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# Cortical cells reveal APP as a new player in the regulation of GABAergic neurotransmission.

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# Summary

Alzheimer's disease (AD) is the most prevalent form of dementia characterized by severe memory deficits. One of the main hallmarks of AD are senile plaques, which contain a core of a small peptide termed amyloid beta peptide (Aβ). Aβ is derived from the proteolytic cleavage of its precursor, the amyloid precursor protein or APP. Besides producing Aβ, APP has been assigned many physiological functions. Amongst these, APP and/or its catabolites were shown to influence excitatory and inhibitory neurotransmissions. Cognition and learning are tightly regulated by a balance between excitatory and inhibitory neurotransmissions. Disruption in this balance leads to memory impairments such as observed in AD. In the adult central nervous system, γ-amino-butyric acid (GABA) is a major inhibitory neurotransmitter. While in the adult brain GABAergic signaling is hyperpolarizing and inhibitory, in the developing brain, GABA acts as a depolarizing and sometimes even excitatory neurotransmitter. GABAergic inhibition is mainly mediated by activation of ionotropic $GABA_A$ receptors that primarily allow the bidirectional flux of $Cl^-$ ions. Therefore, the nature of GABAergic neurotransmission, depolarizing versus hyperpolarizing, is essentially determined by intracellular concentration of $Cl^-$ ($[Cl^-]_i$). In the central nervous system, the two main cation-chloride cotransporters regulating the $[Cl^-]_i$ are $Na^+$-$K^+$-$2Cl^-$ cotransporter 1 (NKCC1) allowing the entrance of $Cl^-$ and $K^+$-$Cl^-$ cotransporter 2 (KCC2), allowing the efflux of chloride.

Here we have investigated the impact of APP expression on NKCC1 and KCC2 expression levels with an emphasis on GABAergic neurotransmission. We observed that overexpression human APP (hAPP) is able to decrease KCC2 expression both *in vitro* and *in vivo*. Furthermore, the decrease in KCC2 expression observed *in vitro* was associated with a less inhibitory and less hyperpolarizing GABA signaling. In addition, our study unveiled an involvement of the juxta-/transmembrane domain of hAPP in this regulation. Expression of KCC2 was also shown to be decreased in the cortex of sporadic AD patients. Our results outline a new important role of APP in KCC2 expression regulation which could influence GABAergic neurotransmission. This information advances our understanding of the physiological function of APP and sheds light on new mechanisms that could contribute to AD pathology.

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# Abbreviations

| | |
|---|---|
| $[Ca^{2+}]_i$ | intracellular $Ca^{2+}$ concentration |
| $[Cl^-]_i$ | intraneuronal $Cl^-$ concentration |
| AD | Alzheimer's disease |
| ADAM10 | a disintegrin and metalloproteinase domain 10 |
| AICD | APP intracellular domain |
| AMPA | alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| Aph-1 | anterior pharynx-defective 1 |
| APLP1 & 2 | APP like proteins 1 and 2 |
| ApoE | apolipoprotein E |
| APP | amyloid precursor protein |
| AQP1 | aquaporin 1 |
| Aβ | β-amyloid peptide |
| BDNF | brain-derived neurotrophic factor |
| CCC | cation chloride cotransporter |
| Cdk5 | cyclin-dependent kinase 5 |
| CNS | central nervous system |
| CTFα | C-terminal α fragment |
| CTFβ | C-terminal β fragment |
| DIV | day(s) *in vitro* |
| DS | Down syndrome |
| EAAT | excitatory amino acid transporter |
| EEG | electroencephalography |
| EGFR | early growth factor receptor |
| Egr4 | early growth response 4 |
| EPSC | excitatory postsynaptic current |
| EPSP | excitatory postsynaptic potential |
| ERK1 & 2 | extracellular signal-regulated kinases 1 and 2 |

| | |
|---|---|
| FAD | familial autosomal dominant Alzheimer's disease |
| FMR1 | fragile X mental retardation 1 |
| GABA | γ-aminobutyric acid |
| GAD | glutamic acid decarboxylase |
| GAT | GABA transporter |
| GSK3β | glycogen synthase kinase 3 β |
| hAPP | human amyloid precursor protein |
| hrGFP | human recombinant green fluorescent protein |
| IPSP | inhibitory postsynaptic potential |
| KAI1 | tetraspanin family gene 1 |
| KCC1, 2, 3, 4 | $K^+$-$Cl^-$ cotransporter 1,2,3,4 |
| KPI | Kunitz-type proteolysis inhibitor |
| LTP | long term potentiation |
| MAP | microtubule-associated protein |
| MCI | Mild Cognitive Impairments |
| MeCP2 | methyl CpG binding protein 2 |
| MEG | magnetoencephalography |
| NCC | $Na^+$-$Cl^-$ cotransporter |
| Neto2 | neuropilin and tolloid like-2 |
| NFT | neurofibrillary tangle |
| NGF | nerve growth factor |
| NICD | notch intracellular domain |
| NKCC1 & 2 | $Na^+$-$K^+$-$2Cl^-$ cotransporter 1 and 2 |
| NMDA | N-methyl-D-aspartate |
| NRSE | neuron-restrictive silencing element |
| NRSF | neuron-restrictive silencing factor |
| PAM | protein associated with Myc |
| Pen-2 | presenilin enhancer 2 |
| PS1 & 2 | presenilin 1 and 2 |

