Dietary Protein Source Influences Brain Inflammation and Memory in a Male Senescence-Accelerated Mouse Model of Dementia

# Dietary Protein Source Influences Brain Inflammation and Memory in a Male Senescence-Accelerated Mouse Model of Dementia

Sabrina Petralla[1] • Cristina Parenti[1] • Valentina Ravaioli[1] • Irene Fancello[1] • Francesca Massenzio[1] • Marco Virgili[1] • Barbara Monti[1] • Emiliano Pena-Altamira[1,2]

[1] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

[2] Present address: Basic and Clinical Neuroscience Department, King's College London, Maurice Wohl Clinical Neuroscience Institute, London, UK

## Abstract

Dementia is a pathological condition characterized by a decline in memory, as well as in other cognitive and social functions. The cellular and molecular mechanisms of brain damage in dementia are not completely understood; however, neuroinflammation is involved. Evidence suggests that chronic inflammation may impair cognitive performance and that dietary protein source may differentially influence this process. Dietary protein source has previously been shown to modify systemic inflammation in mouse models. Thus, we aimed to investigate the effect of chronic dietary protein source substitution in an ageing and dementia male mouse model, the senescence-accelerated mouse-prone 8 (SAMP8) model. We observed that dietary protein source differentially modified memory as shown by inhibitory avoidance testing at 4 months of age. Also, dietary protein source differentially modified neuroinflammation and gliosis in male SAMP8 mice. Our results suggest that chronic dietary protein source substitution may influence brain ageing and memory-related mechanisms in male SAMP8 mice. Moreover, the choice of dietary protein source in mouse diets for experimental purposes may need to be carefully considered when interpreting results.

**Keywords** Diet · Ageing · Dementia · Neuroinflammation · Gliosis

## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| AMPK | AMP-activated protein kinase |
| BDNF | Brain-derived neurotrophic factor |
| CX3CL1 | Fractalkine |
| GAPDH | Glyceraldehyde 3-phosphate dehydrogenase |
| GFAP | Glial fibrillary acidic protein |
| IBA1 | Anti-ionized calcium-binding adapter molecule |
| IL | Interleukin |
| LC3B | Autophagy-related protein LC3B |
| MAG | Myelin-associated glycoprotein |
| MCI | Mild cognitive impairment |
| NDDs | Neurodegenerative diseases |
| OLIG2 | Oligodendrocyte transcription factor 2 |
| SAMP8 | Senescence-accelerated mouse-prone 8 |
| OPC | Oligodendrocyte precursor cell |
| SDS-PAGE | Sodium dodecyl sulphate polyacryalmide-gel electrophoresis |
| TGFB | Transforming growth factor beta |
| TGFBR1 | TGFB receptor 1 |
| TGFBR2 | TGFB receptor 2 |
| TNFA | Tumour necrosis factor alpha |
| TREM2 | Triggering receptor expressed on myeloid cells 2 |

## Introduction

Dementia is a condition characterized by memory loss, as well as by a decline in cognitive and social function. The most common cause of dementia is Alzheimer's disease (AD), which leads initially to the loss of short-term memory and eventually loss of long-term memory, as well as behavioural and emotional alterations [1]. It is often preceded by a prodromic state defined as mild cognitive impairment (MCI), displaying light cognitive alteration [2]. Increasing

evidence suggests the involvement of neuroinflammation in AD and other neurodegenerative diseases (NDDs). Several inflammatory mediators, including complement activators and inhibitors, reactive oxygen species, cytokines, chemokines, and inflammatory enzymes, are expressed and released by activated microglia, reactive astrocytes and neurons in AD [3–6]. Moreover, an early involvement of inflammation has been confirmed in the MCI phase [7].

Microglia are immune cells that have multiple roles during brain development, homeostasis and disease and are classically associated with neuroinflammation in brain diseases and infection [8, 9]. Moreover, microglia can be broadly classified into the M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes. M1 microglia produce interleukins (ILs) 1β and 6, tumour necrosis factor alpha (TNFA), as well as nitric oxide, superoxide and reactive oxygen species. By contrast, M2 microglia produce ILs 4, 10 and 13 as well as transforming growth factor beta (TGFB). Evidence suggests that M1/M2 subtypes are in a dynamic equilibrium in vivo [10].

Astrocytes are star-shaped cells that play multiple physiological roles in the brain parenchyma [11]. Reactive astrogliosis has been documented in AD brains [2]. It is characterized by astrocyte hypertrophy, proliferation and increased expression of intermediate filament proteins, such as glial fibrillary acidic protein (GFAP) [12]. While this response could be considered protective [13], reactive astrocytes may also exacerbate inflammation by releasing an array of cytokines, chemokines, complement proteins and nitric oxide [14].

Immune status can influence brain function in humans. The Hoorn Study evidenced that increased levels of inflammatory plasma markers (TNFA, IL-6, IL-8, C-reactive protein) were associated with cognitive decline [15]. Also, diet may influence immune status since a meta-analysis of 46 studies associated positively animal-based diets with increased levels of inflammatory biomarkers (mainly C-reactive protein), while plant-based diets showed no association [16]. In addition, dietary protein source has shown to play a role in inflammation. Substitution of soy protein for casein prevented an inflammatory response induced in the liver of rats fed a high fructose diet [17]. In addition, dietary soy protein protected a collagen-induced arthritis rat model by inhibiting the production of TNFA and IL-6 [18]. Also, wild-type mice fed a 50% casein-protein diet showed compromised capillary integrity and function in the blood-brain barrier and peripheral inflammation, when compared to wild-type mice fed a 50% soy-protein diet [19].

Among mouse models used to study brain ageing and age-associated cognitive decline, senescence-accelerated mouse-prone 8 (SAMP8) mice have been widely used [20–22]. SAMP8 mice are non-transgenic mice that show accelerated ageing and therefore reduced lifespan [23]. They display a peak in muscle mass at 7 months and a gradual decline, due to muscle atrophy, at 8 months [24, 25]. SAMP8 mice manifest cognitive defects in the Morris water maze at 8 months of age [20] and at 4 months of age in the passive avoidance task [26], as well as reduced anxiety-like behaviour in the elevated plus-maze test at 4 months of age [21]. Moreover, SAMP8 mice display astrogliosis, microglial activation, tau hyperphosphorylation and beta-amyloid deposition [27–30].

In this study, we aimed to investigate whether different dietary protein sources at a standard commercial mouse feed percentage (18% of the total diet) could influence brain inflammation and memory in male SAMP8 mice and support the use of nutritional intervention strategies for the prevention/delay of age-associated cognitive decline and dementia.

## Material and Methods

### Animals and Dietary Treatments

SAMP8 mice were obtained from Envigo Laboratories (Huntingdon, Cambridgeshire, UK). The colony was maintained by mating littermates. Mice were kept in a humidified and temperature-controlled facility and had access to food and water ad libitum. Only males were used in experiments for this study. All experiments were authorized by the University of Bologna Ethical Committee and the Italian Ministry of Health (Protocol number 845/2016) in accordance with Italian and EU regulations on animal welfare (Directive 2010/63/EU).

Male mice from all litters were randomly assigned and fed different diets from 1 to 4 months of age. Diets were prepared according to Sreeja and colleagues [17] with slight modifications (see Supplementary Table 1). Unflavoured protein isolates were commercially available (Myprotein, Norwich, UK). The actual amount of protein per 100 g of isolate was considered: soy protein isolate, 90% of protein content; pea protein isolate, 75% of protein content; bovine casein protein isolate, 80% of protein content; beef protein isolate, 97% of protein content. Corn starch, peanut oil and wheat bran were all commercially available. The multivitamin and mineral complex used was Multicentrum (1 g/100 g of diet; Pfizer, Latina, Italy) to match as closely as possible the vitamin and mineral contents according to Sreeja and colleagues [17]. All diets were isocaloric and isoproteic at 18% of the total diet weight, as other commercially available diets. Male mice were also fed Teklad 2018 global rodent diet (Envigo, San Pietro al Natisone, Italy) as a standard commercially available diet, which also contains 18% protein. Soy protein was used as the control diet for comparisons because commercial diets contain it (e.g. the standard diet used in this study), while pea protein is not contained in commercial diets. However, we tested pea protein as a second plant protein source that would still provide all essential amino acids, although with a different profile compared to soy protein (see Supplementary Table 2).

Food consumption was monitored weekly from 1 to 4 months of age for all dietary groups. Daily week consumption per mouse was estimated by subtracting the amount of food left uneaten after 1 week from the amount initially weighed and dividing it by the number of mice per cage. All animals were weighed when they were 4 months old.

## Behavioural Testing: Inhibitory Avoidance

Four-month-old male SAMP8 mice were tested for inhibitory avoidance, a commonly used behavioural task to investigate learning and memory processes in rodents [31, 32], by using an Ugo Basile inhibitory avoidance apparatus (Gemonio, Italy). A total of 15 animals per dietary group were tested. We followed a previously published single-trial inhibitory avoidance protocol for SAMP8 mice [26]. Briefly, on day 1, animals were put into the lit apparatus chamber and allowed to explore. Ten seconds later, the sliding door between both the light and dark chamber opened and animals moved into the dark apparatus chamber where they received an electric shock (0.5 mA for 3 s). Twenty-four hours after the learning procedure (day 2), animals were once again put into the lit chamber and none of them received an electric shock when moving into the dark chamber. The amount of time taken to move into the dark chamber (latency time) was recorded. A maximum latency time cutoff of 300 s was used [26].

## Sample Preparation and Protein Quantification for Western Blot Analysis

Mice were euthanised by using a $CO_2$ chamber followed by cervical dislocation and tissue dissection. Tissue was snap frozen with liquid nitrogen and stored at − 80 °C until used. Frontal cortex and hippocampus samples were collected, considering their documented role in cognitive function, as well as in memory codification and consolidation [33, 34]. Tissue samples were weighed and homogenized in tissue lysis buffer (50 mM Tris, pH 7.4, 1 mM EDTA, 1% SDS, protease inhibitor cocktail, phosphatase inhibitor cocktail; all from Sigma-Aldrich, St. Louis, MO, USA) at a 1:10 tissue weight/lysis buffer volume ratio with a Potter homogenizer (30 strokes at 1000 rpm) while kept on ice. Homogenates were then sonicated with a Branson SFX250 tip sonicator (Danbury, CT, USA) for 5 s at 10% power output. Total protein sample content was determined by using the Lowry quantification method [35]. Samples were aliquoted and stored at − 80 °C until used.

## Western Blotting

Fifty micrograms of total protein from each sample were mixed with Laemli electrophoresis loading buffer (1 M Tris-HCl, pH 6.8; 20% sodium dodecyl sulphate; 0.4 μL/mL glycerol; 2 g/L bromophenol blue and 2 M dithiothreitol; all from Sigma-Aldrich) and resolved in 12% acrylamide gels through SDS-PAGE. Resolved proteins were transferred onto nitrocellulose membranes for 2 h at a constant current of 400 mA. Membranes were then blocked in PBS-0.1% Tween 20–5% skimmed milk (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h and then incubated overnight with primary antibodies against ionized calcium-binding adapter molecule 1 (IBA1) antibody, triggering receptor expressed on myeloid cells 2 (TREM2), fractalkine (CX3CL1), tumour necrosis factor-alpha (TNFA), glial fibrillary acidic protein (GFAP), oligodendrocyte transcription factor 2 (OLIG2), myelin-associated glycoprotein (MAG), brain-derived neurotrophic factor (BDNF), TGFB 1, TGFB 2, TGFB receptor 1 (TGFBR1), TGFB receptor 2 (TGFBR2), AMP-activated protein kinase (AMPK), autophagy-related protein LC3 (LC3B) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) diluted in PBS-0.1% Tween 20–5% skimmed milk (see Supplementary Table 3 for details). The day after, membranes were washed 3× for 10 min with PBS-0.1% Tween 20 and then incubated with HRP-linked secondary antibodies diluted in PBS-0.1% Tween 20–5% skimmed for 1 h 30 min (see Supplementary Table 3 for details). Membranes were then washed 3× for 10 min with PBS-0.1% Tween 20 and then 1× for 5 min with PBS, incubated with the chemiluminiscence reagent Clarity ECL (Biorad), and images were acquired with a Biorad Chemidoc imager. Densitometric analysis was performed by using Biorad Image Lab software (Version 6.0.0).

