It has also been shown that IFN $\gamma$  has  
35  
36 opposing functions in AD brain, whereby overexpression of IFN $\gamma$  in the hippocampus  
37  
38 augments neuroinflammation and worsens A $\beta$  burden, but abrogates tau pathology and  
39  
40 enhances synaptic markers and neurogenesis (61). Further experimentation should determine  
41  
42 whether the increased IFN $\gamma$  in brains of APP/PS1 mice here represents a component of  
43  
44 pathogenic neuroinflammation or an up-regulation of protective processes. A significant  
45  
46 negative correlation was identified here between IFN $\gamma$  levels and novel object recognition  
47  
48 memory in APP/PS1 mice, in line with a recent study demonstrating improved hippocampal  
49  
50 synaptic plasticity and cognitive performance in mice deficient for IFN $\gamma$  (62), suggesting that  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 increased IFN $\gamma$  in the brain may impair cognitive function in aged APP/PS1 mice. The  
3  
4 increase in IFN $\gamma$  is mirrored by a comparable increase in anti-inflammatory IL-4 in brains of  
5  
6 APP/PS1 mice, which likely reflects an attempt to suppress the Th1 response. Interestingly  
7  
8 IFN $\gamma$  has also been implicated in attenuation of insulin signaling (63) and may be similarly  
9  
10 associated with the brain insulin resistance in the present study. Although we failed to detect  
11  
12 significant correlations between brain levels of IFN $\gamma$  and IRS-1 pSer<sup>616</sup>, this potential  
13  
14 mechanism certainly warrants further exploration.  
15

16  
17 Previous studies have detected increased IL-1 $\beta$  in the brains of APP/PS1 mice (64,  
18  
19 ~~65) in agreement with the present report. The present report, however, detected a non-~~  
20  
21 ~~significant trend towards an increase in IL-1 $\beta$  in the brains of APP/PS1 mice, possibly due to~~  
22  
23 ~~a parallel, age-related elevation of IL-1 $\beta$  in wild-type mice. It has been demonstrated that A $\beta$~~   
24  
25 ~~stimulates IL-1 $\beta$  production and secretion via NLRP3-dependent cleavage of pro-IL-1 $\beta$  by~~  
26  
27 ~~caspase 1 (66, 67). It is also interesting to note that IL-1 $\beta$  is the only one of all cytokines~~  
28  
29 ~~measured that was increased in both aged APP/PS1 and wild-type mice, compared to young~~  
30  
31 ~~wild types.~~ This suggests that IL-1 $\beta$  is involved with neuroinflammation that accompanies  
32  
33 normal aging, while IFN $\gamma$  and IL-4 are not part of the normal process of aging, but are  
34  
35 components of the neuroinflammatory processes associated with AD, since these were  
36  
37 elevated in aged APP/PS1 mice, compared to both young and old wild types.  
38  
39

40  
41 Expression of mTOR and ERK2 was ~~reduced-comparable~~ in brains of aged APP/PS1  
42  
43 mice, compared to ~~young~~ wild types. Extracellular signal-regulated kinase 2 (ERK2)  
44  
45 signaling facilitates learning and memory (68, 69), suggesting that impaired cognitive  
46  
47 function in aged mice may be due, in part to reduced expression of ERK2 in the brain.  
48  
49 Dineley *et al.* (70) showed that A $\beta$  reduces ERK2 activity and that ERK2 expression is  
50  
51 down-regulated in brains of 20 month-old AD mice, ~~similar to results presented here.~~  
52  
53 Similarly, dysregulation of signaling downstream of mTOR has been demonstrated in post-  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 mortem brain tissue from AD patients (71), ~~consistent with the reduced mTOR expression in~~  
3 ~~APP/PS1 brain shown here~~. Signaling through mTOR contributes to synaptic plasticity,  
4  
5 learning and memory (72, 73). It has also been shown that insulin promotes neurogenesis,  
6  
7 dendrite and synapse formation by signaling through IRS-mediated activation of mTOR (74,  
8  
9 75). Amyloid- $\beta$  ( $A\beta$ ) perturbs mTOR signaling in neurons (76) and mTOR inhibition impairs  
10  
11 hippocampal LTP in an AD mouse model (77). Our results suggest that expression of insulin  
12 signaling components is comparable in aged APP/PS1 and wild-type mice. Disruption of  
13 insulin signaling in the brain may be involved in the pathophysiology of AD and may  
14 contribute to cognitive impairments associated with aging, a proposition that should be  
15 further probed in future studies.  
16  
17  
18  
19  
20  
21  
22

