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Effect of C5a/C5aR on Glutamate Receptors and Glutamate Transporters expression in AD model mice

FINAL DEGREE PROJECT

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Biochemistry and Molecular Biology Degree

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ABSTRACT

Background: Alzheimer's disease, a clinically progressive dementia characterized by the accumulation of amyloid- β protein, neurofibrillary tangles, and neuronal loss, is the leading cause of age-related cognitive dysfunction in the world today. The disease is also associated with neuroinflammation, activation of microglia and astrocytes, and evidence of activation of the complement system.

The Tenner Lab previously showed significant deficits in behavior in C5aGFAP Arctic mice and improvement in Arctic C5aRKO. Among amyloid beta plaques and activated microglia, there were only significant differences seen in the activated microglia, stained by its marker CD45. The IHC data did not correlate with the differences seen in the behavior of these models. Glutamate is a major excitatory neurotransmitter in the central nervous system and is known to be involved in synaptic transmission and plasticity, neuronal growth, and learning and memory. To further determine correlates of the loss of cognitive ability in the AD mouse models, we study neuronal markers involved in memory and learning, focusing on those involved in glutamate signaling pathways.

Methods: In the present study we validated previous findings of the amount of fibrillar plaques and recruited microglia using thioflavine staining and CD45 immunohistochemistry, respectively, in 7 months Arctic/ArcticC5aGFAP and in 8.5 months Arctic/ArcticC5aRKO. We then analyzed the expression of the glutamate receptor, GluR1, and its phosphorylated form, pGluR1, and two transporters, GLT1 and VGLuT1, in hippocampal lysates of these transgenic mouse models of AD (8.5m ArcticC5aRKO) by Western Blot.

Results: Significant decreased expression of GluR1 and pGluR1 was observed in the Arctic model which is in agreement with deficits in memory and learning seen in AD. We confirmed that there are no significant differences in fibrillar plaques or microglia staining between genotypes in 8.5 months ArcticC5aRKO and 7months ArcticC5aGFAP groups.

Conclusion: On the basis of these results, we conclude that significant increase in pGluR1 levels of ArcticC5aRKO (compared to Arctic) suggests that the lack of C5aR is beneficial in this AD model. In addition, significant lower expression level of GluR1 in Arctic (compared to WT) agrees with poor cognition of AD model.

INTRODUCCTION

I. Alzheimer disease

Alzheimer's disease (AD) is the most common neurodegenerative pathology of aging, accounting for 50 to 75% of all cases of dementia in the elderly (Ferri et al., 2009), and currently, has no cure (Wang et al., 2014). AD is characterized by development of a progressive neuropsychiatric disease that is involved in gradual memory impairment, loss of acquired skills and emotional disturbances (Lee et al., 2010) .

As AD progresses over the years, it results in immobilization and emaciation, and finally death due to pneumonia (Lee et al., 2010) .The number of cases of dementia worldwide in 2010 was estimated to be about 35.6 million. The number is expected to almost double every 20 years and, 65.7 million in 2030, and 115.4 million in 2050 (Ferri et al., 2009).

The pathology present in AD is characterized by the presence of A β senile plaques, neurofibrillary tangles (NFTs) that involve hyperphosphorylated tau proteins, extensive neuron loss and inflammatory glia (Fonseca et al., 2009). Studies in both AD patients and transgenic mice models of AD suggest that the cause is made by multiple factors (Veerhuis et al., 2011), because the presence of A β plaques is not enough to diagnose AD (Aizenstein et al., 2008). These abnormalities chiefly take place in brain areas that regulate memory (the limbic system) and acquired skills (the frontal, parietal and temporal cortexes) (Fig. 1). Therefore, as the disease advances, symptoms get worse and the patient suffers irritability, confusion, mood swings, language breakdown, long-term memory loss, and senses decline (Waldemar et al., 2007).

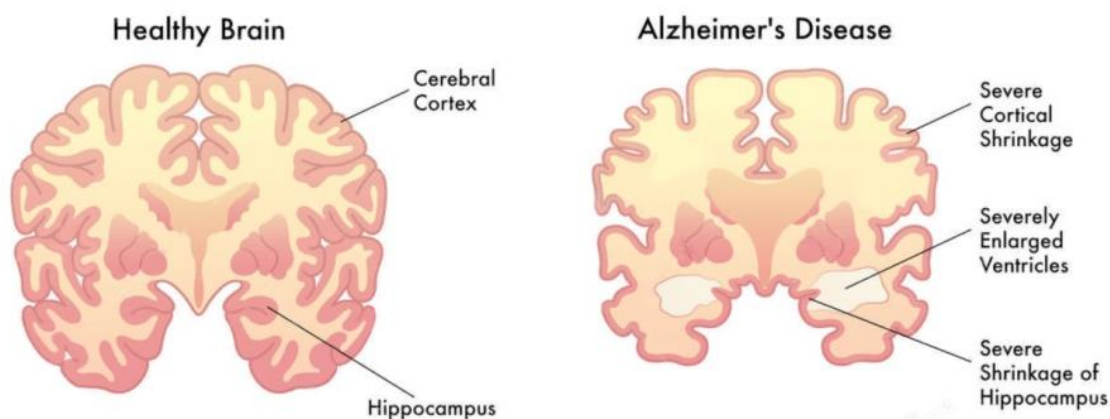


Figure 1. Schematic representation of massive cell loss in Alzheimer's disease (Alexandra Marine & General Hospital 2014).

Amyloid β

A β protein is a small fragment derived from the processing of the larger transmembrane amyloid precursor protein (APP). APP is suspected to be involved in A β accumulation, neuronal growth, survival and postinjury repair (Turner et al., 2003;Priller et al., 2006)

Evidence of an inflammatory response to A β deposition has been accumulating since the 1980s (Eikelenboom and Stam, 1982b) and multiple investigators have attempted to define the role such inflammation plays in disease development (Bonifati and Kishore, 2007). A β accumulation

in parenchyma and blood vessels causes microglial migration and promotes inflammatory responses against the aggregates (Akiyama et al., 2000;Kitazawa et al., 2004).

In contrast to non-demented elderly individuals, who may also contain pools of A β deposits and some low level inflammation, brains of AD patients have fibrillar A β plaques and these plaques show extensive deposition of components of the complement system (Afagh et al., 1996;Zanjani et al., 2005)

Healthy human bodies have clearance mechanisms for A β peptides available, and the generated A β peptides are equivalent to the cleared A β peptides. A deficient clearance of A β appears in the sporadic forms of AD (90% of all AD cases), and the generation and clearance of A β peptides are out of balance (Huang and Jiang, 2009). Increasingly, A β peptides will accumulate and form senile plaques (Zhou et al., 2005) (Fig. 2).

Aggregates of A β are able to play the role of pro-inflammatory molecules causing the activation of different microglia and induce the production of inflammatory mediators such as reactive oxygen species (ROS), nitric oxide (NO), tumor necrosis factor (TNF)- α , Interleukins (IL-1 β , IL-6, IL-18), and prostaglandins (e.g., Prostaglandin E2), that provokes neuronal death (Akiyama et al., 2000;Kitazawa et al., 2004) and exacerbates the disease pathology (Table I). These arguments suggest that A β accumulation directly leads to neuronal damage and indirectly leads to activation of inflammatory systems, contributing to disease progression (Lee et al., 2010). This process can be aggravated by the disturbed balance between complement activators and complement regulatory proteins (Veerhuis et al., 2011).

Table I. Molecules and products released from microglia and astrocytes that are reportedly associated with AD (Veerhuis et al., 2011).

Microglia	Astrocytes
Complement proteins	Complement proteins
Complement inhibitors	Complement inhibitors
A β	A β
Cytokines and chemokines	Cytokines and chemokines
IL-1	IL-1
TNF- α	TNF- α
IL-6	IL-6
IL-18	IL-18
MIP-1	S100
ROS	COX-2
MHC II	

Tau protein

Tau is a microtubule-associated protein known for its stabilization of microtubules in axons. Recently, it has proved that tau participates in synaptic function and that this can be disrupted in Alzheimer's disease (Frandsen et al., 2014). In the AD brain, tau is abnormally hyperphosphorylated and its aggregation form is the major subunit of paired helical filaments (PHFs), which form neurofibrillary tangles (NFTs) in AD (Grundke-Iqbal et al., 1986)(Fig. 2).

Hyperphosphorylation is regulated by several kinases that phosphorylate specific sites of tau in vitro, and its accumulation in neurons is one of the main pathologic hallmarks in AD (Ferrer et al., 2005). Phosphorylation of tau protein contributes to its dysfunction in binding to microtubules and promotes microtubule assembly (Lu et al., 1999). Altered tau protein dissociates from the microtubules and aggregates to form insoluble fibers in the brains of AD patients (Huang and Jiang, 2009). The NFTs finally choke the affected neurons and facilitate cell death by acting as a space-occupying lesion.

In addition, it is believed that hyperphosphorylated tau protein induce direct events such as up-regulation or aberrant activation of tau kinases, down-regulation of phosphatases, covalent modifications of tau proteins, and indirect events such as A β -mediated toxicity, oxidative stress and inflammation (Ballatore et al., 2007; Iyer et al., 2010; McNaull et al., 2010)

Studies of the correlation of the cognitive impairment to the histopathological changes have consistently demonstrated that the number of NFTs correlates best with the presence and the degree of dementia in AD (Gong et al., 2010).

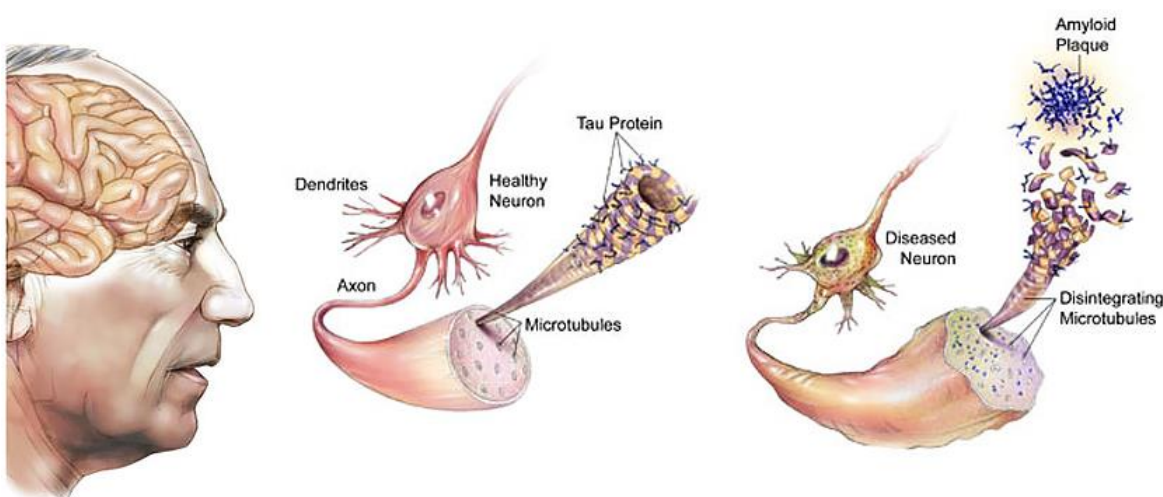


Figure 2. Presence of both amyloid- β plaque deposits and elevated levels of altered Tau protein, interfering with normal thought and memory (Alzheimer's Association, 2013 [www.alz.org]).