## Immunofluorescence Staining

Mice were anaesthetised with 2% isoflurane and transcardially perfused with PBS and 4% paraformaldehyde in phosphate buffer (0.194 M $Na_2HPO_4$, 0.026 M $NaH_2PO_4$; all from Sigma-Aldrich). Brains were then stored overnight in 4% paraformaldehyde/phosphate buffer at 4 °C. The day after, brains were stored in 18% sucrose/phosphate buffer (Sigma-Aldrich) for at least 24 h and then stored at − 80 °C until needed. For immunofluorescence analysis, 40 μm brain sections were obtained by using a microtome with a freezing unit (Micro HM400, Microm, Berlin, Germany). Sections were washed 3× for 10 min in PBS and if required by the primary antibody, incubated at 80 °C for 30 min in 10 mM calcium citrate buffer, pH 6.0, for antigen retrieval (Sigma Aldrich) and washed again 3× for 10 min in PBS. Sections were then washed 3× for 10 min in PBS-0.1% Triton X-100 (Sigma-Aldrich). Sections were then blocked by incubating in PBS-0.1% Triton X-5% normal goat serum (Sigma-Aldrich) for 1 h except for IBA1 blocked in PBS-0.1% Triton X-5% BSA (Sigma-Aldrich) and then incubated overnight with primary antibodies against IBA1 (which required antigen retrieval) diluted in PBS-0.1% Triton X-2% BSA or against GFAP, OLIG2, DCX (doublecortin) all diluted in PBS-0.1% Triton

X-2% goat serum (see Supplementary Table 4 for details). The next day, sections were washed 3× for 10 min with PBS-0.1% Triton X and then incubated with fluorophore-conjugated secondary antibodies, all diluted in PBS-0.1% Triton X-2% goat serum (for GFAP, OLIG2 and DCX) or PBS-0.1% Triton X-2% BSA (for IBA1) for 2 h (see Supplementary Table 4 for details). Sections were then washed 3× for 10 min with PBS-0.1% Triton X and 1× for 5 min with PBS. In order to stain cell nuclei, sections were incubated with Hoechst 33258 (Sigma-Aldrich) diluted in PBS (final concentration 2 μg/ml) for 5 min. Sections were then washed 1× for 5 min in PBS and then mounted on glass slides, after which cover slips were mounted with Ultra Cruz mounting medium (Santa Cruz Biotechnology), air-dried for 10 min and stored at 4 °C until imaged.

### Confocal Microscopy Analysis, Fluorescence Intensity Analysis and Cell Counting

For brain section confocal microscopy, images were acquired with a Nikon EZ-C1 confocal microscope with a × 10 and × 40 objectives by using the z-stack function. A total of 40 stacks of 1 μm each were acquired. Three-dimensional image reconstruction was performed with Fiji (ImageJ2) by using the z-project plugin and the SUM function. Cell count and fluorescence intensity were analysed by using respectively the manual cell counter, subtract background, adjust threshold and measure plugin of Fiji ImageJ2 software, considering always the same square area of frontal cortex and hippocampus from SAMP8 mice.

### Statistics

Inhibitory avoidance results were analysed by using two-way ANOVA followed by Tukey's post hoc test comparing the dietary groups, as the first factor, and the behavioural test as the second one (training vs learning), as previously reported by Bambah-Mukku [36] and Contestabile [37]. Western blotting results were analysed by using one-way ANOVA followed by Dunnet's post hoc test whereas immunofluorescence results were analysed by Student's *t* test. All statistical analyses were performed with GraphPad Prism 4. All results are expressed as the mean ± standard error and defined statistically significant when $p < 0.05$.

## Results

### Food Consumption, Body Weight and Cognitive Performance

In order to evaluate the effect of dietary protein source on cognitive performance and neuroinflammation in ageing, male SAMP8 mice were fed diets containing soy or pea or casein or beef-protein from 1 to 4 months of age. Measured average daily food consumption was 4–5 g per animal in the different dietary groups ($n = 15$ per dietary group; Fig. 1a). No significant differences were found between the different dietary groups ($p > 0.05$). On the other hand, measured average body weight per animal at 4 months of age was 24.0–25.0 g ($n = 15$ per dietary group, Fig. 1b). Mice fed the beef-protein diet showed slightly decreased weight compared to the soy-protein diet group, which was also statistically significant ($p < 0.01$). Mice from the different groups were subjected to inhibitory avoidance ($n = 15$ per dietary group, Fig. 1c), a widely used cognitive task for assessing hippocampal-dependent memory [38, 39]. After a delay of 24 h, mice fed the soy-protein diet showed the highest latency time (290 ± 14 s), while mice fed the casein and beef-protein diets showed the lowest latency times (210 ± 19 s and 215 ± 18 s, respectively). These differences were also statistically significant ($p < 0.01$ for the casein-protein group and $p < 0.05$ for the beef-protein group), when compared to mice fed the soy-protein diet, but not when compared to the standard diet. Also, mice fed the pea-protein diet and the standard diet (2018 Teklad) showed similar performance during inhibitory avoidance.

A smaller cohort of mice ($n = 6$ per dietary group) was also used to test fear conditioning, in the 5 dietary groups (Supplementary Fig. 1). No statistically significant differences between the groups were observed ($p > 0.05$), although a lower freezing time was evident for mice fed the casein-protein diet compared to mice fed the soy or pea-protein diets. Because the standard diet contained a mixture of soy, wheat and corn as protein source, we decided not to test it further, since we aimed to test the effect of single dietary protein sources.

### Neuroinflammation and Gliosis

In order to evaluate whether cognitive deficits in mice exposed to animal protein-based diets could influence microglial activation, frontal cortex and hippocampal extracts from mice fed the soy, pea, casein or beef-protein diets were analysed through Western blotting ($n = 6$ per dietary group). We therefore focused on microglial and inflammation-associated markers. IBA1 protein expression analysis and relative densitometries of frontal cortex and hippocampus extracts (Fig. 2a–d) showed a statistically significant increase only in the hippocampus of the casein-protein diet group compared to the soy-protein diet group (Fig. 2c, d; $p < 0.05$). We then aimed to further analyse microglial activation by performing IBA1 immunofluorescence staining of frontal cortex and hippocampus sections in the soy and casein-protein groups only, since these displayed the most contrasting results in cognitive testing (Fig. 2e–l, $n = 4$ per dietary group). The casein-protein diet group showed a statistically significant increase of IBA1-

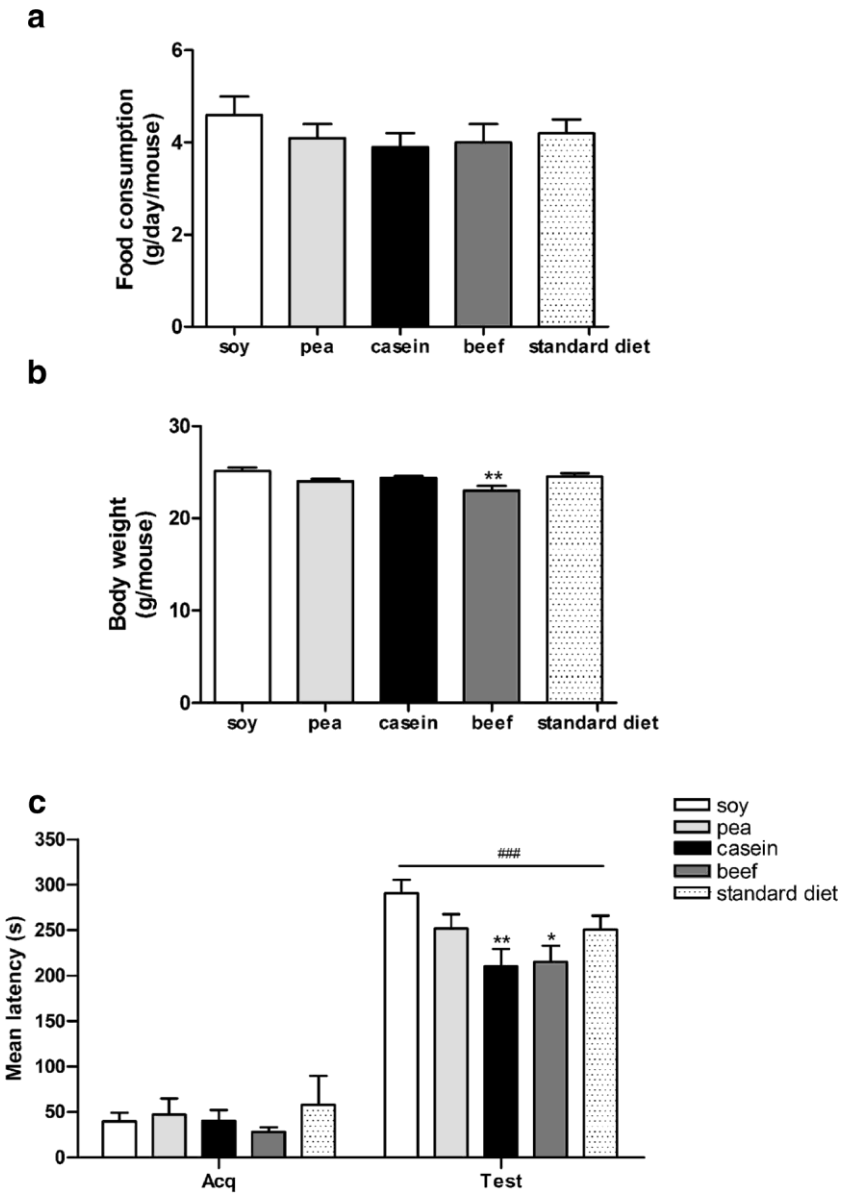


Fig. 1 Effect of dietary protein source on food consumption (a), body weight (b) and cognitive performance during the inhibitory avoidance test (acquisition/training and retention latencies/learning after 24-h test) (c) in 4-month-old male SAMP8 mice. Values are the mean ± SE of 15 animals per dietary group: *$p <$ 0.5, **$p < 0.01$, compared to the soy-protein diet group; ###$p <$ 0.001 compared Acq (training) to test (learning after 24 h): one-way ANOVA for food consumption (a) and body weight (b) followed by Dunnet's post hoc test, two-way ANOVA for the inhibitory avoidance test (c) followed by Tukey's post hoc test.

positive signal in hippocampus tissue sections (Fig. 2i, j,l and Supplementary Fig. 2d; $p < 0.05$) compared to the soy-protein diet group (Fig. 2f, g, l and Supplementary Fig. 2b). No significant differences were detected in frontal cortex sections (Fig. 2e, h, k and Supplementary Fig. 2a, c). To further elucidate the observed microglial activation, we analysed the expression of TREM2. Protein analysis and relative densitometries of TREM2 in frontal cortex (Fig. 3a, b) and hippocampus (Fig. 3e, f) extracts, showed a statistically significant decrease in the frontal cortex of the casein and beef-protein diet groups compared to the soy-protein diet group (Fig. 3a, b; $n = 6$ per dietary group, $p < 0.05$ for the casein-protein diet group and $p < 0.01$ for the beef-protein diet group). We also aimed to investigate whether the previously mentioned microglial changes could influence communication with neurons. Total CX3CL1 protein level analysis and relative densitometries of frontal cortex (Fig. 3a, c) and hippocampal (Fig. 3e, g) samples showed a statistically significant decrease in the frontal cortex of the casein-protein diet group compared to the soy-protein diet group (Fig. 3c; $n = 6$ per dietary group, $p < 0.001$).