23         Synaptophysin staining was reduced in the polymorphic layer of the dentate gyrus of  
24  
25 APP/PS1 mice. Several previous studies have shown that synapse density is decreased in the  
26  
27 brains of APP/PS1 mice (78-80). These studies did not consider the discrete cellular layers of  
28  
29 the hippocampus and may have overlooked subtle variation in synapse density between  
30  
31 subregions (78-80). However, one report showed that synaptophysin levels in the  
32  
33 hippocampus of 7 and 17 month-old APP/PS1 mice were similar to age-matched wild types  
34  
35 (81), which more closely aligns with the findings of the present study. It has also been shown  
36  
37 in Tg2576 mice, that synaptophysin levels were no different from controls at 3, 9, 14 and 19  
38  
39 months of age (82), while Xu *et al.* (45) reported age-related decline in hippocampal synaptic  
40  
41 spine density in C57Bl/6 mice. Results of the present report reflect a similar pattern, with  
42  
43 synapse density being comparable to wild types in 15-18 month-old APP/PS1 mice. Since  
44  
45 synapse density in the polymorphic layer was reduced in aged transgenic mice, it is  
46  
47 reasonable to suggest that this subregion was selectively susceptible to synaptotoxicity  
48  
49 associated with AD neuropathology.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 A limitation of the present study is the absence of young cohorts of wild type and  
3 APP/PS1 mice. Based on evidence from the literature, it is likely that peripheral insulin  
4 sensitivity, cognitive function and synapse density were influenced by aging. Future studies  
5 should include groups of young wild type and transgenic mice in order to more robustly  
6 characterize the differences between AD pathology and changes associated with normal  
7 aging, throughout the lifecourse. Another limitation of the present report is that cytokines and  
8 mRNA were measured in whole hemi-brains, while analysis of immunohistochemistry was  
9 performed on brain sections allowing for quantification within discrete brain regions. This  
10 means that comparing the results of our biochemical assays with our immunohistochemical  
11 data is difficult. Future studies will analyse mRNA and associated proteins and activation  
12 states in discrete brain regions to allow for more accurate demarcation of differences between  
13 APP/PS1 mice and wild types with regard to insulin signaling dysregulation and  
14 inflammation the brain.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28

29 Nevertheless, this study has demonstrated memory deficits and neuroinflammation in  
30 aged APP/PS1 and wild type mice. Astrocyte accumulation, IL-1 $\beta$  and IRS-1 pSer<sup>616</sup> levels  
31 were increased in the brain of APP/PS1 mice in the absence of systemic insulin insensitivity  
32 or glucose intolerance. Pharmacological agents targeting impaired insulin signaling and  
33 inflammation in the brain may prove efficacious in treating AD, a suggestion requiring  
34 further investigation.  
35  
36  
37  
38  
39  
40  
41  
42  
43

#### 44 **Acknowledgements**

45 Some of the data has been submitted, in abstract form, to the 2017 Southern California  
46 Alzheimer's Disease Centers Research Symposium at the University of California, Beckman  
47 Center of the National Academies of Sciences & Engineering, 100 Academy Way, Irvine,  
48 CA, USA 92617  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## **Funding**

This work was supported by the Department of Education and Learning, Northern Ireland.

## **Declaration / Conflict of Interest**

All authors declare that there is no duality of interest associated with their contribution to this manuscript.

## **Author Contribution Statement**

PMcC conceived the study, participated in the analysis and interpretation of data, drafted the manuscript and revised it critically for intellectual content. PD participated in data generation, analysis and interpretation and drafted the manuscript and revised it critically for intellectual content. AE participated in data generation and analysis. All authors approved the final version of the manuscript. PD is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## **References**

1. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* (London, England). 2016;388(10053):1459-544.
2. Querfurth HW, LaFerla FM. Alzheimer's disease. *The New England journal of medicine*. 2010;362(4):329-44.
3. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 2011;7(3):263-9.
4. De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. *Sub-cellular biochemistry*. 2012;65:329-52.