II. Mouse models of AD

The ability to generate transgenic organisms exists for many organisms such as rats, mice, fish, fly, or worms, and, all of which allow, introduction of genetic modifications. Although attempts to model AD in invertebrates systems such as *Drosophila* and *C. elegans*, they have had less effect than models in mammalian systems. Among vertebrates, mice are undoubtedly the species most used (Elder et al., 2010).

AD is considered as good disease for modeling in transgenic mouse, because it has a well-characterized disease and also has well-defined pathological features. Transgenic modeling has taken advantage of mutations in the amyloid precursor protein (APP) that cause Alzheimer's disease.

Nowadays, transgenic mouse models exist that replicate different parts of AD dementia. Although there is no AD model that entirely mimics the human disorder, the models have

helped significant insights into the pathophysiology of β -amyloid toxicity, in particular with the effects of different β -amyloid sequences and the pathogenic function of β -amyloid oligomers. These models will continue to play pivotal functions in preclinical studies, to have an essential role in the progression of AD therapies that are at present in clinical assays, and be used as instruments for creating profound perception of the Alzheimer's disease biological basis (Elder et al., 2010).

Overexpression of a human APP transgene containing the Swedish FAD mutation was produced in 1996 by Hsiao and colleagues. These mice, named Tg2576, exhibited human APP at levels at least 5 times more than levels of the endogenous mouse APP, and $A\beta$ levels ($A\beta_{40-42}$) increased with age. PDAPP mice expressed age-dependent amyloid aggregation, which resulted in Thioflavine staining positive plaques. The Tg2576 mouse has been the most extensively studied transgenic AD model (Elder et al., 2010).

Although the different transgenic models have many likenesses, they distinguish in some aspects; one could be the amyloid- β plaque deposition. For example, TgCRND8 mice, which express many APP mutations, exhibit parenchymal amyloid deposition at 3 months of age (Chishti et al., 2001). APP23 mice, in which express Thy-1 promoter to lead the Swedish mutation, are notable for their prominent vascular amyloid deposition. PDAPP and Tg2576 mice also differ in some aspects; for example, in Tg2576 mice, $A\beta_{40}$ and $A\beta_{42}$ are increased proportionately, whereas in PDAPP mice, $A\beta_{42}$ is disproportionately increased. Tg2576 mice are also known for their giant plaques and exhibit more vascular amyloid deposition, which is widely absent in PDAPP mice. In Tg2576 mice, most $A\beta$ deposits in dense cored plaques and Tg2576 mice have few diffuse deposits of amyloid (Elder et al., 2010).

A triple-transgenic model of AD (3xTg-AD) has been generated to exhibit plaque and tangle pathology, as well as synaptic dysfunction. This model is unique from previous models as it expresses 3 dementia-related transgenes and demonstrates a clear age-dependent onset of AD neuropathology (Sterniczuk et al., 2010).

Transgenic mouse models mimic a range of AD-related pathologies. Table II shows some of the most widely studied mouse models. These models have indicated novel insights into the pathophysiology as well as new therapeutic approaches.

Table II. Selected Examples of AD Mouse Models (Elder et al., 2010).

Line	Promoter	FAD Mutation	Amyloid Pathology	Reference
PDAPP	PDGF	APP- Indiana	Parenchymal plaques at 6–9 months of age	Games et al., 1995
Tg2576	PrP	APP-Swedish	Parenchymal plaques by 11–13 months of age with some vascular amyloid	Hsiao et al., 1996
APP23	Thy-1	APP-Swedish	Parenchymal plaques by 6 months of age and prominent vascular deposition of amyloid	Calhoun et al., 1999
TgCRND8	PrP	APP-Swedish + Indiana	More aggressive parenchymal plaque pathology present by 3 months of age	Chishti et al., 2001
APP-Dutch	Thy-1	APPE693Q associated with hereditary cerebral hemorrhage with Dutch-type amyloidosis	Vascular deposition of amyloid with few parenchymal plaques	Herzig et al., 2004

PS1M146V	PDGF	PS1M146V	Elevated A β 42 without plaque pathology	Duff et al., 1996
PSAPP	PS1M1146V x Tg2576	PS1M146V + APP-Swedish	Earlier and more extensive plaque pathology in comparison with Tg2576 alone	Holcomb et al., 1998
3xTg	Thy-1.2 and native mouse	Transgenes containing Thy-1.2– driven APP-Swedish and tau P301L were coinjected onto a homozygous mutant presenillin PS1M146V knock-in background.	Parenchymal plaques by 6 months of age combined with tau pathology by 12 months of age	Oddo et al., 2003

A β , β -amyloid; **AD**, Alzheimer's disease; **APP**, amyloid precursor protein; **FAD**, familial Alzheimer's disease; **PDAPP**, platelet-derived growth factor promoter driving amyloid precursor protein; **PDGF**, platelet-derived growth factor β ; **PrP**, prion protein; **PS1**, presenilin 1; **PSAPP**, presenilin/amyloid precursor protein; **Tg**, transgenic.

Arctic model mouse

The 'Arctic' mutation, which is located in the A β sequence (E22G), is associated with early onset Alzheimer disease. *In vitro*, Arctic-mutant A β forms provoke protofibrils as well as fibrils more effectively, in larger amounts, and faster than wild-type A β (Cheng et al., 2004).

To compare wild-type mice and mice carrying the human APP transgene with the Arctic mutant *in vivo*, transgenic mouse models expressing Arctic-mutant human amyloid precursor proteins (hAPP) were generated and aged. The Arctic mutation introduced into hAPP minigene causes increase levels of A β ₁₋₄₂ (which in humans causes early-onset autosomal dominant forms of familial Alzheimer's disease (FAD), promotes the formation of neurotoxic A β assemblies, accelerate the appearance of phenotypic differences, and allows for comparison of hAPP-Arc mice with the wild-type A β (Cheng et al., 2004).

III. Inflammation in AD

The brain has been thought to be an immune privileged organ, because the blood-brain barrier (BBB) avoids the entrance of blood borne cells and proteins into the central nervous system (CNS). Consequently, the clearance of invasive microorganisms, senescent cells, aged and glycosylated proteins, in order to keep a healthy environment for neuronal and glial cells, is in great measure restricted to the innate immune system (Veerhuis et al., 2011).

Evidence of neuroinflammation as a considerable component in the evolution of AD has been accumulating since the 1990's and immune activation in the brain has been identified as a potential target for therapeutic intervention (Craft et al., 2006; Hu et al., 2007), although the presence of beneficial as well as detrimental effects requires care in selection of targets (Lucin and Wyss-Coray, 2009; Gasparini et al., 2005)

Inflammatory molecules associated with AD inflammation include the pentraxin acute-phase proteins, the classical and alternative cascades of the complement system, as well as chemokines and cytokines (Tuppo and Arias, 2005).

IV. Complement pathway

Complement cascade is a powerful mechanism of innate immunity (Walport, 2001), recognizing danger, providing defense through the discriminating self from non-self (Ricklin et al., 2010), opsonization and lysis of pathogenic microbes (Monk et al., 2007). Activation of complement, which is composed of over 30 soluble and membrane-associated proteins present in the blood, can occur through three pathways, namely, classical, lectin, and alternative, each triggered by different molecules. All three complement pathways converge at the formation of the enzyme C3 convertase that can cleave component C3 creating C3b (the ligand of CR1, also known as CD35) and C3a activation products (Van Beek et al., 2001; Boos et al., 2005), and causing a cascade of further cleavage and activation events.

The larger C3b fragment, a major effector factor of the C pathway, acts as an opsonin and with other agents can assemble the C5 convertase, which enables further activation of the cascade leading to production of the chemotactic C5a fragment and the initiation of the membrane attack pathway (MAC), consisting of C5b, C6, C7, C8, and polymeric C9 (Fig. 3). Thereby, when the cascade is fully activated, MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which leads to osmotic lysis of the target cell and the invasive microorganisms (Veerhuis et al., 2011).

Complement proteins and activation products have been found associated with neuropathology in AD (Fonseca et al., 2011). The association of complement factors with amyloid deposits in AD was first described in immunohistochemical studies in the early '80s (Eikelenboom and Stam, 1982a); (Ishii and Haga, 1984). In vitro studies have proven the activation of both classical and alternative complement cascades by fibrillar A β (Rogers et al., 1992; Jiang et al., 1994) (Querfurth and LaFerla, 2010; Jiang et al., 1994) .

The development of monoclonal antibodies enhance the specific detection of C activation products and the use of component specific knockout mice to validate antibodies used in mouse models of neurodegeneration further bolstered the validity of the reports of C component association with fibrillar A β plaques, so providing consistent evidence that amyloid plaques can activate complement in vivo (Eikelenboom et al., 1989); (Rogers et al., 1992).