In order to expand our investigation, we analysed other neuroinflammation markers not confined only to microglia. TNFA protein analysis and relative densitometries of frontal cortex (Fig. 3a, d) and hippocampus (Fig. 3e, h) samples showed a statistically significant increase in the hippocampus of the casein-protein diet group compared to the soy protein-diet group (Fig. 3h, $n = 6$ per dietary group, $p < 0.001$). Given its association with neuroinflammation, we also evaluated reactive astrogliosis through GFAP analysis [40]. Total GFAP protein expression analysis and relative densitometries showed no significant differences in frontal cortex nor hippocampal extracts (Fig. 4a–d; $n = 6$ per dietary group). To further

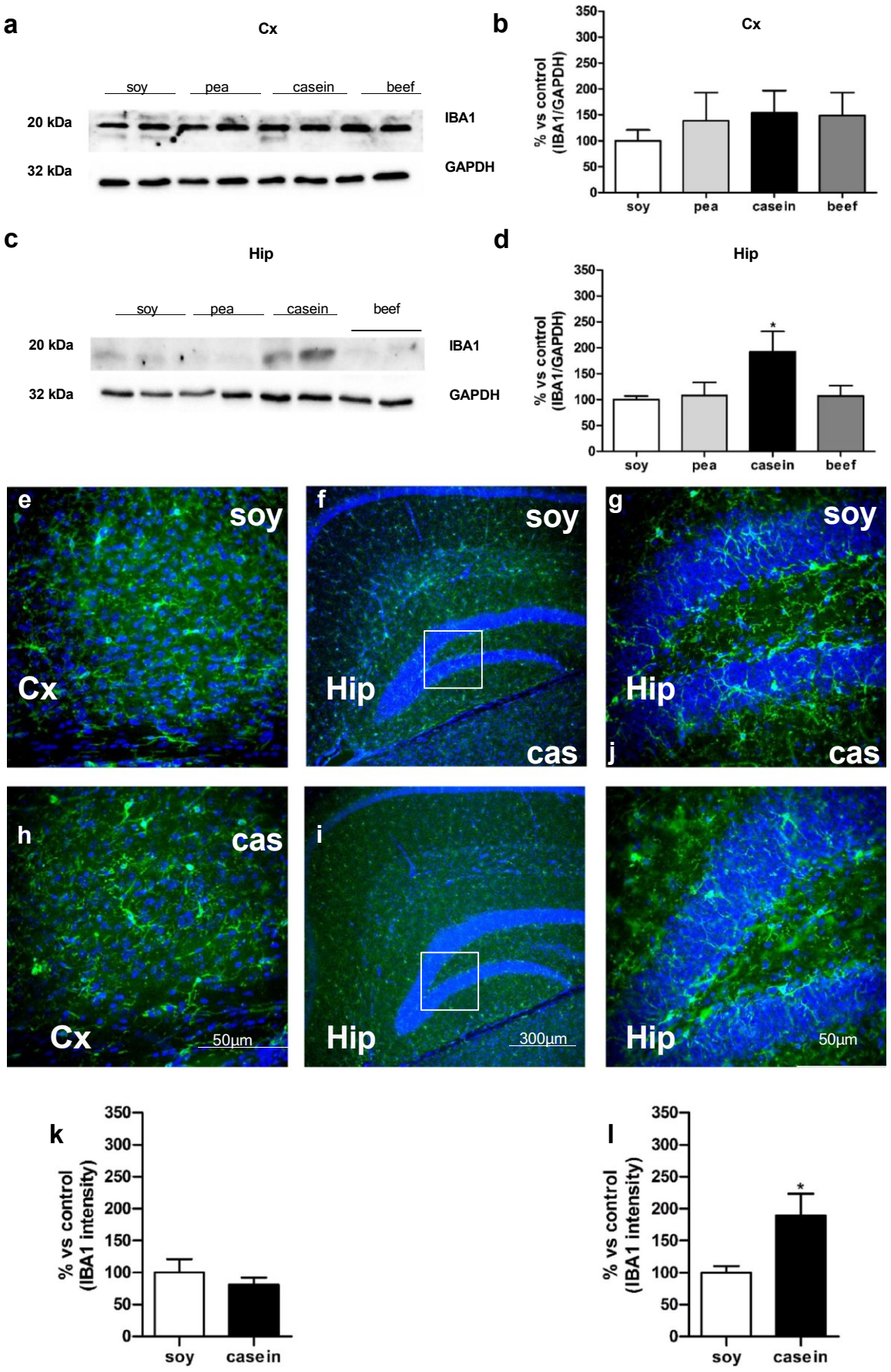
a
Cx
soy
pea
casein
beef
20 kDa
32 kDa
IBA1
GAPDH
b
Cx
% vs control (IBA1/GAPDH)
soy
pea
casein
beef
c
Hip
d
Hip
e
soy
Cx
f
soy
Hip
cas
g
soy
Hip
j
cas
h
cas
Cx
50µm
i
Hip
300µm
Hip
50µm
k
% vs control (IBA1 intensity)
soy
casein
l
% vs control (IBA1 intensity)
soy
casein

Fig. 2 Analysis of microglial marker expression in 4-month-old male SAMP8 mice fed different diets. Western blot analysis and relative densitometries of IBA1 expression in the frontal cortex (a, b) and hippocampus (c, d) of male SAMP8 mice. Immunofluorescence analysis and relative quantification (e–l) of IBA1 expression in the frontal cortex of male SAMP8 mice fed the soy (e, k) and casein (h, k) protein diets and in the hippocampus of male SAMP8 mice fed the soy (f, g, l) and casein (i, j, l) protein diets. Values are the mean ± SE of 6 animals for Western blot analysis and of 4 animals for immunofluorescence analysis, $*p < 0.05$ vs soy-protein diet, Student's $t$ test.

explore reactive astrogliosis, we performed GFAP immunostaining in frontal cortex (Fig. 4e, h, k) and hippocampus (Fig. 4f, g, i, j, l) sections. GFAP signal intensity showed a statistically significant increase in the frontal cortex of mice fed the casein-protein diet (Fig. 4h, k and Supplementary Fig. 1g; $n = 4$ per dietary group, $p < 0.01$) compared to mice fed the soy-protein diet (Fig. 4e, k and Supplementary Fig. 1e). No significant differences were detected in hippocampus sections (Fig. 4f, g, i, j, l and Supplementary Fig. 2F, H).

Considering the increased evidence of myelination involvement in dementia [41, 42], we also analysed oligodendrocyte-associated markers. OLIG2 protein expression analysis and relative densitometries of frontal cortex (Fig. 5a, b) and hippocampal (Fig. 5d, e) samples showed statistically decreased levels in the frontal cortex of the casein and beef-protein diet groups compared to the soy-protein diet group (Fig. 5a, b; $n = 6$ per dietary group, $p < 0.05$). We also performed OLIG2 immunofluorescence staining in frontal cortex (Fig. 5g, j, m) and hippocampus (Fig. 5h, i, k, l, n) tissue sections from the soy and casein-protein diet groups. A statistically significant increase was detected in the hippocampus of the casein-protein group (Fig. 5k, l, n; $n = 4$ per dietary group, $p < 0.01$) compared to the soy-protein group (Fig. 5h, i, n). MAG protein analysis and relative densitometries of frontal cortex (Fig. 2a, c) and hippocampus (Fig. 2d, f) showed a statistically significant decrease in the cortex of male SAMP8 mice fed the casein-protein diet (Fig. 5a, c; $n = 6$ per dietary group, $p < 0.05$) and in the hippocampus of the beef-protein group (Fig. 5d, f, $p < 0.05$) compared to the respective soy-protein group.

## Neurogenesis and Growth Factor Expression

We aimed to investigate whether differences in behavioural performance could influence neurogenesis. To this aim, we analysed adult neurogenesis by performing immunofluorescence staining against the immature-neuron marker DCX (Fig. 6e–l). However, we did not observe significant differences in neurogenesis neither in the frontal cortex (Fig. 6e, h, k and Supplementary Fig. 2I, K) nor in the hippocampus (Fig. 6f, g, i, j, l and Supplementary Fig. 2J, L) of 4-month-old SAMP8 mice.

We then aimed to analyse the expression of growth factors involved in neuronal survival and glial function. We focused on one of the most important trophic factors involved in neuronal survival, BDNF [43]. However, BDNF protein expression analysis and relative densitometries of frontal cortex and hippocampus samples (Fig. 6d; $n = 6$ per dietary group) showed no significant differences in any of the dietary groups. On the other hand, we analysed the expression of TGFB factors and receptors, involved in oligodendrocyte precursor cell (OPC) proliferation and differentiation [44, 45], neuronal plasticity and survival [46, 47] and microglial maturation [48]. TGFB1 protein expression and relative densitometries in frontal cortex and hippocampus samples (Supplementary Fig. 3A-B, G-H, $n = 6$ per dietary group) showed a statistically significant increase in the frontal cortex of the pea-protein diet group compared to the soy-protein diet group (Supplementary Fig. 3A, B; $p < 0.05$). No significant changes in TGFB2 expression were detected (Supplementary Fig. 3A, C, G, I). In addition, TGFBR1 and TGFBR2 protein expression and relative densitometries in frontal cortex and hippocampus extracts (Supplementary Fig. 3D-L; $n = 6$ per dietary group) showed a statistically significant increase in TGFBR1 levels in the hippocampus of the casein and beef-protein diet groups (Supplementary Fig. 3J, K). No other statistically significant differences in TGFBR1 nor TGFBR2 protein expression were detected. Interestingly, a pattern of lower TGFBR2 bands was detected in the soy-protein diet group.

## Autophagy-Related Marker Expression

We analysed the expression of the autophagy-associated markers AMPK and LC3B. AMPK protein expression analysis and relative densitometries showed decreased levels in the frontal cortex of the beef-protein diet group. This decrease was statistically significant (Supplementary Fig. 4A, B; $n = 6$ per dietary group, $p < 0.05$). No other significant differences in AMPK levels were detected. In a similar way, LC3B protein expression analysis and relative densitometries showed decreased levels in the frontal cortex of the beef-protein diet group compared to the soy-protein diet group (Supplementary Fig. 4A, C) ($n = 6$ per dietary group, $p < 0.05$). On the other hand, we observed a statistically significant increase in LC3B levels in the hippocampus of the pea-protein diet group compared to the soy-protein diet group ($p < 0.05$; Supplementary Fig. 3D, F).

A summary of all protein expression results is reported in Supplementary Tables 5 and 6.