- 1  
2 5. Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age,  
3 neuropathology, and dementia. *The New England journal of medicine*.  
4 2009;360(22):2302-9.
- 5 6. Malek-Ahmadi M, Perez SE, Chen K, Mufson EJ. Neuritic and Diffuse Plaque  
6 Associations with Memory in Non-Cognitively Impaired Elderly. *Journal of Alzheimer's*  
7 *disease : JAD*. 2016;53(4):1641-52.
- 8 7. Meraz-Rios MA, Toral-Rios D, Franco-Bocanegra D, Villeda-Hernandez J, Campos-  
9 Pena V. Inflammatory process in Alzheimer's Disease. *Front Integr Neurosci*. 2013;7:59.
- 10 8. Rezai-Zadeh K, Gate D, Town T. CNS infiltration of peripheral immune cells: D-  
11 Day for neurodegenerative disease? *Journal of neuroimmune pharmacology : the official*  
12 *journal of the Society on NeuroImmune Pharmacology*. 2009;4(4):462-75.
- 13 9. Schilling M, Besselmann M, Leonhard C, Mueller M, Ringelstein EB, Kiefer R.  
14 Microglial activation precedes and predominates over macrophage infiltration in  
15 transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone  
16 marrow chimeric mice. *Experimental neurology*. 2003;183(1):25-33.
- 17 10. Fu Y, Hsiao JT, Paxinos G, Halliday GM, Kim WS. ABCA7 Mediates Phagocytic  
18 Clearance of Amyloid-beta in the Brain. *Journal of Alzheimer's disease : JAD*.  
19 2016;54(2):569-84.
- 20 11. El-Shimy IA, Heikal OA, Hamdi N. Minocycline attenuates Abeta oligomers-  
21 induced pro-inflammatory phenotype in primary microglia while enhancing Abeta  
22 fibrils phagocytosis. *Neuroscience letters*. 2015;609:36-41.
- 23 12. Leszek J, Barreto GE, Gasiorowski K, Koutsouraki E, Avila-Rodrigues M, Aliev G.  
24 Inflammatory Mechanisms and Oxidative Stress as Key Factors Responsible for  
25 Progression of Neurodegeneration: Role of Brain Innate Immune System. *CNS &*  
26 *neurological disorders drug targets*. 2016;15(3):329-36.
- 27 13. Steardo L, Jr., Bronzuoli MR, Iacomino A, Esposito G, Steardo L, Scuderi C. Does  
28 neuroinflammation turn on the flame in Alzheimer's disease? Focus on astrocytes.  
29 *Frontiers in neuroscience*. 2015;9:259.
- 30 14. Gabuzda D, Yankner BA. Physiology: Inflammation links ageing to the brain.  
31 *Nature*. 2013;497(7448):197-8.
- 32 15. Maher FO, Martin DS, Lynch MA. Increased IL-1beta in cortex of aged rats is  
33 accompanied by downregulation of ERK and PI-3 kinase. *Neurobiology of aging*.  
34 2004;25(6):795-806.
- 35 16. Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, et al.  
36 Exaggerated neuroinflammation and sickness behavior in aged mice following  
37 activation of the peripheral innate immune system. *FASEB journal : official publication*  
38 *of the Federation of American Societies for Experimental Biology*. 2005;19(10):1329-  
39 31.
- 40 17. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, et al.  
41 Complement and microglia mediate early synapse loss in Alzheimer mouse models.  
42 *Science (New York, NY)*. 2016;352(6286):712-6.
- 43 18. Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, et al. Demonstrated  
44 brain insulin resistance in Alzheimer's disease patients is associated with IGF-1  
45 resistance, IRS-1 dysregulation, and cognitive decline. *The Journal of clinical*  
46 *investigation*. 2012;122(4):1316-38.
- 47 19. Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H,  
48 et al. An anti-diabetes agent protects the mouse brain from defective insulin signaling  
49 caused by Alzheimer's disease- associated Abeta oligomers. *The Journal of clinical*  
50 *investigation*. 2012;122(4):1339-53.
- 51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
20. Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiology of aging*. 2010;31(2):224-43.
  21. Liu Y, Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. *The Journal of pathology*. 2011;225(1):54-62.
  22. McClean PL, Parthasarathy V, Faivre E, Holscher C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011;31(17):6587-94.
  23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>(-Delta Delta C(T))</sup> Method. *Methods (San Diego, Calif)*. 2001;25(4):402-8.
  24. Clarke JR, Lyra ESNM, Figueiredo CP, Frozza RL, Ledo JH, Beckman D, et al. Alzheimer-associated Abeta oligomers impact the central nervous system to induce peripheral metabolic deregulation. *EMBO molecular medicine*. 2015;7(2):190-210.
  25. Pedros I, Petrov D, Allgaier M, Sureda F, Barroso E, Beas-Zarate C, et al. Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. *Biochimica et biophysica acta*. 2014;1842(9):1556-66.
  26. Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *American journal of physiology Endocrinology and metabolism*. 2008;295(6):E1323-32.
  27. Agouni A, Owen C, Czopek A, Mody N, Delibegovic M. In vivo differential effects of fasting, re-feeding, insulin and insulin stimulation time course on insulin signaling pathway components in peripheral tissues. *Biochemical and biophysical research communications*. 2010;401(1):104-11.
  28. Jimenez-Palomares M, Ramos-Rodriguez JJ, Lopez-Acosta JF, Pacheco-Herrero M, Lechuga-Sancho AM, Perdomo G, et al. Increased Abeta production prompts the onset of glucose intolerance and insulin resistance. *American journal of physiology Endocrinology and metabolism*. 2012;302(11):E1373-80.
  29. Catalano KJ, Bergman RN, Ader M. Increased susceptibility to insulin resistance associated with abdominal obesity in aging rats. *Obesity research*. 2005;13(1):11-20.
  30. Yamamoto M, Otsuki M. Effect of inhibition of alpha-glucosidase on age-related glucose intolerance and pancreatic atrophy in rats. *Metabolism: clinical and experimental*. 2006;55(4):533-40.
  31. Romanatto T, Fiamoncini J, Wang B, Curi R, Kang JX. Elevated tissue omega-3 fatty acid status prevents age-related glucose intolerance in fat-1 transgenic mice. *Biochimica et biophysica acta*. 2014;1842(2):186-91.
  32. Houtkooper RH, Argmann C, Houten SM, Canto C, Jenning EH, Andreux PA, et al. The metabolic footprint of aging in mice. *Scientific reports*. 2011;1:134.
  33. Lipina C, Vaanholt LM, Davidova A, Mitchell SE, Storey-Gordon E, Hambly C, et al. CB1 receptor blockade counters age-induced insulin resistance and metabolic dysfunction. *Aging cell*. 2016;15(2):325-35.
  34. Park D, Lee EK, Jang EJ, Jeong HO, Kim BC, Ha YM, et al. Identification of the dichotomous role of age-related LCK in calorie restriction revealed by integrative analysis of cDNA microarray and interactome. *Age (Dordrecht, Netherlands)*. 2013;35(4):1045-60.