| | |
|---|---|
| PSEN1 | gene encoding presenilin 1 |
| PSEN2 | gene encoding presenilin 2 |
| PV | parvalbumin |
| sAPPα | soluble APP α fragment |
| sAPPβ | soluble APP β fragment |
| SGZ | subgranular zone |
| SREBP | sterol regulatory element-binding protein |
| SVZ | subventricular zone |
| TGN | transgolgi network |
| TMS | transcranial magnetic stimulation |
| TrkB | tropomyosin-related kinase B receptor |
| USF1 & 2 | upstream stimulating factors 1 & 2 |
| VDCC | voltage-dependent $Ca^{2+}$ channel |
| VGAT | vesicular GABA transporter |
| VGLUT | vesicular glutamate transporter |
| VIP | vasoactive intestinal peptide |
| VZ | ventricular zone |

# Introduction

## I. Alzheimer's Disease

### 1.1 General overview

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder mainly affecting elderly characterized by a severe memory loss and cognitive deficits. It is considered that the prevalence of this disease doubles every 5 years after the age of 65. Up to 38% of individuals over 85 years and older suffer from AD (Hebert et al., 2013; Querfurth and LaFerla, 2010). Due to steadily increasing life expectancy worldwide, the current number of AD cases, estimated at 46.8 million in 2015 is supposed to triplicate by 2040 reaching 131.5 million of cases (Alzheimer's Disease International, 2015). This dramatic increase is supposed to result from a rise in cases in low and middle-income countries rather than in their high-income counterparts. Cognitive impairment is a major contributor to disability and dependence in the older population. While aged individuals can remain reasonably independent even with marked physical impediments, a cognitive decline quickly compromises their ability to carry out complex but essential daily life tasks. Therefore, support from a caregiver is often required and represents an important cost burden for the public health system. To date, only treatments aiming to temporarily stabilize symptoms exist, however there is no medication available to slow down or reverse the progression of the disease. Therefore, further research is needed to elucidate the pathophysiological mechanisms underlying AD and to identify novel treatment targets.

### 1.2 Clinical symptoms and stages

AD is the most common cause of dementia defined as a decline in memory, language, problem-solving and other cognitive skills resulting in a person's disability to perform daily activities. Even though AD symptoms are highly variable, the most common initial symptom is dampening of short-term memory performance. Later on, as memory deficits become more

prominent, a functional decline appears with visual and language impairments followed by neuropsychiatric symptoms like changes in personality, depression, apathy and aggressiveness. AD progresses following three main stages: the first stage is asymptomatic and generally lasts between 10 and 20 years, the second stage is termed Mild Cognitive Impairments (MCI) and is paired with first memory deficits and personality changes, the third stage also referred as dementia is associated with a severe cognitive decline followed by death. Although AD shortens life span, it is not usually a direct cause of death. Rather, AD patients die from complications of the pathology such as infections, in particular pneumonia, and blood clots. Indeed, as the disease progresses, individuals lose weight and become unable to perform basic activities like bathing, eating, dressing, which, when taken together, weakens their immune responses and increases their risk of infections and blood vessels problems. Furthermore, these patients have difficulties swallowing and they often inhale food, which makes them more prone to develop pneumonia (Association Alzheimer, 2016; Masters et al., 2015).

## 1.3 Historical background

The disease was discovered in 1906 by Dr Alois Alzheimer, a German neuropsychiatrist. His patient Auguste D. was a 51-year-old woman admitted to a psychiatric hospital for "delerium and frenzied jealousy of her husband." Following post-mortem analysis of her brain, Dr Alzheimer reported 2 abnormal lesion types termed *senile plaques* and *neurofibrillary tangles* that are until now considered as major hallmarks of AD (Alzheimer et al., 1995). In November 1906, Dr Alzheimer put together these observations and presented them as a newly discovered disease to the Society of Southwest German Psychiatrists in Tubingen.

Senile plaques and neurofibrillary tangles were further analysed only 50 years later. The filamentous structure of these lesions was described in the 60s based on an electron microscopy analysis (Kidd, 1964; Terry, 1963). Later, in the 80s, major components of these lesions were discovered: β-amyloid peptide (Aβ) in senile plaques (Glenner et al., 1984) and Tau protein in neurofibrillary tangles (Brion et al., 1985).

