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Expression profile of plasticity-related mRNAs in the cortex and hippocampus of young and aged rats and of 3xTg and wild type mice

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Ce mémoire intitulé:

Expression profile of plasticity-related mRNAs in the cortex and hippocampus of aged and young rats and of 3xTg and wild type mice

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Résumé

De récents travaux ont mis en évidence que des dysfonctionnements dans l'expression de gènes impliqués dans la plasticité synaptique contribuent aux déclins cognitifs qu'on observe chez les gens âgés et à la progression de la maladie d'Alzheimer. Notre étude avait comme objectif d'étudier le profil d'expression d'ARNm spécifiques impliqués dans la plasticité synaptique chez des rats jeunes et âgés et chez des souris transgéniques 3xTg et WT.

Des expériences en qRT-PCR ont été effectuées dans des extraits de cortex et d'hippocampe de rats jeunes et âgés et de souris 3xTg et WT, respectivement. Les résultats ont démontré une augmentation significative de l'expression d'ARNm MAP1B, Stau2, BDNF, CREB et AGO2 principalement dans l'hippocampe (régions CA1-CA3) des souris 3xTg comparé aux souris WT. Une diminution significative a également été observée pour l'ARNm αCaMKII dans le cortex des souris 3xTg comparé aux souris WT. Contrairement à ces observations, aucun changement n'a été observé pour l'expression de gènes impliqués dans la plasticité synaptique chez les rats âgés comparé aux rats jeunes.

Ces résultats démontrent qu'un dysfonctionnement existe réellement au début de la maladie d'Alzheimer dans l'expression de gènes spécifiques impliqués dans la plasticité synaptique et contribue potentiellement à la progression de la maladie en engendrant un déséquilibre entre la LTP et la LTD. De plus, les différences d'expressions sont particulièrement observées dans l'hippocampe (régions CA1-CA3) ce qui est consistant avec les études sur la progression de la maladie d'Alzheimer puisqu'il est connu que la région CA1 de l'hippocampe est la plus vulnérable à l'apparition de la maladie. Ces résultats permettent une meilleure compréhension des événements moléculaires qui deviennent dérégulés à l'apparition de la maladie d'Alzheimer.

Mots-clés : Plasticité synaptique, ARNm, déclins cognitifs, maladie d'Alzheimer, rats jeunes, rats âgés, souris 3xTg, souris WT, cortex, hippocampe

Abstract

Recent work has demonstrated that dysregulations in the expression profile of plasticity-related genes in specific brain regions contribute to age-related cognitive decline and Alzheimer's disease. The aim of this study was to determine the expression profile of a subset of plasticity-related mRNAs in different regions of the brain of young and aged rats as well as 3xTg and wild type (WT) mice.

qRT-PCR experiments were performed in extracts of cortex and hippocampus of young and aged rats and of 3xTg and WT mice, respectively. Results demonstrated significant increases in the expression of MAP1B, Stau2, CREB, BDNF, and AGO2 mRNAs, especially in the hippocampus (CA1-CA3 fields) of 3xTg mice compared to WT mice. A significant decrease was also observed in the expression of αCaMKII mRNA in the cortex of 3xTg mice compared to WT mice. On the other hand, no significant changes were observed in the expression of plasticity-related genes in the hippocampus of aged rats compared to young rats.

These results confirm that alterations in gene expression occur at the onset of AD and possibly contribute to the progression of the disease by causing an imbalance between long-term potentiation and long-term depression. In addition, patterns of significant altered gene expression, especially in the hippocampus (CA1-CA3 fields) of 3xTg mice are consistent with the progression of AD whereby the hippocampus (CA1 region) is most vulnerable at the onset of the disease. These results provide a better understanding of the molecular events that first become disturbed in AD.

Keywords : Synaptic plasticity, mRNAs, cognitive decline, Alzheimer disease, young rats, aged rats, 3xTg mice, WT mice, cortex, hippocampus

Contents

Résumé i
Abstract ii
Contents iii
List of tables v
List of figures vi
Remerciements viii
1 Introduction 1
1.1 Memory 1
1.2 Memory and the brain 2
1.3 Brain structures involved in different types of memories 3
1.3.1 Structural organization of the medial temporal lobe 3
1.4 Synaptic plasticity 5
1.4.1 Structure of a neuron 5
1.4.2 Neuronal network in the hippocampus 7
1.4.3 Long-term potentiation and long-term depression 7
1.4.4 Transcriptional and translational regulations of plasticity related mRNAs and proteins 15
1.5 Aging and cognitive decline 17
1.6 Alzheimer's disease 21
1.7 Hypothesis, aims, and rationale 24
1.7.1 Hypothesis 24
1.7.2 Aims 25
1.7.3 Rationale 25
2 Experimental procedures 28
2.1 Animals 28
2.1.1 Young and aged rats 28
2.1.2 3xTgmice and WT mice 28

2.2 RNA isolation 29
2.3 Quantitative PCR 29
3 Results 34
3.1 Expression profile of plasticity-related genes in different brain regions of 3xTg mice and WT mice 35
3.1.1 Hippocampus: CA1-CA3 fields 35
3.1.2 Hippocampus: Dentate gyrus 41
3.1.3 Cortex 44
3.2 Expression profile of plasticity-related genes in the cortex and hippocampus of aged 24-month old rat and young 3-month old Fisher 344 rats 49
3.2.1 Hippocampus 49
3.2.2 Cortex 50
4 Discussion 53
4.1 Alzheimer's disease 53
4.1.1 LTD and plasticity-related mRNAs 54
4.1.2 LTP and plasticity-related mRNAs 57
4.2 Aging 62
5 Conclusion 64
5.1 Perspectives 65
Bibliography 67

List of tables

Table I Sequence information of the *rattus norvegicus* primer pairs for use in quantitative real-time PCR 31

Table II Sequence information of the *mus musculus* primer pairs for use in quantitative real-time PCR 32

Table III *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to aldolase C 36

Table IV *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to Ywhaz 38

Table V *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to GAPDH 40

Table VI *p*-value: Statistical significance in the dentate gyrus of 3xTg and WT mice relative to aldolase C 42

Table VII *p*-value: Statistical significance in the cortex of 3xTg and WT mice relative to aldolase C 45

Table VIII *p*-value: Statistical significance in the cortex of 3xTg and WT mice relative to Ywhaz 47

Table IX *p*-value: Statistical significance in the hippocampus of young and aged Fisher 344 rats relative to aldolase C 49

Table X *p*-value: Statistical significance in the cortex of young and aged Fisher 344 rats relative to aldolase C 51

List of figures

Figure 1 Types of memories 2
Figure 2 Anatomical representation of the hippocampus 4
Figure 3 Structure of a neuron 6
Figure 4 Pyramidal neurons of CA1-CA3 fields 7
Figure 5 E-LTP, L-LTP, and LTD 8
Figure 6 Lesions in Alzheimer’s disease 22
Figure 7 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: Aldolase C) 37
Figure 8 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: Ywhaz) 39
Figure 9 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: GAPDH) 41
Figure 10 Normalized expression level of mRNAs in the dentate gyrus of 3xTg and WT mice (reference gene: Aldolase C) 43
Figure 11 Normalized expression level of mRNAs in the cortex of 3xTg and WT mice (reference gene: Aldolase C) 46
Figure 12 Normalized expression level of mRNAs in the cortex of 3xTg and WT mice (reference gene: Ywhaz) 48
Figure 13 Normalized expression of mRNAs in the hippocampus of young and aged rats (reference gene: Aldolase C) 50
Figure 14 Normalized expression of mRNAs in the cortex of young and aged rats (reference gene: Aldolase C) 52

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Abbreviation list

%	Percentage
^{o}C	Degree Celsius
µl	Microliter
3xTg	Triple transgenic model of Alzheimer disease
AD	Alzheimer's disease
AGO2	Argonaute 2
Akt	Protein kinase B
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
AMPAR	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid receptor
APP	Amyloid precursor protein
APP_{swe}	APP swedish mutation
APT1	Acyl-protein thioesterase 1
Arc	Activity-regulated cytoskeleton-associated
Aβ	Amyloid-beta
Bax	Bcl-2–associated X protein
Bcl-2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
BIRC3 and 4	Baculoviral IAP Repeat-Containing 3 and 4
CA1/CA2/CA3	*Cornu Ammonis* 1/2/3
Ca^{2+}	Calcium ions 2+
CaMKII	Calcium/calmodulin protein kinase II
αCaMKII	CaMKII-alpha
cDNA	Complementary DNA
CO_2	Carbon dioxide
CPE	Cytoplasmic polyadenylation element

CPEB	Cytoplasmic polyadenylation element binding protein
CRE	cAMP responsive element
CREB	cAMP responsive transcription factor
CycA	Cyclin A
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dsRNA	Double stranded ribonucleic acid
E-LTP	Early long-term potentiation
EPSP	Excitatory postsynaptic potential
F-actin	Filamentous-actin
FAD	Familial Alzheimer disease
FMRP	Fragile X mental retardation protein
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GluR2	Metabotropic glutamate receptor 2
HBSS	Hank's balanced salt solution
HFS	High frequency stimulation
hnRNP A2	Heterogeneous nuclear ribonucleoprotein A2
Hz	Hertz
IEG	Immediate early gene
KO	Knock-out
LFS	Low frequency stimulation
LIMK1	LIM domaine kinase 1
L-LTP	Late long-term potentiation
LTD	Long-term depression
LTP	Long-term potentiation
MAP1B	Microtubule associated protein 1b
MAP2	Microtubule associate protein 2
MAPK	Mitogen-activated protein kinase
Mg^{2+}	Magnesium ion 2+

mM	Millimolar
miRISC	miRNA-induced silencing complex
miRNA	micro-ribonucleic acid
mRNA	Messenger ribonucleic acid
NCBI	National Center for Biotechnology Information
NFT	Neurofibrillary tangles
Ng	Nanogram
Nm	Nanometer
NMDA	*N*-Methyl-D-aspartic acid
NMDAR	*N*-Methyl-D-aspartic acid receptor
NR1/NR2	*N*-Methyl-D-aspartic acid receptor subunit ½
PKA	Protein kinase A
Pkcz	Atypical protein kinase C
Poly(A)	Polyadenylation
PP1	Protein phosphatase 1
Pri-miRNA	Primary-miRNA
PRP	Plasticity related protein
$PS1_{M146V}$	Presenilin-1 mutation : M146V
PSD95	Postsynaptic density protein 95
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNase	Ribonuclease
RNP	Ribonucleoprotein
RT-qPCR	Real-time reverse transcription polymerase chain reaction
S	Seconds
SNAP25	Synaptosomal-associated protein 25
Stau1	Staufen 1
Stau2	Staufen 2
SYBR	Synergy brands

Tau_{P301L}	Tau mutation : P301L
Thy1.2	Thymocyte antigen 1.2
Trkβ	Tropomyosin receptor kinase Beta
UTR	Untranslated region
VAMP2	Vesicle-associated membrane protein 2
VDCC	Voltage-dependent calcium channels
WT	Wild-type
Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
Zif268	Zinc finger protein 225

1 Introduction

1.1 Memory

Memory, the process of encoding, consolidating, storing, and retrieving knowledge, is achieved through the plasticity of the human brain: the ability of neurons to modify their connections to make certain neural circuits more efficient (1). With aging, as much as 60% of the population is affected by learning and memory impairments of varying severity (2). These can range from mild cognitive decline to more severe cases such as Alzheimer's disease (AD). Consequently, the aging population affected suffers from diminished quality of life and independence and inadvertently, imposes costly and time consuming demands on society and the health care system.

At the onset of cognitive impairment, specific regions of the brain are especially vulnerable and as a result a specific type of memory, termed episodic memory, is impaired (3). This type of hippocampus dependent long-term memory enables the consolidation and recollection of information in a determined temporal and spatial context. Initially, various areas of the neocortex process visual, auditory and somatic characteristics of information which are then integrated by the hippocampus (4,5). Repetition via the hippocampal-neocortical loop allows strengthening of associations among new elements. Eventually the neocortex associates these various properties itself to reconstruct a memory.

At the molecular level, the ability to strengthen or weaken neuronal associations is referred to as synaptic plasticity (1). The synaptic transmission between neurons can either be enhanced by activity via long-term potentiation or depressed by activity via long-term depression. Morphological and cellular modifications ensue due to the actions of several plasticity-related mRNAs and proteins and can last from milliseconds to long-lasting alterations. The precise transcriptional and translational regulations as well as the localization of these mRNAs and proteins are crucial in maintaining an optimal synaptic transmission between neurons. Throughout the lifespan, apparent age-related changes occur in the expression profile of these mRNAs and proteins and can contribute to cognitive decline by engendering a dysregulation in synaptic transmission (2,6,7).

1.2 Memory and the brain

Memory is a broad term that encompasses both short-term memory, which can last several seconds to several minutes, and long-term memory which may be stored for an unlimited duration (8). Long-term memory is divided into explicit memory (declarative) and implicit memory (nondeclarative) – (figure 1) (9,10). The fundamental difference between these types of memories lies in the conscious effort required in remembering things and facts (explicit) and the unconscious effort required in remembering procedural skills or emotional responses (implicit). Explicit memory is further subdivided into semantic and episodic memories which include the knowledge of facts as well as the spatial and temporal context of these facts, respectively.

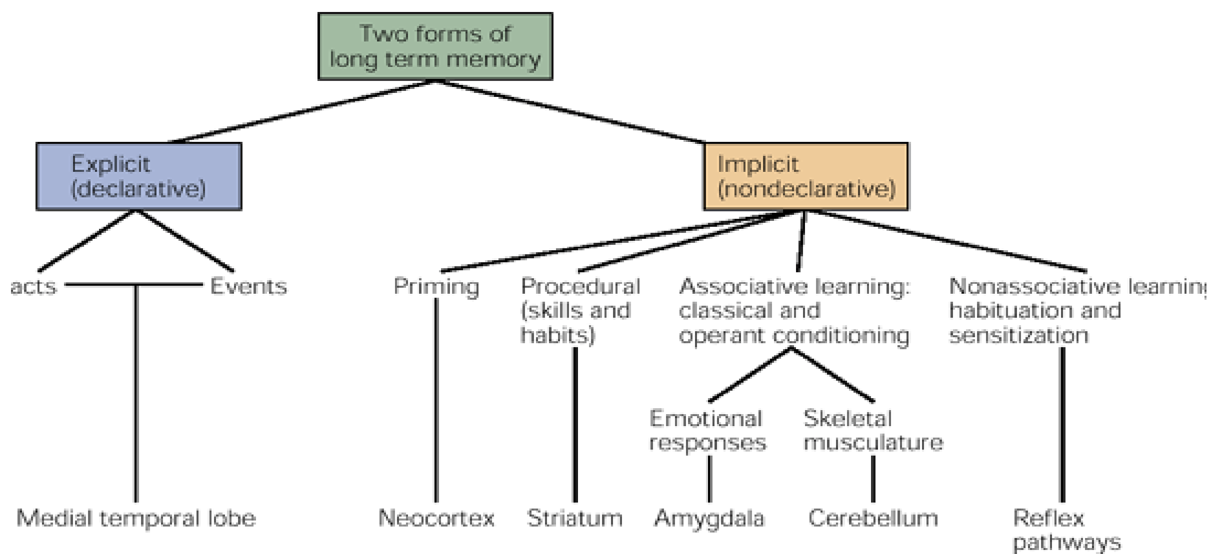


Figure 1 Types of memories. Various forms of memory can be classified as explicit or implicit memory. Adapted from (1).

1.3 Brain structures involved in different types of memories

Various brain structures are involved in the acquisition, consolidation, and recollection of the different memory subtypes. Whereas non-declarative memory relies mostly on the neostriatum and cerebellum, declarative memory is processed through the medial temporal lobe (11-13). This region contains the hippocampus and connected areas such as the entorhinal, perirhinal and parahippocampal cortex. The importance of the medial temporal lobe in declarative memory consolidation is best described by Nadel and O'Keefe's cognitive map theory which suggests that episodic memory, a subtype of declarative memory, is dependent on the hippocampus (14).

The first and best studied case exemplifying Nadel and O'Keefe's theory is the case of patient H.M. Due to a severe case of seizures, patient H.M. underwent bilateral surgery to remove portions of his temporal lobe including the hippocampus. After surgery, H.M. retained intact reasoning, motor skills, short-term memory as well as long-term memory for events that occurred prior to surgery, but he could no longer create new long-term memories (15). A similar phenomenon is observed in patients with damage to the hippocampus and in elderly suffering from cognitive impairments (16-18). In support of these observations, the discovery of hippocampal place cells in animals further validates the cognitive map theory. The firing of these neurons helps orient animals in their environment by providing them with a so-called "cognitive map" (19).

1.3.1 Structural organization of the medial temporal lobe

At the structural level, the anatomical organization of the medial temporal lobe corroborates its importance in the encoding and consolidation of episodic memory. A unidirectional path of various linked structures enables the processing of information in an efficient and logical manner. Essentially, neocortical inputs that process unimodal sensory information about object features converge in the perirhinal cortex and lateral entorhinal area (20). Alternatively, polymodal spatial information converges in the parahippocampal

cortex and medial entorhinal area. Subsequently, this information reaches the dentate gyrus and converges mainly in the hippocampus. In the hippocampus, the mossy fibre pathway projects the information to the CA3 field which relays the information to the CA1 field via the Schaffer collateral pathway. Ultimately, the information enters the subiculum and is sent back to the deep layers of the entorhinal cortex where the information was originally processed (figure 2) (20,21).