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
  - 61
  - 62
  - 63
  - 64
  - 65
35. Gu XH, Xu LJ, Liu ZQ, Wei B, Yang YJ, Xu GG, et al. The flavonoid baicalein rescues synaptic plasticity and memory deficits in a mouse model of Alzheimer's disease. *Behavioural brain research*. 2016;311:309-21.
36. Yang YJ, Zhao Y, Yu B, Xu GG, Wang W, Zhan JQ, et al. GluN2B-containing NMDA receptors contribute to the beneficial effects of hydrogen sulfide on cognitive and synaptic plasticity deficits in APP/PS1 transgenic mice. *Neuroscience*. 2016;335:170-83.
37. Zhou D, Liu H, Li C, Wang F, Shi Y, Liu L, et al. Atorvastatin ameliorates cognitive impairment, Abeta1-42 production and Tau hyperphosphorylation in APP/PS1 transgenic mice. *Metabolic brain disease*. 2016;31(3):693-703.
38. Qiu J, Dunbar DR, Noble J, Cairns C, Carter R, Kelly V, et al. Decreased Npas4 and Arc mRNA Levels in the Hippocampus of Aged Memory-Impaired Wild-Type But Not Memory Preserved 11beta-HSD1 Deficient Mice. *Journal of neuroendocrinology*. 2016;28(1).
39. Mechan AO, Wyss A, Rieger H, Mohajeri MH. A comparison of learning and memory characteristics of young and middle-aged wild-type mice in the IntelliCage. *Journal of neuroscience methods*. 2009;180(1):43-51.
40. Benice TS, Rizk A, Kohama S, Pfankuch T, Raber J. Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity. *Neuroscience*. 2006;137(2):413-23.
41. Fol R, Braudeau J, Ludewig S, Abel T, Weyer SW, Roederer JP, et al. Viral gene transfer of APP $\alpha$  rescues synaptic failure in an Alzheimer's disease mouse model. *Acta neuropathologica*. 2016;131(2):247-66.
42. Xiao Q, Shi R, Yang W, Zou Y, Du Y, Zhang M, et al. Time-Dependent Increase of Chitinase1 in APP/PS1 Double Transgenic Mice. *Neurochemical research*. 2016;41(7):1604-11.
43. Barreto G, Huang TT, Giffard RG. Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. *Journal of neurosurgical anesthesiology*. 2010;22(3):214-9.
44. Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Molecular brain*. 2016;9:11.
45. Xu B, Sun A, He Y, Qian F, Liu L, Chen Y, et al. Running-induced memory enhancement correlates with the preservation of thin spines in the hippocampal area CA1 of old C57BL/6 mice. *Neurobiology of aging*. 2017;52:106-16.
46. Cheng D, Low JK, Logge W, Garner B, Karl T. Novel behavioural characteristics of female APP<sup>Swe</sup>/PS1<sup>DeltaE9</sup> double transgenic mice. *Behavioural brain research*. 2014;260:111-8.
47. O'Leary TP, Brown RE. Visuo-spatial learning and memory deficits on the Barnes maze in the 16-month-old APP<sup>swe</sup>/PS1<sup>dE9</sup> mouse model of Alzheimer's disease. *Behavioural brain research*. 2009;201(1):120-7.
48. Gallagher JJ, Minogue AM, Lynch MA. Impaired performance of female APP/PS1 mice in the Morris water maze is coupled with increased Abeta accumulation and microglial activation. *Neuro-degenerative diseases*. 2013;11(1):33-41.
49. McManus RM, Higgins SC, Mills KH, Lynch MA. Respiratory infection promotes T cell infiltration and amyloid-beta deposition in APP/PS1 mice. *Neurobiology of aging*. 2014;35(1):109-21.
50. Duffy AM, Holscher C. The incretin analogue D-Ala2GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience*. 2013;228:294-300.