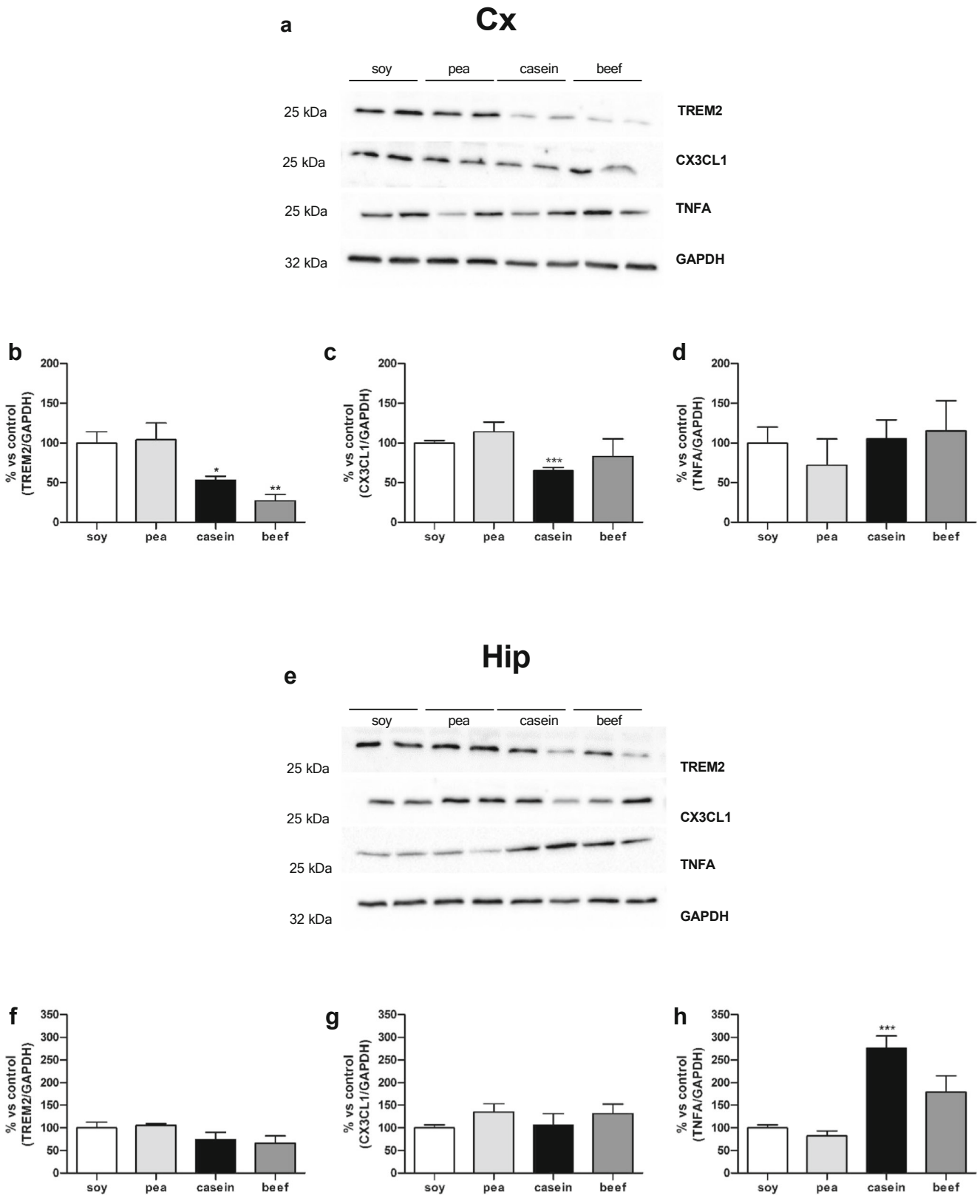


Fig. 3 Analysis of inflammation-associated marker expression in 4-month-old male SAMP8 mice fed different diets. Western blot analysis and relative densitometries of TREM2 (a, b), CX3CL1 (a, c) and TNFA (a, d) expression in the frontal cortex and TREM2 (e, f), CX3CL1 (e, g) and TNFA (e, h) expression in the hippocampus of male SAMP8 mice. Values are the mean ± SE of 6 animals, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs soy-protein diet, Student's *t* test.

## Discussion

Here, we evaluated the effect of chronic dietary regimens, in which the protein component is provided by soy/pea protein (plant origin) or by casein/beef protein (animal origin) without changing other components or caloric con- tent, on hippocampal-dependent memory in male SAMP8 mice. Increasing evidence shows differences between males and females including regional differences in microglial phenotype and activation [49]. Despite this, in this study, we focused on male mice, partly to reduce the number of animals used, since female mice may show a higher latency time in the inhibitory avoidance test, when compared to males [50]. Oral administration of soy pep- tides to SAMP8 mice has been previously shown to pre- vent cognitive decline [51]. Moreover, administration of bovine casein-derived peptides prevented cognitive de- cline in an acute model of AD [52]. However, no study has been published on the effect of dietary protein source on memory in SAMP8 mice, which may better represent sporadic forms of dementia. The differences observed in memory retention among the different dietary groups could be partly explained by the amount of isoflavones contained in soy, particularly genistein and daidzein, which are not present in the other dietary protein sources used, although the amount of isoflavones in soy-protein isolate is much lower compared to whole soybeans [53]. Isoflavones are known to have several biological effects, including the enhancement of cognitive function [54, 55]. Therefore, even small amounts of isoflavones in the diet could still have a positive effect on cognitive functions. On the other hand, it could be that the casein and beef- protein diets worsen cognitive performance, partly due to the induction of an increased inflammatory status and in- creased disruption of the blood-brain-barrier, which would allow peripheral inflammatory mediators enter the brain and thus compromise cognitive function [19]. While the four dietary protein sources used in this study provide all essential amino acids, there are differences in the amounts of each single amino acid they provide (see Supplementary Table 1). Thus, we cannot exclude that these amino acid content differences could affect the re- sults we observed. In fact, methionine restriction has been associated with reduced inflammatory maker levels in rats [56]. Casein and beef protein contain around 500% and 300% higher methionine levels, respectively, compared to soy and pea protein; thus, chronic methionine differences could also impact memory in male SAMP8 mice by influencing inflammation [57, 58]. This is of particular importance to our study, given the involvement of neuro- inflammation in AD and other neurodegenerative dis- eases. Neuroinflammation, due to microglial activation, can influence memory consolidation and may be modulated by diet [59, 60]. We therefore focused on microglial and inflammation-associated markers. In fact, the increased protein expression and immunofluorescence signal of IBA1, a calcium-binding EF protein that is up-regulated in activated microglia and macrophages [61], which we detected in the hippocampus of male SAMP8 mice fed the casein-protein diet, suggest that microglial activation may already be taking place at 4 months of age. Moreover, the reduction in cortical TREM2 protein levels, a phagocytosis receptor expressed by M2- phenotype microglia, suggests that microglial cells may have started shifting towards a pro-inflammatory M1-like phenotype at 4 months of age in the animal-protein diet groups. To investigate whether the previously mentioned microglial changes could influence communication with neurons, we analysed the expression of CX3CL1, which is normally expressed by neurons. CX3CL1 plays an im- portant role in the crosstalk between neurons and microg- lia, the latter expressing CX3CR1, the receptor that binds CX3CL1 [62]. In addition, while CX3CL1 is membrane- bound, it can be cleaved by secretases and the intracellu- lar domain left after cleavage can be transported back into the nucleus and stimulates neurogenesis [63]. Furthermore, we analysed other neuroinflammation markers not confined only to microglia, such as TNFA, a cytokine that is produced by several cell types in the body. In the brain, the main sources are microglia and astrocytes, although neurons can also release TNFA dur- ing extreme pathological conditions such as ischaemia [64]. In pathological conditions, microglia and astro- cytes release large amounts of TNFA which contribute remarkably to neuroinflammation [65, 66]. Moreover, TNFA can display anti-neurogenic effects in vitro and in the adult brain by blocking cell division and inducing progenitor cell death [67, 68]. Thus, the decreased levels of intracellular CX3CL1 fragment in the cortex and the increased levels of TNFA protein in the hippo- campus we observed, suggest that neurogenesis could be impaired during later stages of the disease in these areas, at least in male SAMP8 mice fed the casein- protein diet. Neurogenesis in the adult brain decreases with ageing [69], as well as in animal models of de- mentia, including SAMP8 mice [70, 71]. On the other hand, strategies that increase the number of newly formed neurons, such as exposure to an enriched envi-ronment, improve behavioural performance in mice [72]. Here, we observed a slightly decreasing trend in DCX staining both in the cortex and hippocampus of 4- month-old male SAMP8 mice fed the casein diet, al- though this was not statistically significant. Nonetheless, a recent study in AD patients showed that adult human hippocampal neurogenesis drops dramati- cally compared to healthy age-matched controls [73].

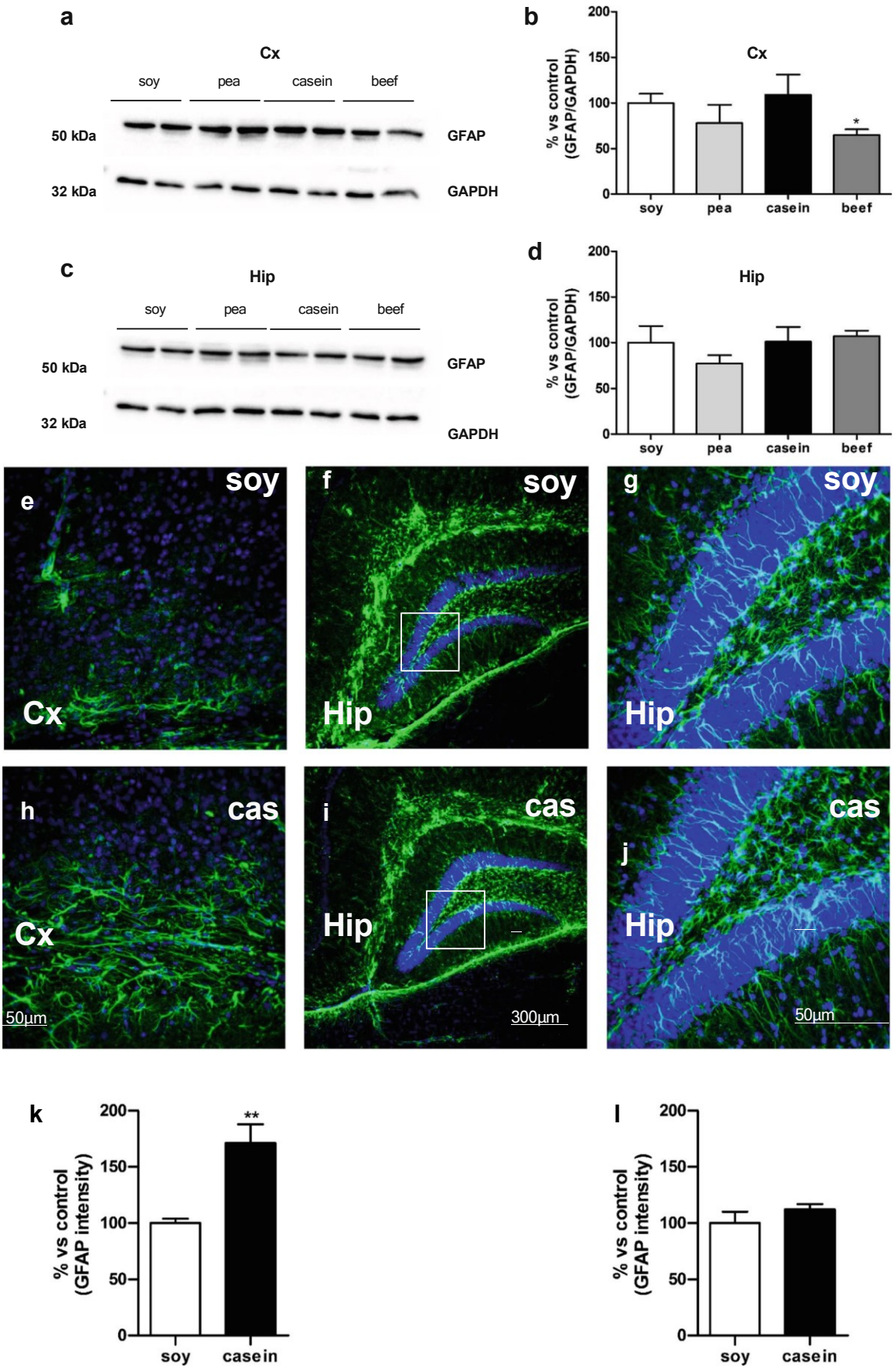
a
Cx
soy
pea
casein
beef
50 kDa
32 kDa
GFAP
GAPDH
b
Cx
% vs control (GFAP/GAPDH)
200
150
100
50
0
*
soy
pea
casein
beef
c
Hip
soy
pea
casein
beef
50 kDa
32 kDa
GFAP
GAPDH
d
Hip
% vs control (GFAP/GAPDH)
200
150
100
50
0
soy
pea
casein
beef
e
soy
Cx
f
soy
Hip
g
soy
Hip
h
cas
Cx
50µm
i
cas
Hip
300µm
j
cas
Hip
50µm
k
% vs control (GFAP intensity)
200
150
100
50
0
**
soy
casein
l
% vs control (GFAP intensity)
200
150
100
50
0
soy
casein