Furthermore, the distribution pattern and the degree of diffusion of these AD hallmarks has been associated with different pathological stages of the disease (Braak and Braak, 1996) (**Fig.1**). Typically, senile plaques precede the appearance of neurofibrillary tangles and emerge

first in the frontal and temporal lobes spreading to the rest of the cortex, the hippocampus and the limbic system. The hippocampus and the limbic system are particularly vulnerable in AD and are the main structures involved in memory formation and consolidation (see Annex Fig.1 for the structure of the hippocampus). The neurofibrillary tangles first appear in the entorhinal region and in later stages are apparent in different structures of the limbic system as well as in the cortex.

## 1.4 Etiology

The great majority of AD cases (more than 95%) appear in individuals above 60 years old and are termed late-onset AD or sporadic cases. Their trigger is not yet clearly identified but one major risk factor is well admitted - age. Nevertheless, a few environmental factors like lower education level, physical inactivity, mid-life hypertension and obesity, type II diabetes, depression and smoking have been described to favor the occurrence of the disease (Baumgart et al., 2015; Masters et al., 2015). Furthermore, a family history of affected close relatives is not uncommon among sporadic AD patients and therefore indicates a probable existence of underlying genetic risk factors. The only well-established genetic risk factor for AD is the ε4 allele of apolipoprotein E (ApoE ε4). There are three ApoE alleles: ε2, ε3 and ε4, the ApoE ε3 being the most abundant (Kanekiyo et al., 2014). While, the ε2 allele is considered to be protective for AD (Corder et al., 1994), inheriting one copy of ApoE ε4 increases the risk of developing AD approximately by 3 fold (Farrer et al., 1997; Strittmatter et al., 1993), Furthermore, the ApoE ε4 allele has been estimated to account for approximately 50% of sporadic AD cases (Ashford, 2004). ApoE is involved in lipoprotein metabolism and transport. Although, the precise role of ApoE in AD pathogenesis remains unclear, several evidences indicate that ApoE could contribute to Aβ clearance, therefore preventing Aβ aggregation and formation of senile plaques (Kanekiyo et al., 2014).

A minority of AD cases (less than 1%) are autosomal dominant inherited cases (FAD), which are characterized by a much earlier age of onset - around 45 years. They are due to autosomal dominant mutations identified in 3 genes: amyloid precursor protein (*APP*); presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*), catalytic subunits of the γ-secretase complex involved in

APP processing and Aβ production (for further details see below). It is now well known that all of these mutations result in overproduction and/or formation of aberrant forms of Aβ (Tanzi and Bertram, 2005).

In addition, some very rare genetic cases of AD are due to a triplication of the *APP* gene locus, which also leads to greater Aβ production (Rovelet-Lecrux et al., 2006). Furthermore, Down's syndrome individuals that have an extra copy of chromosome 21 and therefore carry three copies of *APP* gene located on chromosome 21, also exhibit increased Aβ production as well as pathological changes that resemble AD detectable starting from the age of 35 (Wisniewski et al., 1985).

## 1.5 Histopathological hallmarks of AD

### 1.5.1 Neurofibrillary tangles and protein tau

Neurofibrillary tangles (NFTs) are filamentous intraneuronal inclusions composed of hyperphosphorylated protein Tau. They can be found not only in AD but in several other pathologies named tauopathies like for example frontotemporal dementias. In AD, tangle pathology spreads in the brain following a predetermined spatiotemporal pattern along anatomically connected pathways. Indeed, NFTs first appear in the transentorhinal region and the entorhinal cortex, followed by the CA1 region of the hippocampus (**Fig.1**; see **Fig.2** for the structure of the hippocampus). In the next stages, they continue spreading through the limbic structures such as the subiculum of the hippocampal formation and the amygdala. In the final stages of the disease, the whole cortex is affected by NFTs resulting in an impairment of associative, sensory and motor functions (Braak and Braak, 1997). Furthermore, the importance of NFTs in AD pathology is highlighted by the fact that the severity of cognitive impairment correlates with tangle progression and neuronal loss rather than with the appearance of senile plaques (Arriagada et al., 1992).