- 1
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9
  - 10
  - 11
  - 12
  - 13
  - 14
  - 15
  - 16
  - 17
  - 18
  - 19
  - 20
  - 21
  - 22
  - 23
  - 24
  - 25
  - 26
  - 27
  - 28
  - 29
  - 30
  - 31
  - 32
  - 33
  - 34
  - 35
  - 36
  - 37
  - 38
  - 39
  - 40
  - 41
  - 42
  - 43
  - 44
  - 45
  - 46
  - 47
  - 48
  - 49
  - 50
  - 51
  - 52
  - 53
  - 54
  - 55
  - 56
  - 57
  - 58
  - 59
  - 60
  - 61
  - 62
  - 63
  - 64
  - 65
51. Jin JL, Liou AK, Shi Y, Yin KL, Chen L, Li LL, et al. CART treatment improves memory and synaptic structure in APP/PS1 mice. *Scientific reports*. 2015;5:10224.
52. Yun HM, Jin P, Park KR, Hwang J, Jeong HS, Kim EC, et al. Thiocremone Potentiates Anti-Oxidant Effects to Improve Memory Dysfunction in an APP/PS1 Transgenic Mice Model. *Molecular neurobiology*. 2016;53(4):2409-20.
53. Chakrabarti S, Munshi S, Banerjee K, Thakurta IG, Sinha M, Bagh MB. Mitochondrial Dysfunction during Brain Aging: Role of Oxidative Stress and Modulation by Antioxidant Supplementation. *Aging and disease*. 2011;2(3):242-56.
54. Manczak M, Jung Y, Park BS, Partovi D, Reddy PH. Time-course of mitochondrial gene expressions in mice brains: implications for mitochondrial dysfunction, oxidative damage, and cytochrome c in aging. *Journal of neurochemistry*. 2005;92(3):494-504.
55. Yarchoan M, Toledo JB, Lee EB, Arvanitakis Z, Kazi H, Han LY, et al. Abnormal serine phosphorylation of insulin receptor substrate 1 is associated with tau pathology in Alzheimer's disease and tauopathies. *Acta neuropathologica*. 2014;128(5):679-89.
56. Zhang B, Tang XC, Zhang HY. Alternations of central insulin-like growth factor-1 sensitivity in APP/PS1 transgenic mice and neuronal models. *Journal of neuroscience research*. 2013;91(5):717-25.
57. Galea E, Morrison W, Hudry E, Arbel-Ornath M, Bacskai BJ, Gomez-Isla T, et al. Topological analyses in APP/PS1 mice reveal that astrocytes do not migrate to amyloid-beta plaques. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(51):15556-61.
58. Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM. The effect of amyloid associated proteins on the expression of genes involved in amyloid-beta clearance by adult human astrocytes. *Experimental neurology*. 2012;233(1):373-9.
59. Lopez-Gonzalez I, Schluter A, Aso E, Garcia-Esparcia P, Ansoleaga B, F LL, et al. Neuroinflammatory signals in Alzheimer disease and APP/PS1 transgenic mice: correlations with plaques, tangles, and oligomeric species. *Journal of neuropathology and experimental neurology*. 2015;74(4):319-44.
60. Abbas N, Bednar I, Mix E, Marie S, Paterson D, Ljungberg A, et al. Up-regulation of the inflammatory cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP(SWE) transgenic mice. *Journal of neuroimmunology*. 2002;126(1-2):50-7.
61. Mastrangelo MA, Sudol KL, Narrow WC, Bowers WJ. Interferon- $\gamma$  differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. *The American journal of pathology*. 2009;175(5):2076-88.
62. Monteiro S, Ferreira FM, Pinto V, Roque S, Morais M, de Sa-Calcada D, et al. Absence of IFN $\gamma$  promotes hippocampal plasticity and enhances cognitive performance. *Translational psychiatry*. 2016;6:e707.
63. McGillicuddy FC, Chiquoine EH, Hinkle CC, Kim RJ, Shah R, Roche HM, et al. Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. *The Journal of biological chemistry*. 2009;284(46):31936-44.
64. Xuan AG, Pan XB, Wei P, Ji WD, Zhang WJ, Liu JH, et al. Valproic acid alleviates memory deficits and attenuates amyloid-beta deposition in transgenic mouse model of Alzheimer's disease. *Molecular neurobiology*. 2015;51(1):300-12.
65. Guo HB, Cheng YF, Wu JG, Wang CM, Wang HT, Zhang C, et al. Donepezil improves learning and memory deficits in APP/PS1 mice by inhibition of microglial activation. *Neuroscience*. 2015;290:530-42.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
66. Salminen A, Ojala J, Suuronen T, Kaarniranta K, Kauppinen A. Amyloid-beta oligomers set fire to inflammasomes and induce Alzheimer's pathology. *Journal of cellular and molecular medicine*. 2008;12(6a):2255-62.
  67. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493(7434):674-8.
  68. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. *Nature reviews Neuroscience*. 2004;5(3):173-83.
  69. Satoh Y, Endo S, Ikeda T, Yamada K, Ito M, Kuroki M, et al. Extracellular signal-regulated kinase 2 (ERK2) knockdown mice show deficits in long-term memory; ERK2 has a specific function in learning and memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007;27(40):10765-76.
  70. Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD. Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: In vitro and in vivo mechanisms related to Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21(12):4125-33.
  71. Yates SC, Zafar A, Hubbard P, Nagy S, Durant S, Bicknell R, et al. Dysfunction of the mTOR pathway is a risk factor for Alzheimer's disease. *Acta neuropathologica communications*. 2013;1:3.
  72. Sosanya NM, Cacheaux LP, Workman ER, Niere F, Perrone-Bizzozero NI, Raab-Graham KF. Mammalian Target of Rapamycin (mTOR) Tagging Promotes Dendritic Branch Variability through the Capture of Ca<sup>2+</sup>/Calmodulin-dependent Protein Kinase II alpha (CaMKIIalpha) mRNAs by the RNA-binding Protein HuD. *The Journal of biological chemistry*. 2015;290(26):16357-71.
  73. Garza-Lombo C, Gonsebatt ME. Mammalian Target of Rapamycin: Its Role in Early Neural Development and in Adult and Aged Brain Function. *Frontiers in cellular neuroscience*. 2016;10:157.
  74. Lee CC, Huang CC, Hsu KS. Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 signaling pathways. *Neuropharmacology*. 2011;61(4):867-79.
  75. Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, et al. Ezh2 regulates adult hippocampal neurogenesis and memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014;34(15):5184-99.
  76. Chen TJ, Wang DC, Chen SS. Amyloid-beta interrupts the PI3K-Akt-mTOR signaling pathway that could be involved in brain-derived neurotrophic factor-induced Arc expression in rat cortical neurons. *Journal of neuroscience research*. 2009;87(10):2297-307.
  77. Ma T, Hoeffler CA, Capetillo-Zarate E, Yu F, Wong H, Lin MT, et al. Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. *PloS one*. 2010;5(9).
  78. Liu SJ, Yang C, Zhang Y, Su RY, Chen JL, Jiao MM, et al. Neuroprotective effect of beta-asarone against Alzheimer's disease: regulation of synaptic plasticity by increased expression of SYP and GluR1. *Drug design, development and therapy*. 2016;10:1461-9.
  79. Ostapchenko VG, Chen M, Guzman MS, Xie YF, Lavine N, Fan J, et al. The Transient Receptor Potential Melastatin 2 (TRPM2) Channel Contributes to beta-Amyloid Oligomer-Related Neurotoxicity and Memory Impairment. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2015;35(45):15157-69.