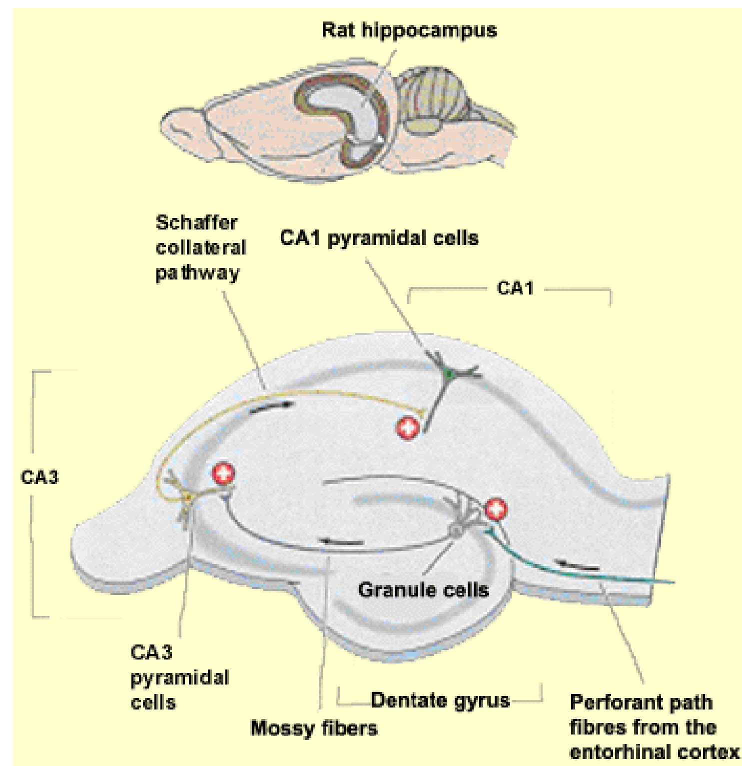


Figure 2 Anatomical representation of the hippocampus. Adapted from (22).

Under this system-level consolidation model, the hippocampus is depicted as part of a retrieval network for recently acquired memories (5). As associations amongst the different elements of a memory are formed and strengthened via repetition, recent memories become gradually transferred to neocortical circuits for long-term storage. Consequently, the

reorganized information can be retrieved independently of the hippocampus upon recall (4,5).

1.4 Synaptic plasticity

It is important to understand memory acquisition at the level of individual cells. Information flows through the different structures of the brain and through the subfields within these structures via neurons that communicate by means of chemical synapses. The chemical messages conveyed between neurons result in functional alterations at existing excitatory synapses (1). This creates changes in the efficacy of communication or in other words, in the excitability of a neuron, for a short period of time. For persistent changes to occur, anatomical alterations consisting of the loss or growth of new synaptic connections, must take place. The process of strengthening particular neural circuits through the modification of synapses is referred to as synaptic plasticity. Donald Hebb best describes this process by postulating that “if neuron A connects to neuron B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (23).

1.4.1 Structure of a neuron

As aforementioned, the hippocampus is compartmentalized in a way that allows for the laminar organization of neurons which provides an optimal layout for a presynaptic ‘neuron A’ to persistently fire a postsynaptic ‘neuron B’. An efficient synaptic transmission between neurons is possible due to the highly specialized structure and function of a neuron.

A neuron contains four distinct regions consisting of a cell body, dendrites, an axon, and axon terminals (figure 3) (24). Synaptic signals are received as neurotransmitters from synaptic boutons on the axon terminals of a presynaptic ‘neuron A’ and form a synaptic connection with the cell body and dendrites of a postsynaptic ‘neuron B’. Chemical signals are rapidly propagated via the axon of the postsynaptic neuron and if they create a

sufficiently large excitation within the neuron, the signal is transmitted to other neurons in a similar manner.

As for the multiple dendrites found on most neurons, they are replete with synaptic spines that are specialized in receiving chemical signals owing to particular receptors embedded in the cell membrane. Activation of these receptors causes a series of molecular reactions that lead to the transcription and synthesis of mRNAs and proteins, respectively. The cell body is the main site for protein synthesis but local translation of mRNAs can also take place in dendrites at synapses. The cell body also contains the nucleus where gene activation and transcription take place. Newly synthesized proteins and transcribed mRNAs can be assembled and transported to active synapses in vesicles or multiprotein particles in dendrites along tracks known as microtubules.

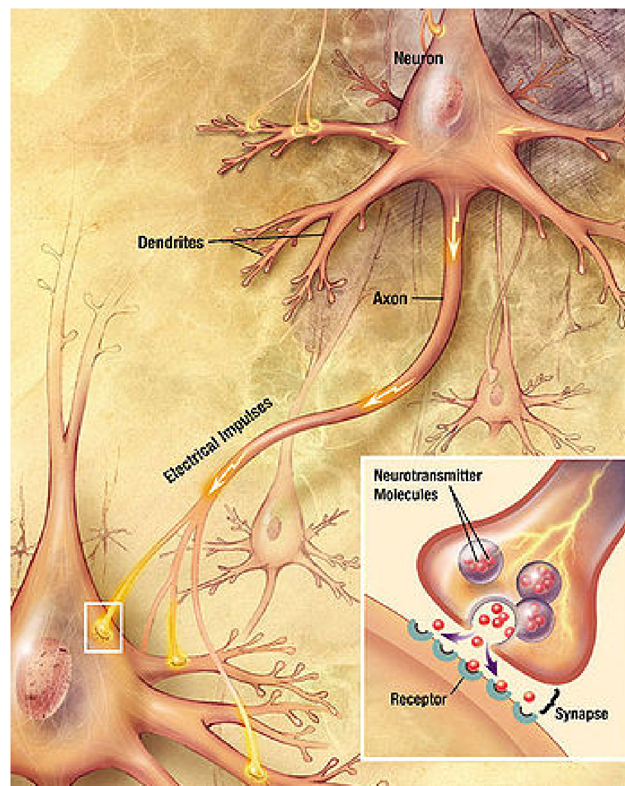


Figure 3 Structure of a neuron. Adapted from (25)

1.4.2 Neuronal network in the hippocampus

In the hippocampus, the chemical messages are transmitted by pyramidal neurons situated in a single continuous layer in the CA1 and CA3 subfields (figure 4) (1,26). About five thousand CA3 pyramidal cell axons converge onto the dendritic spines of a single CA1 cell (1). Unlike many parts of the brain where both excitatory and inhibitory synapses are present on a single spine of a cell, the CA1 region of the hippocampus is solely comprised of cells with one excitatory synapse per spine. The shape of the spine enables the confinement of the molecular reactions that occur during a synaptic transmission thereby, allowing each spine to function as a distinct biochemical region. Therefore, each spine can participate in strengthening a specific neural circuit during learning and memory.

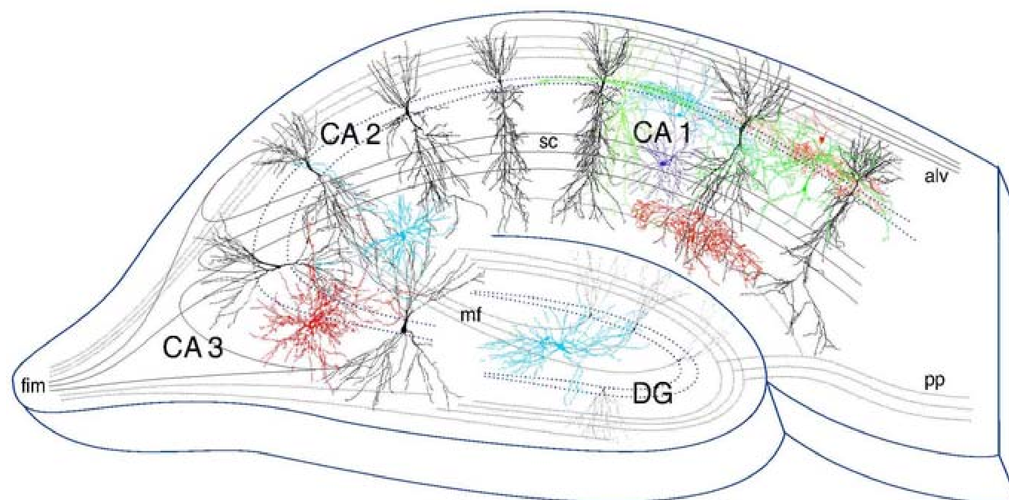
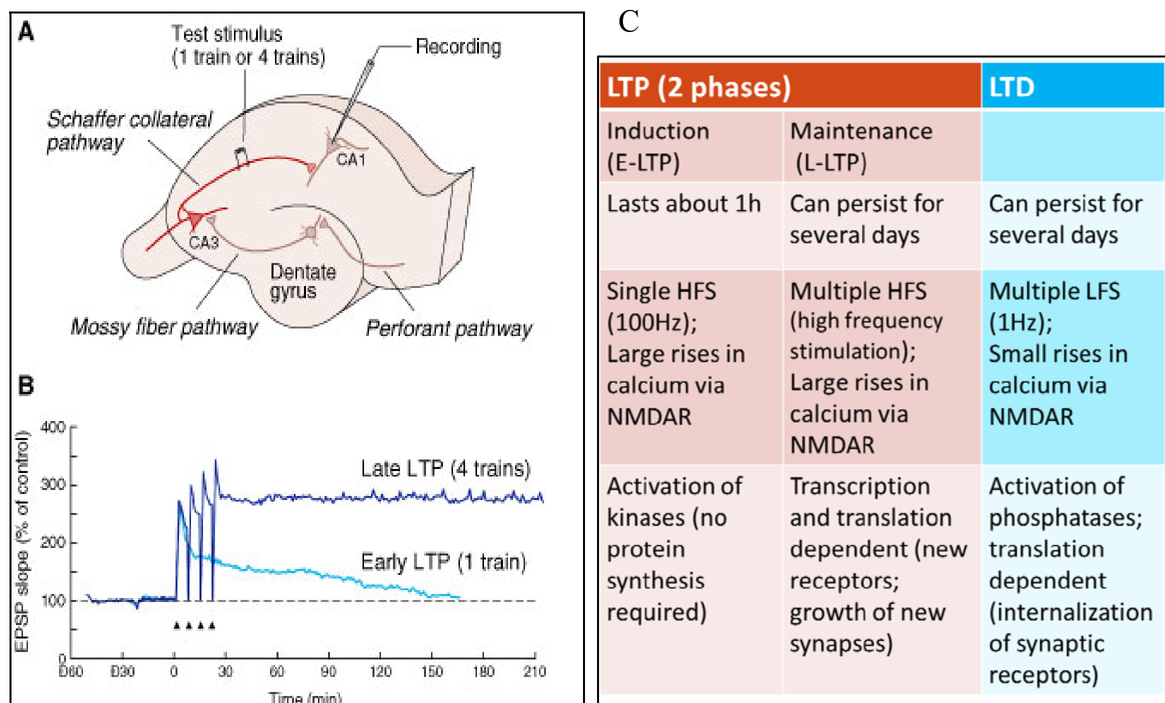


Figure 4 Pyramidal neurons of CA1-CA3 fields. Adapted from (27).

1.4.3 Long-term potentiation and long-term depression

The strengthening of a neural circuit for learning and memory is dependent on the different forms of synaptic plasticity that exist. For one, long-term potentiation (LTP) is described as the enhancement in synaptic transmission between neurons and is broken down into an early (E) and a late (L) component (1). On the other hand, long-term

depression is defined as the diminution of a synaptic transmission by activity. The regional anatomy of the hippocampus is ideal for the physiological recordings of LTP and LTD. By stimulating afferent axons of presynaptic neurons in the CA3 region, researchers can record excitatory postsynaptic potentials from the cell bodies of postsynaptic neurons in the CA1 region. By doing so, the physiological and temporal activities of E-LTP, L-LTP and LTD can be assessed, respectively. From these recordings, it was established that LTP involves an induction phase (E-LTP) that lasts about one hour after stimulation and a maintenance phase (L-LTP) that can persist for several days as can LTD (figure 5).



LTP (2 phases)		LTD
Induction (E-LTP)	Maintenance (L-LTP)	
Lasts about 1h	Can persist for several days	Can persist for several days
Single HFS (100Hz); Large rises in calcium via NMDAR	Multiple HFS (high frequency stimulation); Large rises in calcium via NMDAR	Multiple LFS (1Hz); Small rises in calcium via NMDAR
Activation of kinases (no protein synthesis required)	Transcription and translation dependent (new receptors; growth of new synapses)	Activation of phosphatases; translation dependent (internalization of synaptic receptors)

Figure 5 E-LTP, L-LTP, and LTD. A) Experimental setup for studying LTP in the CA1 region of the hippocampus. The Schaffer collateral pathway is stimulated electrically and the response of the pyramidal population of neurons is recorded. B) Comparison of early and late LTP in a pyramidal neuron in the CA1 region of the hippocampus. The graph is a plot of the slope (measure of synaptic efficacy) of the excitatory postsynaptic potential in the cell as a function of time. Apdated from (1). C) Characteristics of E-LTP, L-LTP, and LTD, respectively.

Multiple molecular mechanisms can explain the electrophysiological phenomena of LTP and LTD. Firstly, E-LTP does not require protein synthesis whereas L-LTP requires both mRNA transcription and translation (28-32). In fact, studies have demonstrated that if either RNA or protein synthesis is blocked in the CA1 area of the hippocampus, long-term memory is disrupted but not short-term memory (33-35). As for LTD, it solely depends on translation. Despite these differences, LTP and LTD share a common feature in that they both require specific glutamate receptors in order to take place. The main receptors are known as the NMDA (*N*-Methyl-D-aspartic acid) and AMPA (2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid) receptors which often co-localize on individual synaptic spines (1).

1.4.3.1 Early long-term potentiation

E-LTP, is initiated upon a single strong stimulation that causes the release of the neurotransmitter glutamate from a presynaptic bouton into the synapse (1). The release of glutamate can be increased by brain derived neurotrophic factor (BDNF) and conversely, glutamate increases the transcription and secretion of BDNF (36). Glutamate crosses the synapse and binds to receptors on the postsynaptic spine (1). At first, only the AMPA receptors open their channels to allow a flow of ions into the postsynaptic neuron. NMDA receptors remain closed due to their unique characteristic of being both ligand-gated and voltage-dependent, a result of ion channel block by extracellular Mg^{2+} ions. Once the voltage change induced by the flow of ions through AMPA receptors is large enough, Mg^{2+} ions dissociate from their binding sites on the NMDA receptor channels. Subsequently, co-activation by two ligands, glutamate and glycine, triggers the opening of NMDA receptors resulting in a large calcium influx in the postsynaptic neuron.

When calcium enters the postsynaptic cell a series of chemical reactions occur that eventually lead to the activation of plasticity-related target genes. The cascade of events includes the activation of protein kinases such as calcium/calmodulin protein kinase II (CaMKII) and atypical protein kinase C (Pkcz). The protein kinases work in synergy to

phosphorylate AMPA receptors thus, increasing their conductance to ions and their responsiveness to glutamate (29,37-40).

When CaMKII is activated, it is capable of transiently autophosphorylating and its activity becomes independent of Ca^{2+}/calmodulin (41). If this Ca^{2+}/calmodulin independent postsynaptic activity is inhibited, LTP induction is blocked, but not LTP maintenance (42). Furthermore, similar to the actions of CaMKII and Pkcz, BDNF participates in increasing NMDA single-channel open probability (43,44) and in regulating the expression of NMDA receptor subunits in hippocampal neurons (45,46). This is achieved by propagation of an intracellular signalling pathway mediated by the binding of BDNF to its receptor Trkβ tyrosine kinase (47). As a result, the excitability of the synapse is temporarily increased during a subsequent synaptic transmission. The changes that occur during E-LTP are short-lived and within one hour, basal conditions are restored.

1.4.3.2 Late long-term potentiation

For persistent changes to occur, repeated strong synaptic stimulations (32) must take place for E-LTP to translate into L-LTP. L-LTP can also be induced at a "tagged" synapse if "tagging" is accompanied by the capture of "plasticity related proteins" (PRPs) (48). The concept of "synaptic tagging" was suggested by Frey and Morris, based on their observation that the induction of L-LTP at a synapse stimulates the synthesis of PRPs. PRPs can be captured at a second weakly stimulated synapse, termed tagged synapse, to convert a weak short-lived signal into a signal strong enough to generate L-LTP providing that the capture occurs within a specific time frame. Synaptic tagging also ensures that only activated synapses become modified via PRPs.

The best PRP candidate currently known is BDNF (49). Induction of a weak synaptic stimulation accompanied by elevated BDNF expression is sufficient to generate L-LTP (50,51). However, this is not the case if BDNF is delivered 70 min after stimulation (52) since the lifetime of a tag is about 1 to 2 hours (53). "Tags" must meet several criteria that include being activated by weak stimulation that induces E-LTP only, being activated

independent of protein synthesis, and being capable of interacting with PRPs for L-LTP. Under this definition, a great potential "tag" is Trkβ; the receptor for BDNF.

Regardless of the mechanism by which L-LTP is induced, it lasts about 1 to 3 hours and requires the translation of dendritically localized mRNAs independent of transcription (53,54). The transcription independent induction phase of L-LTP is followed by the transcription dependent maintenance phase which occurs about >4 hours after L-LTP induction (55). The time lag between the induction and maintenance phase results from the time required to activate the nuclear transcription of plasticity-related mRNAs, and their assembly and transport to the active synapse for local translation. These events must be tightly regulated by various proteins.