Fig. 4 Analysis of astrocytic marker expression in 4-month-old male SAMP8 mice fed different diets. Western blot analysis and relative densitometries of GFAP expression in the frontal cortex (a, b) and hippocampus (c, d) of SAMP8 mice. Immunofluorescence analysis and relative quantification (e–l) of GFAP expression in the frontal cortex of male SAMP8 mice fed the soy (e, k) and casein (h, k) protein diets and in the hippocampus of male SAMP8 mice fed the soy (f, g, l) and casein (i, j, l) protein diets. Values are the mean ± SE of 6 animals for Western blot analysis and of 4 animals for immunofluorescence analysis, $*p < 0.05$, $**p < 0.01$ vs soy-protein diet Student's *t* test

With our current results, we cannot conclude whether microglial activation at this stage is beneficial or detrimental. However, recent evidence obtained through magnetic resonance imaging and positron emission tomography imaging, shows that microglial activation may have a beneficial effect in the early stage of AD. Namely, microglial activation was positively associated with higher hippocampal volume and higher grey matter volume in patients with MCI [74]. In addition, astrogliosis has previously been reported in the brain of 4-month-old male SAMP8 mice by immunolabelling against GFAP [27], which we also observed in the cor- tex of mice fed the casein-protein diet through immuno- fluorescence analysis. While astrogliosis and increased blood-brain barrier dysfunction have previously been re- ported in wild-type mice fed a 50% casein-protein diet [19], here we detected astrogliosis in the hippocampus and cortex of 4-month-old male SAMP8 mice fed a lower percentage of dietary protein, namely 18% of casein-protein in the diet, further supporting an effect of animal-protein diets towards gliosis and neuroinflammatory conditions. This in turn could influ- ence memory in male SAMP8 mice.

Considering the changes described above in microglia and astrocytes and the increased evidence of myelination involvement in dementia [41, 42], we also analysed oligodendrocyte-associated markers. While oligodendrocytes have not been extensively studied in AD, they represent up to 75% of glial cells in the brain [75]. However, evidence of demyelination/aberrant myelination in AD has been reported, specifically in the hippocampus and the anterior cingulate [76]. It has also been reported that OLIG2 upregulation, a transcription factor required for oligodendrocyte specification and differentiation [77, 78], identifies oligodendrocyte progenitor cells responding to demyelination in the CNS [78]. However, we revealed a slight but significant decrease in OLIG2 expression by Western blotting in the frontal cortex of mice fed the casein and beef-protein diets, which could indicate that an attempt to remyelinate is being halted. On the other hand, we cannot exclude that OLIG2 is upregulated in other brain cells at this stage. However, OLIG2 immunofluorescence staining revealed a significant increase in OLIG2-positive cells in the hippocampal dentate gyrus of animals fed the casein-protein diet, which could suggest an attempt to remyelinate is taking place locally in this brain region. Also, reduced MAG levels in the brain of AD patients have been associated with white matter ischaemia during lifetime [79]. Therefore, to further elucidate the involvement of oligodendrocytes, we also analysed the expression of MAG, a component of myelin important for maintaining axonal integrity and axon-glia contact [80]. While we observed reduced MAG levels by Western blotting in the frontal cortex and hippocampus of 4-month-old male SAMP8 mice in the casein and beef-protein diet groups, we did not evaluate small vessel disease. Reduced expression of MAG and OLIG2 has been observed in the white matter of the frontal lobe in humans with AD, suggesting that oligodendrocytopathy may be a part of AD [81]. Although 4-month-old SAMP8 mice may represent the early stages of AD, the exacerbation of inflammation (decreased expression of TREM2 and CX3CL1 in the cortex) observed with the casein and beef diets may accelerate the decrease of OLIG2-positive cells and the expression of myelin associated proteins such as MAG, as observed in more advanced stages of AD in humans. On the other hand, TGFB factors and their receptors play a significant role in oligodendrocyte precursor cell (OPC) proliferation and differentiation [44, 45], as well as in neuronal plasticity and survival [46, 47]. Moreover, TGFB signalling plays an important role in microglial maturation [48] and loss of TGFB signalling contributes to increased microglial activation [82].

Decreased hippocampal TGFB1 levels have been previously reported in an AD mouse model, where intraventricular infusion of TGFB1 restored synaptic plasticity and memory loss [83]. However, we did not detect any decrease in TGFB1 levels in 4-month-old male SAMP8 mice. Interestingly, the pea-protein diet group showed increased TGFB1 levels. It has been previously shown in rodents that TGFB1 levels increase in the prefrontal cortex during learning, after the first training session and then return to normal levels [84]. On the other hand, the same study showed that TGFB1 levels decrease in the left hippocampus but increase in the right hippocampus after learning. Here, the whole hippocampus was used for Western blotting analysis. Increased TGBF1 levels after learning may persist longer in mice fed the pea protein diet, compared to the other dietary groups where TGFB1 levels return to basal levels. Also, mice were sacrificed one day after the second day of learning (on day 3); thus, this could have influenced the expression of TGFB1. On the other hand, increased TGFB2 levels have been reported in the frontal cortex and hippocampus of AD patients, which seems to be an indication of neuronal death [85, 86]. However, we observed no significant differences in TGFB2 levels. This could depend on the fact that all human brain samples analysed were from patients in the end stage of AD, whereas our results were obtained in 4-month-old male SAMP8 mice, which represent an early stage of the disease. In the CNS, TGFBR2 is predominantly

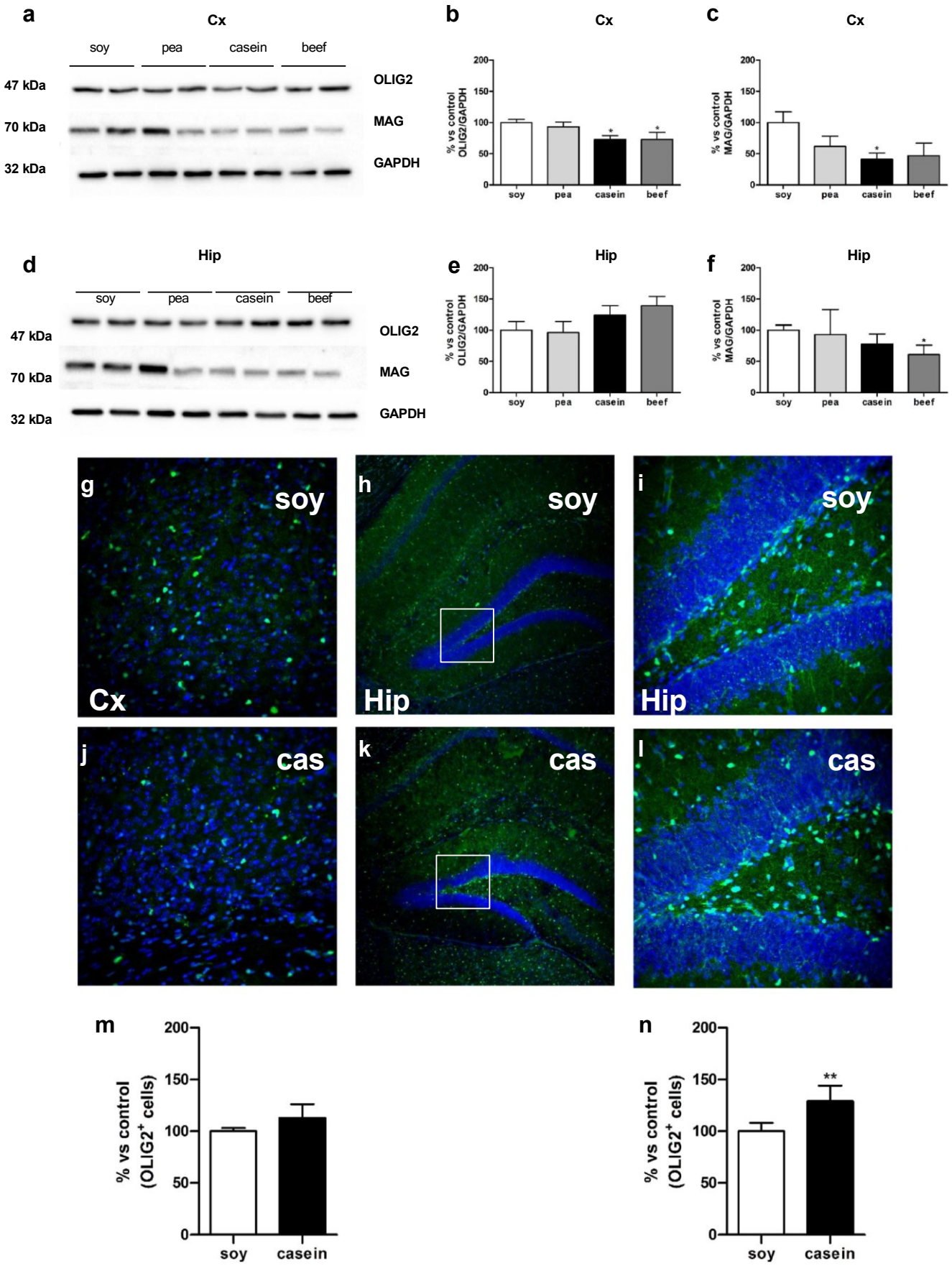
a
Cx
soy
pea
casein
beef
47 kDa
70 kDa
32 kDa
OLIG2
MAG
GAPDH
b
Cx
% vs control OLIG2/GAPDH
c
Cx
% vs control MAG/GAPDH
d
Hip
e
Hip
% vs control OLIG2/GAPDH
f
Hip
% vs control MAG/GAPDH
g
soy
Cx
h
soy
Hip
i
soy
Hip
j
cas
k
cas
l
cas
m
% vs control (OLIG2+ cells)
n
% vs control (OLIG2+ cells)

Fig. 5 Analysis of oligodendrocyte-associated marker expression in 4-month-old male SAMP8 mice fed different diets. Western blot analysis and relative densitometries of OLIG2 (a, b) and MAG (a, c) expression in the frontal cortex and OLIG2 (d, e) and MAG (d, f) expression in the hippocampus (c, d) of male SAMP8 mice. Immunofluorescence analysis and relative quantification (g–n) of OLIG2 expression in the frontal cortex of male SAMP8 mice fed the soy (g, m) and casein (j, m) protein diets and in the hippocampus of male SAMP8 mice fed the soy (h, i, n) and casein (k, l, n) protein diets. Values are the mean ± SE of 6 animals for Western blot analysis and of 4 animals for immunofluorescence analysis, $*p < 0.05$, $**p < 0.01$ vs soy-protein diet, Student's *t* test

expressed by neurons, compared to glial cells [87]. However, both TGFBR1 and TGFBR2 expression appear to be important for OPC differentiation and consequent myelination [88]. Thus, alterations in TGFB signalling could affect neuronal survival, microglial activation and oligodendrocyte differentiation into myelinating oligodendrocytes. Increased TGFBR1 and TGFBR2 levels have been previously detected in reactive glia in the frontal cortex of AD patients compared to healthy controls [89]. Here, we observed a statistically significant increase of TGFBR1 in the hippocampus of 4-month-old SAMP8 mice fed the casein and beef-protein diets, which could influence increased glial activation, myelination alterations and ultimately cognitive performance, namely memory. Moreover, hyperactivation of TGFB signalling in astrocytes has been associated with increased blood-brain barrier dysfunction in aging rodents and humans [90]. Increased TGFBR1 expression in the hippocampus of mice fed the casein and beef-protein diets could be associated with astrocyte activation, which we detected in the hippocampus of 4-month-old male SAMP8 mice fed the casein-protein diet through GFAP immunofluorescence, potentially secondary to blood-brain barrier dysfunction [19]. While we did not detect astrocyte activation in male SAMP8 mice fed the beef-protein diet, this might have as well been detected by GFAP immunofluorescence, which we did not perform. The increased TNFA expression we observed in the hippocampus of male SAMP8 mice fed the casein and beef-protein diets may in turn exacerbate astrocyte activation and influence TGFB receptor signalling. On the other hand, albumin has been reported to bind directly to TGFB receptors, induce their internalisation and activate TGFB signalling [91]. If increased blood-brain barrier dysfunction is present in male SAMP8 mice fed the casein and beef-protein diets, favouring albumin transvasation and accumulation in astrocytes [90], this could partly explain the increased expression of TGFBR1 and TGFBR2 without increased TGFB1 expression, at least at 4 months of age. However, since TGFBR2 expression increases also in male SAMP8 mice fed the pea-protein diet, with the currently available data, we are unable to provide an explanation for this and further analyses will be required.