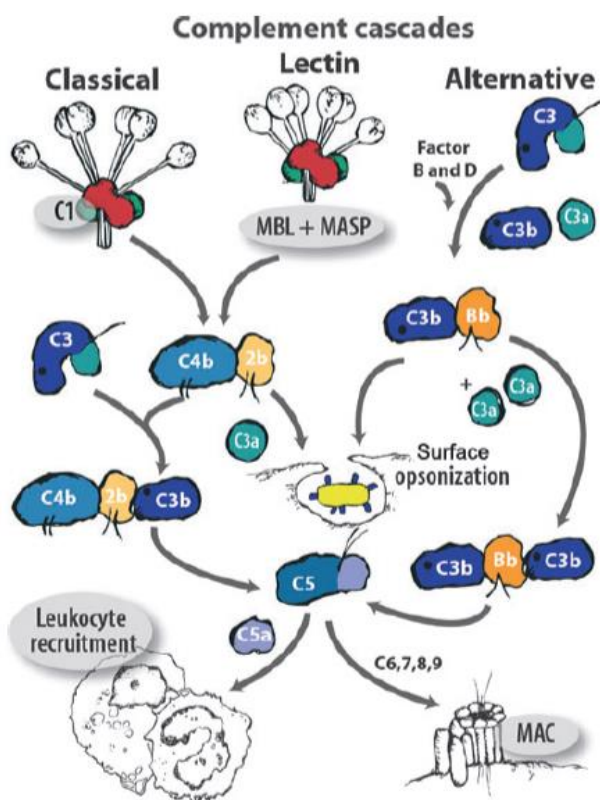


Figure 3. Model of complement activation and effector functions. The three complement activation pathways result in the effector functions of surface opsonization [C3b deposits on the pathogen (yellow)], leukocyte recruitment (C3a and C5a), and pathogen lysis by formation of the C5b-9 complex in membranes (MAC) (Alexander et al., 2008).

C5a/C5aR

C5a, a fragment generated by complement pathway activation, is chemotactic for glia (Yao et al., 1990). Increasing evidence suggests that C5a-C5aR interaction has a detrimental effect in neuroinflammatory diseases either indirectly via microglia activation (Ingersoll et al., 2010) or directly on neurons (Farkas et al., 1998).

C5a receptor, also identified as CD88, is a seven-transmembrane domain receptor, and its binding to C5a fragment provokes intracellular calcium mobilization and activation of different signaling pathways (Monk et al., 2007). Neutrophils, eosinophils, and macrophages express C5aR and these cells will migrate from the peripheral region to the inflammatory area in response to a gradient of C5a (Ballatore et al., 2007).

There are reports of C5a neuroprotective effects (Mukherjee et al., 2010), but increasing evidence suggests that C5a-CD88 interaction has a negative role in the progression of AD either directly on neurons or indirectly via microglia activation (Veerhuis et al., 2011).

A recent relevant study (Fonseca et al., 2009) reported that the C5a receptor antagonist, PMX205, lead to a significantly reduction of neuropathology in two murine models of AD, Tg2576 and 3xTg, suggesting a detrimental role for C5a/C5aR in AD (Ager et al., 2010).

V. Glutamate

Glutamate is one of the most important neurotransmitters for adequate brain function. It is involved in multiple functions, including synaptic transmission, neuronal differentiation, synaptic plasticity and learning and memory. Glutamate-mediated toxicity has been implicated in the neurodegeneration observed in Alzheimer's disease. This neurotransmitter has an important role in AD pathology, because high concentrations of extracellular glutamate released as a result neural injury and cause further neurotoxicity. In addition, glutamate transport dysfunction may increase susceptibility to glutamate toxicity, thereby contributing to neuronal cell injury and death (Scott et al., 2002).

Nearly all excitatory neurons in the central nervous system are glutamatergic, and it is estimated that over half of all brain synapses release this agent. The glutamatergic neurons are located in regions that are affected in AD, and in the initial stages damage starts with the pyramidal neurons of the neocortex and glutamate innervated cortical and hippocampal neurons (Revet et al., 2013).

The function of the glutamatergic system is to transform nerve impulses into a chemical stimulus by controlling the concentration of glutamate at the synapse. In presynaptic neurons, the vesicular glutamate transporters, VGLUT1 and VGLUT2 (1), maintain the level of the amino acid stored in vesicles, and when the synapse is activated (neuron is depolarized), glutamate is released into the synaptic cleft where it binds glutamate receptors (GluR) on pre and postsynaptic neurons.

There are two families of glutamate receptors at the plasma membrane of neurons, (2) ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors. The iGluR family is also divided into 3 classes of receptor, which are based on specific agonists and permeability to different cations; NMDA receptors (NR1, NR2A–D and NR3A–B) are mainly Ca²⁺ ion permeable, while AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR1–4) and KA (kainate; GluR5–7, KA1–2) receptors are mostly permeable to Na⁺ and K⁺ ions (Fig 4. (2)).

Molecular studies have shown that modulation of postsynaptic AMPA receptors by phosphorylation may have a relationship with the expression of synaptic plasticity at central excitatory synapses. It is known from biochemical studies that GluR1 AMPA receptor subunits can be phosphorylated by cAMP-dependent protein kinase A (PKA).

Following synapse activation, (3) most of glutamate is cleared from the synaptic cleft by astrocytes, which express high concentrations of the glutamate transporters GLAST and GLT-1 in rats, the human homologues are the excitatory amino acid transporters 1 and 2 (EAAT1 and 2). Within astrocytes, (4) glutamate is converted to glutamine by the enzyme glutamine synthetase (5) and transported back to neurons, where glutamine is converted back to glutamate by glutaminase and then returned to vesicles in the presynaptic membrane by VGLUT1 and 2. This cycle and transamination of cytosolic aspartate are essential for maintaining glutamate levels in presynaptic terminals (Fig. 4 (3-5)).

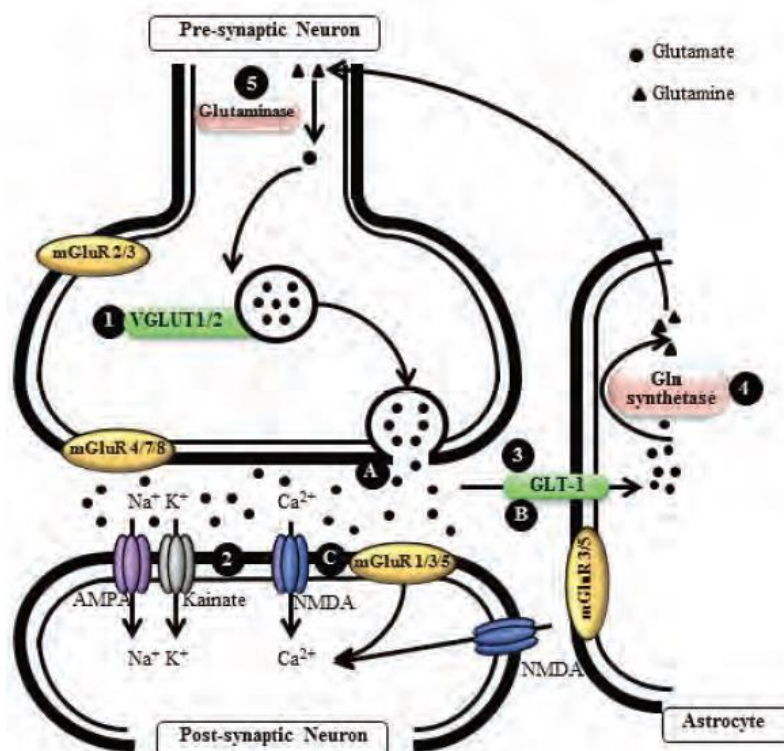


Figure 4. Glutamate cycle: (1) Presynaptic neurons: VGLUT1 and VGLUT2. (2) Postsynaptic neurons: ionotropic iGluRs; [AMPA] and kainate) and metabotropic (mGluRs) receptors. (3) Synaptic Cleft and astrocytes: GLT-1 and GLAST in mice or EAAT1 and 2 in humans. (4) Astrocytes: glutamate is converted to glutamine. (5) Astrocytes and Presynaptic terminals: glutamine is converted back to glutamate.

Many aspects of the glutamate cycle are affected in AD: Amyloid β -related peptides have been shown to (A) increase glutamate release, (B) inhibit clearance of glutamate by astrocytes and (C) affect glutamate receptor activity (Revett et al., 2013).

Deficiencies in a lot of phases of the glutamate cycle have been suggested to occur in AD, causing increased levels of glutamate around the neurons of the synapse (Table III). There have been a number of studies that showed decreased concentrations of VGLUT1 and 2 in the prefrontal cortex of Alzheimer showed subjects (Kashani et al., 2008). More recent investigations also suggest a trend for a decrease in VGLUT1 and VGLUT2 levels in the parietal cortex of patients with AD pathology compared with controls, but they didn't reach significant levels. In addition, a recent study showed that A β accumulates more in VGLUT1/2 terminals than in non-VGLUT terminals, thereby indicating that these peptides could preferably impact presynaptic glutamatergic terminals in the cortex of subjects with Alzheimer Disease (Sokolow et al., 2012).

Studies from animal models of Alzheimer disease have shown that upregulation of cholinergic presynaptic boutons occurs before the involvement of glutamatergic terminals, thus raising the possibility that a compromised cholinergic system may affect the functioning/survival of glutamatergic neurons in the brain (Bell and Claudio, 2006).

A link between glutamate transporter dysfunction, increased extracellular glutamate levels, and onset of excitotoxic neuronal damage has been established in animal models (Rothstein et al., 1996; Rao et al., 2001)

Table III. Alterations in the glutamate system in brains of people with Alzheimer disease (Revett et al., 2013).

Component of glutamate system	Alteration in the brain
VGLUT1	Reduced protein levels before cell loss and onset of pathology
VGLUT2	Reduced protein levels before cell loss and onset of pathology
GLAST/EAAT1	Reduced protein at early clinical stages
GLT-1/EAAT2	Reduced protein levels at early clinical stages
Glutamine synthetase	Reduced protein levels
NMDA receptor	Increased protein levels in mild cognitive impairment
NR1	Reduced protein levels
NR2A–B	Reduced protein levels
NR2C–D	Unaffected
NR3	Not assessed
AMPA receptor	Variable results indicate that there is initial increase at very early stages before a reduction in GluR1 and increased GluR2/3 at later stages
GLUR1–3	
GLUR4	Not assessed
Kainate receptor	Reduced receptor binding
GLUR5–7	Reduced expression
Metabotropic receptor	
mGluR 1	Reduced protein levels
mGluR 2	Increased protein levels
mGluRs 3–7	Not assessed

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; **mGluR**, metabotropic glutamate receptor; **NMDA**, N-methyl-d-aspartate.

Phospho-Glutamate Receptor (pGluR)

Ionic glutamate receptors are ligand-gated ion channels that mediate the rapid excitatory neurotransmission in the mammalian CNS (Roche et al., 1996), and are known to play a role in learning and memory (Snyder et al., 2000). In addition, glutamate receptors are crucial for different forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), two well established cellular models of learning and memory (Bliss and Collingridge, 1993).

Protein phosphorylation is one of the most important mechanisms in the regulation of cellular function and considerable evidence suggests that glutamate receptors are modulated by phosphorylation (Nicoll and Malenka, 1995). It is also demonstrated that protein kinase and phosphatase activity in neurons modulates glutamate receptor ion channel function (Roche et al., 1994).