1  
2 80. Zhang Y, Huang LJ, Shi S, Xu SF, Wang XL, Peng Y. L-3-n-butylphthalide Rescues  
3 Hippocampal Synaptic Failure and Attenuates Neuropathology in Aged APP/PS1 Mouse  
4 Model of Alzheimer's Disease. *CNS neuroscience & therapeutics*. 2016.

5 81. Minkeviciene R, Ihalainen J, Malm T, Matilainen O, Keksa-Goldsteine V,  
6 Goldsteins G, et al. Age-related decrease in stimulated glutamate release and vesicular  
7 glutamate transporters in APP/PS1 transgenic and wild-type mice. *Journal of*  
8 *neurochemistry*. 2008;105(3):584-94.

9 82. King DL, Arendash GW. Maintained synaptophysin immunoreactivity in Tg2576  
10 transgenic mice during aging: correlations with cognitive impairment. *Brain research*.  
11 2002;926(1-2):58-68.  
12

### 13 **Figure Legends**

14  
15 **Figure 1. Learning and memory in aged APP/PS1 and wild type mice.** The acquisition

16  
17 training phase of the Morris water maze (MWM) involved four training sessions per day

18  
19 over four consecutive days, followed by a probe trial on the fifth day, 24 hours following the

20  
21 final training session. Escape latency during the training phase is shown (A), as is the

22  
23 proportion of time spent in each quadrant during the probe trial by 15-18 month-old wild

24  
25 type (solid line with circles; D) and APP/PS1 (dotted line with squares; G) mice. Reversal

26  
27 water maze acquisition training began 24 hours following the MWM probe trial and

28  
29 consisted of four consecutive days with four training sessions per day, followed by a reversal