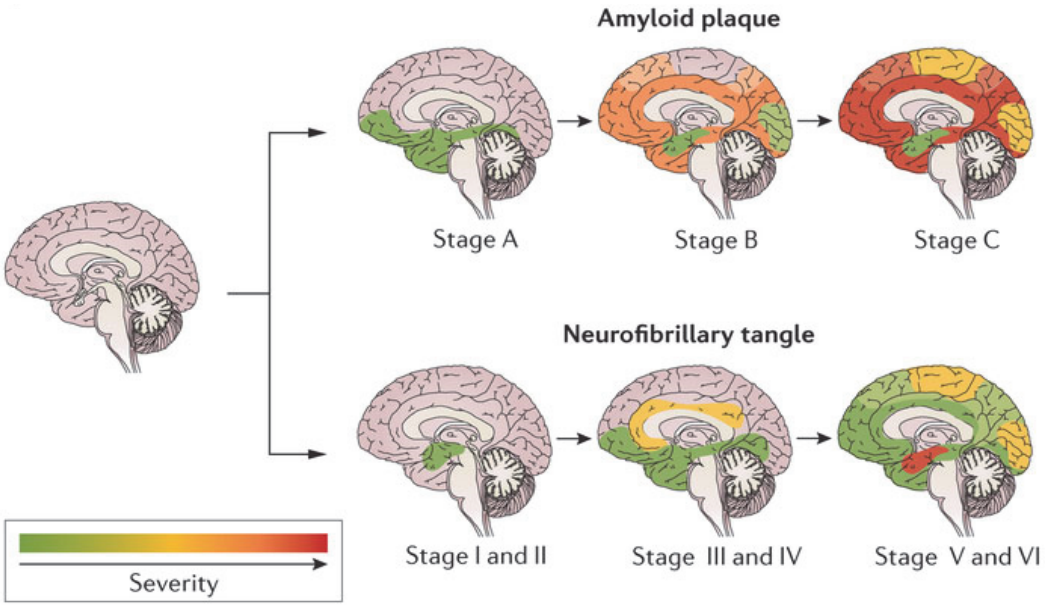



**Fig. 1. Distribution of AD hallmarks and different stages of the disease.** There are three main stages of progression for senile plaques: Stage A, with lesions mainly found in the basal parts of the frontal, temporal, and occipital lobes; Stage B, with lesions in all isocortical association areas and in the limbic system; and Stage C, with numerous and widespread lesions all over primary isocortical areas and in the limbic system. The neurofibrillary tangles appear first in the transentorhinal region and the entorhinal cortex, followed by the CA1 region of the hippocampus (stages I-II) (see **Fig.2** for the structure of the hippocampus). Next, they develop in the limbic structures such as the subiculum of the hippocampal formation, the amygdala and also in the thalamus (stage III- IV). Finally, they spread to all isocortical areas (stage V-VI). (Adapted from (Masters et al., 2015))

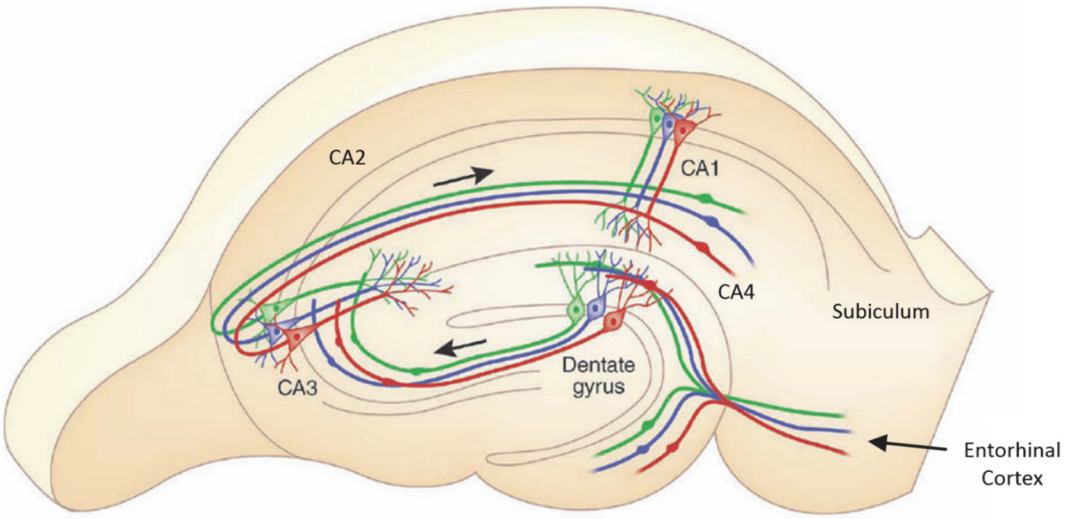



**Fig. 2. Structure and circuitry of the hippocampus.** Simplified hippocampal circuitry forms a loop that starts and ends in the entorhinal cortex and comprises several essential projection areas (Dentate Gyrus, CA3, CA1, Subiculum) containing neuronal somas. Pyramidal neurons from the entorhinal cortex project to rows of cells in the dentate gyrus. Neurons from the dentate gyrus project to the CA3 region. CA3 cells in turn form synapses with neurons in the CA1 region. Pyramidal cells of the CA1 send their projects to the subiculum. The subiculum terminates the loop by projecting back to the entorhinal cortex. CA2 and CA4 are secondary areas that also contain neuronal somas and receive projections from various areas within this loop (i.e. CA2 receives projections from the entorhinal cortex, CA3 and the dentate gyrus; CA4 forms synapses with the dentate gyrus and CA3 neurons) (Adapted from (Moser, 2011)).