Modulation of postsynaptic AMPA receptors in the brain by phosphorylation plays a pivotal function in the expression of synaptic plasticity at central excitatory synapses. It is known from biochemical studies that GluR1 receptor subunits can be phosphorylated within their C-terminus by cAMP-dependent protein kinase A (PKA) and also that modulated phosphorylation regions of the AMPA receptor GluR1 subunit are all in the C terminal (Roche et al., 1996).

Glutamate and amyloid β -mediated toxicity

Chronic exposure to A β peptides can induce toxicity in a different cell lines and in human cultured neurons (Revett et al., 2013). The mechanisms associated with amyloid β toxicity are not clearly defined, but seem to be involved in intracellular calcium alterations, production of free radicals, phosphorylation of tau protein, and activation of caspase and non-caspase pathways that conclude in programmed cell death (Clippingdale et al., 2001).

Amyloid β -induced excitotoxic cell death was first reported in the early 1990s, with initial reports indicating that prolonged exposure of amyloid β peptides with glutamate induced greater cell death than exposure to amyloid β or glutamate alone in mouse cortical neuronal cultures. Many studies have suggest that amyloid β toxicity is mediated by glutamate-mediated excitotoxicity, which implicate activation of the NMDA receptors, leading to elevated intracellular Ca²⁺, and consequently stimulation of enzyme cascade causing cell death (Koh et al., 1990).

HYPOTHESIS AND OBJECTIVES

In the presence of A β C5a induces an inflammatory response that enhances neurodegeneration and can affect the expression of the receptor GluR1 and its phosphorylated form, pGluR1, and the glutamate transporters, GLT1 and VGluT1.

C5a was shown to have a detrimental effect in two transgenic mouse models (Tg2576 and 3xTg) since the treatment with a C5aR antagonist improved pathology and behavior (Fonseca et al., 2009). Further studies using the Arctic AD transgenic models either with a deletion of C5aR (ArcticC5aR) or overexpressing C5a (Arctic C5aGFAP) showed significant deficits in behavior in ArcticC5aGFAP mice and improvement in ArcticC5aRKO compared to the Arctic genotype. However, no differences in amyloid-beta plaques between genotypes were observed. Only small significant differences were seen in the microglia, stained by its marker CD45 (Cole et al., 2014, in preparation). However, these small differences in inflammation may not account for the differences seen in the behavior of these models.

The behavior data suggest that C5a may contribute to loss of cognitive ability, but the molecular mechanisms are not yet identified. Based on the relevant role of glutamate receptor 1 and its phosphorylated form, pGluR1, in synaptic plasticity, and learning and memory, the aim of our study is to analyze their expression as well as the expression of the glutamate transporters, VGLUT1 and GLT1, in the Arctic model of AD overexpressing C5a or with a deletion of C5aR in comparison with the Arctic model.

Objectives

- Analyze the plaque pathology (fibrillar amyloid and microglia) by immunostaining and image analysis, in the same animals that will be studied by western blot for glutamate receptors and transporters (Arctic/ArcticC5aRKO at 8.5 months and Arctic/ArcticC5aGFAP at 7 months).
- Determine the expression of the Glutamate receptors (GluR1 and pGluR1) and the Glutamate transporters (GLT1 and VGluT1) in 7 months wild type, transgenic C5aGFAP mice and ArcticC5aGFAP and 8.5 months wild type, C5aR knock out (KO) and ArcticC5aRKO transgenic AD mouse models by Western Blot.

MATERIALS AND METHODS

Transgenic mouse model

The AD Arctic model was used in these studies expressing human amyloid precursor protein (APP) transgene containing the Indiana mutation (V717F), Swedish double mutation (K670N + M671L), and Arctic mutation (E22G) in the APP gene. The Arctic mouse model is characterized by accelerated disease with (Thioflavin staining β -amyloid fibril sheet) plaques starting 2-3 months of age as the E22G mutation promotes A β fibril formation (Cheng et al. 2004). The Arctic mice (provided by Dr. Lennart Mucke) were crossed with mice containing the transgene with the coding location of the C5a fragment under control of the GFAP promoter.

This approach generated the experimental groups: wild type (WT) (GFAP/C5a^{-/-} Arctic^{-/-}), C5a GFAP (C5a^{+/-} Arctic^{-/-}), Arctic (GFAP/C5a^{-/-} Arctic^{+/-}), and Arctic C5aGFAP (C5a^{+/-} Arctic^{+/-}). Furthermore, Arctic mice were crossed to mice with a homozygous lack of C5aR^{-/-}. The filial 1 (F1) Arctic mice were crossed to C5aR^{-/-} to produce Arc^{+/-} C5aR^{-/-} mice, which were subsequently bred to C5aR^{-/-} to produce littermates of C5aR^{-/-} mice with and without the Arctic APP transgene. Ultimately, the experimental groups produced were: Arctic^{-/-} C5aR^{+/+}, Arctic^{-/-} C5aR^{-/-}, Arctic^{+/-} C5aR^{+/+} and Arctic^{+/-} C5aR^{-/-}.

Tissue Collection and Immunohistochemistry

Mice were anesthetized with isoflurane gas (3.5%) for approximately 1 minute. Then, mice were perfused with phosphate buffered saline (PBS). The brains were dissected at 4°C on ice with half of the tissue rapidly frozen on dry ice, and the other half fixed overnight in 4% paraformaldehyde-PBS. After 24 hours, the tissue was saved in storage solution (PBS 0.02% Na Azide) and preserved at 4°C.

Thioflavine and CD45 staining

Staining procedures were performed using free floating coronal sections (40 μ m) cut with a vibratome. For fibrillar β amyloid labeling, sections were stained with 1% thioflavine S (Sigma, St. Louis, MO), differentiated with 50% ethanol, rehydrated, and coverslipped with Vectashield (Vector Laboratories).

For CD45 immunostaining vibratome sections (coronal, 40 μ m) were sequentially incubated with 3% H₂O₂/ 10% methanol/TBS (0.1 M Tris, 0.85% NaCl, pH 7.5–7.6) for 20 min at room temperature to block endogenous peroxidase, and with 2% BSA/0.1% Triton TBS for 1 hr at room temperature to block nonspecific binding. Primary (1:1,000 CD45, Serotec 1mg/ml) and secondary (biotinylated anti-goat Vector, 1:300) antibodies were diluted in the same blocking solution. Primary antibody incubation was overnight at 4°C. Primary antibodies were detected with biotinylated secondary antibodies against the corresponding species (1 hr at room temperature) followed by ABC complex and DAB (Vector Laboratories, Burlingame, CA) used following the manufacturer's instructions for peroxidase labeling.

Thioflavine and CD45 stainings were observed under a Zeiss Axiovert-200 inverted microscope (Carl Zeiss, Thornwood, NY), and images were acquired with a Zeiss AxioCam high-resolution digital color camera using Axiovision 3.1 software.

Digital images were analyzed using KS300 software (Carl Zeiss). Images were acquired with Zeiss Axiocam high-resolution digital color camera (1300 x 1030 pixels) using Axiovision 4.6 software. The same software was used to quantify the digital pictures. Percent of immunopositive area (% Field Area, immunopositive area/total image area x 100) was determined for the Thioflavine or CD45 staining by averaging 2 or 6 images, respectively, of the hippocampus region from 2 sections of each animal. Digital images were obtained using the same settings and the segmentation parameters constant within a range per given marker and experiment. The mean value of the % Field Area for each marker in each animal was averaged per genotype group with the number of animals per group indicated in figure legends. All quantitative comparisons for a given marker were performed on sections stained simultaneously per group age. One way ANOVA statistical analysis was used to analyze differences in plaque area and glial reactivity, among the animals groups (ArcticC5aGFAP and ArcticC5aRKO).

Western Blot

Fresh frozen half brain (cortex and hippocampus) was homogenized in Tissue Protein Extraction Reagent (Pierce), containing protease inhibitors (complete Mini) (Roche Diagnostics, Indianapolis, IN, USA). Homogenates were briefly centrifuged at 14,000g at 4°C for 30 minutes. Supernatant was collected and protein concentration was determined with the BCA protein assay (Pierce, Rockford, IL, USA). Samples (all 10µg per lane loaded) were run in 10% SDS polyacrylamide gel under reducing conditions (100mM DTT).

Proteins were transferred from the gel to PVDF membranes (polyvinylidene difluoride, Millipore Corporation, Bedford, MA, USA) at 350mA for 3 hours. Membranes were blocked with 5% dry milk in 0.1% Tween/TBS (Tris Buffer Saline) for 1 hour, and then incubated with primary antibodies for 2h at RT at the dilutions indicated (Table IV). After washing, blots were incubated with corresponding peroxidase HRP-labeled secondary antibodies (1:7,000 – 1:10,000 dilution) for 1 hour. Labeling was detected using the ECL system (GE Healthcare, Pittsburg, PA, USA). Blots were stripped following the manufacturer's instructions (Amersham) with stripping buffer (Restore™ PLUS Western Blot Stripping Buffer, Thermo Scientific) before probing with the next primary antibody.

Finally, blots were probed with anti β -actin antibody (1:10,000 dilution Sigma) followed by anti-mouse HRP-labeled secondary antibody (Jackson Immunoresearch, West Grove, PA 1:10,000 dilution) and detected with ECL, following same procedures as above to verify equal loading.

Quantification of the bands obtained with the digital camera was done using Image J (National Institutes of Health) software. Each sample was tested in two Western blots in independent experiments. Data were analyzed using single ANOVA statistics.