Autophagy is a physiological process regulating protein degradation and cytoplasmic turnover of cellular components [92]. Moreover, autophagy plays a crucial role in the central nervous system and its impairment can lead to neuronal death [93]. Alterations of the autophagic flux have been observed in NDDs, including AD [94]. Alteration of the autophagy process has previously been reported in SAMP8 mice as shown by increased LC3B expression in the hippocampus and frontal cortex of 7- and 12-month-old mice [95]. Moreover, BECLIN expression, another marker associated with autophagy activity, showed first an increase at 7 months of age and then a decrease at 12 months of age in SAMP8 mice. This suggests that the autophagy flux may increase in earlier phases of AD, perhaps as a protective mechanism and then decline with ageing/later phases of AD. The decrease in expression levels of LC3B protein in 4-month-old male SAMP8 mice fed the beef-protein diet may suggest that autophagy could be already impaired in the early phases of dementia due to this dietary protein source. On the other hand, the pea-protein diet may exert a protective effect by increasing LC3B expression in the hippocampus of male SAMP8 mice. The reduction in total AMPK levels we observed in male SAMP8 mice fed the casein and beef-protein diets could be another indicator of reduced autophagic flux, although we did not analyse the levels of activated AMPK, phosphorylated on threonine 172 [96]. Moreover, it is known that AMPK is one of the kinases responsible for tau phosphorylation [97]. However, we cannot speculate on the effects that reduced total AMPK levels could have on tau phosphorylation.

One of the main limitations of our study is the fact that only male mice were used for experiments. Increasing evidence shows clearly differences between the male and female rodent brain, particularly regarding microglial phenotype, activation as well as transcriptional and translational differences [49, 98]. Moreover, male and female mice display differential neuroinflammatory responses [99]. Therefore, our results cannot be generalised, and the same experiments should be performed in female mice to obtain a wider picture of the effect of dietary protein source.

In conclusion, the results show how dietary protein source alone can impact memory, inflammation and myelination in male SAMP8 mice. This could be due partly to the presence of isoflavones and methionine restriction or increased blood-brain barrier permeability to inflammatory factors. Moreover, we cannot exclude the direct involvement of the gut microbiome, although further studies are needed to support this. The different diets used in this study were fed as a preventative strategy from 1 month of age. It would be valuable to test the potential therapeutic effects of the soy-protein diet on mice previously fed the casein or beef-protein diets, before symptom onset. The protein source in commercially available diets for mouse model research may be, among others, soy based, casein based, a mixture of soy, casein and

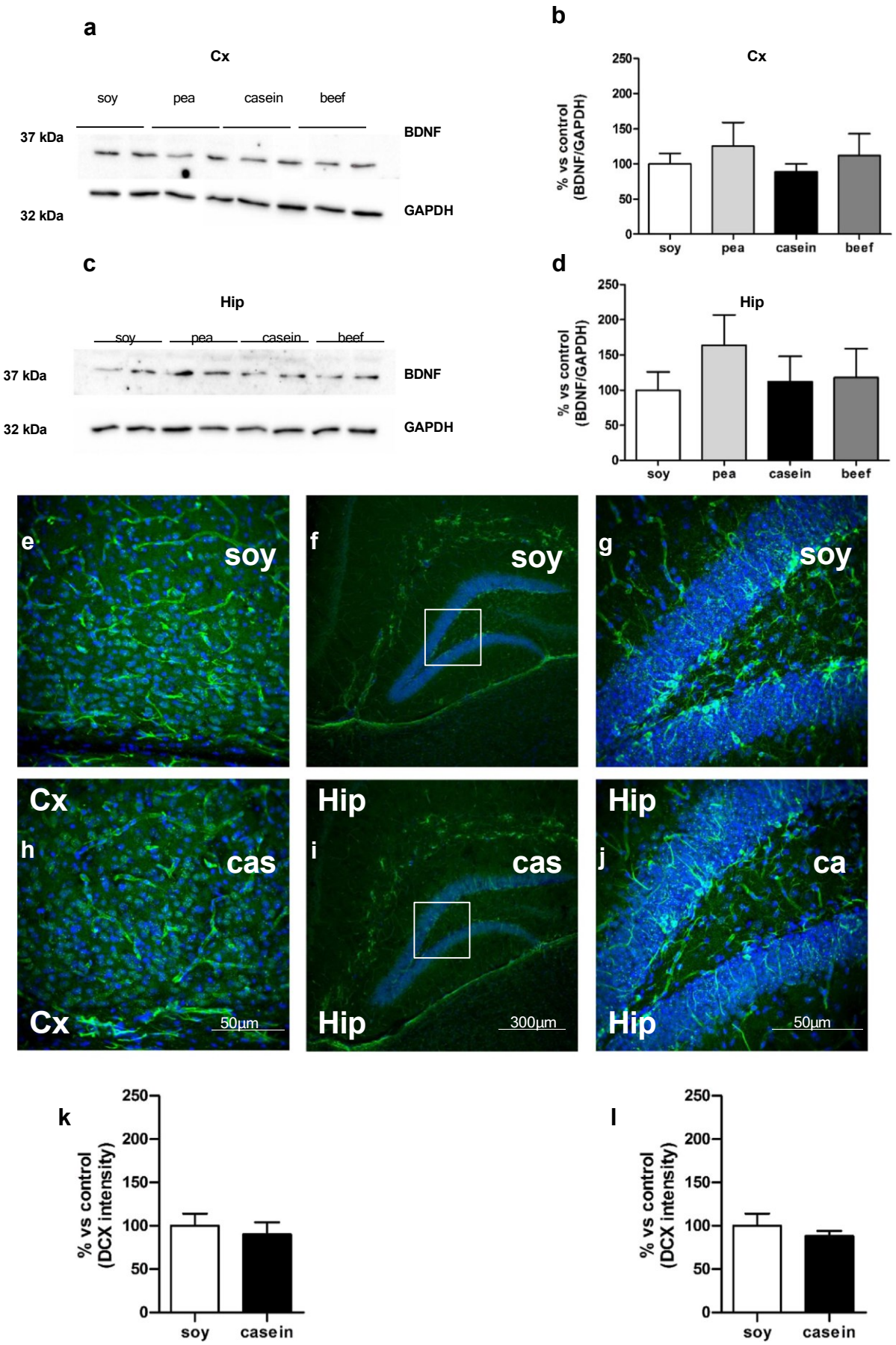
a
Cx
soy
pea
casein
beef
37 kDa
BDNF
32 kDa
GAPDH
b
Cx
% vs control (BDNF/GAPDH)
250
200
150
100
50
0
soy
pea
casein
beef
c
Hip
soy
pea
casein
beef
37 kDa
BDNF
32 kDa
GAPDH
d
Hip
% vs control (BDNF/GAPDH)
250
200
150
100
50
0
soy
pea
casein
beef
e
soy
f
soy
g
soy
Cx
h
cas
Cx
50µm
Hip
i
cas
Hip
300µm
Hip
j
ca
Hip
50µm
k
% vs control (DCX intensity)
250
200
150
100
50
0
soy
casein
l
% vs control (DCX intensity)
250
200
150
100
50
0
soy
casein

Fig. 6 Analysis of neurotrophic factor expression and neurogenesis in 4-month-old male SAMP8 mice fed different diets. Western blot analysis and relative densitometries of BDNF expression in the frontal cortex (a, b) and hippocampus (c, d) of male SAMP8 mice. Immunofluorescence analysis and relative quantification (e–l) of DCX expression in the frontal cortex of male SAMP8 mice fed the soy (e, k) and casein (h, k) protein diets and in the hippocampus of male SAMP8 mice fed the soy (f, g, l) and casein (i, j, l) protein diets. Values are the mean ± SE of 6 animals for Western blot analysis and of 4 animals for immunofluorescence analysis, Student's *t* test

fish protein or a mixture of plant-based proteins. Therefore, care should be taken when interpreting results depending on the dietary protein source chosen, particularly regarding diseases where inflammation and memory are affected.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-020-02191-y.

Acknowledgements We thank Miss Monia Bentivogli for her skilful technical assistance and Mr Alessandro Casoni for contributing to maintain the mouse colony. We also thank Professor Antonio Contestabile for reading the manuscript and giving valuable feedback.

Author contributions Author responsibilities were as follows: BM and EPA designed the research; SP, CP, VR, IF, FM and EPA conducted the research; SP, CP, VR, IF and EPA analysed the data; SP, BM, MV and EPA wrote the manuscript and were primarily responsible for final content; all authors contributed to and have approved the final manuscript.

Funding EPA was supported with a research fellowship from Fondazione Umberto Veronesi, Italy, to perform this study. BM receives support from the University of Bologna. Fondazione Umberto Veronesi and the University of Bologna had no further role in study design; data collection, analysis and interpretation; writing of the manuscript and in the decision to submit the manuscript for publication.

## Compliance with ethical standards

Animal welfare was guaranteed throughout this study by guidelines set by the Italian Health Ministry. All experiments were authorized by the University of Bologna Ethical Committee and the Italian Ministry of Health (protocol number 845/2016) in accordance with Italian and EU regulations on animal welfare (Directive 2010/63/EU).

Conflict of Interest The authors declare that they have no conflicts of interest.