30  
31 probe trial on the fifth day. Illustrated are training phase escape latency (B) and time spent in

32  
33 each quadrant during the reversal probe trial by wild type (E) and APP/PS1 (H) mice. For

34  
35 the novel object recognition task, recognition index, a measure of the percentage of time

36  
37 spent exploring either object, is illustrated in the acquisition phase (C) during exposure to

38  
39 two identical objects, and the test phase (F), in the presence of one familiar (black bars) and

40  
41 one novel (white bars) object. \* $p < 0.05$ , \*\* $p < 0.01$  APP/PS1 vs. wild type; ~~Data represent~~

42  
43 ~~mean  $\pm$  SEM for 13-15 mice per group,~~ two-way repeated measures ANOVA with

44  
45 Bonferroni's *post-hoc* test (A, B), ordinary one-way ANOVA with Dunnett's *post-hoc* test

46  
47 (D, E, G, H), multiple *t* tests with Holm-Šidák's *post-hoc* test (C, F). Data represent mean  $\pm$

48  
49 SEM for 13-15 mice per group.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 **Figure 2. A $\beta$  deposition and IRS-1 pSer<sup>616</sup> in the cerebral cortex and dentate gyrus of**  
5 **aged APP/PS1 and wild type mice.** Representative images (10x magnification) are shown  
6 that depict A $\beta$  staining in the cerebral cortex (A) and dentate gyrus (B) of 15-18 month old  
7 wild type mice and the cerebral cortex (E) and dentate gyrus (F) of age-matched APP/PS1  
8 mice. Also shown is an exemplary magnified image (20x magnification) of A $\beta$  staining in  
9 brains of wild type (C) and APP/PS1 (G) mice. Quantification of A $\beta$  immunopositivity in  
10 the cortex (D) and dentate gyrus (H) of 15-18 month old APP/PS1 and wild type mice is also  
11 shown. Representative images (20x magnification) are also shown that depict IRS-1 pSer<sup>616</sup>  
12 staining in the cerebral cortex (I) and dentate gyrus (J) of 15-18 month old wild type mice  
13 and the cerebral cortex (M) and dentate gyrus (N) of age-matched APP/PS1 mice. Also  
14 shown are exemplary magnified images (40x magnification) from wild type (K) and  
15 APP/PS1 (O) mice. Quantification of IRS-1 pSer<sup>616</sup> immunopositivity in cortex (L) and  
16 dentate gyrus (P) of 15-18 month old APP/PS1 and wild type mice is also illustrated.  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

\* $p < 0.05$ , \*\*\*\* $p < 0.0001$ , Student's  $t$  test. Data represent mean  $\pm$  SEM for 6 per group.

**Figure 3. Oxidative stress and astrocytes in the cerebral cortex and dentate gyrus of**  
**aged APP/PS1 and wild type mice.** Representative images (20x magnification) are shown  
that depict the 8-oxoguanine staining in cerebral cortex (A) and dentate gyrus (B) of 15-18  
month old wild type mice and cerebral cortex (E) and dentate gyrus (F) of age-matched  
APP/PS1 mice. Also shown are exemplary magnified images (40x magnification) from wild  
type (C) and APP/PS1 (G) mice. Quantification of 8-oxoguanine immunopositivity in cortex  
(D) and dentate gyrus (H) of 15-18 month old APP/PS1 and wild type mice is also  
illustrated. Representative images (20x magnification) are also shown that depict GFAP  
staining in the cerebral cortex (I) and dentate gyrus (J) of 15-18 month-old wild type mice

1  
2 and the cerebral cortex (M) and dentate gyrus (N) of age-matched APP/PS1 mice. Also  
3 shown are exemplary magnified images (100x magnification) from wild type (K) and  
4 APP/PS1 (O) mice. Quantification of GFAP immunopositivity in cortex (L) and dentate  
5 gyrus (P) of 15-18 month old APP/PS1 and wild type mice is also shown. \*\*\* $p < 0.001$   
6 Student's *t* test. Data represent mean  $\pm$  SEM for 6 per group.  
7  
8  
9  
10  
11  
12  
13

14 **Figure 4. Synapse density is decreased in the polymorphic layer of the dentate gyrus in**  
15 **APP/PS1 mice.** Illustrated are representative images depicting synaptophysin staining of  
16 brain sections from 15-18 month-old wild type (A, B, C) and APP/PS1 (D, E, F) mice. A  
17 and D show the polymorphic layer (PL), granule cell layer (GCL) and molecular layer (ML)  
18 of the dentate gyrus. C and D show the stratum radiatum (SR), stratum pyramidale (SP) and  
19 stratum oriens (SO) of the hippocampus, while B and E show the inner (IC) and outer (OC)  
20 cerebral cortex. Also illustrated is quantification of synaptophysin optical density values for  
21 the polymorphic layer, granule cell layer and molecular layer of the dentate gyrus and the  
22 stratum radiatum, stratum pyramidale and stratum oriens of the hippocampus, inner and outer  
23 cortex of 15-18 month-old APP/PS1 and wild type mice (G). \* $p < 0.05$  Student's *t* tests.  
24 Data represent mean  $\pm$  SEM for 6 per group.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39