Table IV: List and information of antibodies primary and secondary studied

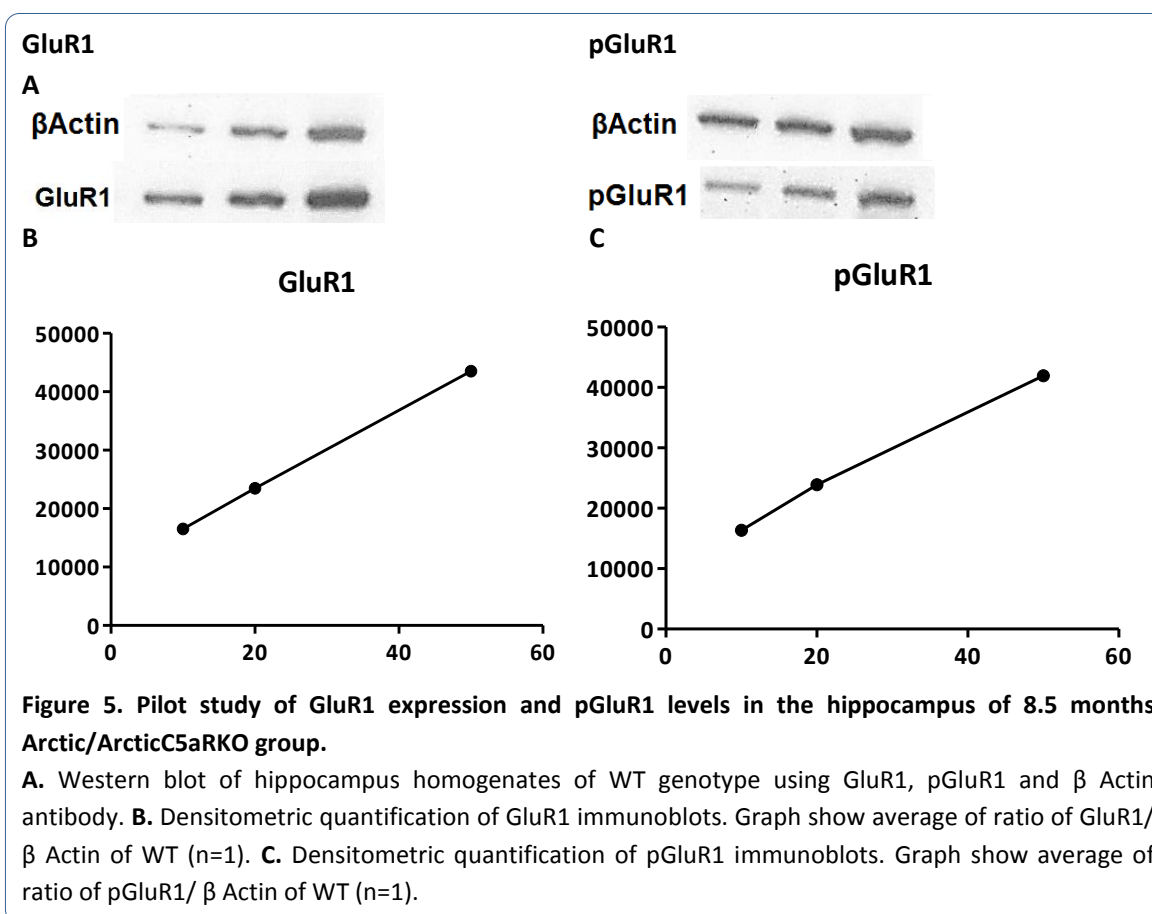
	Antibody	Dilution	Supplier
Primary	Rabbit Anti-GluR1	1:1,000	Millipore, Temecula, CA
Secondary	Donkey Anti-Rabbit IgG (H+L)	1:10,000	Jackson Immunoresearch, West Grove, PA

Primary	Rabbit Anti-pGluR1	1:1,000	Millipore, Temecula, CA
Secondary	Donkey Anti-Rabbit IgG (H+L)	1:10,000	Jackson Immunoresearch, West Grove, PA
Primary	Mouse Anti-VGluT1	1:500	Millipore, Temecula, CA
Secondary	Donkey Anti-Mouse IgG (H+L)	1:7,000	Jackson Immunoresearch, West Grove, PA
Primary	Guinea Pig Anti-GLT1	1:500	Millipore, Temecula, CA
Secondary	Donkey Anti-Guinea-Pig IgG (H+L)	1:10,000	Jackson Immunoresearch, West Grove, PA

Anti-GluR1, Anti-Glutamate receptor 1; **Anti-pGluR1**, Anti-phospho-GluR1; **Anti-VGluT1**, Anti-Vesicular Glutamate Transporter 1; **Anti-GLT1**, Anti-Glutamate Transporter, Glial.

RESULTS

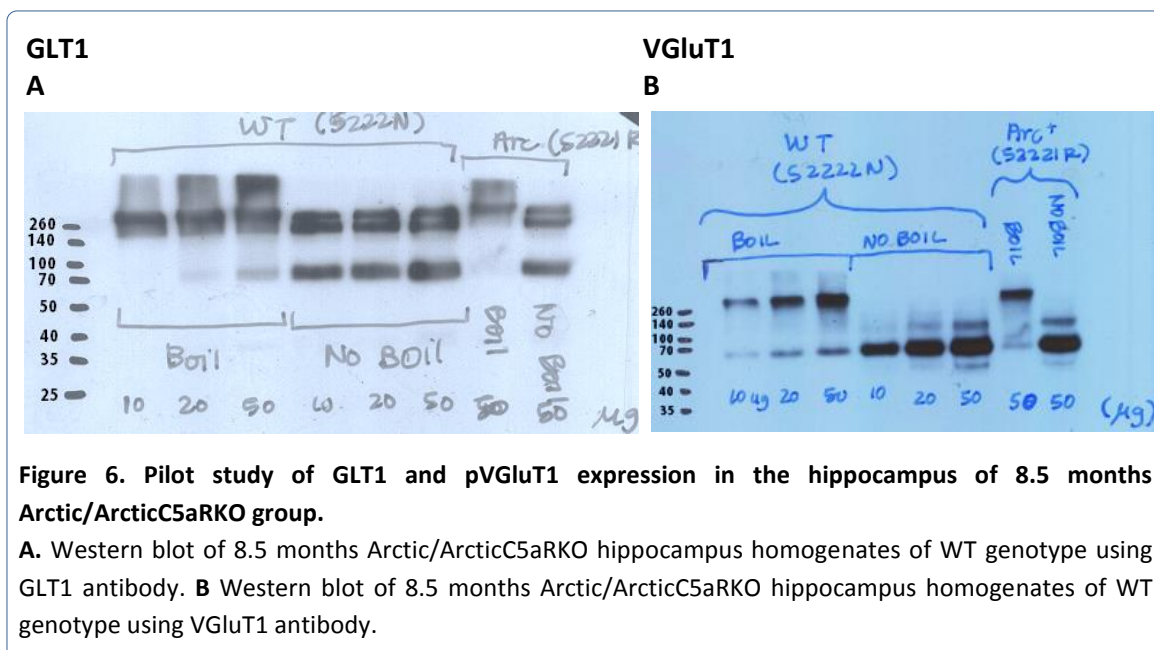
10µg of protein is the best condition for GluR1 and pGluR1 and is sufficient to visualize the expression levels of both markers



To test the optimal protein of GluR1 and phosphorylated (Ser845) GluR1, Western blot of hippocampal lysates were performed using 10, 20 and 50µg of loading protein. Densitometric quantification of GluR1 (Fig. 5B) and pGluR1 band (Fig. 5C) showed notable increase proportional to the respective concentrations 10, 20 and 50µg.

In summary, the best condition is 10µg of protein because is sufficient to visualize GluR1 and pGluR1 levels.

No boil condition (RT) and 10 μ g of loading protein are the best conditions for GLT1 and VGLuT1 proteins



To study the optimal protein load and treatment (boil/no boil) of GLT1 and VGLuT1, Western blot of hippocampal lysates were performed using 3 different concentrations 10, 20 and 50 μ g of loading protein, half of the samples were boiled at 100°C and the other half were left at RT. Two animals were tested: Wild type and Arctic model mice.

The developed films (Fig. 6A and 6B) show that no boil condition (RT) and 10 μ g of protein are the best conditions. 10 μ g of protein is sufficient to visualize the expression of both proteins. Boiling (100°C for 2 min) make the proteins aggregate, which causes them to stay at high MW. In short, these pilots indicate that the optimal protein load is 10 μ g of loading protein and the treatment is no boil (RT).

No significant differences in fibrillar plaques or microglia staining observed between genotypes in 8.5 months ArcticC5aRKO and 7months ArcticC5aGFAP groups

8.5 months Arctic/ArcticC5aRKO

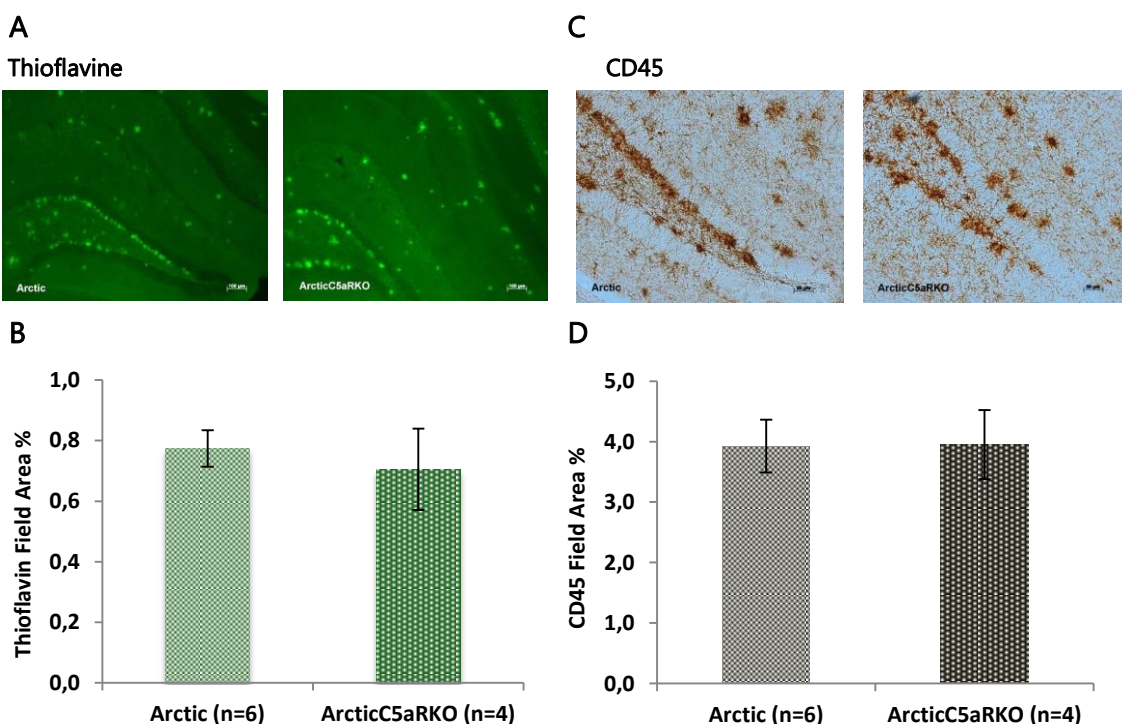


Figure 7. Thioflavine and CD45 stainings in the hippocampus of 8.5 months Arctic/ArcticC5aRKO.