## References

1. Kozlov S, Afonin A, Evsyukov I, Bondarenko A (2017) Alzheimer's disease: as it was in the beginning. Rev Neurosci 28(8):825–843. https://doi.org/10.1515/revneuro-2017-0006
2. Broussard GJ, Mytar J, Li RC, Klapstein GJ (2012) The role of inflammatory processes in Alzheimer's disease. Inflammopharmacology 20(3):109–126. https://doi.org/10.1007/s10787-012-0130-z
3. Ishizuka K, Kimura T, Igata-yi R, Katsuragi S, Takamatsu J, Miyakawa T (1997) Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease. Psychiatry Clin Neurosci 51(3):135–138. https://doi.org/10.1111/j.1440-1819.1997.tb02375.x
4. Ho L, Pieroni C, Winger D, Purohit DP, Aisen PS, Pasinetti GM (1999) Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. J Neurosci Res 57(3):295–303. https://doi.org/10.1002/(SICI)1097-4547(19990801)57:3<295::AID-JNR1>3.0.CO;2-0
5. Calingasan NY, Erdely HA, Altar CA (2002) Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury. Neurobiol Aging 23(1):31–39. https://doi.org/10.1016/s0197-4580(01)00246-9
6. Ager RR, Fonseca MI, Chu SH, Sanderson SD, Taylor SM, Woodruff TM, Tenner AJ (2010) Microglial C5aR (CD88) expression correlates with amyloid-beta deposition in murine models of Alzheimer's disease. J Neurochem 113(2):389–401. https://doi.org/10.1111/j.1471-4159.2010.06595
7. Parbo P, Ismail R, Hansen KV, Amidi A, Mårup FH, Gottrup H, Brændgaard H, Eriksson BO et al (2017) Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. Brain 140(7):2002–2011. https://doi.org/10.1093/brain/awx120
8. Wake H, Moorhouse AJ, Miyamoto A, Nabekura J (2013) Microglia: actively surveying and shaping neuronal circuit structure and function. Trends Neurosci 36(4):209–217. https://doi.org/10.1016/j.tins.2012.11.007
9. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I (2018) Disease-associated microglia: a universal immune sensor of neurodegeneration. Cell 173(5):1073–1081. https://doi.org/10.1016/j.cell.2018.05.003
10. Tang Y, Le W (2016) Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol 53(2):1181–1194. https://doi.org/10.1007/s12035-014-9070-5
11. Kaminsky N, Bihari O, Kanner S, Barzilai A (2016) Connecting malfunctioning glial cells and brain degenerative disorders. Genomics Proteomics Bioinformatics 14(3):155–165. https://doi.org/10.1016/j.gpb.2016.04.003
12. Shentu YP, Hu WT, Zhang Q, Huo Y, Liang JW, Liuyang ZY, Zhou H, Wei H et al (2019) CIP2A-promoted astrogliosis induces AD-like synaptic degeneration and cognitive deficits. Neurobiol Aging 75:198–208. https://doi.org/10.1016/j.neurobiolaging.2018.11.023
13. Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, Nodin C, Ståhlberg A et al (2008) Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab 28(3):468–481. https://doi.org/10.1038/sj.jcbfm.9600546
14. Fuller S, Münch G, Steele M (2009) Activated astrocytes: a therapeutic target in Alzheimer's disease? Expert Rev Neurother 9(11):1585–1594. https://doi.org/10.1586/ern.09.111
15. Heringa SM, van den Berg E, Reijmer YD, Nijpels G, Stehouwer CD, Schalkwijk CG, Teerlink T, Scheffer PG et al (2014) Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population - the Hoorn Study. Psychoneuroendocrinology 40:108–118. https://doi.org/10.1016/j.psyneuen.2013.11.011
16. Barbaresko J, Koch M, Schulze MB, Nöthlings U (2013) Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 71(8):511–527. https://doi.org/10.1111/nure.12035
17. Sreeja S, Geetha R, Priyadarshini E, Bhavani K, Anuradha CV (2014) Substitution of soy protein for casein prevents oxidative modification and inflammatory response induced in rats fed high

fructose diet. ISRN Inflamm 2014:641096. Published 2014 Apr 15. https://doi.org/10.1155/2014/641096
18. Mohammad Shahi M, Rashidi MR, Mahboob S, Haidari F, Rashidi B, Hanaee J (2012) Protective effect of soy protein on collagen-induced arthritis in rat. Rheumatol Int 32(8):2407–2414. https://doi.org/10.1007/s00296-011-1979-7
19. Snelson M, Mamo JCL, Lam V, Giles C, Takechi R (2017) Differential effects of high-protein diets derived from soy and casein on blood-brain barrier integrity in wild-type mice. Front Nutr 4:35. Published 2017 Jul 24. https://doi.org/10.3389/fnut.2017.00035
20. Miyamoto M, Kiyota Y, Yamazaki N, Nagaoka A, Matsuo T, Nagawa Y, Takeda T (1986) Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol Behav 38(3):399–406. https://doi.org/10.1016/0031-9384(86)90112-5
21. Miyamoto M, Kiyota Y, Nishiyama M, Nagaoka A (1992) Senescence-accelerated mouse (SAM): age-related reduced anxiety-like behavior in the SAM-P/8 strain. Physiol Behav 51(5):979–985. https://doi.org/10.1016/0031-9384(92)90081-c
22. Zhang X, Li G, Guo L, Nie K, Jia Y, Zhao L, Yu J (2013) Age-related alteration in cerebral blood flow and energy failure is correlated with cognitive impairment in the senescence-accelerated prone mouse strain 8 (SAMP8). Neurol Sci 34(11):1917–1924. https://doi.org/10.1007/s10072-013-1407-8
23. Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, Tomita Y, Yasuhira K et al (1981) A new murine model of accelerated senescence. Mech Ageing Dev 17(2):183–194. https://doi.org/10.1016/0047-6374(81)90084-1
24. Guo AY, Leung KS, Siu PMF, Qin JH, Chow SKH, Qin L, Li CY, Cheung WH (2015) Muscle mass, structural and functional investigations of senescence-accelerated mouse P8 (SAMP8) Exp Anim. 2015;64(4):425-33. https://doi.org/10.1538/expanim.15-0025
25. Derave W, Eijnde BO, Ramaekers M, Hespel P (2005) No effects of lifelong creatine supplementation on sarcopenia in senescence-accelerated mice (SAMP8). Am J Physiol Endocrinol Metab 289(2):E272–E277. https://doi.org/10.1152/ajpendo.00039.2005
26. Yagi H, Akiguchi I, Ohta A, Yagi N, Hosokawa M, Takeda T (1998) Spontaneous and artificial lesions of magnocellular reticular formation of brainstem deteriorate avoidance learning in senescence-accelerated mouse SAM. Brain Res 791(1-2):90–98. https://doi.org/10.1016/s0006-8993(98)00070-5
27. Nomura Y, Yamanaka Y, Kitamura Y, Arima T, Ohnuki T, Oomura Y, Sasaki K, Nagashima K et al (1996) Senescence-accelerated mouse. Neurochemical studies on aging. Ann N Y Acad Sci 786:410–418. https://doi.org/10.1111/j.1749-6632.1996.tb39080.x
28. Amano T, Nakanishi H, Oka M, Yamamoto K (1995) Increased expression of cathepsins E and D in reactive microglial cells associated with spongiform degeneration in the brain stem of senescence-accelerated mouse. Exp Neurol 136(2):171–182. https://doi.org/10.1006/exnr.1995.1094
29. Canudas AM, Gutierrez-Cuesta J, Rodríguez MI, Acuña-Castroviejo D, Sureda FX, Camins A, Pallàs M (2005) Hyperphosphorylation of microtubule-associated protein tau in senescence-accelerated mouse (SAM). Mech Ageing Dev 126(12):1300–1304. https://doi.org/10.1016/j.mad.2005.07.008
30. Del Valle J, Duran-Vilaregut J, Manich G, Casadesús G, Smith MA, Camins A, Pallàs M, Pelegrí C et al (2010) Early amyloid accumulation in the hippocampus of SAMP8 mice. J Alzheimers Dis 19(4):1303–1315. https://doi.org/10.3233/JAD-2010-1321
31. Gold PE (1986) The use of avoidance training in studies of modulation of memory storage. Behav Neural Biol 46(1):87–98. https://doi.org/10.1016/s0163-1047(86)90927-1
32. Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, Bambah-Mukku D, Blitzer RD, Alberini CM (2011) A critical role for IGF-II in memory consolidation and enhancement. Nature 469(7331):491–497. https://doi.org/10.1038/nature09667
33. Preston AR, Eichenbaum H (2013) Interplay of hippocampus and prefrontal cortex in memory Curr Biol 9;23(17):R764-R773. doi: https://doi.org/10.1016/j.cub.2013.05.041
34. Snow PJ (2016) The structural and functional organization of cognition. Front Hum Neurosci 10:501. https://doi.org/10.3389/fnhum.2016.00501
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275
36. Bambah-Mukku D, Travaglia A, Chen DY, Pollonini G, Alberini CM (2014) A positive autoregulatory BDNF feedback loop via C/EBPβ mediates hippocampal memory consolidation. J Neurosci Off J Soc Neurosci 34(37):12547–12559. https://doi.org/10.1523/JNEUROSCI.0324-14.2014
37. Contestabile A, Peña-Altamira E, Virgili M, Monti B (2016) Zinc supplementation in rats impairs hippocampal-dependent memory consolidation and dampens post-traumatic recollection of stressful event. Eur Neuropsychopharmacol 26(6):1070–1082. https://doi.org/10.1016/j.euroneuro.2015.12.041
38. Boccia M, Freudenthal R, Blake M, de la Fuente V, Acosta G, Baratti C, Romano A (2007) Activation of hippocampal nuclear factor-kappa B by retrieval is required for memory reconsolidation. J Neurosci 27(49):13436–13445. https://doi.org/10.1523/JNEUROSCI.4430-07.2007
39. Krawczyk MC, Blake MG, Baratti CM, Romano A, Boccia MM, Feld M (2015) Memory reconsolidation of an inhibitory avoidance task in mice involves cytosolic ERK2 bidirectional modulation. Neuroscience 294:227–237. https://doi.org/10.1016/j.neuroscience.2015.03.019
40. Beach TG, Walker R, McGeer EG (1989) Patterns of gliosis in Alzheimer's disease and aging cerebrum. Glia 2(6):420–436. https://doi.org/10.1002/glia.440020605
41. Desai MK, Mastrangelo MA, Ryan DA, Sudol KL, Narrow WC, Bowers WJ (2010) Early oligodendrocyte/myelin pathology in Alzheimer's disease mice constitutes a novel therapeutic target. Am J Pathol 177(3):1422–1435. https://doi.org/10.2353/ajpath.2010.100087
42. Cai Z, Xiao M (2016) Oligodendrocytes and Alzheimer's disease. Int J Neurosci 126(2):97–104. https://doi.org/10.3109/00207454.2015.1025778
43. Hofer MM, Barde YA (1988) Brain-derived neurotrophic factor prevents neuronal death in vivo. Nature 331(6153):261–262. https://doi.org/10.1038/331261a0
44. McKinnon RD, Piras G, Ida JA Jr, Dubois-Dalcq M (1993) A role for TGF-beta in oligodendrocyte differentiation. J Cell Biol 121(6):1397–1407. https://doi.org/10.1083/jcb.121.6.1397
45. Zhang Y, Zhang J, Navrazhina K, Argaw AT, Zameer A, Gurfein BT, Brosnan CF, John GR (2010) TGFbeta1 induces Jagged1 expression in astrocytes via ALK5 and Smad3 and regulates the balance between oligodendrocyte progenitor proliferation and differentiation. Glia 58(8):964–974. https://doi.org/10.1002/glia.20978
46. Poulsen KT, Armanini MP, Klein RD, Hynes MA, Phillips HS, Rosenthal A (1994) TGF beta 2 and TGF beta 3 are potent survival factors for midbrain dopaminergic neurons. Neuron 13(5):1245–1252. https://doi.org/10.1016/0896-6273(94)90062-0
47. Caraci F, Gulisano W, Guida CA, Impellizzeri AA, Drago F, Puzzo D, Palmeri A (2015) A key role for TGF-β1 in hippocampal synaptic plasticity and memory. Sci Rep 5:11252. https://doi.org/10.1038/srep11252
48. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B et al (2014) Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci 17(1):131–143. https://doi.org/10.1038/nn.3599