The first regulatory proteins involved in propagating the signal for the onset of the maintenance phase of L-LTP include a wide range of protein kinases such as Pkcz, CaMKII, and protein kinase A (PKA). Activation of these kinases initiates a molecular pathway that leads to the phosphorylation and thereby, the activation of cAMP responsive transcription factor CREB (56,57). The first kinase identified for CREB activation is PKA. Upon synaptic stimulation, PKA becomes activated through dissociation of its subunits (57). The released catalytic subunits can translocate to the nucleus where they can phosphorylate CREB (56,57). CREB is responsible for the activation of genes implicated in synaptic structural and functional remodelling as well as in modulation of intrinsic excitability (58). When specific CREB isoforms (CREBα/Δ) are knocked out in mice, spatial and contextual memories are impaired (59). These findings are further supported by numerous other studies demonstrating the role of CREB during memory formation (60-63).

1.4.3.2.1 Transcription

During synaptic plasticity, transcription is mediated by the concerted action of a multitude of transcription factors, such as CREB, that mediate the transcription of specific genes upon L-LTP induction. The immediate early gene Arc (activity-regulated cytoskeleton associated protein) has been extensively studied for its role in synaptic plasticity. It is one of the first genes activated at the onset of synaptic transmission and its

RNA and protein rapidly localize to dendrites and spines after activition (64,65). It is required for L-LTP and memory consolidation (66,67) as demonstrated in a study in which Arc KO mice exhibited enhanced E-LTP while L-LTP was blocked in both the dentate gyrus in vivo and in the CA1 region of acute hippocampal slices (68). Rapid Arc expression is necessary for the modulation of postsynaptic density expansion as well as for growth of postsynaptic dendritic spines by promoting F-actin stabilization through major regulators of F-actin dynamics (69).

1.4.3.2.2 Assembly and transport

Newly transcribed mRNAs must be assembled into ribonucleoprotein (RNP) transport granules in the nucleus before being transported to distal active synapses. Mechanisms must be in place to ensure that the correct mRNAs are assembled and transported in specific particles for their precise trafficking to active synapses. The particles in which the proteins and their cognate mRNAs are packaged include other proteins; some involved in the translational repression of the mRNAs during transport, others implicated in associating with microtubules (70,71), and more as components of the translational machinery (72).

Transport of mRNAs from the cell body to dendrites requires intact microtubules (70). The regulation of microtubule assembly into a functional cytoskeletal network is highly dependent on the functions of microtubule associated proteins such as microtubule associated protein 1B and 2 (MAP1B and MAP2) (73). A disruption in the functions of MAP1B and/or MAP2 affects the trafficking of new mRNAs and proteins critical for the maintenance of LTP (74) .

Additionally, various proteins are involved in selecting their respective mRNAs by recognizing *cis*-acting sequences in the 3′-untranslated region (UTR) of the mRNAs (75) and enabling the transport of these mRNAs to their final destination (76). Some of these mRNA-binding proteins include hnRNP A2 in cortical and hippocampal rat neurons (77),

zipcode-binding protein involved in the transport and translation of β-actin mRNA in rat dendrites (78) as well as Staufen proteins.

The double stranded RNA (dsRNA)-binding proteins Staufen 1(Stau1) and Staufen 2 (Stau2) are found in distinct RNPs in the cell body and dendrites of mammalian neurons (79-82) where they each regulate the microtubule mediated transport of a variety of mRNAs (79,83-86). In fact, down-regulation of Stau1 and Stau2 by siRNA reduces the amounts of RNA in dendrites of neurons. For example, down-regulation of Stau1 reduces the amount of αCaMKII at synapses (81) while down-regulation of Stau2 reduces the amount of β-actin (87) and of a reporter with the 3′-UTR at synapses (88).

1.4.3.2.3 Translation

Once at the active synapse, the silenced mRNAs are de-repressed and translated into proteins that participate in the growth of new synapses and in the formation of new receptors (BDNF and its receptor Trkβ, αCaMKII, Arc, etc – see previous paragraphs). CPEB (see section 1.3: post-transcriptional modifications) and possibly Stau1 are some of the proteins responsible in regulating the local translation of a subset of mRNAs in an activity-dependent manner (89-92). For Stau1, it was demonstrated that it increases the translation of a specific subset of mRNAs by binding to their 5’ end (89).

It can be inferred from the above information that LTP is a complex phenomenon. It includes a variety of steps in which specific regulatory proteins participate in ensuring that an effective synaptic transmission can take place between neurons. However, it is the determination of the interaction between LTP and LTD in a neuron that will ensure that a distinctive neural circuit will be reinforced for enhanced memory and learning.

1.4.3.3 Long-term depression

Similarly to LTP, LTD is a complex molecular event but unlike LTP, transcription is not required since pre-localized mRNAs are translationally activated upon LTD induction (93). LTD is initiated upon multiple low frequency stimulations that cause small

rises in calcium via NMDA receptors (94). The low calcium concentration is responsible for the distinctive cascade of events that prompts the activation of protein phosphatases during LTD compared to protein kinases activated during LTP (95). A few advances have been made regarding the precise phosphatases that are activated during LTD. It has been demonstrated that postsynaptic addition of phosphatase inhibitors that primarily target calcineurin or protein phosphatase 1 (PP1) prevent LTD (96). Protein phosphatases are involved in the de-phosphorylation of AMPA receptors thus reducing their open channel probability (97). In addition, the change in phosphorylation is accompanied by the internalization of these receptors (98-100).

Besides its role in LTP, Arc was found to be crucial for LTD (101,102) by facilitating endocytosis of AMPA receptors through its interaction with endocytic proteins (66,103). This event is inhibited upon LTD induction by acute blockade of new Arc synthesis (101). In reference to LTD, Arc mRNA translation is believed to be regulated by the fragile X mental retardation protein (FMRP) (104) known to be a key player in LTD (105). Indeed, activation of Arc is absent in *Fmr1* KO mice (102). FMRP associates with a subset of mRNAs, including Arc, and possibly acts as a negative regulator of translation. This theory is based on the observation that in the absence of FMRP, there is excess protein synthesis that leads to the increased internalization of AMPA receptors and exaggerated LTD (106).

Furthermore, other candidates, including MAP1B that co-localizes with FMRP in dendrites of cultured neurons (107), are thought to play equally important roles in the internalization of AMPA receptors during LTD (104,108). It was shown that the rapid synthesis of MAP1B is linked to the internal trafficking of AMPA receptors (109) and that the basal expression of MAP1B is augmented in hippocampal slices of Fmr1 KO (110). Conversely, the expression of MAP1B in dendrites is reduced in the absence of Stau2. Specifically, Stau2 knockdown, but not Stau1 knockdown, with siRNA in primary hippocampal neurons blocks LTD and decreases the endogenous expression of MAP1B protein as well as the level of a reporter with the MAP1B 3'UTR that is transported to dendrites (88). It also prevents the dissociation of this reporter from Stau2 mRNA transport

granules. Consequently, translational activation of MAP1B is inhibited thus, preventing MAP1B dependent AMPAR internalization required for efficient LTD. Therefore, Stau2 is thought to be required for the loading of MAP1B mRNA into transport granules, for its transport within these granules to active synapses, and for its local translational activation by way of dissociation with MAP1B mRNA.

All of the events described for LTD and LTP rely on the precise and accurate function of plasticity related proteins. Any disruptions in the expression and activity of a protein can hinder the efficacy of a synaptic transmission. For that reason, strict transcriptional and translational regulations of plasticity-related mRNAs and proteins exist.

1.4.4 Transcriptional and translational regulations of plasticity related mRNAs and proteins

Transcriptional and translational regulations are crucial in preventing ectopic and/or altered expression of synaptic plasticity-related mRNAs and proteins which could otherwise result in various defects such as fragile X syndrome (caused by the absence of Fmr1 gene expression) (111), Alzheimer's disease (caused by accumulation of Aβ peptides due to disruption of APP metabolism) (112), and more. Translational repression via RNA-binding proteins and microRNAs (miRNA) as well as polyadenylation of mRNAs has emerged as important post-transcriptional regulations (113,114). Similarly, post-translational modifications such as phosphorylation, adenylation, and methylation are crucial in altering the activity of a protein (115-117).

1.4.4.1 Post-transcriptional modifications

Transcription is the first regulatory step in gene expression. Following transcription, mRNAs are assembled in ribonucleoprotein transport granules for their export from the nucleus to the cytosol. During transport, mRNA translation is silenced by repressors associating to specific recognition sequences in the 3'UTR of a mRNA (118-122) or by the presence of an inhibitory RNA structure in the 5′-UTR to block translation initiation (123,124).

A great example is the cytoplasmic polyadenylation element protein (CPEB) that regulates the polyadenylation (poly(A)) of the 3' end of a subset of mRNAs. These mRNAs contain CPE recognition sequences in their 3'UTR that bind to CPEB (125). Translation can only be initiated when CPEB becomes phosphorylated by a protein kinase Aurora (116). This induces CPEB to interact with proteins involved in the recruitment of a poly(A) polymerase for the process of polyadenylation (117). This step is thought to increase translation (126). A likely target of polyadenylation during synaptic plasticity is the 3'UTR CPE-containing N-actin mRNA in Aplysia (127). Polyadenylation of this mRNA was shown to occur in response to stimulation with serotonin (128,129). Furthermore, it was demonstrated in mice that αCaMKII contains two CPEs in its 3′UTR (130) thereby, making it another potential candidate to be regulated by CPEB via the addition of a poly(A) tail.

miRNAs also take part in the tight spatial regulation of mRNA translation in neuronal dendrites (131). miRNAs represent an extensive class of small non-coding RNAs that act as post-transcriptional regulators of gene expression. They have been discovered in a plethora of tissues, including the nervous system and fittingly, have a widespread role at various stages of synaptic development and during synapse function and plasticity. In post-mitotic neurons, many miRNAs are associated with translation regulatory complexes (132) where one miRNA can control the fate of a few hundred different synapse-relevant mRNAs simultaneously (133).

miRNAs result from a multi-step maturation process starting with the transcription of miRNAs into primary miRNAs (pri-miRNAs) (134,135). Cleavage of pri-miRNAs produces precursor hairpin miRNAs which are exported to the cytoplasm to be further processed by the RNase Dicer. The end product is a mature single-stranded miRNA loaded into a multi-protein miRNA-induced silencing complex (miRISC). An important component of this complex with regard to synaptic function is the Argonaute protein. The miRNA within the complex is guided to target mRNAs in order to extensively bind, albeit imperfectly, to sequences usually present within the 3'UTR of these mRNAs. This results in the translational repression and/or degradation of the mRNAs in question.

In particular, miR-134 is known for its role in suppressing the translation of LIMK1 mRNA in dendrites of mature hippocampal neurons. LIMK1 is a kinase that promotes actin polymerization and spine growth (136). The miR-134-LIMK1 association seems to be regulated by synaptic activity whereby increased levels of BDNF release LIMK1 from the inhibitory effects of miR-134. miR-138 is another miRNA with a role in spine morphogenesis. miR-138 acts on the depalmitoylation catalytic enzyme APT1 which participates in modulating the actin cytoskeleton of dendritic spines (137).

1.4.4.2 Post-translational modifications

Phosphorylation is a major post-translational modification that, in most cases, induces the activation of a protein. The constitutively expressed CREB protein is well known for being subjected to activation through phosphorylation (115). In the context of synaptic transmission, a signalling pathway originating from activation of NMDA receptors, leads to an increase in cAMP. In turn, cAMP activates PKA and the mitogen-activated protein kinase (MAPK) and both proteins translocate to the nucleus where they phosphorylate an activator form of CREB (138,139). This activator form is responsible for the stimulation of gene expression for target genes containing a cAMP responsive element (CRE) (140).

The regulatory mechanisms described must remain functional for the maintenance of a healthy cognitive status. Cognitive decline associated with aging and neurodegenerative diseases arises when disturbances occur in the expression and/or function of plasticity-related mRNAs and proteins that bring about changes in the efficacy of synaptic transmission.

1.5 Aging and cognitive decline

Aging is associated with learning and memory impairments, especially hippocampus-dependent spatial memories. Unfortunately, due to the limited knowledge about the etiology of cognitive impairment associated with aging, few pharmaceutical

interventions have proven to be effective in preventing and/or treating age-related memory loss. Currently, it is established that during normal aging, various other structural modifications are attributed to cognitive decline despite the lack of significant neurodegeneration. Neurons in the cerebral cortex and especially in the medial temporal structures undergo attrition of dendritic branches, loss of dendritic spines and loss of synapses (141-143). These changes contribute to the decreased efficiency in relaying information through the perforant, mossy fibre, and Shaffer collateral pathways in aged rodents not to mention that they are likely a result of dysregulations in the transcription and translation of plasticity-related mRNAs and proteins. Such disruptions lead to defects in cytoskeletal integrity, microtubule-dependent transport, synaptic functions, and neurotransmission (6,7,144-146).

The effects of these modifications can be observed in experimental settings. Importantly, with regards to hippocampus-dependent spatial memory, there is ample evidence that aged rats that undergo the Morris water maze test show deficits in recalling the path to the hidden platform after several trials (when the elapsed time between trials is long) when compared to young rats (147-155). Additional electrophysiological studies substantiate these findings by providing LTP and LTD recordings in rodent models of healthy aging that suggest perturbations in hippocampal plasticity during aging. These recordings display decreased basal synaptic transmission (156), enhanced LTP threshold (157), decreased LTP maintenance (158), altered LTP mechanisms (159), and increased chances of LTP reversal (160) in aged rodents compared to young ones. There also appears to be facilitated LTD and hence, not surprisingly, an imbalance in synaptic transmission whereby LTD prevails over LTP (157,161,162).

Each one of the altered electrophysiological recordings observed in aged rodents is associated to defects that occur at the molecular level. For example, significant decreases in proteins such as SNAP25, syntaxin 1, VAMP2, and synaptophysin (2,163) critical in the docking and fusion of presynaptic vesicles, and in the scaffold protein PSD95 (7,164,165), have been observed in aged rodents. These proteins are essential for the formation of a functional synaptic contact. Causally, a decrease in their expression can help explain the

diminution in basal synaptic transmission and in LTP maintenance in aged rodents. More specifically, it may provoke slowed endocytic replenishment and/or abolished exocytosis of synaptic vesicle pools as well as impaired receptor aggregation (PSD95) upon prolonged or intense stimulation of synaptic transmission (166,167).

Furthermore, perturbations in LTP maintenance can arise from defects in the expression or function of individual mRNAs and/or proteins. Immediate early genes (IEG) such as Arc represent an important class of transcription factors that are transiently and rapidly activated to regulate the expression of target genes involved in synaptic plasticity (168,169). One such IEG is zif268. Normally, Zif268 expression increases during hippocampus-dependent spatial orientation tasks (170) but in aged rodents that express spatial memory impairments, resting levels of zif268 mRNAs are decreased in the CA1 and CA2 areas of the hippocampus and in the neocortex (171). These results demonstrate that zif268 expression may play an important role in the maintenance of intact spatial memory of aged rodents.

A dysfunction in the activity of an individual protein can also impair synaptic transmission. The previously discussed CREB protein (sections 1.2 and 1.3) shows phosphorylation dependent activity upon LTP induction. There is evidence for the absence of change in the resting expression of CREB in the hippocampal CA1 region and dentate gyrus of aged rodents with cognitive decline when compared to young rodents (172,173). In contrast, there is a reduced amount of phosphorylated CREB activity that is noticeable in the same aged rodents. Over-expression of CREB in aged rodents can partly rescue age-related memory changes (174). This demonstrates the great importance in the activation status of CREB for its effective role in synaptic plasticity. The decreased activity of CREB may be partially responsible for the increased LTP reversal observed in aged rats due to decreased activation of target genes important in maintaining LTP.

Another salient study offered enlightenment for the facilitated LTD observed in aged rodents suffering from cognitive impairments. VanGuilder H.D., *et al*. demonstrated, in hippocampal slices of aged rats, an increased expression of hippocalcin, a protein involved in facilitating LTD through calcium sensitive dynamics (2). Moreover, alterations

in the subunit composition of AMPA and NMDA receptors may exacerbate LTD prevalence. The subunit subtypes of NMDA and AMPA receptors have differing kinetics that dictate whether LTP or LTD will take place (175-178). For example, the GluR2 AMPA receptor subunit prevents calcium permeability through the receptor channel. Aged cognitively-impaired rodents show a decrease in the GluR2 subunit which renders AMPA receptors more permeable to calcium. Increased calcium permeability augments susceptibility to excitotoxicity and postsynaptic calcium concentrations determine the strength and direction of synaptic transmission (179-182). Therefore, altered calcium homeostasis can result in a wider range of low-frequency activity patterns thereby, triggering excessive LTD (183-185).

As for NMDA receptors, they are made up of an obligatory NR1 subunit and two additional NR2 subunits: NR2A and NR2B (186). The NR2A or NR2B subunit governs at different developmental stages (187,188). One study determined that the expression profiles for both types of NR2 subunits shift in a similar manner in both aged rodents suffering from cognitive impairment and young rodents (7). However, they found an increase in NR1 subunits in aged rodents. These researchers speculated that the additional NR1 subunits may represent non-functional subunits that could bring about the internalization of NMDA receptors. Conversely, another study observed an increased NR2A/NR2B ratio in the hippocampus of aged memory-impaired mice as compared to young adults (189). This led to changes in the induction thresholds for LTP. Despite the differences between studies, the outcome remains the same. Altogether, these findings provide insights into possible mechanisms underlying the predominance of LTD over LTP as well as the enhanced LTP threshold observed in aged cognitively- impaired rodents.