Protein Tau is an essential component of NFTs. It is a microtubule-associated protein (MAP) that was found to play a role in the promotion of microtubule assembly and stability back in the 70s (Weingarten et al., 1975). More precisely, it was shown that Tau is involved in regulating motor-driven axonal transport by facilitating the assembly and stabilizing α and β-tubulin heterodimers (Drechsel et al., 1992; Gustke et al., 1994; Weingarten et al., 1975). Additionally, Tau is a major MAP since it accounts for approximately 80% of total MAPs (Wang et al., 2014b).

Human Tau is encoded by the *MAPT* gene found on chromosome 17 and is composed of 16 exons. In the adult brain, alternative splicing of exons 2, 3 and 10 gives rise to six different tau isoforms which are natively unfolded and lack a precise 3D structure (Schweers et al., 1994). These isoforms essentially differ by their number of N-terminal domains (N) and C-terminal

microtubule binding repeats (R). They can contain 0, 1 or 2 N domains and 3 or 4 R repeats (Brandt and Lee, 1993; Sergeant et al., 2005) (**Fig.3**). The R domains influence Tau microtubule binding affinity, with 4R Tau being the strongest binder of microtubules. Tau has approximately 80 phosphorylation sites mainly located near the R repeats (Stoothoff and Johnson, 2005). Phosphorylation of Tau at most of these sites decreases its affinity for microtubules (Wang et al., 1995).

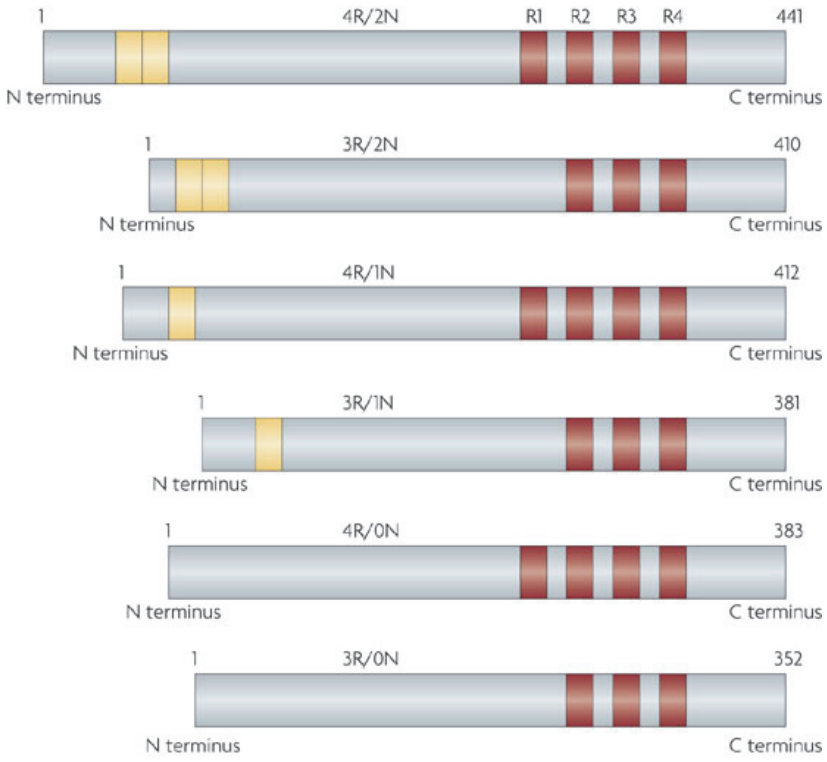



**Fig.3. Different Tau isoforms.** Tau isoforms differ by the number of N-terminal domains (N) (yellow) and microtubule binding repeats (R) (red). They can contain 0, 1 or 2 N domains and 3 or 4 R repeats. (Ballatore et al., 2007).

A lot of work has been done on Tau phosphorylation since it plays an important role in AD pathology and is also apparent in other tauopathies. In AD, phosphorylation of tau is highly increased and leads to a dissociation of Tau from the microtubules, consequently favoring Tau aggregation and formation of paired helical filaments (Alonso et al., 1996). This causes a disruption of the microtubule network, which results in an impairment of axonal transport and synaptic functioning (Buee et al., 2000) followed by neurotoxicity (Fath et al., 2002).