A.C. Representative images of thioflavine (green) (A) and CD45 (brown) staining (C) in hippocampus brain sections at 8.5 months Arctic and ArcticC5aRKO. Scale bar 100 μ m (left panel) and 50 μ m (right panel). **B.D.** Image analysis of thioflavine staining (B) and CD45 immunoreactivity (D) in hippocampus of 8.5 months ArcticC5aRKO. Mean of each animal is the average of two sections (stained in two independent assays, each of which contained animals from each genotype) in which most or all the target area was analyzed (2 images per section in Thioflavine staining and 6 images per section in CD45 staining). Bars represent the average \pm SE of n mice per genotype. Thioflavine: Arctic n=6, and ArcticC5aRKO n=4; CD45: Arctic n=6, and ArcticC5aRKO n=4.

The presence of A β plaques have been reported in transgenic mice of AD. These plaques contain diffuse and fibrillar amyloid peptide. Fibrillar amyloid can be labelled by thioflavine, a reagent that stains proteins in β sheet conformation. Representative pictures showed in Figure 6A, and image analysis using Axiovision software of sections from multiple animals demonstrated that the Thioflavine field area % (Fig. 7B) show no significant differences in the staining of fibrillar A β plaques between the genotypes in hippocampus of brain sections confirming results obtained with previous group of the same genotypes.

Quantification of microglia staining (using the CD45 marker) (Fig. 7C) in the hippocampus of animals at 8.5 months indicated that CD45 reactivity had similar expression in all animals and that there were no differences observed between groups (Fig. 7D), indicating that the deletion of C5aR did not affect the level of CD45 immunoreactivity seen around the plaques in the Arctic/ArcticC5aRKO.

7months Arctic/ArcticC5aGFAP

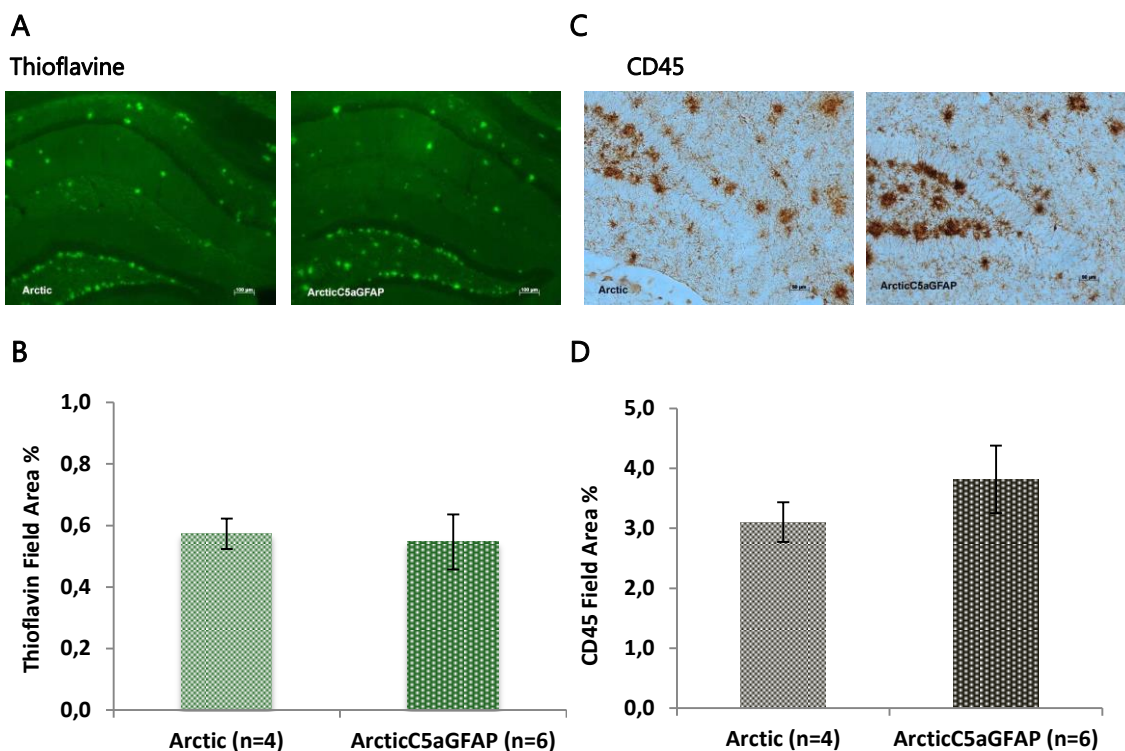


Figure 8. Thioflavine and CD45 stainings in the hippocampus of 7 months Arctic/ArcticC5aGFAP.

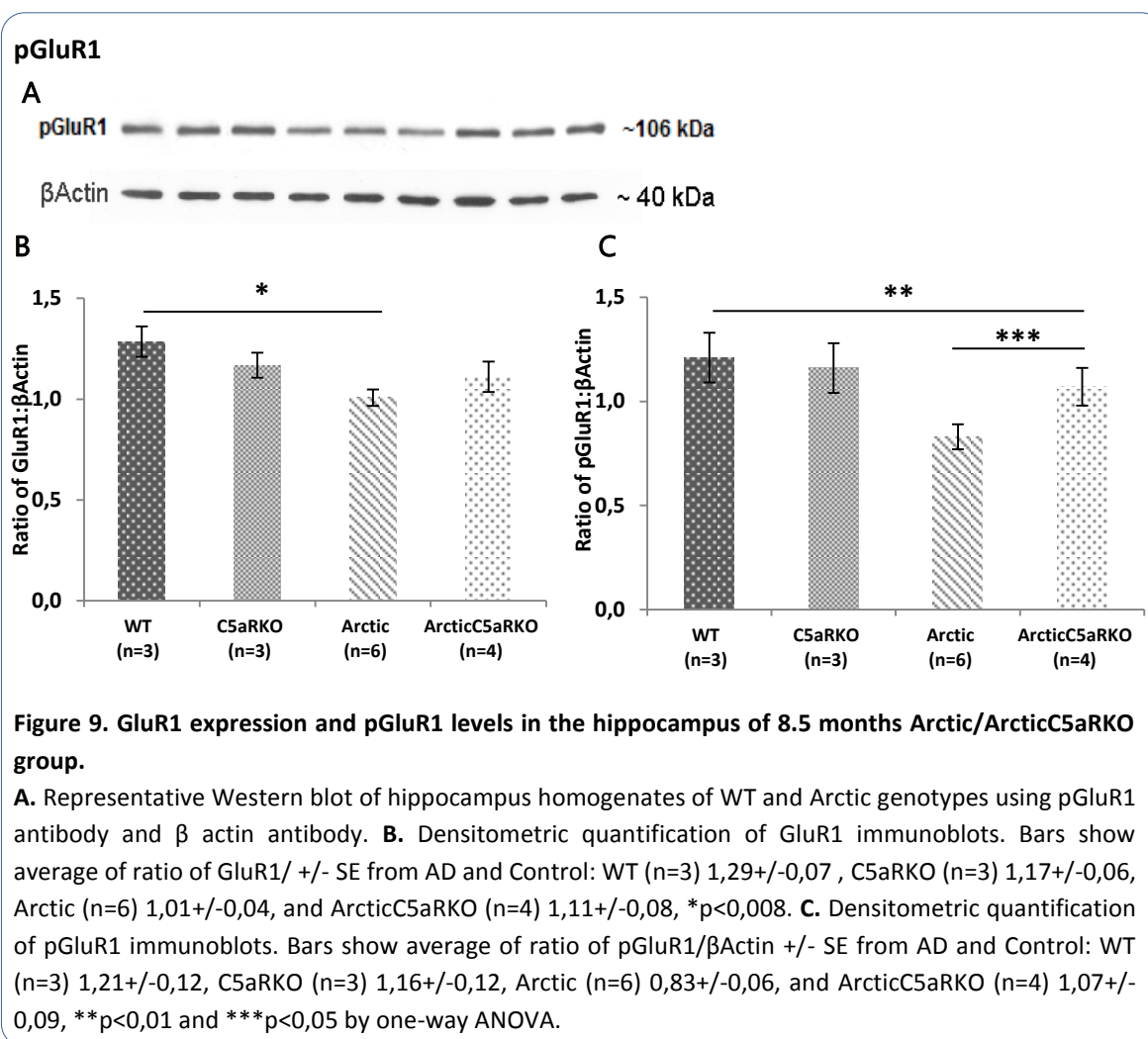
A. C. Representative photomicrographs of thioflavine (green) (A) or CD45 (brown) (C) staining of brain sections from 7 months Arctic and ArcticC5aGFAP. Scale bar 100 μ m (left panel) and 50 μ m (right panel).
C. D. Image analysis of thioflavine staining (B) or CD45 immunoreactivity (D) in hippocampus of 7 months ArcticC5aGFAP mouse model. Mean of each animal is the average of two sections (stained in two independent assays, each of which contained animals from each genotype) in which most or all the area of study was analyzed (2 images per section in Thioflavine staining and 6 images per section in CD45 staining). Bars represent the average \pm SE of n mice per genotype. Thioflavine: Arctic n = 5, and ArcticC5aRKO n=4; CD45: Arctic n=5, and ArcticC5aRKO n=4.

The accumulation of Thioflavine stained plaques in animals at ages 7 months was assessed in hippocampus region of control animals and transgenic animals (ArcticC5aGFAP). Representative photomicrographs presented in Figure 8A, and image analysis (Fig. 8B) using Axiovision analysis program (Zeiss) of sections from multiple animals demonstrated that the staining of $\text{fA}\beta$ plaques using Thioflavine showed no significant differences between the genotypes in this model mouse of AD, relative to WT.

Similarly, levels of immunohistochemical CD45 reactivity (Fig. 8C) (a general marker of microglial reactivity) showed a trend for an increase in ArcticC5aGFAP compared with Arctic after quantification, but the difference between the genotypes is not statistically significant (Fig. 8D).

The thioflavine and CD45 results are in agreement with the results obtained with a previous cohort of animals with the same genotype and age with the exception that CD45 trend for increase reached statistical significance previously.