NMDA receptors in the hippocampus are also susceptible to altered calcium homeostasis; (162,190-192) a process that is regulated by two different calcium sources during synaptic activity. Voltage-dependent calcium channels (VDCCs) as well as intracellular calcium stores release calcium during synaptic transmissions and their activity is altered in aged hippocampal neurons (193,194). For instance, aged rodents display greater numbers of VDCCs in the CA1 region of their hippocampus (195) and thus, cause

an imbalance towards an increased dependence on a form of LTP that relies on VDCCs rather than NMDA receptors (196-198). Consistent with this, the activity of NMDA receptors is reduced in the hippocampus of aged rats (199). Such disturbances have important implications for LTP induction which henceforth, requires a greater stimulus to be triggered via the NMDA receptors. This represents an altered mechanism by which LTP can take place and by which its threshold can be increased.

All of the aforementioned alterations observed in cognitively-impaired aged rodents occur in the absence of significant neural loss. It is difficult to imagine the increased detrimental effects that neurodegeneration can have on the structural, cellular, and molecular processes that take place during synaptic transmission. These processes will be reviewed for a more severe form of cognitive decline exhibiting neurodegeneration that is, Alzheimer's disease (AD).

1.6 Alzheimer's disease

Alzheimer's disease is a fatal neurodegenerative disease afflicting over 35 million people worldwide and representing 70% of dementia cases in the world. Its prevalence is constantly rising as a consequence of the general increase in the aging population which is expected to quadruple by 2050 in the United States (200). Despite the ongoing studies and our better understanding of the disease, there are currently no effective treatments available to cure Alzheimer's disease. Its emergence is most often sporadic and in a population aged over 65 years. However, about 5% of cases present with a less common form known as familial Alzheimer disease (FAD) which usually strikes sooner (201). FAD is the result of mutations in specific genes including the amyloid precursor protein (APP), presenilin 1, and presenilin 2 genes. Regardless of its cause of onset, Alzheimer disease has detrimental effects on the quality of life of those affected and imparts costly burdens to the public health sector.

At the neuropathological level, Alzheimer's disease is characterized by cerebral atrophy which is especially apparent in the hippocampus but also affects the neocortex

(202). In addition there are manifestations of neuronal cell death, neuroinflammation, synapse loss, and the accumulation of two specific types of lesions. These lesions first appear in the medial temporal lobe (203,204) and consist of extracellular senile plaques made up of aggregated amyloid-beta (Aβ) peptides (205) and intracellular neurofibrillary tangles (NFT) (206) composed of hyper-phosphorylated forms of the tau protein (figure 6) (207).

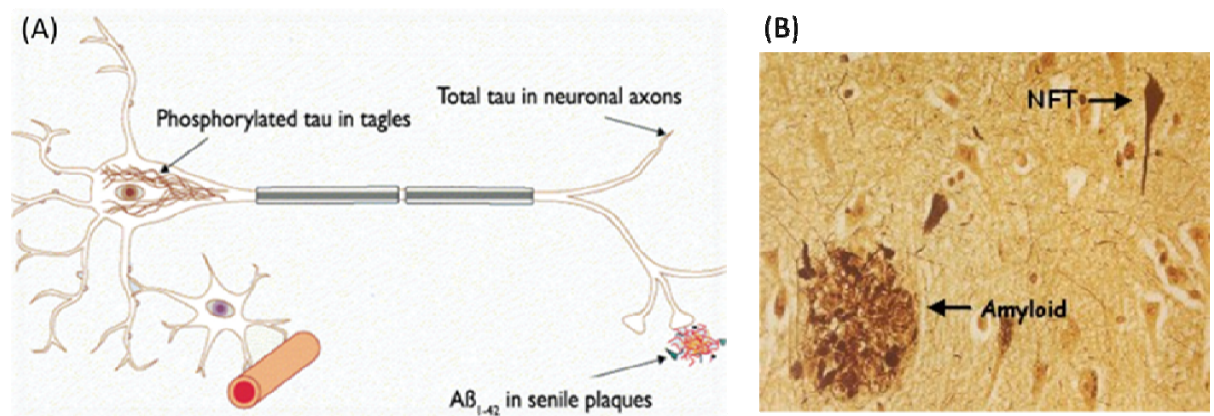


Figure 6 Lesions in Alzheimer's disease. A) Schematic representation of senile plaques and neurofibrillary tangles. Adapted from (208). (B) Histological representation of senile plaques (Amyloid) and neurofibrillary tangles (NFT). Adapted from (209).

NFT arise from a dysregulation in the phosphorylation state of tau (210). Tau is a microtubule-associated protein that participates in the stabilization and regulation of microtubule dynamics necessary for neurite outgrowth, morphogenesis and axonal microtubule-dependent transport. In its de-phosphorylated state, tau's affinity for microtubules is reduced thereby, promoting their depolymerisation. In contrast, in its phosphorylated state, tau binds to microtubules and enhances their stability (211,212). Hyperphosphorylation of tau in Alzheimer's disease hinders the microtubule network and promotes self-assembly of tau into paired helical filaments (212,213).

On the other hand, Aβ peptides present in senile plaques are cleavage products of APP (214). A dysfunction in the metabolism of APP processing results in excessive Aβ fibril accumulation of 39-42 amino acids long. The 40 (Aβ40) and 42 amino acid residues (Aβ42) are the most prevalent while the Aβ42 fibril has a higher propensity to form aggregates. The translational regulation of APP is carried out by FMRP which when bound to APP mRNA, represses its translation (215). FMRP may therefore play an important role in the pathogenesis of Alzheimer's disease if its expression is disrupted as seen in fragile X mental disorder.

Similar to the electrophysiological recordings described for cognitive decline (see section 1.4) the recordings observed for Alzheimer's disease display inhibited LTP (216) and facilitated LTD in the presence of oligomeric Aβ in hippocampal synapses (217). The mechanisms underlying the disruptions in LTP and LTD include partial inhibition of NMDA receptor activity due to Aβ deposition (218) which provokes decreased calcium influx through the NMDA channels. The resultant disruption of calcium homeostasis causes the activation of calcineurin; a key player involved in the internalization of NMDA and AMPA receptors (219,220). Internalization of NMDA receptors also occurs via dephosphorylation of NR2B subunits by oligomeric Aβ induced-phosphatases (221).

Furthermore, reduced calcium concentration limits downstream events such as activation of plasticity related proteins including CaMKII, MAPK, and Akt/protein kinase B (222). All of these events lead to Aβ mediated dendritic spine loss and altered LTP and LTD mechanisms.

Other plasticity-related proteins downstream of receptor activation are dysregulated in Alzheimer's disease. The previously discussed CREB protein (section 1.2: late long-term potentiation) is downregulated in the brains of Alzheimer mice (223). As a result, its mRNA and protein levels are lowered thereby, decreasing the phosphorylation-dependent activation of the protein. The underlying cause appears to be the oxidative stress caused by the accumulation of Aβ fibrils. In the presence of antioxidants, the adverse effects of oxidative stress on CREB are prevented and so CREB can resume its normal function as an activator of target genes. Under stress conditions, the target genes under the control of

CREB are also affected. CREB target proteins such as BDNF, and anti-apoptotic proteins Bcl-2, BIRC3, and BIRC4 are downregulated while the pro-apoptotic protein Bax is increased in Alzheimer mice. Therefore, decreased CREB activity ultimately leads to neuronal apoptosis, a major hallmark of Alzheimer's disease.

In addition, miRNAs may also play an important role in the translational regulation of APP in Alzheimer's disease. Various miRNAs have been recognized to bind the 3'UTR of APP mRNA (224-226). In human brains with Alzheimer, the level of miR-106b is significantly reduced (225). However, there is no convincing evidence of a correlation between miRNA levels and APP in Alzheimer tissues. However, regulation of APP by miRNAs has not been ruled out. In rat hippocampal neurons, silencing of AGO2, a RISC protein involved in the miRNA pathway (see section 1.3) was shown to increase APP protein levels (226). Further investigations demonstrated the effects of the brain-enriched miR-10 on APP levels. Inhibition of endogenous miR-101 increased APP levels whereas overexpression of miR-101 significantly reduced APP levels and consequently Aβ aggregates decreased as well. In agreement with these observations, miR-101 is downregulated in the cerebral cortex of patients afflicted with Alzheimer's disease (227,228).

As can be deduced from the above information, Alzheimer's disease exhibits similar dysfunctions in mRNA and protein expression, in protein activity and in transcriptional and translational regulations as observed in age-related cognitive decline. The main differences are the presence of senile plaques and neurodegeneration in Alzheimer's disease compared to memory impairments in elderly. Despite these differences, the key players involved in synaptic plasticity are equally affected in both cases.

1.7 Hypothesis, aims, and rationale

1.7.1 Hypothesis

The expression of plasticity-related mRNAs will be altered in aged rodents and in transgenic rodent models showing early stages of AD. More specifically, the expression of

mRNAs mainly involved in LTD will increase whereas the expression of mRNAs mainly involved in LTP will decrease and in this manner, cause an imbalance between LTP and LTD whereby LTD will prevail over LTP. The disturbances in mRNA expression will be most prevalent in the hippocampus (CA1-CA3 fields) than in other regions of the brain such as the dentate gyrus or the cortex. In addition, the genes affected will differ from one brain region to the other.

1.7.2 Aims

1) To study the expression of plasticity-related mRNAs in the hippocampus (CA1-CA3 fields), dentate gyrus, and cortex of 3xTg mice showing early stages of AD and their wild-type counterparts by means of RT-qPCR experiments.

2) To study the expression of plasticity-related mRNAs in the hippocampus and cortex of young (3 months old) and aged (24 months old) Fisher 344 rats by means of RT-qPCR experiments.

1.7.3 Rationale

The morphological modifications of neurons that occur in the cerebral cortex and more notably in the medial temporal structures where the hippocampus is located (141-143), are the outcome of disturbances in the transcription and translation of plasticity-related mRNAs and proteins which together, results in a less efficient synaptic plasticity within these structures (6,7,144,145,229). During aging (157,161,162) and AD (216,217), these defects cause an imbalance between LTP and LTD whereby LTD starts prevailing over LTP. Therefore, the expression of mRNAs mainly involved in LTD should increase to help explain the increase observed in LTD whereas the expression of mRNAs mainly involved in LTP should decrease to help explain the inhibition of LTP observed in these pathologies.

Additionally, during age-related cognitive decline and AD, progressive cognitive decline and neurodegeneration (in the case of AD) occur in a sequential process across

brain tissues, with the temporal lobe being affected earlier than the frontal part of the cortex (3,203). Thus, changes in the expression of plasticity-related genes most likely appear at a later stage in the cortex than in the hippocampus. Furthermore, Kaiwen He *et al.* (230) demonstrated that the mechanisms for synaptic plasticity might diverge depending on the brain region. Therefore, the genes that become dysregulated in age-related cognitive decline and AD might differ across brain tissues and for different sub-regions within these tissues.

For example, the hippocampus contains distinctive anatomical regions consisting of the CA1 field, CA3 field, and the dentate gyrus all of which possess unique molecular and biophysical properties (231). Whereas the CA1-CA3 sub-regions are made up of four layers of pyramidal cells, the dentate gyrus is made up of three layers of cells with granule cells representing the major cell type. These cells have the ability to proliferate throughout the lifespan by means of neurogenesis. This process is thought to be important for learning and memory (232). Neurogenesis declines with age (233-235) and therefore, might be associated to cognitive impairments as demonstrated in various studies (236-238). The defects in neurogenesis observed in these studies likely reflect changes in gene expression. The differences between the sub-regions within the hippocampus result in characteristic region-specific electrophysiological properties. These arise from the fact that each field exhibits a specific synaptic response to plasticity-related genes normally expressed in all regions of the hippocampus (239,240).

At the molecular level, each hippocampal sub-region has been shown to display unique transcription and protein expression patterns (241,242). Additionally, analysis of the hippocampal regions in aged cognitively impaired animals compared to young and aged cognitively unimpaired animals revealed that changes in gene expression were more pronounced in the CA3 field than in the CA1 field and dentate gyrus of the hippocampus (243). Similarly, in transgenic AD mice, the Schaffer collateral pathway (pathway from CA1 to CA3 field) of the hippocampus exhibits diminished LTP whereas the Mossy fibre pathway (from the dentate gyrus to the CA3 field) is slightly enhanced (244). This supports a unique role for each sub-region within the hippocampus in learning and memory.

For all of the aforementioned reasons, qRT-PCR analyses must be performed separately in the cortex and sub-regions (dentate gyrus and CA1-CA3 fields) of the hippocampus in 3xTg and WT mice and in the cortex and hippocampus of young and aged rats.

2 Experimental procedures

2.1 Animals

Experiments were conducted in accordance with the guidelines of the Canadian Council of Animal care and were approved by the "Comité de déontologie de l'expérimentation sur les animaux (CDEA)" of "Université de Montréal".

2.1.1 Young and aged rats

Four young (3 months) and four aged (24 months) Fisher 344 rats obtained from Harlan Laboratory were used for qPCR analysis. Animals were euthanized in a CO_2 chamber one at a time and cortices and hippocampi were dissected within thirty minutes after death. Dissection was performed under RNAse free conditions and tissues were maintained in cold Hank`s balanced salt solution (HBSS: Potassium chloride 5.33 mM, potassium phosphate monobasic 0.441 mM, sodium chloride 137.93 mM, sodium phosphate dibasic anhydrous 0.338 mM, D-Glucose 5.56 mM, Phenol Red 0.0266 mM, sodium pyruvate 1 mM, Hepes 10 mM). Once dissected, tissues were immediately placed in Trizol Reagent (Invitrogen) and kept on ice. Tissues were then homogenized using a polytron and immediately subjected to RNA isolation or stored at -80^0C until use.

2.1.2 3xTgmice and WT mice

The hippocampi, dentate gyrus, and cortices of the right brain hemisphere of three transgenic Alzheimer (3xTg) mice and three control wild-type (WT) mice were obtained from Karl Fernandes laboratory in the "départment de pathologie et biologie cellulaire à l'Université de Montréal". The generation of 3xTg mice has been described previously (245). Briefly, 3xTg mice were produced from co-microinjection of two independent transgenes encoding human APP_{swe} and human tau_{P301L} into single-cell embryos harvested from homozygous mutant $PS1_{M146V}$ knock-in mice. The APP_{swe} and tau_{P301L} transgenes are both under the control of the neuron-specific mouse Thy1.2 regulatory element. The non-transgenic WT mice used have the same background as the 3xTg mice (C57BL6/129SVJ)

and were littermates of the original $PS1_{M146V}$ knock-in mice. Housing conditions were identical for 3xTg and WT mice.

The brain tissues (Hippocampus (CA1-CA3 fields), dentate gyrus, and cortex) of each of the 3xTg and WT mice were dissected from the brains of the mice once they had reached seven months of age. At this age, cognitive impairments started to be apparent in the 3xTg mice. Immediately after dissection, the brain tissues were stored at -80^0C. Upon reception, the frozen tissues were deposited in Trizol Reagent (Invitrogen) and homogenized using a polytron. RNA isolation was performed immediately afterwards or stored at -80^0C until use.

2.2 RNA isolation

All tissue samples were homogenized with a polytron in Trizol Reagent (Invitrogen), according to the protocol of the manufacturers. RNA concentration was determined using DO readings at 260 nm and 280 nm in a spectrophotometer (Thermo Fisher Scientific). RNA samples were diluted in DEPC water (RNAse free water) to obtain a uniform concentration of 250 ng/µL across samples. The quality of the RNA dilutions was determined with 1% agarose gel electrophoresis. Samples were subjected to reverse transcription (RT) or kept at -80^0C until additional analysis.

2.3 Quantitative PCR

Prior to qPCR reactions, synthesis of cDNA was achieved using the GeneAmp RNA PCR kit (Applied Biosystems) following the guidelines of the manufacturer. For the cortex and hippocampus samples of young and aged rats and for the cortex samples of WT and 3xTg mice, the starting concentration of RNA used for cDNA synthesis was 750 ng/µL and for the dentage gyrus and hippocampus samples of WT and 3xTg mice the starting concentration of RNA was 200 ng/µL. Subsequently, qPCR reactions were performed

using the LightCycler 480 SYBR Green I Master. For each reaction, 3 µL of cDNA was added to a mix containing 3 µL PCR-grade water, 2 µL of 10X PCR primers, and 10 µL of 2X SYBR Green Master Mix (Roche) for a total of 20 µL to obtain a final concentration of 1X for the PCR primers and SYBR Green Master Mix. Primers were designed using Integrated DNA Technology Primer Quest (http://www.idtdna.com/Scitools/Applications/Primerquest/), blasted using NCBI Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized at Integrated DNA Technology. Primer sequences are listed in Table I and Table II. Cycling conditions were 5 min at 95^0C, followed by 50 cycles of 10 s at 95^0C, 20 s at 60^0C, and 30 s at 72^0C. A melting curve followed the cycling conditions for identification of the specificity of the amplified products by fluorescence measurement every 1^0C from 72^0C to 95^0C.