The pathological hyperphosphorylation of Tau is due to an imbalance in neuronal kinase and phosphatase activities. Two kinases highly involved in Tau phosphorylation in AD are glycogen synthase kinase 3 β (GSK3β) and cyclin-dependent kinase 5 (Cdk5) (Liu et al., 2004).

### 1.5.2 Senile plaques

Senile plaques are extracellular lesions defined by the presence of a central core containing a 4-kDa peptide with a beta-pleated sheet structure termed Aβ (Masters et al., 1985). Although often observed in the brains of normal aged individuals (Villemagne et al., 2011), when they are present together with NFTs they are a sign of AD. The pattern of appearance of plaques in AD is quite unpredictable and doesn't correlate well with the degree of cognitive decline (McLean et al., 1999).

Aβ is a cleavage product of its precursor protein: amyloid precursor protein or APP (Glenner et al., 1984; Masters et al., 1985). Although, Aβ exists in the form of soluble monomers, it can also aggregate to form insoluble oligomers or insoluble β-sheet fibrils, the latter being confined to senile plaques. The most abundant form of Aβ is composed of 40 amino acids (Aβ40), however, it is the 42 amino-acid form (Aβ42) that is considered to be the most pathologically relevant since it is this form that is majorly found in senile plaques.
It is noteworthy that senile plaques are complex structures containing a mix of various components besides Aβ. Indeed, a corona of large dystrophic neurites encircles the plaques. In addition, on the outer side of the plaques can be found numerous activated microglial cells and sparse reactive astrocytes (Perl, 2010). Other components of senile plaques include heparan sulfate proteoglycan, apolipoprotein E, aluminum, complement cascade actors like α-1-antichymotrypsin, lysosomal proteases and anti-oxidant enzymes (Atwood et al., 2002).

## 1.6 The Amyloid Cascade Hypothesis

Since its announcement in 1992, the amyloid cascade hypothesis has been considered as the most valid explicative hypothesis of AD (Hardy and Higgins, 1992) (**Fig.4**). The strongest evidence supporting this hypothesis comes from the study of inherited forms of AD where mutations in presenilins and APP result in increased Aβ production favoring its aggregation

and initiation of the disease. Four chronologically and hierarchically connected events form the basis of this theory. First, Aβ levels increase due to its overproduction or impaired clearance (event 1) eventually leading to its accumulation in the form of toxic species (event 2). Next, these toxic species trigger hyperphosphorylated tau aggregation and formation of NFTs (event 3). Finally, all these events are followed by neuronal death (event 4). The validity of this hypothesis has been however questioned, since there was evidence that senile plaques can appear with age in healthy individuals and do not correlate well with the degree of cognitive decline. Based on this, the original amyloid cascade hypothesis has been revised (Hardy and Selkoe, 2002), and it was postulated that it's the non-fibrillary oligomeric forms of Aβ that would be the primary cause of cognitive decline in AD (Selkoe, 2008). This assumption was mainly based on two important studies, the first one showing that memory impairments precede the appearance of fibrillary forms of Aβ in transgenic mouse models of AD (Billings et al., 2005) and the second one identifying a strong positive correlation between soluble levels of Aβ and the severity of the dementia (McLean et al., 1999) . It is worth mentioning that often it is the longer form of Aβ, Aβ42, which is considered to be at the center of the amyloid cascade. However, this matter is a subject of debate since there is not only one toxic species of Aβ. Longer forms of Aβ exist too and it's more the aggregating properties of these different Aβ peptide species that need to be taken into consideration when assessing their pathological relevance.