49. Osborne BF, Turano A, Caulfield JI, Schwarz JM (2019) Sex- and region-specific differences in microglia phenotype and characterization of the peripheral immune response following early-life infection in neonatal male and female rats. Neurosci Lett 692:1–9. https://doi.org/10.1016/j.neulet.2018.10.044
50. Yokota S, Suzuki Y, Hamami K, Harada A, Komai S (2017) Sex differences in avoidance behavior after perceiving potential risk in mice. Behav Brain Funct 13(1):9. https://doi.org/10.1186/s12993-017-0126-3
51. Katayama S, Imai R, Sugiyama H, Nakamura S (2014) Oral administration of soy peptides suppresses cognitive decline by induction of neurotrophic factors in SAMP8 mice. J Agric Food Chem 62(16):3563–3569. https://doi.org/10.1021/jf405416s
52. Min LJ, Kobayashi Y, Mogi M, Tsukuda K, Yamada A, Yamauchi K, Abe F, Iwanami J et al (2017) Administration of bovine casein-derived peptide prevents cognitive decline in Alzheimer disease model mice. PLoS One 12(2):e0171515. https://doi.org/10.1371/journal.pone.0171515
53. Bhagwat S, Haytowitz DB, Holden JM (2008) USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav
54. File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H (2001) Eating soya improves human memory. Psychopharmacology 157(4):430–436. https://doi.org/10.1007/s002130100845
55. López P, Sánchez M, Perez-Cruz C, Velázquez-Villegas LA, Syeda T, Aguilar-López M, Rocha-Viggiano AK, Del Carmen S-LM et al (2018) Long-term genistein consumption modifies gut microbiota, improving glucose metabolism, metabolic endotoxemia, and cognitive function in mice fed a high-fat diet. Mol Nutr Food Res 62(16):e1800313. https://doi.org/10.1002/mnfr.201800313
56. Wanders D, Ghosh S, Stone KP, Van NT, Gettys TW (2014) Transcriptional impact of dietary methionine restriction on systemic inflammation: relevance to biomarkers of metabolic disease during aging. Biofactors 40(1):13–26. https://doi.org/10.1002/biof.1111
57. Detzel CJ, Petschow BW, Johnson N, Weaver EM (2016) Comparison of the amino acid and peptide composition and postprandial response of beef, hydrolyzed chicken, and whey protein nutritional preparations. Funct Foods Health Dis 6(10):612–626
58. Gorissen S, Crombag J, Senden J, Waterval W, Bierau J, Verdijk LB, van Loon L (2018) Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids 50(12):1685–1695. https://doi.org/10.1007/s00726-018-2640-5
59. Cope EC, LaMarca EA, Monari PK, Olson LB, Martinez S, Zych AD, Katchur NJ, Gould E (2018) Microglia play an active role in obesity-associated cognitive decline. J Neurosci 38(41):8889–8904. https://doi.org/10.1523/JNEUROSCI.0789-18.2018
60. Carey AN, Gildawie KR, Rovnak A, Thangthaeng N, Fisher DR, Shukitt-Hale B (2019) Blueberry supplementation attenuates microglia activation and increases neuroplasticity in mice consuming a high-fat diet. Nutr Neurosci 22(4):253–263. https://doi.org/10.1080/1028415X.2017.1376472
61. Hendrickx DAE, van Eden CG, Schuurman KG, Hamann J, Huitinga I (2017) Staining of HLA-DR, Iba1 and CD68 in human microglia reveals partially overlapping expression depending on cellular morphology and pathology. J Neuroimmunol 309:12–22. https://doi.org/10.1016/j.jneuroim.2017.04.007
62. Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN et al (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95(18):10896–10901. https://doi.org/10.1073/pnas.95.18.10896
63. Fan Q, Gayen M, Singh N, Gao F, He W, Hu X, Tsai LH, Yan R (2019) The intracellular domain of CX3CL1 regulates adult neurogenesis and Alzheimer's amyloid pathology. J Exp Med 216(8):1891–1903. https://doi.org/10.1084/jem.20182238
64. Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC, Feuerstein GZ (1994) Tumor necrosis factor-alpha expression in ischemic neurons. Stroke 25(7):1481–1488. https://doi.org/10.1161/01.str.25.7.1481
65. Lau LT, Yu AC (2001) Astrocytes produce and release interleukin-1, interleukin-6, tumour necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. J Neurotrauma 18(3):351–359. https://doi.org/10.1089/08977150151071035
66. Riazi K, Galic MA, Kuzmiski JB, Ho W, Sharkey KA, Pittman QJ (2008) Microglial activation and TNFalpha production mediate altered CNS excitability following peripheral inflammation. Proc Natl Acad Sci U S A 105(44):17151–17156. https://doi.org/10.1073/pnas.0806682105
67. Cacci E, Claasen JH, Kokaia Z (2005) Microglia-derived tumour necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. J Neurosci Res 80(6):789–797. https://doi.org/10.1002/jnr.20531
68. Iosif RE, Ekdahl CT, Ahlenius H, Pronk CJ, Bonde S, Kokaia Z, Jacobsen SE, Lindvall O (2006) Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. J Neurosci 26(38):9703–9712. https://doi.org/10.1523/JNEUROSCI.2723-06.2006
69. Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18(9):3206–3212. https://doi.org/10.1523/JNEUROSCI.18-09-03206.1998
70. Faure A, Verret L, Bozon B, El Tannir El Tayara N, Ly M, Kober F, Dhenain M, Rampon C et al (2011) Impaired neurogenesis, neuronal loss, and brain functional deficits in the APPxPS1-Ki mouse model of Alzheimer's disease. Neurobiol Aging 32(3):407–418. https://doi.org/10.1016/j.neurobiolaging.2009.03.009
71. Gang B, Yue C, Han N, Xue H, Li B, Sun L, Li X, Zhao Q (2011) Limited hippocampal neurogenesis in SAMP8 mouse model of Alzheimer's disease. Brain Res 1389:183–193. https://doi.org/10.1016/j.brainres.2011.03.039
72. Kempermann G, Gast D, Gage FH (2002) Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 52(2):135–143. https://doi.org/10.1002/ana.10262
73. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med 25(4):554–560. https://doi.org/10.1038/s41591-019-0375-9
74. Femminella GD, Dani M, Wood M, Fan Z, Calsolaro V, Atkinson R, Edginton T, Hinz R et al (2019) Microglial activation in early Alzheimer trajectory is associated with higher gray matter volume. Neurology 92(12):e1331–e1343. https://doi.org/10.1212/WNL.0000000000007133
75. von Bartheld CS, Bahney J, Herculano-Houzel S (2016) The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. J Comp Neurol 524(18):3865–3895. https://doi.org/10.1002/cne.24040
76. Ota M, Sato N, Kimura Y, Shigemoto Y, Kunugi H, Matsuda H (2019) Changes of myelin organization in patients with Alzheimer's disease shown by q-space myelin map imaging. Dement Geriatr Cogn Dis Extra 9(1):24–33. Published 2019 Feb 6. https://doi.org/10.1159/000493937
77. Zhu X, Zuo H, Maher BJ, Serwanski DR, LoTurco JJ, Lu QR, Nishiyama A (2012) Olig2-dependent developmental fate switch of NG2 cells. Development 139(13):2299–2307. https://doi.org/10.1242/dev.078873

78. Fancy SP, Zhao C, Franklin RJ (2004) Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol Cell Neurosci 27(3):247–254. https://doi.org/10.1016/j.mcn.2004.06.015
79. Barker R, Wellington D, Esiri MM, Love S (2013) Assessing white matter ischemic damage in dementia patients by measurement of myelin proteins. J Cereb Blood Flow Metab 33(7):1050–1057. https://doi.org/10.1038/jcbfm.2013.46
80. Kinter J, Lazzati T, Schmid D, Zeis T, Erne B, Lützelschwab R, Steck AJ, Pareyson D et al (2013) An essential role of MAG in mediating axon-myelin attachment in Charcot-Marie-Tooth 1A disease. Neurobiol Dis 49:221–231. https://doi.org/10.1016/j.nbd.2012.08.009
81. Ferrer I, Andrés-Benito P (2020) White matter alterations in Alzheimer's disease without concomitant pathologies. Neuropathol Appl Neurobiol. https://doi.org/10.1111/nan.12618 Advance online publication
82. Zöller T, Schneider A, Kleimeyer C, Masuda T, Potru PS, Pfeifer D, Blank T, Prinz M et al (2018) Silencing of TGFβ signalling in microglia results in impaired homeostasis. Nat Commun 9(1):4011. Published 2018 Oct 1. https://doi.org/10.1038/s41467-018-06224-y
83. Hu Y, Chen W, Wu L, Jiang L, Liang N, Tan L, Liang M, Tang N (2019) TGF-β1 restores hippocampal synaptic plasticity and memory in Alzheimer model via the PI3K/Akt/Wnt/β-catenin signaling pathway. J Mol Neurosci 67(1):142–149. https://doi.org/10.1007/s12031-018-1219-7
84. Arkhipov VI, Pershina EV, Levin SG (2019) The role of anti-inflammatory cytokines in memory processing in a healthy brain. Behav Brain Res 367:111–116. https://doi.org/10.1016/j.bbr.2019.03.053
85. Noguchi A, Nawa M, Aiso S, Okamoto K, Matsuoka M (2010) Transforming growth factor beta2 level is elevated in neurons of Alzheimer's disease brains. Int J Neurosci 120(3):168–175. https://doi.org/10.3109/00207450903139689
86. Chong JR, Chai YL, Lee JH, Howlett D, Attems J, Ballard CG, Aarsland D, Francis PT et al (2017) Increased transforming growth factor β2 in the neocortex of Alzheimer's disease and dementia with Lewy bodies is correlated with disease severity and soluble Aβ42 load. J Alzheimers Dis 56(1):157–166. https://doi.org/10.3233/JAD-160781
87. Tesseur I, Zou K, Esposito L, Bard F, Berber E, Can JV, Lin AH, Crews L et al (2006) Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. J Clin Invest 116(11):3060–3069. https://doi.org/10.1172/JCI27341
88. Palazuelos J, Klingener M, Aguirre A (2014) TGFβ signaling regulates the timing of CNS myelination by modulating oligodendrocyte progenitor cell cycle exit through SMAD3/4/FoxO1/Sp1. J Neurosci 34(23 ):7917– 7930. https://doi.org/10.1523/JNEUROSCI.0363-14.2014
89. Lippa CF, Flanders KC, Kim ES, Croul S (1998) TGF-beta receptors-I and -II immunoexpression in Alzheimer's disease: a comparison with aging and progressive supranuclear palsy. Neurobiol Aging 19(6):527–533. https://doi.org/10.1016/s0197-4580(98)00089-x
90. Senatorov VV Jr, Friedman AR, Milikovsky DZ, Ofer J, Saar-Ashkenazy R, Charbash A, Jahan N, Chin G et al (2019) Blood-brain barrier dysfunction in aging induces hyperactivation of TGFβ signaling and chronic yet reversible neural dysfunction. Sci Transl Med 11(521):eaaw8283. https://doi.org/10.1126/scitranslmed.aaw8283
91. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A et al (2009) Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. J Neurosci 29(28 ):8927– 8935. https://doi.org/10.1523/JNEUROSCI.0430-09.2009
92. Marzella L, Ahlberg J, Glaumann H (1981) Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. Virchows Arch B Cell Pathol Incl Mol Pathol 36(2-3):219–234. https://doi.org/10.1007/bf02912068
93. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441(7095):880–884. https://doi.org/10.1038/nature04723
94. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM (2005) Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol 64(2):113–122. https://doi.org/10.1093/jnen/64.2.113
95. Ma Q, Qiang J, Gu P, Wang Y, Geng Y, Wang M (2011) Age-related autophagy alterations in the brain of senescence accelerated mouse prone 8 (SAMP8) mice. Exp Gerontol 46(7):533–541. https://doi.org/10.1016/j.exger.2011.02.006
96. Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG (1996) Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. J Biol Chem 271(44):27879–27887. https://doi.org/10.1074/jbc.271.44.27879
97. Domise M, Didier S, Marinangeli C, Zhao H, Chandakkar P, Buée L, Viollet B, Davies P et al (2016) AMP-activated protein kinase modulates tau phosphorylation and tau pathology in vivo. Sci Rep 6:26758. https://doi.org/10.1038/srep26758
98. Guneykaya D, Ivanov A, Hernandez DP, Haage V, Wojtas B, Meyer N, Maricos M, Jordan P et al (2018) Transcriptional and translational differences of microglia from male and female brains. Cell Rep 24(10):2773–2783.e6. https://doi.org/10.1016/j.celrep.2018.08.001
99. Rossetti AC, Paladini MS, Trepci A, Mallien A, Riva MA, Gass P, Molteni R (2019) Differential neuroinflammatory response in male and female mice: a role for BDNF. Front Mol Neurosci 12:166. https://doi.org/10.3389/fnmol.2019.00166