Gene	Primer	Sequence
AldolaseC	Forward Reverse	5’-AACCTCAATGCCATCAACCGATGC-3’ 5’-TCATCTCGGCCCGCTTGATAAACT-3’
AGO2	Forward Reverse	5’-TCAGCCAGGGATCACATTCATCGT-3’ 5’-AGTCAAACTCAGTTGGGTGGGTGA-3’
Arc	Forward Reverse	5’-AGAGCTGAAGGTGAAGACAAGCCA-3’ 5’-ACCTTAAGGCTCCTGCAAGGTTCT-3’
BDNF	Forward Reverse	5’-AGGGCAGTTGGACAGTCATTGGTA-3’ 5’-TTCAACTCTCATCCACCTTGGCGA-3’
αCaMKII	Forward Reverse	5’-AACACCAAGAAGCTCTCAGCCAGA-3’ 5’-ACTGTAATACTCCCGGGCCACAAT-3’
CPEB	Forward Reverse	5’-TGACACAGATGCCTACACTTGGGT-3’ 5’-AAGGCACTGTATGGCAACTGCAAG-3’
CREB	Forward Reverse	5’-AAATGACAGTTCAAGCCCAGCCAC-3’ 5’-TGCACTAAGGTTACAGTGGGAGCA-3’
FMRP	Forward Reverse	5’-AGGCAGTTGGTGCCTTCTCTGTAA-3’ 5’-AGCTGCCTTGAACTCTCCAGTTGT-3’
MAP1B	Forward Reverse	5’-TCCCAGACCACAGCGATTCATTCT-3’ 5’-ACAGGCTTCGTCCTTCCTGTTCTT-3’
MAP2	Forward Reverse	5’-GCAGCGCCAATGGATTTCCATACA-3’ 5’-TCCGTTGATCCCGTTCTCTTTGGT-3’
NMDAR1	Forward Reverse	5’-TGAGGCTTCACAGAAGTGCGATCT-3’ 5’-TCGCATTCCTGATACCGAACCCAT-3’
Pkcz	Forward Reverse	5’-ACTATGGCCTGGACAACTTCGACA-3’ 5’-AGACAGCAGAAGCGGGTTGATGTA-3’
Stau1	Forward Reverse	5’-TGCACTGTGCGTGAAACTGGAAAG-3’ 5’-ATTAAACTGCTGTCCTCCCACGGA-3’
Stau2	Forward Reverse	5’-GGCAATGAAGTTGCGACTGGAACA-3’ 5’-AGGCTTTGGACCACTCCATCCTTT-3’
Trkβ	Forward Reverse	5’-TTCCGAGGTTGGAACCTAACAGCA-3’ 5’-TTGCCGTTCTTCAGAAACGCCTTG-3’

Table I Sequence information of the *rattus norvegicus* primer pairs for use in quantitative real-time PCR

Gene	Primer	Sequence
AldolaseC	Forward Reverse	5'- GGCCTAGCAATGCACATCCCATTT-3' 5'-TGAGATGTGAGATGACTTGGCGCT-3'
AGO2	Forward Reverse	5'-AGGACAGCATGCTCTTTGAGACCT-3' 5'-TGACAAAGGCCACACGGAACTAGA-3'
Arc	Forward Reverse	5'-TGCCTACATGGGTTCCAAGACACT -3' 5'-TGGCTTGTCTTCACCTTCAGCTCT-3'
BDNF	Forward Reverse	5'- CACAGACGGCTCCTGCCAATTTAT-3' 5'-TTTCTGATGCTCAGGAACCCAGGA-3'
αCaMKII	Forward Reverse	5'-ACACACTCACACCACTTCCTTCCA-3' 5'-TCCATACAAGAGCCAAACCCAGGA-3'
CPEB	Forward Reverse	5'-TGACACAGATGCCTACACTTGGGT -3' 5'-GGCAACTGCAGAATGAACGAACCA-3'
CREB1	Forward Reverse	5'-GGAATCTGGAGCAGACAACC -3' 5'-ATAACGCCATGGACCTGGAC -3'
CycA	Forward Reverse	5'-TACCTGCCTTCACTCATTGCTGGA-3' 5'-ATTGACTGTTGGGCATGTTGTGGC-3'
FMRP	Forward Reverse	5'-ATCAAGCTGGAGGTGCCAGAAGAT-3' 5'-ATGTCAATCAACATGTGGGCTCGC-3'
GAPDH	Forward Reverse	5'-TCAACAGCAACTCCCACTCTTCCA-3' 5'-ACCCTGTTGCTGTAGCCGTATTCA-3'
MAP1B	Forward Reverse	5'-TACCGTGAAAGTTGAGTCCGTGCT-3' 5'-AGCAAACTGGGTGCTAGGGTACTT-3'
MAP2	Forward Reverse	5'-ATCCTGGTGCCCAGTGAGAAGAAA-3' 5'-AGGTCCGGCAGTGGTTGGTTAATA-3'
NMDAR1	Forward Reverse	5'-GGAGCGGGTAAACAACAGCAACAA-3' 5'-AATGGTCAGGCCCTGGTACTTGAA-3'
Pkcz	Forward Reverse	5'-TGCCAGGTTCTATGCTGCTGAGAT-3' 5'-TAATGTGTCCGTCGGCATCAAGGA-3'
Stau1	Forward Reverse	5'-CACAGCCAATTCTCATGCTGGCTT-3' 5'-TCATGTGCAGTAGGAAGCACTGGT-3'
Stau2	Forward Reverse	5'-TCAGATCCAGCAAGCCAGAAAGGA-3' 5'-TGTTCCAGTCGCAACTTCATTGCC-3'
Trkβ	Forward Reverse	5'-ATGTTGCTGACCAAAGCAATCGGG-3' 5'-GCCAAACTTGGAATGTCTCGCCAA-3'
Ywhaz	Forward Reverse	5'-GTGCTTCCAAGTCGCTGTTGGTTT-3' 5'-CATGGCCACCAAGTTGGAATGGTT-3'

Table II Sequence information of the *mus musculus* primer pairs for use in quantitative real-time PCR

Standard curves were calculated from cDNA stock solutions for each primer pair tested for the chosen target and control genes (non-diluted, 10^{-1}; 10^{-2}; 10^{-3} and 10^{-4}). Triplicates were performed in each case. To obtain the relative expression of target genes in the hippocampi and cortices of young and aged rats and in the hippocampi (CA1-CA3 fields), dentate gyrus, and cortices of WT and 3xTg mice, corresponding Ct values were obtained from duplicates and normalized according to the chosen control genes stably expressed in the brain (Aldolase C for young and aged rats, and aldolase C, Ywhaz (246) and GAPDH (246) for 3xTg and WT mice). The mean relative expression of 13 target genes was calculated for 2 groups where group 1 refers to young rats and group 2 refers to aged rats and was calculated separately for the two subgroups. These subgroups consist of different areas of the brain including the cortex and the hippocampus.

Similarly, the mean relative expression of 14 genes was calculated for a third and fourth group referring to WT mice and 3xTg mice, respectively. In this case, the calculations were performed separately for three subgroups consisting of different brain regions including the cortex, the hippocampus CA1-CA3 fields), and the dentate gyrus. Statistics were performed using the 'R' program.

Normalized data were tested for mean differences using the Welch two sample student t-test. More specifically, the mean difference in mRNA expression was compared for group 1 and group 2 for each subgroup described and for group 3 and group 4 for each subgroup described. All data are presented as the mean ± standard error, which is indicated by bars in the figures and a value of $p < 0.05$ was considered to be significant.

3 Results

To study the expression of genes related to synaptic function and memory processes in aging and AD, we performed qRT-PCR analyses. For AD, the mice used were 7-month old 3xTg transgenic mice that demonstrated impaired working memory performance compared with nontransgenic littermates (245). Analyses were performed in three different brain regions including the cortex, hippocampus (CA1-CA3 fields), and dentate gyrus. In order to evaluate age-related cognitive decline, 3-month and 24-month old Fisher 344 rats were compared. Analyses were performed in the cortex and hippocampus of these rats.

Several plasticity-related genes could become disrupted during aging and AD including genes involved in cytoskeletal integrity, microtubule-dependent transport, synaptic functions, and neurotransmission. This could contribute to alterations in neuronal structure that are associated with age-related cognitive decline and AD. For this reason, fourteen different genes involved in the various processes of synaptic plasticity were chosen for investigation (Arc, AGO2, BDNF, αCaMKII, CPEB, CREB1, FMRP, MAP1B, MAP2, NMDAR1, Pkcz, Stau1, Stau2, and Trkβ).

The expression of the mRNAs studied was normalized using three different reference genes (Aldolase C, Ywhaz, and GAPDH) in AD for improved data normalization. On the other hand, only aldolase C was used for normalization in young and aged rats. Initially, the expression level of these genes and various other potential candidate reference genes including, β-actin, CycA, synaptophysin, and calnexin were tested for their stable expression in the brains of aged rats and 3xTg mice compared to young rats and WT mice, respectively. The three control genes chosen were the ones with the most stable expression across individuals and with relevant supporting literature for their use as reference genes in aging and AD.

Aldolase C is a glycolytic enzyme expressed at high levels in the adult brain (247) with a predominant expression in neuronal cells (248,249) as is the case for many of the genes under investigation such as Arc (250), BDNF (251) and its receptor Trkβ (252), FMRP (253), and αCaMKII (254). The expression of aldolase C was found to be stable in aged rats compared to young rats (2). Additionally, it is a neuronal gene that contains a

housekeeping-like promoter (255), and it has not been reported to participate in synaptic plasticity and/or in the progression of cognitive decline and AD. This helps support the notion that it is a good reference gene for the conditions studied.

As for Ywhaz and GAPDH, they were used in conjunction with aldolase C since they are expressed in both neuronal and non-neuronal cells. Despite the fact that all genes under investigation are expressed in neuronal cells, it is possible that they are equally expressed in non-neuronal cells (ex: MAP1B (256), MAP2 (257), Pkcz (258), Stau1, and Stau2 (82) and may even become overexpressed in glial cells such as astrocytes (ex: CREB (259) under conditions of excitotoxically damaged hippocampi as can be seen in AD (260). In addition, Ywhaz and GAPDH are both housekeeping genes that have been validated by geNorm application as two of the most stable genes in AD (246) thereby, making them suitable to use as reference genes.

3.1 Expression profile of plasticity-related genes in different brain regions of 3xTg mice and WT mice

3.1.1 Hippocampus: CA1-CA3 fields

The expression of plasticity-related genes was first studied in the hippocampus (CA1-CA3 fields) using aldolase C as a reference gene. Results from the qRT-PCR analysis revealed a significant increase in the expression of four of the genes studied in 3xTg mice compared to WT mice. These genes were BDNF (p = 0.0104), CREB1 (p = 0.0228), MAP1B (p = 0.0084), and Stau2 (p = 0.0370) (Table III). BDNF and CREB1 both participate predominantly in the induction and maintenance of LTP by promoting synthesis of new NMDA receptors (45,46) and increasing the conductance (43,44) through these receptors and by activating the transcription of plasticity-related genes (58), respectively. Stau2 and MAP1B on the other hand, participate mainly in LTD maintenance. More precisely, Stau2 regulates the transport and possibly the translation of MAP1B (88); a protein critical for the maintenance of cytoskeletal integrity (73). The expression of the

other genes investigated did not change significantly between groups ($p > 0.05$). The normalized differential expression of all genes investigated in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice is depicted in figure 7.

mRNA	Hippocampus	
	t-value	p-value
Arc	-1.9181	0.1951
AGO2	-1.4905	0.2653
BDNF	-5.0001	0.0104
αCamKII	-2.1880	0.0975
CPEB	-2.9104	0.0584
CREB1	-4.6123	0.0228
FMRP	-2.1607	0.0969
MAP1B	-6.9121	0.0084
MAP2	-0.0028	0.9980
NMDAR1	0.0055	0.9959
Pkcz	-0.5477	0.6139
Stau1	-0.0101	0.9927
Stau2	-3.1004	0.0370
Trkβ	0.2818	0.7921

Table III *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to aldolase C. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to aldolase C (Significant difference if $p < 0.05$ highlighted in yellow).

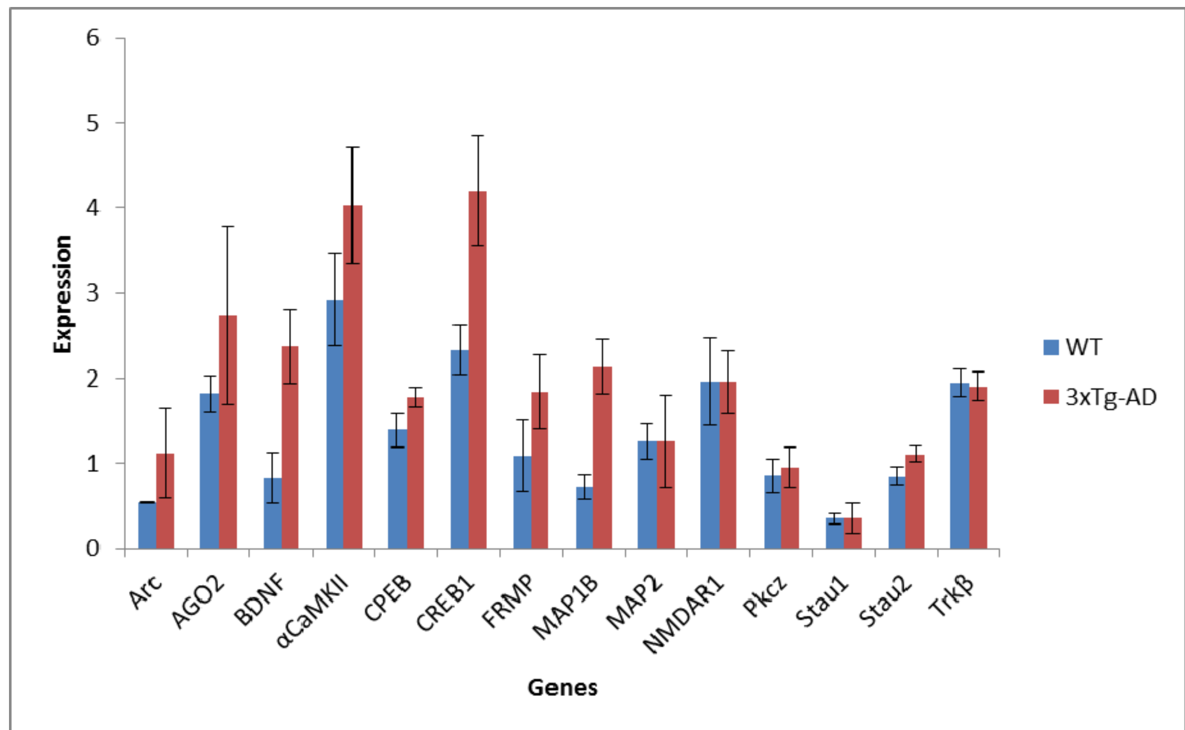


Figure 7 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: Aldolase C). Significant increases were observed for the expression of BDNF, CREB1, MAP1B, and Stau2 mRNAs ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

qRT-PCR experiments were repeated using Ywhaz as a reference gene. Upon analysis of results, a significant increase (Table IV) was observed for the expression of BDNF ($p = 0.0222$) and MAP1B ($p = 0.0171$) mRNAs in the hippocampus of 3xTg mice compared to their WT counterpart but not for the expression of Stau2 and CREB1 mRNAs as observed for the first set of qRT-PCR performed using aldolase C as the reference gene. The expression of the other genes studied did not differ significantly between groups ($p > 0.05$). The normalized expression between 3xTg mice and WT mice for all genes is demonstrated in figure 8.

mRNA	Hippocampus	
	t-value	p-value
Arc	-1.5273	0.2226
AGO2	.0.9751	0.3869
BDNF	-3.7141	0.0222
αCamKII	0.7114	0.5487
CPEB	-1.0244	0.3716
CREB1	-2.9384	0.0927
FMRP	-1.2774	0.3035
MAP1B	-5.0235	0.0171
MAP2	1.0375	0.3926
NMDAR1	2.4112	0.0798
Pkcz	1.2563	0.3235
Stau1	0.3359	0.7580
Stau2	-1.1944	0.3014
Trkβ	1.5948	0.2419

Table IV *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to Ywhaz. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to Ywhaz (Significant difference if $p < 0.05$ highlighted in yellow).

