Honours Projects 2014

TITLE OF PROJECT: Consequences of chronic neuroinflammation on the brain: implications on Alzheimer's disease

SUPERVISOR: Prof. Gerald Muench

EMAIL: g.muench@uws.edu.au

CO-SUPERVISOR/S:

1. Dr. Erika Gyengesi
   EMAIL: e.gyengesi@uws.edu.au

2. Dr Tim-Stait-Gardner
   EMAIL: t.stait-gardner@uws.edu.au

3. Prof. William Price
   EMAIL: w.price@uws.edu.au

CAMPUS/S PROJECT IS OFFERED AND CONDUCTED: Campbelltown

BACKGROUND (200 WORDS):

Epidemiological evidence shows that plant-derived foods with anti-inflammatory and anti-oxidant potential protect against Alzheimer’s disease (AD), but it is not clear which compounds are responsible for this positive effect.

Activated, cytokine-overexpressing microglia are near-universal components of Aβ plaques at early and mid stages during the progression of AD, and only decline in end-stage, dense core plaques.

Activated microglia show close associations with tangle-bearing cholinergic neurons in AD. However, transgenic animal models of AD, which overexpressing mutant forms of presenilin, tau and amyloid precursor protein do not show the same variety of pro-inflammatory markers as human AD patients and develop a much weaker neuroinflammatory phenotype.

To establish an animal model in which specific food compounds can be tested, we will use transgenic mice, which show chronic brain inflammation and develop progressive neurological deficits.

We will monitor changes in their behavior, including a decline in cognitive functions. We will also investigate the underlying pathological changes, e.g. how chronic inflammation destroys nerve cells and their connections by using anatomical methods and MRI scans.

Finally, we will investigate to what degree curcumin, a natural anti-oxidant and anti-inflammatory compound, can attenuate inflammation, prevent brain damage, and the loss of memory functions in our mice.

We expect that our model will assist in the selection of potent candidate drugs, which can then be validated in clinical trials with Alzheimer patients

AIM OF STUDY:

The specific aims of this study are:

1. To study the effects of chronic neuroinflammation on the deterioration of memory and cognition in the GFAP-IL-6 overexpressing mice during their lifespan. The changes of the brain structure will be also examined by high resolution Magnetic Resonance Imaging (MRI).

2. To study the effects of neuroinflammation on the morphological structure of the basal forebrain cholinergic system and the interconnected areas using immunohistochemistry.
3. To investigate the effects of a high bioavailable preparation of the anti-inflammatory compound curcumin on brain inflammation, neurodegeneration and cognitive decline.

**METHODS:**

**cognition in the IL-6 overexpressing mice during their lifespan.**

*Research plan:* Physical examination of the animals will be carried out before the start of the behavioural testing, to test their sensory abilities (response to auditory, olfactory, somatosensory, taste, vestibular, and visual stimuli), and muscle strength. The performance of transgenic GFAP-IL6 overexpressing mice in special learning tasks (n =12/group, equal distribution of gender) will be compared in several behavioural tests to that of sex- and age-matched wild type controls (littermates) over time. Tests will be taking place at 3, 6, 9, and 12 months of age and different tests will take place on separate days. Both groups (heterozygous and wild type) will go through the same test on one day and testing times will be matched to exclude variance due to diurnal rhythm for each testing session.

The behavioural tests will include the following:

- **Open field and elevated O-maze** will be used to identify differences between the groups in exploratory behaviour, anxiety and locomotor activity. The open field test measures hyperactivity, exploratory activity, stereotyped rotation, anxiety and memory for context.

- **The novel object recognition test** will be used to evaluate cognition, particularly working and recognition memory. This is one particular object recognition task that is sensitive to age-related deficits and is very suitable to test dementia related deficits.

- **The T-maze Spontaneous Alternation test** will be measuring the willingness of mice to explore new environments. T-maze tasks are characterized and are widely used for cognitive behavioural testing in mice. Many parts of the brain - including the hippocampus, septum, basal forebrain, and prefrontal cortex - are involved in this task.

- **The radial arm maze** will test the spatial memory of the animals. This maze requires the use of working memory to retain information that is important for a short time (within trial information), as well as the use of reference memory to retain the general rules of the task across days.

- **The operant conditioning chamber, or Skinner box,** will be used to investigate different aspects of learning behaviour (classical and operant conditioning). Animals will be trained to (in most cases) press a lever in response to a specific stimulus (sound, light etc.) in order to receive a reward (often food). In some cases, the missing or giving an incorrect or delayed response is followed by a punishment (air puff, minor electric foot shock).

In-between behavioural testing, mice will be scanned by a high resolution MRI machine (located at UWS, Campbelltown campus), in collaboration with Prof. Bill Price and Dr Tim Stait-Gardner (Nanoscale Group, UWS). Repeated scanning of the same animals will shed light about the progressive changes caused by low-grade neuroinflammation, in particular the effects on the white matter of the brain. Myelination will also be examined in Specific aim 2, by using histochemical techniques.

**Specific aim 2. To study the effects of neuroinflammation on the morphological structure of the basal forebrain cholinergic system and its interconnected areas.**

*Research plan:* Immunohistochemical investigation of the expression of cholinergic cell bodies and neuritis, calcium-binding protein containing neurons (parvalbumin), IL-6, GFAP, myelination and overall cell numbers will be carried out on both GFAP-IL6 transgenic mice and their wild type littermates.
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GFAP-IL6 heterozygote transgenic and wild type mice of age from 3 to 12 months (n=7) will be euthanized and transcardinally perfused. Brains will be removed and sectioned at 40 μm with a cryostat the next day in 7 series. Sections will be collected from the entire brain from the olfactory bulb to the cerebellum and analysed by using a research microscope equipped with MBF Biosciences StereoInvestigator to achieve design-based, un-biased quantitative results. On the first series of sections, modified Nissl staining will be performed. Sections of series 2 to 6 will be treated with the following primary antibodies followed by secondary antibodies to visualise cholinergic, parvalbumin, secretagogin, GFAP, IL-6 and IL-6 receptor expressing cells and glia. Myelin silver staining, first described by Gallyas, will be carried out on the last series to visualise myelinated axons.

Specific aim 3. To investigate the effects of anti-inflammatory compound curcumin on brain inflammation, neurodegeneration and synaptic decline.

Research plan: To test the effects of curcumin on our animal model, a group of GFAP-IL6 heterozygous mice (n=8/group, equal distribution of gender) will be treated with Longvida curcumin in the drinking water (Longvida, Vendure Sciences). Levels of curcumin will be measured from the blood and the brain tissue by using HPLC-MS. Animals will undergo the above-mentioned behavioural tests at the age of 6, 9, 12, 15, and 18 months. Additional 5 groups (n=7) of GFAP-IL6 mice will be also continuously treated, but sacrificed for immunohistological experiments described in Aim 2, to investigate the morphological and gene expression changes during the life span caused by continuous consumption of curcumin. Results will then be compared between the treated, the untreated and wild type groups (using our results from Aim 1 and 2).

ETHICS APPLICATION REQUIREMENTS: Animal Research Authority application has been approved by the UWS Animal Ethics committee to conduct animal research, as documented in Animal Research and Teaching Proposal Number: A10057; Title: Anatomical and behavioural studies of the rodent brain during neuroinflammation in IL6-GFAP mice; at the following site: Animal holdings - C Building 30 SoM animal holdings

KEY REFERENCES:

1 Ikegami, S. Behavioral impairment in radial-arm maze learning and acetylcholine content of the hippocampus and cerebral cortex in aged mice. Behav Brain Res 65, 103-111 (1994).