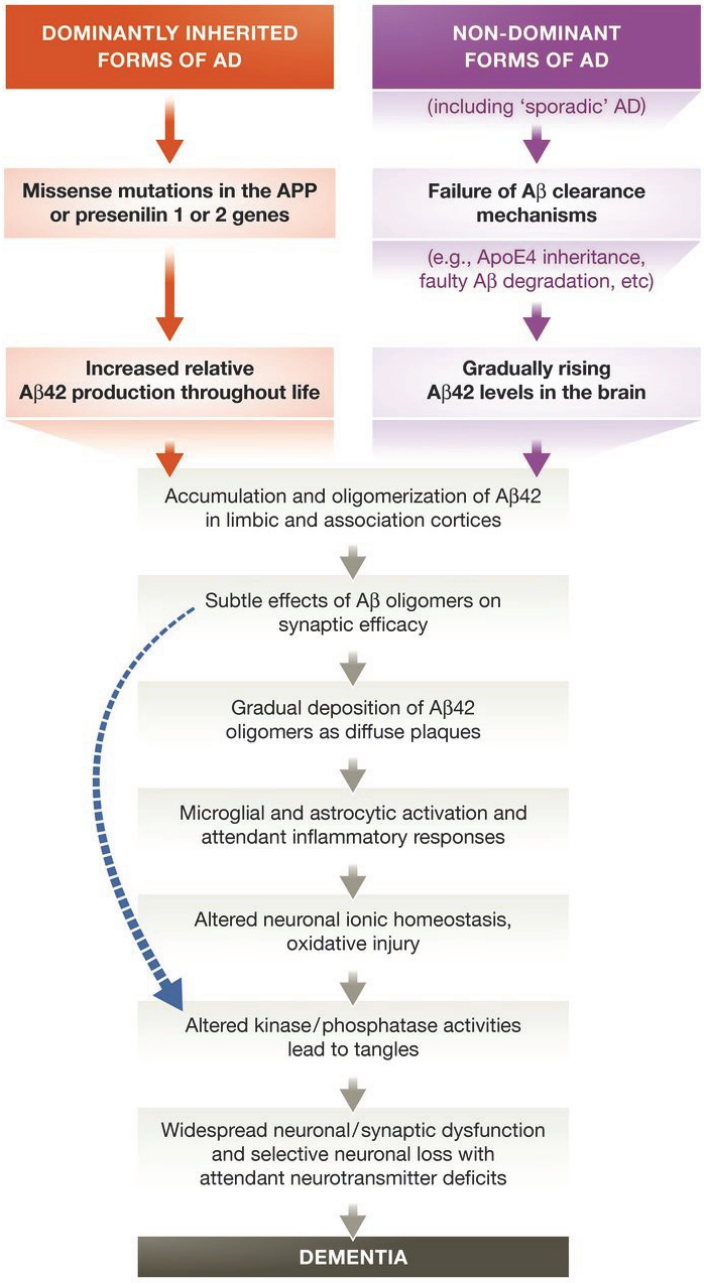



**Fig.4. The amyloid cascade hypothesis.** In this figure is illustrated the sequence of major pathogenic events leading to AD in its genetic or sporadic form. The curved blue arrow suggests that Aβ oligomers can directly harm synapses and neuronal processes, in addition to activating microglia and astrocytes. (Selkoe and Hardy, 2016)

## 1.7 The Amyloid Precursor Protein

### 1.7.1 Genetics and structure

*APP* gene is composed of 18 exons and is located on chromosome 21 (Kang J, 1987). Alternative splicing of exons 2, 7, 8 and 15 results in generation of 10 different isoforms of APP with a length ranging from 365 to 771 amino acids. The three major isoforms include APP695, APP751 and APP770. The APP695 isoform is mainly expressed in the central nervous system (CNS) and is the most important isoform to be found in neurons (Thinakaran and Koo, 2008). It results from splicing of exons 7-8 and lacks the Kunitz-type proteolysis inhibitor domain (KPI) that is present in 751 and 770 isoforms. The latter isoforms are primarily found in many peripheral tissues and fibroblasts (Bayer et al., 1999; Muller et al., 2017).

APP is a member of an evolutionary conserved family of genes that includes for example the *APL1* in Caenorhabditis elegans (Daigle and Li, 1993), *APPL* in Drosophila (Luo et al., 1990) (Martin-Morris and White, 1990), *appa* and *appb* in zebrafish (Musa et al., 2001). In mammals, *APP* gene has paralogs that are also part of the same family, APP like proteins 1 and 2 (APLP1 and APLP2).

The amyloid precursor protein just like its paralogs is a single-pass transmembrane protein with a large extracellular domain and a short intracellular tail (**Fig.5**). The N-terminal extracellular part of APP contains a signal peptide followed by the E1 domain, an acidic domain, KPI domain and the E2 domain. Within the juxtamembrane and the transmembrane domain of APP can be found the Aβ sequence while the cytoplasmic C-terminal tail contains the APP intracellular domain or AICD. This intracellular domain is important for APP internalization since it comprises the YENPTY internalization motif. Furthermore, the E1 and E2 domains as well as the YENPTY motif are among the most evolutionally conserved sequences (Coulson et al., 2000). All APP paralogs share the E1 and E2 domains as well as the acidic domain. In addition, APLPs possess an intracellular domain similar to AICD termed ALID. However, the Aβ sequence is unique to APP and can't be found in APLPs.