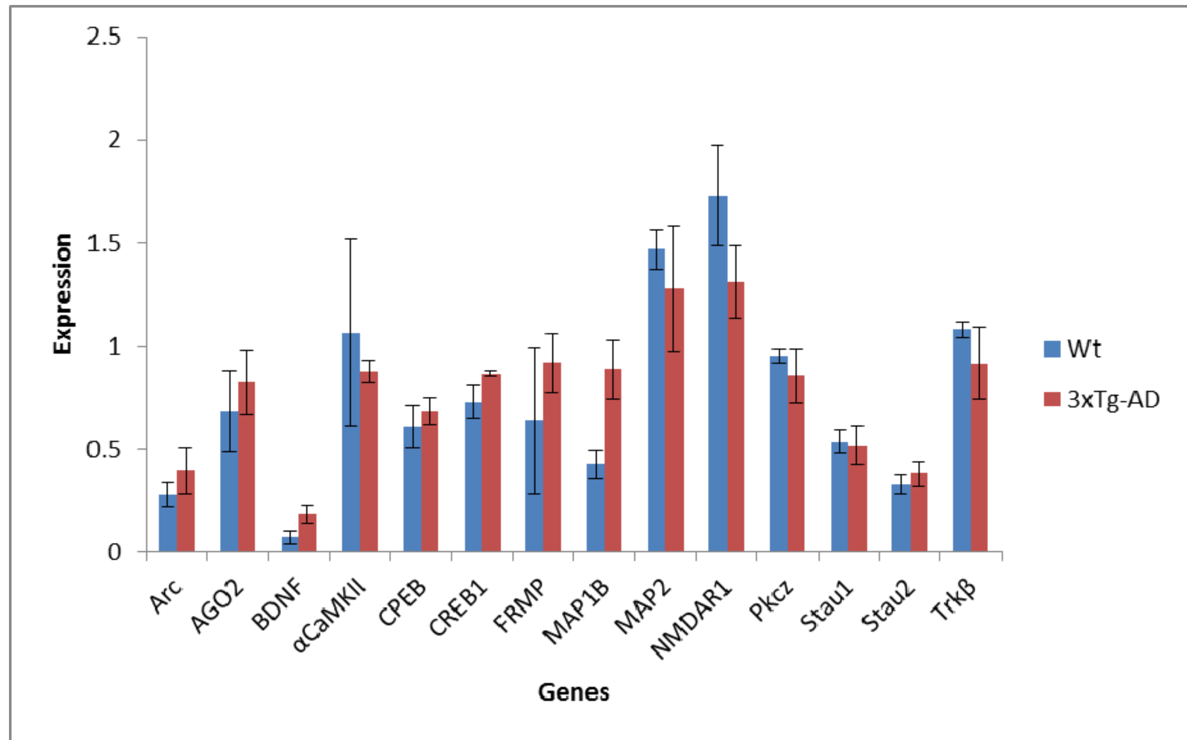


Figure 8 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: Ywhaz). Significant increases were observed for the expression of BDNF and MAP1B mRNAs ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

Finally, the same experiment was performed using a third reference gene; GAPDH. qRT-PCR experiments were performed on 13 selected genes. The expression for MAP1B mRNA increased significantly ($p = 0.0331$) in 3xTG mice compared to WT mice (Table V) as observed for both previous sets of qRT-PCR experiments using aldolase C and Ywhaz as reference genes. The expression of Stau2 mRNA also increased significantly ($p = 0.0127$) similarly to the first experiment using aldolase C as a reference gene. Lastly, AGO2 mRNA expression was significantly increased ($p = 0.0475$) in 3xTg mice compared to WT mice. AGO2 protein has a fundamental role in RNA silencing processes (261) which is important in regulating the expression of genes involved in synaptic plasticity for memory and learning (134-137). The expression of the other plasticity-related genes tested in the

hippocampus did not change significantly between the groups being compared ($p > 0.05$) (figure 9). However, it must be noted that BDNF mRNA expression increased considerably, similar to previous experiments using aldolase C and Ywhaz as reference genes. In this case, the *p*-value for BDNF was calculated at $p = 0.0559$ which is very close to the cut-off value of $p < 0.05$ for a significant difference between groups.

mRNA	Hippocampus	
	t-value	p-value
Arc	-1.6289	0.2263
AGO2	-3.6075	0.0475
BDNF	-2.6879	0.0559
αCamKII	-1.2271	0.2895
CPEB	-1.8037	0.1524
FMRP	-0.8197	0.4619
MAP1B	-4.0154	0.0331
MAP2	1.0474	0.3873
NMDAR1	1.3829	0.2926
Pkcz	0.6849	0.5333
Stau1	-1.7055	0.2082
Stau2	-4.3220	0.0127
Trkβ	1.9484	0.1298

Table V *p*-value: Statistical significance in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to GAPDH. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice relative to GAPDH (Significant difference if $p < 0.05$ highlighted in yellow).

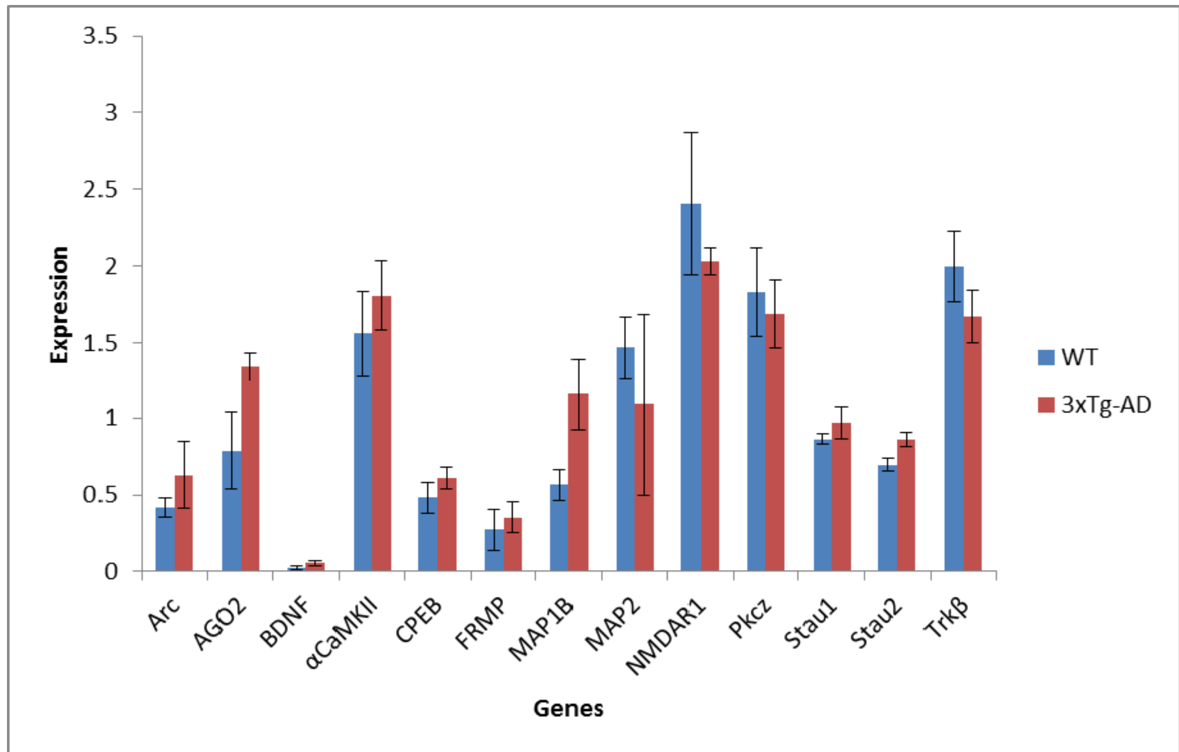


Figure 9 Normalized expression level of mRNAs in the hippocampus (CA1-CA3 fields) of 3xTg and WT mice (reference gene: GAPDH). Significant increases were observed for the expressions of AGO2, MAP1B, and Stau2 mRNAs ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

3.1.2 Hippocampus: Dentate gyrus

Results from the qRT-PCR experiments using aldolase C as a reference gene revealed a significant increase in the expression of MAP1B mRNA ($p = 0.0313$) in the dentate gyrus of 3xTg mice compared to WT mice (Table VI) as observed in the hippocampus (CA1-CA3 fields) for all reference genes used. However, the expression of the other genes studied did not display a significant change between groups ($p > 0.05$) (figure 10).

mRNA	Dentate gyrus	
	t-value	p-value
Arc	-1.0451	0.3947
AGO2	0.3113	0.7759
BDNF	1.0170	0.3988
αCamKII	1.2120	0.3282
CPEB	2.5026	0.0876
CREB1	0.1668	0.8785
FMRP	1.0284	0.3660
MAP1B	-3.7822	0.0313
MAP2	0.8221	0.4621
NMDAR1	0.5964	0.5987
Pkcz	1.2553	0.3161
Stau1	1.2338	0.3226
Stau2	-0.2493	0.8190
Trkβ	-0.5090	0.6559

Table VI *p*-value: Statistical significance in the dentate gyrus of 3xTg and WT mice relative to aldolase C. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the dentate gyrus of 3xTg and WT mice relative to aldolase C (Significant difference if $p < 0.05$ highlighted in yellow).

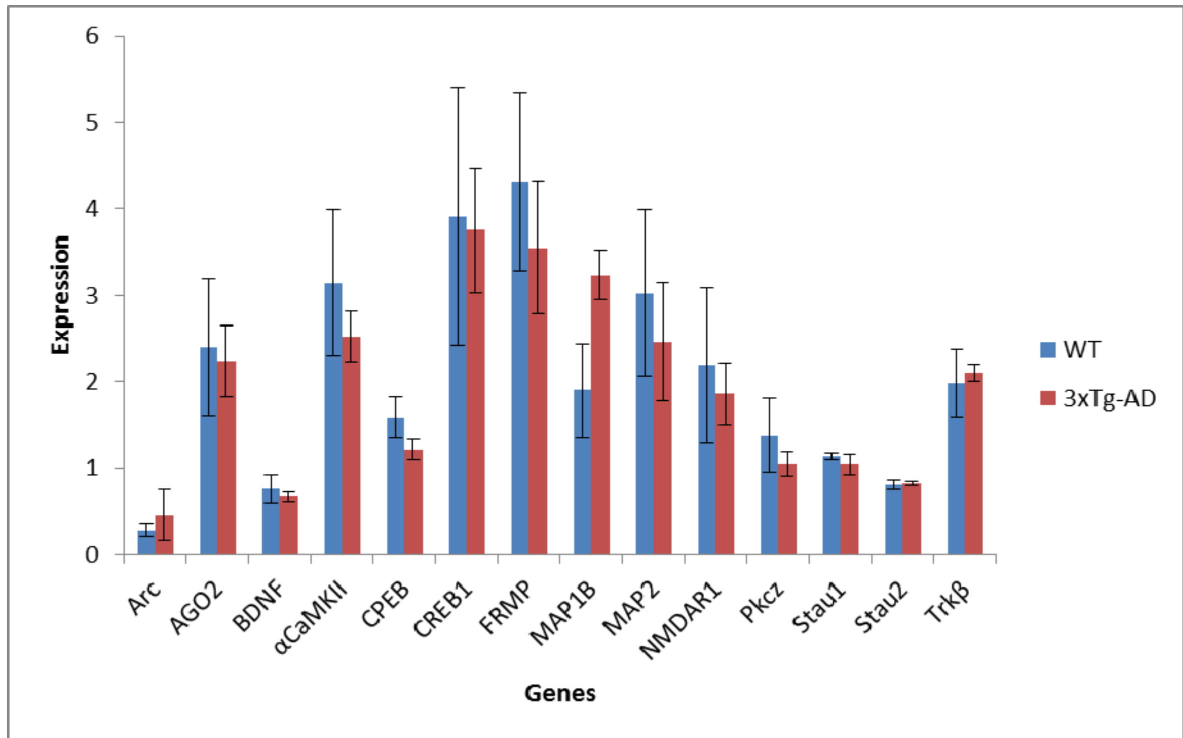


Figure 10 Normalized expression level of mRNAs in the dentate gyrus of 3xTg and WT mice (reference gene: Aldolase C). A significant increase was observed for the expression of MAP1B mRNA ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

In summary, the expression of the genes investigated varied across the sub-regions of the hippocampus with the exception of MAP1B. MAP1B mRNA expression increased significantly in the hippocampus (CA1-CA3 fields) and the dentate gyrus of 3xTg mice compared to WT mice, for all reference genes used. As for Stau2 and BDNF, their mRNA expressions increased significantly in the CA1-CA3 fields of the hippocampus of 3xTg mice compared to WT mice for two out of three reference genes used. Lastly, AGO2 and CREB1 mRNA expression increased significantly in the hippocampus (CA1-CA3 fields) of 3xTg mice compared to WT mice for only one of the reference genes used. This demonstrates how different sub-regions of the same tissue can express different genes in

order to behave as separate units and to generate a specific response upon a synaptic stimulation (239,240).

3.1.3 Cortex

To determine whether the expression of genes is affected to a lesser extent in the cortex compared to the hippocampus at the onset of AD and if different genes become dysregulated, we performed qRT-PCR experiments in the cortex of 3xTg and WT mice. The results normalized using aldolase C demonstrated a significant increase in the expression of MAP1B mRNA ($p = 0.0031$) in 3xTg mice compared to WT mice (Table VII), similar to observations made for the hippocampus (CA1-CA3 fields) and dentate gyrus. On the other hand, a significant decrease was observed for the expression of αCaMKII mRNA ($p = 0.0147$) in the cortex of 3xTg mice compared to WT mice. CaMKII protein becomes activated upon synaptic stimulation and participates in increasing the conductance of AMPAR to facilitate a subsequent synaptic transmission for enhanced LTP (29,37-39). The other genes investigated did not exhibit significant changes in their expression levels ($p > 0.05$) between the groups studied (Figure 11).

mRNA	Cortex	
	t-value	p-value
Arc	-2.129	0.1328
AGO2	0.0330	0.9759
BDNF	-1.4745	0.2148
αCamKII	5.2226	0.0147
CPEB	0.9796	0.3868
CREB1	-0.3831	0.7363
FMRP	-2.0559	0.1092
MAP1B	-9.1744	0.0031
MAP2	0.6320	0.5628
NMDAR1	0.5265	0.6440
Pkcz	0.1733	0.8730
Stau1	-3.3046	0.0696
Stau2	-0.0411	0.9701
Trkβ	-2.4305	0.0719

Table VII *p*-value: Statistical significance in the cortex of 3xTg and WT mice relative to aldolase C. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the cortex of 3xTg and WT mice relative to aldolase C (Significant difference if $p < 0.05$ highlighted in yellow).

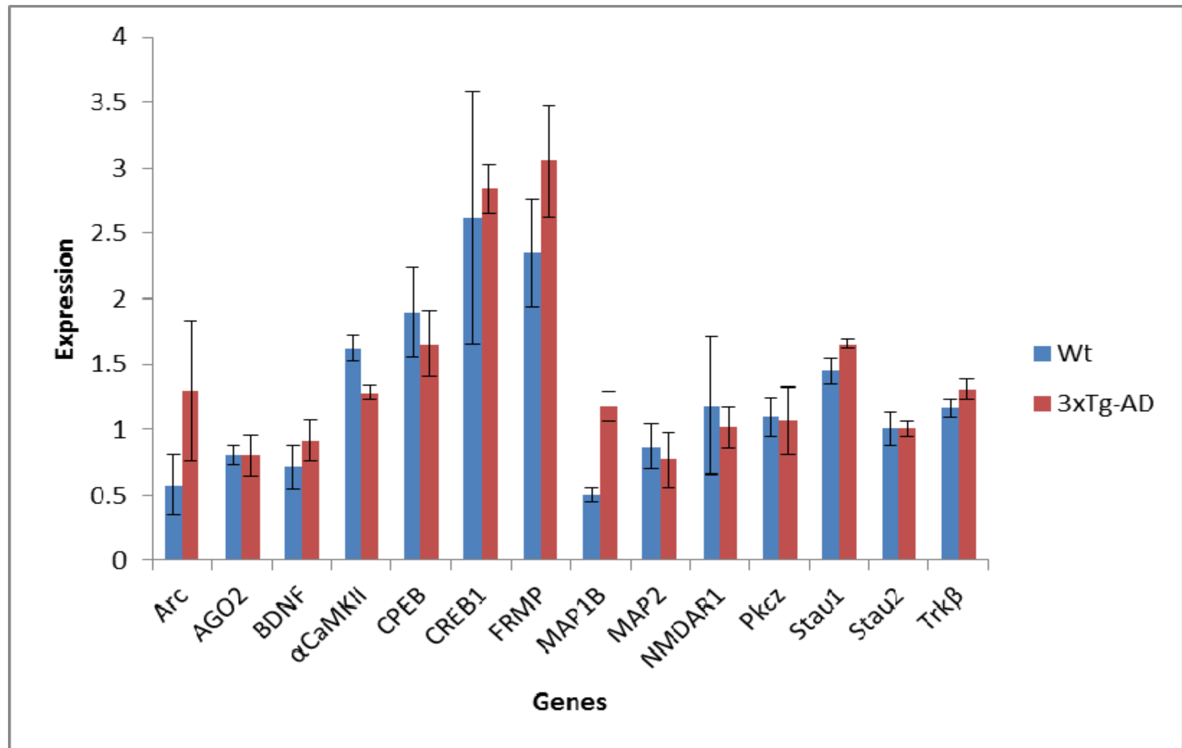


Figure 11 Normalized expression level of mRNAs in the cortex of 3xTg and WT mice (reference gene: Aldolase C). A significant increase was observed for the expression of MAP1B mRNA ($p < 0.05$). A significant decrease was observed for the expression of αCaMKII mRNA. No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

qRT-PCR experiments were repeated in the cortex of 3xTg and WT mice using Ywhaz gene as a reference. In this case, the expression of MAP1B mRNA increased significantly ($p = 0.0008$) in the cortex of 3xTg mice compared to WT mice (Table VIII) as observed when aldolase C was used as the reference gene. However, results obtained for the expression of αCaMKII mRNA using aldolase C were not reproduced when using Ywhaz for normalization. The expression of αCaMKII mRNA and of the other mRNAs under investigation remained relatively stable between groups ($p > 0.05$) (Figure 12).

mRNA	Cortex	
	t-value	p-value
Arc	-2.1070	0.1342
AGO2	0.1283	0.9052
BDNF	-2.6705	0.1083
αCamKII	1.7411	0.1762
CPEB	0.1351	0.8992
CREB1	1.3911	0.2413
FMRP	-0.6429	0.5726
MAP1B	-9.1873	0.0008
MAP2	0.9097	0.4145
NMDAR1	0.7667	0.5158
Pkcz	0.5612	0.6096
Stau1	-1.4043	0.2334
Stau2	0.4978	0.6551
Trkβ	-0.7969	0.4732

Table VIII *p*-value: Statistical significance in the cortex of 3xTg and WT mice relative to Ywhaz. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the cortex of 3xTg and WT mice relative to Ywhaz (Significant difference if $p < 0.05$ highlighted in yellow).