Significant decreased expression of GluR1 and pGluR1 was observed in the Arctic model and significant recovery of the pGluR1 in was observed the Arctic C5aRKO

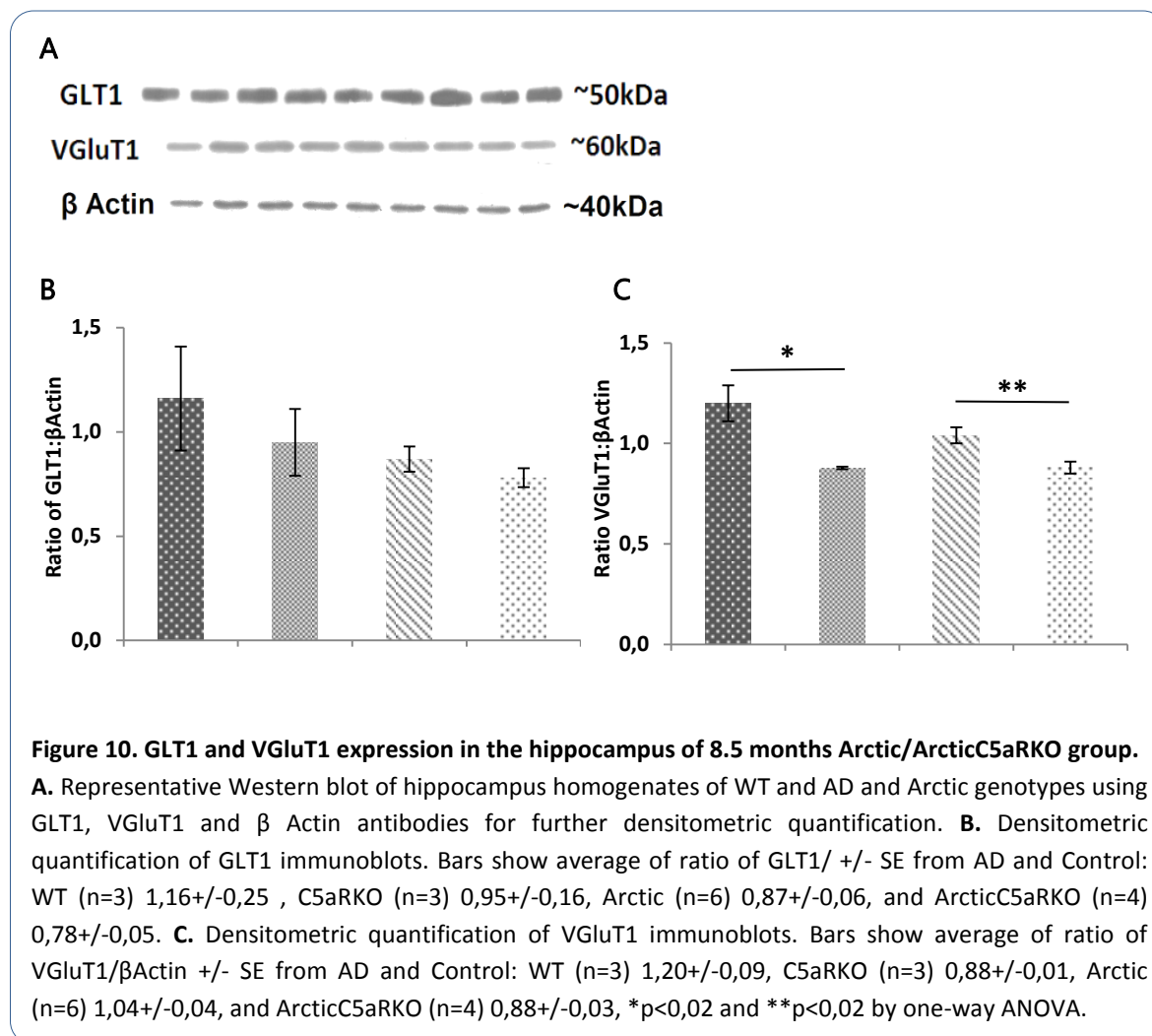


To test the expression of GluR1 and phosphorylated (Ser845) GluR1 Western blot of hippocampal lysates were performed using control WT (n=3), C5aRKO (n=3), Arctic (n=6) and Arctic C5aRKO (n=4) (Table IV).

Densitometric quantification of the GluR1 bands (Fig. 9B) showed a significant decrease (22%, p<0,008) in Arctic compared to WT. In addition, the graph shows a slight trend for an increase in ArcticC5aRKO compared to Arctic, although this difference between the genotypes did not reach statistical significant with this number of animals. The results obtained with pGluR1 membranes (Fig. 9C) showed a significant decrease in Arctic compared to WT (25%, p<0,01) and significant increase in ArcticC5aRKO compared to Arctic (29%, p<0,05).

In summary, GluR1 and pGluR1 receptors are decreased in the Arctic model compared to WT and there is a recovery of pGluR1 receptors in the ArcticC5aRKO.

No differences are seen in the expression levels of glutamate transporter (GLT1) between the genotypes. The VGLuT1 is significantly decreased in the C5aRKO genotypes



This set of Western blots was performed using the same animals as previous WB: control WT (n=3), C5aRKO (n=3), Arctic (n=6) and ArcticC5aRKO (n=4). Hippocampal homogenates were probed with different anti Glutamate Transporters antibodies (anti-GLT1 and anti-VGLuT1, Table IV).

Densitometric quantification of the GLT1 reactive bands (Fig.10) show similar expression in ArcticC5aRKO compared to Arctic. The values obtained indicate no differences of GLT1 levels between the genotypes. In addition, WT has a trend to be higher than the rest of the genotypes mainly due to the variability of the animals.

The results obtained with VGLuT1 membranes (Fig. 10C) showed a significant decrease in ArcticC5aRKO compared to Arctic (26% p<0,03) as well as in C5aRKO compared to WT (28% p<0,02).

In summary, no differences are seen in the GLT1 transporter levels between the genotypes. However, for the VGLuT1 transporter the C5aRKO genotypes have lower levels than the C5aR sufficient mice.

DISCUSSION

The pathology data presented here indicates that the deletion of C5aR or the overexpression of C5a do not cause major changes in fibrillar plaques, or microglia in the Arctic model and cannot support the changes in behavior observed in these models (Cole et al., 2014 in preparation). The results are similar to the ones obtained with previous groups of the same genotypes (7 and 10 months old) that gave no differences in fibrillar amyloid although a small significant change was seen in microglia. This is consistent with the growing body of evidence that while amyloid may be necessary for cognitive loss, it is not sufficient.

This study shows that the neuronal markers GluR1 and its phosphorylated form (Ser845pGluR1) have a significant decrease in the Arctic model at 8.5 months compared to WT. The decreases in GluR1 and pGluR1 support previous data showing decreases in the AMPA receptors in AD (Parameshwaran et al., 2008) and in other transgenic AD models (Palop et al., 2007; Almeida et al., 2005).

The decreases in GluR1 and pGluR1 in the Arctic compared to WT are also in agreement with deficits in memory and learning observed in AD models and in particular the Arctic model at 10 m (Cole et al., 2014, in preparation).

Glutamate-mediated toxicity has been implicated in the neurodegeneration observed in Alzheimer's disease. In particular, glutamate transport dysfunction may increase susceptibility to glutamate toxicity, thereby contributing to neuronal cell injury and death (Scott et al., 2002). In addition, amyloid beta interferes with glutamate transmission by causing synaptic depression through the clearance of glutamate receptors by endocytosis. This process can lead to loss of synapses even before the formation of plaques and is consistent with the deficits in cognitive ability, such as learning and memory, seen in AD. High levels of A β can cause the loss of dendritic spines, interferes with the trafficking of glutamate receptors leading to a decrease of surface expression of AMPA receptors (Parameshwaran et al., 2008).

In comparison to Arctic, the ArcticC5aRKO group showed a significant recovery in pGluR1 expression that is in agreement with the improvements in learning and memory seen in this model at 10 m (Cole et al., 2014, in preparation). This supports our hypothesis that C5a is detrimental since the lack of C5aR restored the level of pGluR1 to that similar to WT, pGluR1 is crucial for the availability of the receptor at the synapse. Phosphorylation of GluR1 (pGluR1) is a very important step to increase the trafficking of the receptor to the synapse playing a key role in long term potentiation (LTP) which is crucial for memory and learning (He et al., 2011).

In contrast, there were no significant differences in astrocyte GLT1 expression levels between the genotypes. The neuronal marker VGluT1 showed significant decreases in expression in C5aRKO genotypes (C5aRKO and ArcticC5aRKO) compared to C5aR sufficient mice (WT and Arctic). Our results suggest that differences in VGluT1 expression may not have a major effect in cognitive ability because previously, behavior of these animals showed that there were no significant differences in the behavior of the WT groups (WT and C5aRKO).

CONCLUSIONS

In summary, the results presented here using the Arctic model mouse of AD deleting C5aR support the correlation of C5a/C5aR with the expression of the neuronal marker, Glutamate receptor (GluR1) and its activated form, phosphorylated GluR1 (pGluR1). These observations

suggest that they could be implicated in the effects of C5a in AD. These findings have improved our understanding of the consequences of C5a and C5aR interaction on the expression of GluR1 and levels of pGluR1 in these transgenic mouse models of AD, and could provide information for future therapeutic treatments for AD, although whether these effects are due to a direct effect of C5a on neurons or to the enhanced inflammatory response by microglia remains to be determined.

Tenner lab continues to analyze the expression of transcription factors CREB, pCREB and C/EBP that could be influenced by glutamate receptors and are involved in memory learning.

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REFERENCE LIST

- Afagh A, Cummings BJ, Cribbs DH, Cotman CW, Tenner AJ (1996) Localization and cell association of C1q in Alzheimer's disease brain. *Exp Neurol* 138:22-32.
- 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* 113:389-401.
- Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, Ziolkowski SK, James JA, Snitz BE, Houck PR, Bi W, Cohen AD, Lopresti BJ, DeKosky ST, Halligan EM, Klunk WE (2008) Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol* 65:1509-1517.
- Akiyama H, Arai T, Kondo H, Tanno E, Haga C, Ikeda K (2000) Cell mediators of inflammation in the Alzheimer disease brain. *Alzheimer Dis Assoc Disord* 14 Suppl 1:S47-S53.
- Alexander JJ, Anderson AJ, Barnum SR, Stevens B, Tenner AJ (2008) The complement cascade: Yin-Yang in neuroinflammation--neuro-protection and -degeneration. *J Neurochem* 107:1169-1187.
- Almeida CG, Tampellini D, Takahashi RH, Greengard P, Lin MT, Snyder EM, Gouras GK (2005) Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis* 20:187-198.
- Ballatore C, Lee VM, Trojanowski JQ (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci* 8:663-672.
- Bell KF, Claudio CA (2006) Altered synaptic function in Alzheimer's disease. *Eur J Pharmacol* 545:11-21.

- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31-39.
- Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. *Mol Immunol* 44:999-1010.
- Boos L, Szalai AJ, Barnum SR (2005) C3a expressed in the central nervous system protects against LPS-induced shock. *Neurosci Lett* 387:68-71.
- Cheng IH, Palop JJ, Esposito LA, Bien-Ly N, Yan F, Mucke L (2004) Aggressive amyloidosis in mice expressing human amyloid peptides with the Arctic mutation. *Nat Med* 10:1190-1192.
- Chishti MA, et al. (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem* 276:21562-21570.
- Clippingdale AB, Wade JD, Barrow CJ (2001) The amyloid-beta peptide and its role in Alzheimer's disease. *J Pept Sci* 7:227-249.
- Craft JM, Watterson DM, Van Eldik LJ (2006) Human amyloid beta-induced neuroinflammation is an early event in neurodegeneration. *Glia* 53:484-490.
- Eikelenboom P, Hack CE, Rozemuller JM, Stam FC (1989) Complement activation in amyloid plaques in Alzheimer's dementia. *Virchows Archiv B Cell Pathol* 56:259-262.
- Eikelenboom P, Stam FC (1982a) Immunoglobulins and complement factors in senile plaques. *Acta Neuropathol* 57:239-242.
- Eikelenboom P, Stam FC (1982b) Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. *Acta Neuropathol* 57:239-242.
- Elder GA, Gama Sosa MA, De GR (2010) Transgenic mouse models of Alzheimer's disease. *Mt Sinai J Med* 77:69-81.
- Farkas I, Baranyi L, Takahashi M, Fukuda A, Liposits Z, Yamamoto T, Okada H (1998) A neuronal C5a receptor and an associated apoptotic signal transduction pathway. *J Physiol* 507 (Pt 3):679-687.
- Ferrer I, Gomez-Isla T, Puig B, Freixes M, Ribe E, Dalfo E, Avila J (2005) Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies. *Curr Alzheimer Res* 2:3-18.
- Ferri C, Sousa R, Albanese E, Ribeiro W, Honyashiki M (2009) World Alzheimer Report 2009 – Executive Summary. In Prince, M. and Jackson, J. (Eds.). *Alzheimer's*.
- Fonseca MI, Ager RR, Chu SH, Yazan O, Sanderson SD, LaFerla FM, Taylor SM, Woodruff TM, Tenner AJ (2009) Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. *J Immunol* 183:1375-1383.

- Fonseca MI, Chu SH, Berci AM, Benoit ME, Peters DG, Kimura Y, Tenner AJ (2011) Contribution of complement activation pathways to neuropathology differs among mouse models of Alzheimer's disease. *J Neuroinflammation* 8:4.
- Frandemiche ML, De SS, Rush T, Borel E, Elie A, Arnal I, Lante F, Buisson A (2014) Activity-dependent tau protein translocation to excitatory synapse is disrupted by exposure to amyloid-beta oligomers. *J Neurosci* 34:6084-6097.
- Gasparini L, Ongini E, Wilcock D, Morgan D (2005) Activity of flurbiprofen and chemically related anti-inflammatory drugs in models of Alzheimer's disease. *Brain Res Brain Res Rev* 48:400-408.
- Gong CX, Grundke-Iqbal I, Iqbal K (2010) Targeting tau protein in Alzheimer's disease. *Drugs Aging* 27:351-365.
- Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem* 261:6084-6089.
- He K, Goel A, Ciarkowski CE, Song L, Lee HK (2011) Brain area specific regulation of synaptic AMPA receptors by phosphorylation. *Commun Integr Biol* 4:569-572.
- Hu W, Ranaivo HR, Roy SM, Behanna HA, Wing LK, Munoz L, Guo L, Van Eldik LJ, Watterson DM (2007) Development of a novel therapeutic suppressor of brain proinflammatory cytokine up-regulation that attenuates synaptic dysfunction and behavioral deficits. *Bioorg Med Chem Lett* 17:414-418.
- Huang HC, Jiang ZF (2009) Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. *J Alzheimers Dis* 16:15-27.
- Ingersoll SA, Martin CB, Barnum SR, Martin BK (2010) CNS-specific expression of C3a and C5a exacerbate demyelination severity in the cuprizone model. *Mol Immunol* 48:219-230.
- Ishii T, Haga S (1984) Immuno-electron-microscopic localization of complement in amyloid fibrils of senile plaques. *Acta Neuropathol* 63:296-300.
- Iyer SS, Ghaffari AA, Cheng G (2010) Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages. *J Immunol* 185:6599-6607.
- Jiang H, Burdick D, Glabe CG, Cotman CW, Tenner AJ (1994) beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. *J Immunol* 152:5050-5059.
- Kashani A, Lepicard E, Poirel O, Videau C, David JP, Fallet-Bianco C, Simon A, Delacourte A, Giros B, Epelbaum J, Betancur C, El MS (2008) Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease. *Neurobiol Aging* 29:1619-1630.
- Kitazawa M, Yamasaki TR, LaFerla FM (2004) Microglia as a Potential Bridge between the Amyloid {beta}-Peptide and Tau. *Ann N Y Acad Sci* 1035:85-103.

- Koh JY, Yang LL, Cotman CW (1990) Beta-amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. *Brain Res* 533:315-320.
- Lee YJ, Han SB, Nam SY, Oh KW, Hong JT (2010) Inflammation and Alzheimer's disease. *Arch Pharm Res* 33:1539-1556.
- Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP (1999) The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* 399:784-788.
- Lucin KM, Wyss-Coray T (2009) Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron* 64:110-122.
- McNaull BB, Todd S, McGuinness B, Passmore AP (2010) Inflammation and anti-inflammatory strategies for Alzheimer's disease--a mini-review. *Gerontology* 56:3-14.
- Monk PN, Scola AM, Madala P, Fairlie DP (2007) Function, structure and therapeutic potential of complement C5a receptors. *Br J Pharmacol* 152:429-448.
- Mukherjee SP, Davoren M, Byrne HJ (2010) In vitro mammalian cytotoxicological study of PAMAM dendrimers - towards quantitative structure activity relationships. *Toxicol In Vitro* 24:169-177.
- Nicoll RA, Malenka RC (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 377:115-118.
- Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55:697-711.
- Parameshwaran K, Dhanasekaran M, Suppiramaniam V (2008) Amyloid beta peptides and glutamatergic synaptic dysregulation. *Exp Neurol* 210:7-13.
- Priller C, Bauer T, Mitteregger G, Krebs B, Kretschmar HA, Herms J (2006) Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci* 26:7212-7221.
- Querfurth HW, LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* 362:329-344.
- Rao VL, Dogan A, Bowen KK, Todd KG, Dempsey RJ (2001) Antisense knockdown of the glial glutamate transporter GLT-1 exacerbates hippocampal neuronal damage following traumatic injury to rat brain. *Eur J Neurosci* 13:119-128.
- Revett TJ, Baker GB, Jhamandas J, Kar S (2013) Glutamate system, amyloid ss peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology. *J Psychiatry Neurosci* 38:6-23.
- Ricklin D, Hajishengallis G, Yang K, Lambris JD (2010) Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 11:785-797.

- Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16:1179-1188.
- Roche KW, Tingley WG, Huganir RL (1994) Glutamate receptor phosphorylation and synaptic plasticity. *Curr Opin Neurobiol* 4:383-388.
- Rogers J, Cooper NR, Webster S, Schultz J, McGeer PL, Styren SD, Civin WH, Brachova L, Bradt B, Ward P, Lieberburg I (1992) Complement activation by beta-amyloid in Alzheimer disease. *Proc Natl Acad Sci* 89:10016-10020.
- Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16:675-686.
- Scott HL, Pow DV, Tannenberg AE, Dodd PR (2002) Aberrant expression of the glutamate transporter excitatory amino acid transporter 1 (EAAT1) in Alzheimer's disease. *J Neurosci* 22:RC206.
- Snyder GL, Allen PB, Fienberg AA, Valle CG, Huganir RL, Nairn AC, Greengard P (2000) Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J Neurosci* 20:4480-4488.
- Sokolow S, Luu SH, Nandy K, Miller CA, Vinters HV, Poon WW, Gyls KH (2012) Preferential accumulation of amyloid-beta in presynaptic glutamatergic terminals (VGluT1 and VGluT2) in Alzheimer's disease cortex. *Neurobiol Dis* 45:381-387.
- Sterniczuk R, Antle MC, LaFerla FM, Dyck RH (2010) Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 2. Behavioral and cognitive changes. *Brain Res* 1348:149-155.
- Tuppo EE, Arias HR (2005) The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol* 37:289-305.
- Turner PR, O'Connor K, Tate WP, Abraham WC (2003) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol* 70:1-32.
- Van Beek J, Nicole O, Ali C, Ischenko A, MacKenzie ET, Buisson A, Fontaine M (2001) Complement anaphylatoxin C3a is selectively protective against NMDA-induced neuronal cell death. *NeuroReport* 12:289-293.
- Veerhuis R, Nielsen HM, Tenner AJ (2011) Complement in the brain. *Mol Immunol* 48:1592-1603.
- Waldemar G, Dubois B, Emre M, Georges J, McKeith IG, Rossor M, Scheltens P, Tariska P, Winblad B (2007) Recommendations for the diagnosis and management of Alzheimer's disease and other disorders associated with dementia: EFNS guideline. *Eur J Neurol* 14:e1-26.
- Walport MJ (2001) Complement. Second of two parts. *N Engl J Med* 344:1140-1144.

- Wang J, Bi W, Cheng A, Freire D, Vempati P, Zhao W, Gong B, Janle EM, Chen TY, Ferruzzi MG, Schmeidler J, Ho L, Pasinetti GM (2014) Targeting multiple pathogenic mechanisms with polyphenols for the treatment of Alzheimer's disease-experimental approach and therapeutic implications. *Front Aging Neurosci* 6:42.
- Yao J, Harvath L, Gilbert DL, Colton CA (1990) Chemotaxis by a CNS macrophage, the microglia. *J Neurosci Res* 27:36-42.
- Zanjani H, Finch CE, Kemper C, Atkinson J, McKeel D, Morris JC, Price JL (2005) Complement activation in very early Alzheimer disease. *Alzheimer Dis Assoc Disord* 19:55-66.
- Zhou J, Fonseca MI, Kaye R, Hernandez I, Webster SD, Yazan O, Cribbs DH, Glabe CG, Tenner AJ (2005) Novel Abeta peptide immunogens modulate plaque pathology and inflammation in a murine model of Alzheimer's disease. *J Neuroinflammation* 2:28.



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