40 **Figure 5. Peripheral insulin sensitivity, glucose tolerance and expression of**  
41 **inflammatory and insulin signaling genes in brains of aged APP/PS1 mice.** Illustrated is  
42 quantification of the expression of genes associated with inflammatory pathways and insulin  
43 signaling in brains of 15-18 month-old APP/PS1 mice (black bars), compared with age-  
44 matched wild type controls (white bars) (A). ~~Also shown is quantification of expression of~~  
45 ~~the same genes in aged APP/PS1 and wild type mice, compared with 17-22 week old wild~~  
46 ~~types (dark grey bars) (B).~~ Also shown are blood glucose levels following insulin injection  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 | (**BE**) and following glucose injection (**CD**). Wild type (solid line with circles) and APP/PS1  
3  
4 mice (dotted line with squares) aged 15-18 months were administered insulin or glucose via  
5  
6 i.p. injection and blood glucose levels were measured at 15, 30 and 60 minutes post-injection.  
7

8 | \* $p < 0.05$ , \*\* $p < 0.01$ . ~~Data represent mean  $\pm$  SEM for 5 per group,  $p$ , ordinary two-way~~  
9  
10 ANOVA with Holm-Šidák's *post-hoc* test (**A**, **B**) or 13-15 per group, two-way repeated  
11  
12 measures ANOVA with Holm-Šidák's *post-hoc* test (**BE**, **CD**). Data represent mean  $\pm$  SEM  
13  
14 for 5 per group.  
15  
16

17  
18  
19 **Figure 6. Cytokine levels in the brains of aged APP/PS1 and wild type mice.** MSD

20  
21 multiplex analysis of 8 cytokines was performed on supernatant extracted from brain tissue.  
22  
23 Protein levels of IFN $\gamma$  (**A**), IL-10 (**B**), IL-1 $\beta$  (**C**), IL-12p70 (**D**), IL-2 (**E**), IL-4 (**F**), IL-5 (**E**),  
24  
25 IL-6 (**G**) and KC/GRO (CXCL1) (**H**) were measured and compared between 15-18 month-

26  
27 old APP/PS1 mice (black bars) and, age-matched wild types (white bars) and younger mice,  
28  
29 aged 17-22 weeks (dark grey bars). \*\* $p < 0.01$ ; Student's  $t$  tests. \*\*\* $p < 0.001$ ,  
30  
31 \*\*\* $p < 0.0001$ . Data represent mean  $\pm$  SEM for 6 per group, ordinary one-way ANOVA  
32  
33 with Holm-Šidák's *post-hoc* test.  
34  
35

36  
37  
38 **Figure 7. Correlations between IFN $\gamma$ , IRS-1 pSer<sup>616</sup> and novel object recognition**

39  
40 **memory in aged APP/PS1 and wild type mice.** Pearson's correlation analysis was  
41  
42 performed between IRS-1 pSer<sup>616</sup> immunopositivity and novel object recognition index (**A**,  
43  
44 **B**), between IFN $\gamma$  and novel object recognition index (**C**) and between IFN $\gamma$  and IRS-1  
45  
46 pSer<sup>616</sup> immunopositivity (**D**, **E**) in wild type (open circles and dotted best fit line) and  
47  
48 APP/PS1 (black squares and solid best fit line) mice. Lines of best fit,  $r$  and  $p$  values were  
49  
50 also added to the graphs. Each data point represents an  $XY$  pair for a total of 6  $XY$  pairs per  
51  
52 genotype on each graph. Significance of correlation was determined using two-tailed  $t$  tests.  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Supplementary Figure 1. Cytokines and gene expression in brains of young wild type mice.** Quantification of expression of inflammatory and insulin signaling genes in the brains of young wild type mice (17-22 weeks old) is shown (black bars), compared to aged wild types (white bars) and APP/PS1 mice (dark grey bars) (A). Also illustrated are brain levels of IFN $\gamma$  (B), IL-1 $\beta$  (C) and IL-4 (D) in young wild type mice, compared with aged wild types and APP/PS1 mice. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, \*\*\*\* $p$ <0.0001; ordinary one-way ANOVA with Holm-Šidák's *post-hoc* test (A) and Student's *t* test (B-D). Data represent mean  $\pm$  SEM for 5 (A) or 6 (B-D) per group.

- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Bold
- Formatted: Font: Not Italic
- Formatted: Font: Not Bold