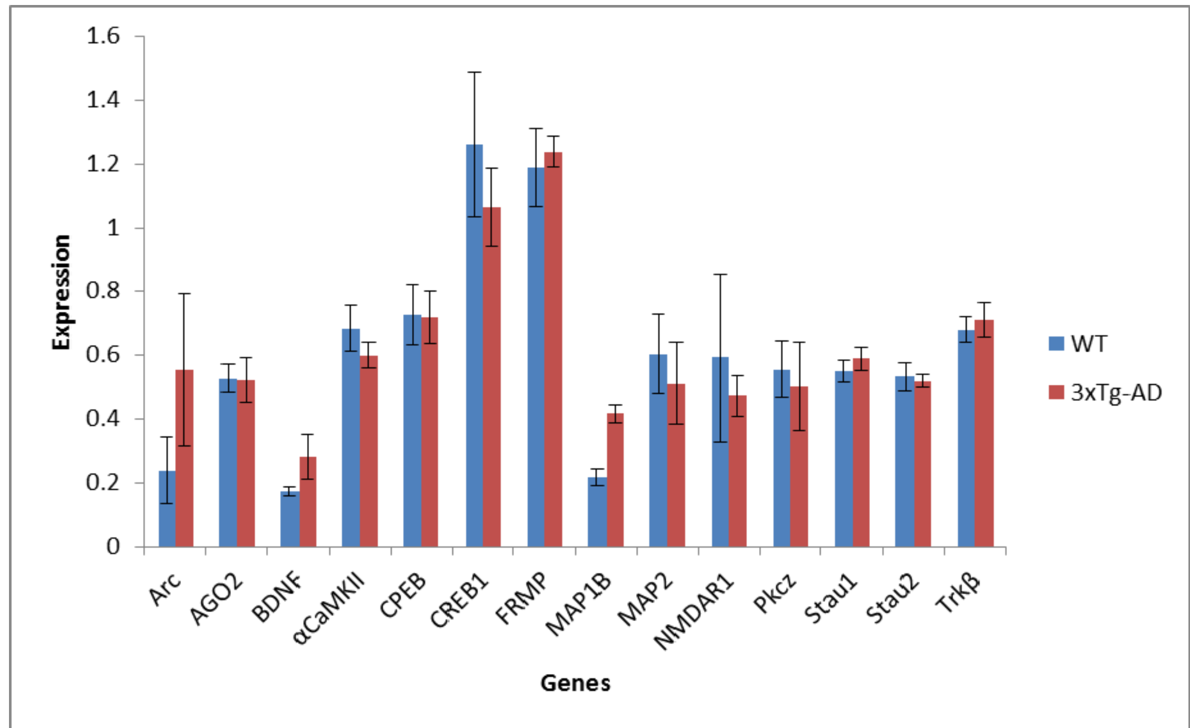


Figure 12 Normalized expression level of mRNAs in the cortex of 3xTg and WT mice (reference gene: Ywhaz). A significant increase was observed for the expression of MAP1B mRNA ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

Therefore, as observed for the dentate gyrus, only MAP1B mRNA expression increased significantly in the cortex of 3xTg mice compared to WT mice for the reference genes used. This helps demonstrate the selective progression of AD whereby the hippocampus (CA1-CA3 fields) is most vulnerable at the onset of the disease followed by the cortex (203).

3.2 Expression profile of plasticity-related genes in the cortex and hippocampus of aged 24-month old rat and young 3-month old Fisher 344 rats

3.2.1 Hippocampus

To establish an age-related difference in expression levels of the plasticity-related mRNAs in the hippocampus of 24-month old rats compared to 3-month old rats, qRT-PCR experiments were performed using aldolase C as a reference gene. No significant differences ($p > 0.05$) were observed in the expression level of the mRNAs studied between the two groups (Table IX). The expression of all mRNAs is depicted in figure 13 for young rats compared to aged rats.

mRNA	Hippocampus	
	t-value	p-value
Arc	0.4165	0.7034
AGO2	1.2178	0.2696
αCamKII	0.3954	0.7135
CPEB	-0.0507	0.9612
CREB	0.1356	0.8968
FMRP	0.3019	0.7736
MAP1B	-0.0757	0.9438
MAP2	-0.5570	0.6058
NMDAR1	0.3084	0.7761
Pkcz	-0.0130	0.9896
Stau1	-0.2066	0.8464
Stau2	1.4302	0.2087
Trkβ	0.6393	0.5467

Table IX *p*-value: Statistical significance in the hippocampus of young and aged Fisher 344 rats relative to aldolase C. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the hippocampus of young and aged Fischer 344 rats relative to aldolase C (Significant difference if $p < 0.05$ highlighted in yellow).

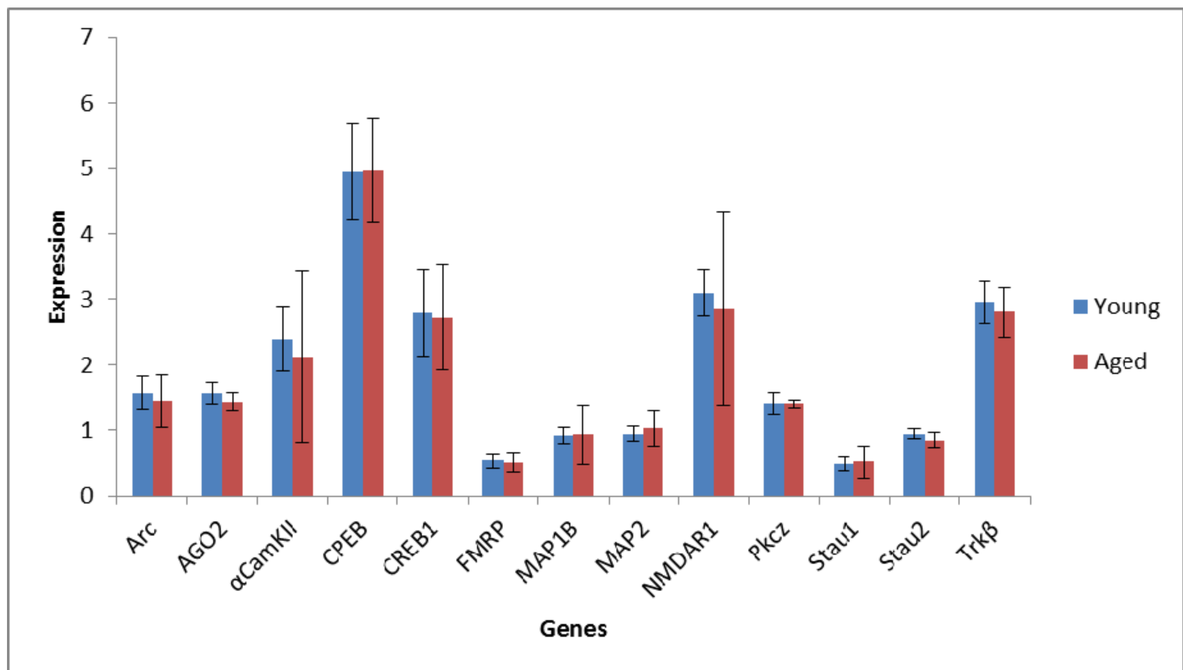


Figure 13 Normalized expression of mRNAs in the hippocampus of young and aged rats (reference gene: Aldolase C). No significant differences were found in the expression of the genes under investigation ($p > 0.05$).

3.2.2 Cortex

The expression of the chosen plasticity-related mRNAs was also tested in the cortex of the young and aged rats using aldolase C as a reference gene. Results from the qRT-PCR experiments revealed a significant increase in CPEB mRNA in aged rats compared to the young rats (Table X). The other genes investigated did not exhibit a significant difference in expression between groups ($p > 0.05$) (Figure 14).

mRNA	Cortex	
	t-value	p-value
Arc	-1.6260	0.1959
AGO2	-0.6602	0.5400
αCaMKII	-0.2073	0.8435
CPEB	-4.7760	0.0050
CREB	-0.2578	0.8071
FMRP	-1.4781	0.1929
MAP1B	-0.6931	0.5170
MAP2	0.0871	0.9337
NMDAR1	-0.450	0.6690
Pkcz	-0.9099	0.4169
Stau1	-0.2693	0.8003
Stau2	-0.3510	0.7384
Trkβ	-0.2063	0.8440

Table X *p*-value: Statistical significance in the cortex of young and aged Fisher 344 rats relative to aldolase C. *p*-value obtained from Welch Two Sample t-test to determine the significance of the difference between the expression levels of plasticity-related mRNAs in the hippocampus of young and aged Fischer 344 rats relative to aldolase C (Significant difference if $p < 0.05$ highlighted in yellow).

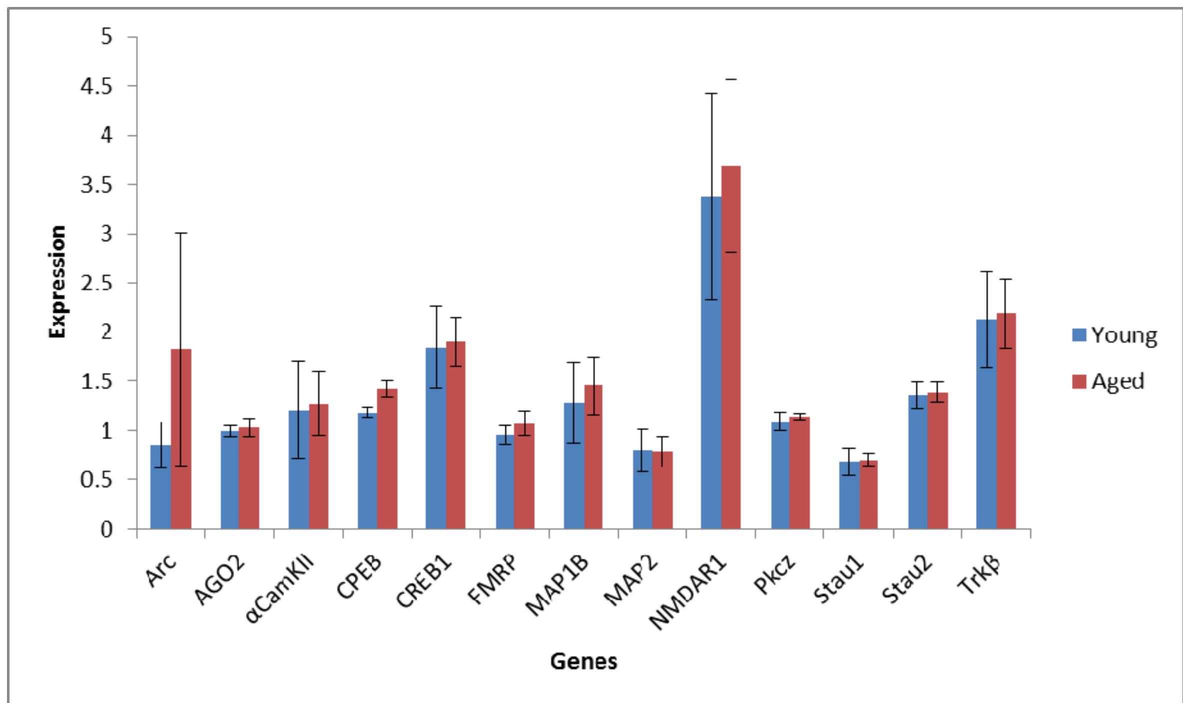


Figure 14 Normalized expression of mRNAs in the cortex of young and aged rats (reference gene: Aldolase C). A significant increase was found in the expression of CPEB mRNA ($p < 0.05$). No significant differences were found in the expression of the other genes under investigation ($p > 0.05$).

It has been established that the hippocampus is most vulnerable during aging (3). However, a significant increase in CPEB expression was only observed in the cortex of aged rats compared to young rats. This might represent another example of the difference in gene expression across brain tissues.

4 Discussion

Age-related cognitive decline of varying severity is common amongst an otherwise healthy aging population but its causes remain to be determined. It has been suggested that alterations in gene expression in the brain might underlie synaptic dysfunction that occurs during normal and pathological aging (262,263). In this study we determined the expression profile of plasticity-related mRNAs in aging and in Alzheimer's disease.

4.1 Alzheimer's disease

AD is characterized by amyloid plaques and neurofibrillary tangles that promote the loss of neurons in areas of the brain involved in learning and memory. However, cognitive and memory impairment precedes neurodegeneration and may even occur before excessive Aβ deposition (214). Under normal conditions, physiological levels of Aβ are essential for synaptic plasticity and memory consolidation (264) whereas, under pathological conditions, toxic soluble oligomeric aggregates of Abeta accumulate and initiate synaptic dysfunction (265). The current hypothesis suggests that cognitive decline and synapse loss in AD correlates with the levels of soluble Abeta rather than the number of amyloid plaques. This new line of thinking emerged from observations that amyloid plaques also occur in individuals with no cognitive impairment (265,266). Furthermore, senile plaque formation is now believed to occur in order to protect neurons from the toxicity of diffusible Abeta oligomers by sequestering them into deposits (267).

Accumulation of soluble Aβ disrupts the molecular pathways that lead to LTP and/or LTD. In fact, it has been demonstrated that Aβ oligomers inhibit LTP and induce LTD by disturbing molecular events and thus, causing gene expression changes in the brain (217). However, it remains to be elucidated which plasticity related genes become dysregulated in the presence of Alzheimer's disease, especially at the onset of the disease. For that reason, we investigated the expression levels of various plasticity related mRNAs in the brain of 3xTg mice (C57BL6/129SVJ) compared to wild type mice at an early stage of Alzheimer's disease when cognitive deficits are apparent especially in the hippocampus but without excessive neuron death (268).

4.1.1 LTD and plasticity-related mRNAs

The results of the qRT-PCR study for Alzheimer's disease revealed significant changes in the expression of specific plasticity-related mRNAs mainly involved in LTD. We observed a significant increase in MAP1B mRNA expression in Alzheimer mice compared to their WT counterpart in all brain regions including the cortex, hippocampus and dentate gyrus relative to all the control genes used. This is in agreement with a study that demonstrated the upregulation of MAP1B mRNA in cultured cortical neurons upon addition of Aβ1-42 peptide (269). This study also provided insight into the overexpression of MAP1B protein in cultured cortical neurons. MAP1B is a minor component of NFT and senile plaque neuritis in AD. Accordingly, the author speculated that aberrant neuritic sprouting processes are induced by the deposition of Aβ or plaque-associated molecules, such as MAP1B, at an early stage of AD before neurodegeneration. To test this hypothesis, an *in vitro* transfection experiment of the MAP1B gene was performed and successively revealed that up-regulation of MAP1B did in fact accelerate disease progression by promoting neurite outgrowth and subsequently, neuronal death.

Conversely, a different study observed diminished expression of MAP1B mRNA in the temporal lobe and occipital lobe of AD brain (270). In the same experiment, levels of MAP2 mRNA were found to be up-regulated in the AD occipital lobe. Both, MAP1B and MAP2 become sequestered by hyperphosphorylated tau which leads to disassembly of microtubules in AD. This results in the breakdown of the neuronal microtubule network. Based on their findings, the authors speculated that the increase in MAP2 served to compensate for the decreased expression of MAP1B to help protect the occipital lobe against cell death during early stages of neurodegeneration in AD. These results are contrary to our observations, where no significant difference was observed in the mRNA expression of MAP2 in AD compared to WT mice. The discrepancy between results for MAP1B and MAP2 mRNA expression could be explained by the fact that we used mice with early stage AD for our experiments, whereas they used brain samples from one human patient with moderate disease stage. It is therefore possible that MAP1B mRNA increases in the initial stages of AD and decreases thereafter.

Interestingly, a study demonstrated an Aβ-dependent activation of caspase-3 and calpain which led to proteolysis of MAP1B (271). Another study demonstrated that Aβ1-42 colocalizes with MAP1B and binds to its N-terminal region in SH-SY5Y human neuroblastoma cells (272). This could explain how the microtubule network becomes disrupted in the early stages of AD preceding caspase activation and neurodegeneration. A possible mechanism is that Aβ1-42 initially binds to MAP1B causing its dissociation from microtubules and in this manner disturbs the cytoskeletal network. Meanwhile, caspase-3 and calpain become activated in an Aβ-dependent manner and target the dissociated MAP1B for degradation. This could help explain how MAP1B mRNA initially increases at the onset of AD but becomes diminished as the disease progresses when proteolysis starts dominating synthesis.

It is logical to assume that as a result of an increase in MAP1B mRNA, the expression of associated regulatory RNA binding proteins must increase accordingly. Indeed, the expression of the mRNA coding for Stau2, a protein involved in regulating the transport of specific mRNAs including MAP1B (88), appears to increase significantly in the hippocampus of AD mice compared to WT mice when both aldolase C and GAPDH are used as control genes. An increase in Stau2 mRNA might lead to increased translation of Stau2 protein and in this manner bring about enhanced transport of MAP1B mRNA. Under normal conditions, both, Stau2 and MAP1B proteins mainly participate in LTD maintenance and so their translation is enhanced upon LTD induction (88,109). It has been demonstrated that LTD starts to prevail over LTP in AD due to internalization of AMPAR (219,220) and decreased current through NMDAR (218). The resulting diminished calcium entry into the postsynaptic neuron triggers the induction of LTD rather than LTP (216,217) which only occurs in the presence of high calcium concentration (1). It is therefore not surprising to observe an increase in the expression levels of Stau2 and MAP1B mRNAs in AD since low calcium concentrations activate LTD-related mRNAs that participate in promoting LTD maintenance.

Two other molecules, Arc and FMRP, involved in LTD maintenance (101,102,105) showed a tendency to increase in the cortex and hippocampus of AD mice compared to WT

mice for all control genes used. However, the differences observed were not significant mostly due to expression variations between mice belonging to the same group. Further studies should be conducted using a greater sample size to determine if a significant increase truly exists for these mRNAs in AD mice compared to WT mice. In our study, the tendency of Arc mRNA to increase is in disagreement with studies that demonstrated a decrease in Arc mRNA levels in the neocortex and hippocampus of aged APP transgenic mice (273) and in the cortex and hippocampus of aged APP + PS1 transgenic mice (274). Both these studies performed RT-qPCR experiments in aged transgenic mice between 25 to 30 months old and 17 to 18 months old, respectively, whereas our experiments were performed in 7 month old transgenic mice. It is therefore possible that an initial increase in Arc mRNA occurs in AD before the disease progresses and neurodegeneration worsens at which point the expression levels of Arc mRNA might drop considerably.

As for FMRP, no studies have been published on its expression level in AD. Nevertheless, it has been demonstrated that FMRP supresses the translation of APP mRNA (112). Therefore, in the absence of FMRP, APP translation is increased and thus, impacts the levels of processed Aβ. Enhanced processing of APP via decreased translational repression by FMRP is a plausible mechanism by which increased Aβ deposition occurs in AD. However, our results suggest that FMRP mRNA increases in AD. The increase in FMRP mRNA might represent a compensatory mechanism at the onset of AD to increase FMRP translation and thereby increase the translational repression of APP. This would help prevent excessive processing of APP. This could also be true for MAP1B. Under normal conditions, FMRP binds to and inhibits MAP1B mRNA (275,276). Therefore an increase in FMRP mRNA might correlate with the increase in MAP1B mRNA that we observed at the onset of AD. Similar to the case of APP, increased FMRP mRNA possibly serves as a compensatory mechanism to help inhibit overexpression of MAP1B mRNA. However, it is also possible that despite an increase in its mRNA expression level, the protein expression level of FMRP protein is decreased or its function is altered. Interestingly, altered FMRP expression and/or function would cause the compensatory mechanisms to fail and consequently, AD would continue to progress. Indeed, an example

of altered FMRP function comes from a study in which the absence of AGO2 prevents FMRP repression of APP translation (112). AGO2 colocalizes with FMRP (277) and it is an argonaute protein that is a component of the miRISC involved in the processing and regulation of miRNAs (134,135).

In fact, FMRP also associates with endogenous miRNAs for the precise translational repression of specific mRNAs (112). If the association between these molecules is altered, the translational regulation via FMRP becomes dysfunctional. For example, we observed that the expression of AGO2 increased significantly in the hippocampus of AD mice when GAPDH was used as the control gene. Such an alteration in mRNA expression could have an impact on FMRP function itself by altering associations between molecules. It is also possible that the miRNAs with which FMRP associates regulate the translation of FMRP mRNA itself. In this case, an imbalance in miRNA expression could inhibit translation of FMRP thereby, explaining the increased expression of FMRP mRNA observed in our experiments and the expected decrease in its protein level.

Altogether, these observations demonstrate the detrimental effects of Aβ on the expression of specific plasticity-related mRNAs mainly involved in LTD. These effects can also be observed for mRNAs mainly involved in LTP. This further contributes to the disruption of synaptic plasticity and as a consequence, to the progression of AD.

4.1.2 LTP and plasticity-related mRNAs

For LTP, functional NMDA and AMPA receptors are crucial in propagating a synaptic transmission to activate the transcription of LTP-related mRNAs. However, increasing evidence suggests that Aβ synaptic dysfunction is attributed to the synaptic removal of AMPA receptors (220,278,279). In a study led by Zhenglin Gu, CaMKII, a molecule essential for trafficking of AMPAR at synapses (280,281), was found to be reduced at synapses by Aβ whereas it was found to be increased in the cytosol of cortical neurons from APP transgenic mice (282). The decrease of CaMKII at synapses led to the

loss of synaptic AMPA receptors. The authors speculated that Aβ might disturb the synaptic distribution of CaMKII by altering intracellular calcium signalling and/or actin cytoskeleton dynamics. These events are required for the synaptic translocation of CaMKII. In our study, we wanted to test the possibility that Aβ might interfere with the transcription of αCaMKII which could also lead to decreased synaptic pools of CaMKII. However, we failed to observe a significant difference in the expression level αCaMKII in most brain tissues and for all the control genes used with the exception of one. αCaMKII was significantly reduced in the cortex of Alzheimer mice compared to WT mice when aldolase C was used as the control gene. In light of these results, it might be more likely that defects occur in the transport and/or translation of the αCaMKII mRNA transcript which would be consistent with the previous findings that Aβ alters CaMKII compartmentalization rather than its transcription.

In addition, the same study (282) failed to observe a change in the synaptic expression of NMDAR. Consistent with these results, we found no significant change in the mRNA expression level of NMDAR1 in Alzheimer mice compared to WT mice for all conditions. Normal NMDAR mRNA expression is crucial in ensuring optimal translation of synaptic NMDAR. This information further suggests that it is predominantly the activity of NMDAR that is altered at the onset of Alzheimer's disease (218) rather than its transcription and/or translation. However, it is also possible that the loss of synaptic AMPAR precedes that of NMDAR. Changes in the transcription and/or translation of NMDAR might occur at a later stage of the disease. In fact, altered calcium homeostasis due to reduced NMDAR activity eventually causes the activation of calcineurin which promotes the internalization of NMDA receptors (219). Removal of NMDA receptors from the synaptic membrane also occurs via oligomeric Aβ-dependent dephosphorylation of NR2B subunits (221). These events likely start occurring at a more advanced stage of Alzheimer's disease which would explain why no differences in mRNA expression levels of NMDAR are observed in the initial stages of AD. This could also be true for the expression of Stau1, Pkcz, and CPEB mRNAs for which no significant differences were observed in 3xTg mice compared to WT mice. It would be interesting to repeat these

experiments at various stages of the disease to establish when and how expression alterations occur.

Dysfunction in NMDAR activity causes decreased current through this receptor and results in a disruption in the molecular chain of events that occur downstream of synaptic stimulation via NMDAR such as CREB activation via MAPK (222). For example, it has been well established that the activity of CREB is reduced in Alzheimer's disease. It has also been suggested that CREB mRNA and protein expression levels decrease in the presence of Aβ (223). These findings are inconsistent with our results which suggest a significant increase in CREB mRNA expression in the hippocampus of Alzheimer mice compared to WT mice when aldolase C is used as the control gene. Although not significant, this tendency is also observed when Ywhaz is used as the control gene. The discrepancy between results obtained could be explained by the fact that we used hippocampal extracts from transgenic Alzheimer mice whereas Pugazhenthi S *et al*. used cultured rat hippocampal neurons to test the effects of Aβ on CREB mRNA expression. Although an increase in CREB mRNA expression is unexpected, it could represent a compensatory mechanism at the onset of Alzheimer's disease to increase the protein level of CREB and subsequently, its activity in order to restore normal LTP.

Normal CREB activity via phosphorylation is crucial in regulating the transcription of CREB specific plasticity-related genes such as BDNF (283). It is logical to assume that the expression of these genes correlates with the expression and activity of CREB. Indeed, in our study, we observed an increase in the CREB target gene BDNF in the hippocampus of Alzheimer mice compared to WT mice when either Ywhaz or aldolase C are used as the control gene. This is consistent with the increase we previously observed for CREB1 mRNA in the early stages of AD. Similar observations were made in a study in which differentiated SH-SY5Y cells treated with Abeta caused up-regulation of BDNF as well as its receptor Trkβ (284). On the other hand, various other studies report a decrease in the mRNA (285-288) and protein (289-291) levels of BDNF in the hippocampus and neocortex of post-mortem samples from AD patients. This observation was also observed for the receptor Trkβ (289,292). These conflicting results could be explained by the varying

severity of Alzheimer's disease in the different studies. The studies demonstrating a decrease in BDNF mRNA and protein expression levels represent late-stage Alzheimer's disease when the activity of CREB has been shown to be greatly reduced via dephosphorylation (223) whereas the one suggesting an increase demonstrate the direct effects of Abeta addition to cultured neurons. The latter study might better reflect our results since our experiments were performed at an early stage of AD.

Alternatively, it has been shown that the maintenance of normal BDNF mRNA levels is mainly mediated by NMDA receptors whereas excessive levels of BDNF are mediated by non-NMDA receptors (293,294). Since it has been demonstrated that NMDAR activity is reduced in AD, it is possible that the activity of non-NMDA receptors is increased to make up for diminished NMDAR activity. Regulation via non-NMDAR pathways could increase BDNF transcription via alternate mechanisms that do not depend on CREB for gene activation and thereby, explain our results that suggest an increase in BDNF mRNA expression in AD. Correspondingly, studies indicate that BDNF increases the mRNA and protein levels of NMDA receptor subunits as well as their delivery to the plasma membrane in cultured hippocampal neurons in order to up-regulate receptor activity (46). Therefore, an attempt to increase BDNF transcription through non-NMDAR could represent a compensatory mechanism to help restore NMDAR activity in the early stages of AD.

The aforementioned molecules predominantly participate in LTP. The increased mRNA expression of CREB and BDNF could represent a failed compensatory mechanism in the early stages of AD to maintain LTP. As the disease progresses, it is possible that their expression levels become severely diminished as demonstrated by other studies in which experiments were conducted in brain samples with late stage AD. The altered expression levels of these molecules might contribute to the diminished prevalence of LTP seen in AD.

For plasticity-related mRNAs principally involved in both LTP and LTD, significant changes in the expression level of specific mRNAs (MAP1B, Stau2, αCaMKII, BDNF, CREB1, and AGO2) under investigation were observed especially in the

hippocampus (CA1-CA3 fields) of 3xTg mice compared to WT mice. These alterations might contribute to the imbalance between LTP and LTD in AD. On the other hand, some of the mRNAs studied showed no significant change in their expression level (Pkcz, CPEB, NMDAR1, MAP2, FMRP, and Stau1) between groups. This demonstrates that the expression changes we observed were specific to a subset of plasticity-related mRNAs and to specific brain regions. If all genes had been impaired and in an equal manner for all brain regions, it could have been attributed to a general loss of neurons. Moreover, the greater number of genes affected in the hippocampus than in the cortex of 3xTg mice is consistent with the progression of AD whereby, in the early stages of the disease, the hippocampus (especially the CA1 and CA3 sub-regions) is most vulnerable (244).

However, the significant changes in mRNA expression for some of the genes studied (ex: CREB1, AGO2) were observed for only one reference gene. Also, for some of the other plasticity-related genes investigated, they exhibited a tendency for altered expression levels (ex: Arc), but a significant difference could not be established. This does not rule out their potential roles in the progression of AD. It is possible that a greater sample size is required to observe a significant difference in the expression level of these mRNAs as well as to reduce the variation between individuals of the same group.
In addition, more accurate results could be obtained by performing experiments in an activated system rather than in unperturbed *in vitro* preparations. Yet, there is a possibility that defects occur in protein expression rather than mRNA expression or in the localization and/or function of mRNAs and proteins. Nonetheless, it is unmistakable that alterations in the expression of specific plasticity-related mRNAs occur at the onset of AD, as observed in our study, and likely contribute to the progression of the disease.

Various possibilities can account for the altered expression levels of the mRNAs discussed in AD. For one, regulation of mRNAs by miRNAs represents a promising avenue for further investigations. miRNAs contribute in governing mRNA turnover and translation rates which are of particular importance for altering the levels of expressed proteins (295). Up to now, several miRNAs have been shown to have altered expression levels in AD and most likely accelerate the progression of the disease. Many of these

miRNAs such as miR-106 and miR-101 may have a direct role in modulating APP expression therefore, potentially participating in excessive amyloid production (224,296,297). Other miRNAs involved in regulating the expression levels of the aforementioned plasticity-related mRNAs in AD pathology have yet to be discovered. miRNAs could represent an important mechanism by which mRNA and consequently, protein expression become altered in AD. This can contribute to the disrupted synaptic function observed in AD.

4.2 Aging

For aging, the results of the qRT-PCR study show that no significant differences were observed for the expression profile of plasticity-related mRNAs in the hippocampus of young rats compared to aged rats. However, various other studies have observed age related changes in the transcription of plasticity-related mRNAs. For example, although Arc mRNA expression in the cortex and hippocampus of young and aged rats was similar for both groups in our experiment, Blalock, E. *et al.* (298) observed a decreased resting Arc expression in the CA1 region of aged rats using microarray analysis. Additionally, Lanahan *et al.* (299) observed changed expression of another IEG during aging. They found that c-fos mRNA expression increases to a greater extent in aged rats compared to young rats upon LTP induction in the hippocampus. Therefore, the possibility that the expression profile of the plasticity-related mRNAs studied changes during aging cannot be ruled out.

Various factors can account for our findings. For one, the cognitive status of the aged rats used in our experiments was not determined. Although aged Fisher 344 rats are a well-known model for examining decreased cognitive decline, there is a possibility that the rats studied had an intact cognitive status. This could explain the lack of differences observed for the expression of the plasticity-related mRNAs under investigation. Assessing the cognitive status of the rats using a memory test such as the Morris water maze prior to

experiments could allow the categorization of rats into memory impaired and memory unimpaired groups for better results. Moreover, the sample size was small making it difficult to draw conclusive data not to mention the large variations in expression between rodents of the same group. Another caveat is that experiments were performed in unperturbed *in vitro* preparations thereby, revealing resting rates of transcription that may not reflect the actual rates occurring when the system is activated. On the other hand, there is a possibility that the transcription of the mRNAs studied remains intact throughout aging and rather, defects occur in the localization of the transcripts and/or in the function and/or localization of the corresponding protein.

5 Conclusion

A greater number of plasticity-related mRNAs exhibit altered expressions in the hippocampus (CA1-CA3 fields) than in the cortex or dentate gyrus of 3xTg mice. This is consistent with the pathology of AD whereby the CA1-CA3 regions of the hippocampus are particularly susceptible at the onset of AD (244). In this region of the brain especially, significant increases occur in the expression levels of MAP1B and Stau2 mRNAs in 3xTg mice compared to WT mice (Table III and table V). Upon LTD induction, MAP1B mRNA is transported to active synapses under the regulation of Stau2 (88). Once released from the translational repression of Stau2, MAP1B locally participates in the internalization of synaptic AMPAR to weaken a subsequent synaptic transmission (109). An increase in the expression of these mRNAs possibly stimulates enhanced translation. Subsequently, the resulting proteins likely participate in excessive internalization of AMPAR and thereby, contribute to the prevalence of LTD over LTP that occurs in AD (216,217). A significant increase in the expression of BDNF, and CREB mRNAs also takes place in the hippocampus (CA1-CA3 fields) of 3xTg mice compared to their WT counterpart (Table III). BDNF and CREB proteins mainly participate in LTP by increasing the synthesis of NMDAR and the conductance of these receptors (46) and by activating the transcription of plasticity-related genes (58), respectively. An increase in the expression of these mRNAs might represent a compensatory mechanism at the onset of AD to restore the balance between LTP and LTD by attempting to enhance LTP. A significant increase was similarly observed for AGO2 mRNA in the hippocampus (CA1-CA3 fields) of 3xTg mice compared to WT mice (Table V). AGO2 participates in the regulation of miRNAs. An increase in its mRNA level might promote enhanced translation of AGO2 and thereby, cause an imbalance in miRNA expression. Consequently, the translational regulation of various plasticity-related mRNAs may become disrupted. Finally, the other plasticity-related mRNAs investigated appear to remain relatively stable between 3xTg and WT mice at the onset of AD in most regions of the brain.

Since very few studies have focussed on studying the expression of genes in the early stages of AD, our experiments have shed light on which plasticity-related mRNAs first exhibit altered expression levels in AD.

As for the expression levels of plasticity-related mRNAs in aged rats compared to young rats, they seem to remain stable between both groups. However, further studies must be conducted in rodents with a pre-established cognitive status (young, aged cognitively impaired, and aged cognitively unimpaired) in order to obtain more conclusive results.

5.1 Perspectives

Various studies have compared the expression of plasticity-related genes between AD and control subjects. However, most of these studies have focussed on moderate to advanced stages of AD when molecular pathways are severely dysregulated and neurodegeneration is prominent in all regions of the brain (270,273,274). This makes it difficult to develop a progressive model for AD and thereby, to develop pharmaceutical interventions aimed at preventing and/or halting the progression of the disease. It would therefore be important to study the expression of various plasticity-related genes at several stages of the disease, especially at the onset of AD, in different brain tissues such as the cortex and sub-regions of the hippocampus to determine which molecular pathways are initially disrupted. This can be achieved using microarrays or qRT-PCR experiments.

Western blots should be performed in parallel in AD and control subjects to determine if there is a discrepancy between protein expressions between groups. This could help determine if dysregulations in AD occur at the transcriptional and/or at the translational level. In the case that disruptions are observed at the translational level, the study of miRNAs is a great approach since miRNAs are known to regulate the translation of mRNAs. Not to mention that several miRNAs display altered expression levels in AD (226-228).

On the other hand, if no changes in gene and protein expressions are observed, FISH and immunohistochemistry experiments should be conducted to determine if defects occur

in the localization of the mRNAs and proteins, respectively. In parallel, the function of the proteins should also be verified by means of proteomics.

The aforementioned studies should also be performed in cognitively impaired, cognitively unimpaired, and young rodents for a large sample size to determine which molecular pathways become disrupted during age-related cognitive decline in the absence of neurodegeneration

Ultimately, better understanding the sequential process through which molecular pathways become dysregulated during age-related cognitive decline and AD and how they become dysregulated, would help provide the necessary tools to create a comprehensive model on the progression of these conditions. This would enable health professionals to develop effective pharmaceutical interventions for the prevention and/or treatment of age-related cognitive decline and AD.

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