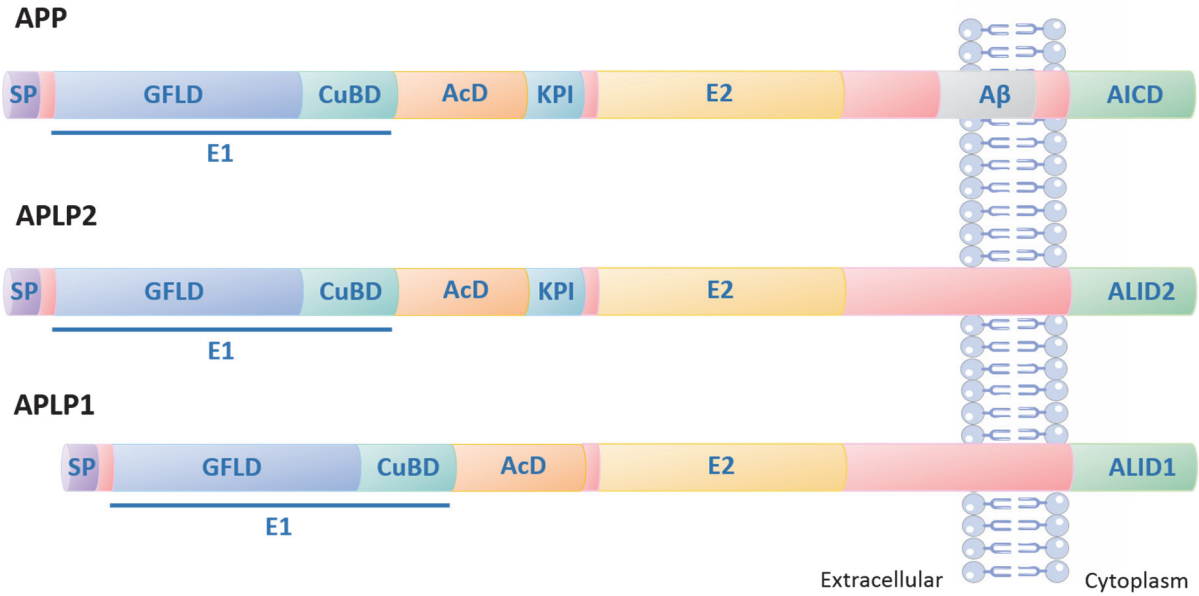



**Fig.5. Structure of APP family proteins: APP, APLP2 and APLP1.** A signal peptide (SP) can be found at their N-terminal of all APP family proteins. It is followed by the Growth factor-like domain (GFLD) and the Copper-binding domain (CuBD) together forming the E1 domain. The E1 domain is followed by the Acidic domain (AcD), the Kunitz-type protease inhibitor domain (KPI) and the E2 domain. APLP1 is the only one that lacks the KPI domain. APP differs from the other members by the Aβ sequence located within its transmembrane domain. Finally, the intracellular domain contains a domain AICD in APP and ALID1 and 2 in APLP1 and APLP2, respectively.

APP and APLP2 are ubiquitously expressed and their expression levels are particularly high in neurons. Moreover, they evolve following similar expression patterns both during embryonic development and in the adult CNS (Slunt et al., 1994; Lorent et al., 1995; Thinakaran et al., 1995). In contrast, APLP1 expression is specific to the nervous system (Lorent et al., 1995). In the brain, APP is abundantly expressed in the cortex and the hippocampus, both in astrocytes and in neurons including excitatory as well as inhibitory subtypes (Muller et al., 2017).

Given the important structural and expression similarities that exist between APP family members, the notion of functional compensation has emerged. This notion was further supported by results obtained in APP/APLP knock-out models where redundant roles of APP and APLPs have been described (Heber et al., 2000).

### 1.7.2 Processing

Ever since APP has been identified as the precursor of Aβ peptide, trying to figure out how this peptide is generated has been an important research subject. For decades, two main processing pathways were described for APP: amyloidogenic and non-amyloidogenic (**Fig.6**). As their names indicate, the first one leads to generation of Aβ while the second one doesn't. The endoproteolytic enzymes involved in these pathways are termed secretases. While in non-neuronal cells the non-amyloidogenic processing of APP is predominant, there is evidence that in neurons considerable amounts of APP would be processed by the amyloidogenic pathway (Muller and Zheng, 2012; Kuhn et al., 2010; Simons et al., 1996).

In the amyloidogenic pathway, processing of APP is initiated by a β-secretase cleavage at the N-terminus of the Aβ sequence generating an APP extracellular fragment sAPPβ. In its non-amyloidogenic counterpart, APP is cleaved by α-secretase within the Aβ region preventing its generation and triggering the release of a respective soluble fragment sAPPα. The β-secretase cleavage is performed by aspartyl proteases BACE1 or 2 (Vassar et al., 2014) while the metalloprotease ADAM10 is the major α-secretase in the brain (Prox et al., 2013; Kuhn et al., 2010). No matter the pathway concerned, the remaining membrane-anchored C-terminal fragments generated after the β- and α-secretase cleavage, CTFβ and CTFα, respectively, are further processed by the γ-secretase. In case of the amyloidogenic pathway, cleavage of CTFβ leads to the release of Aβ and APP intracellular domain or AICD. AICD is also a common product to the non-amyloidogenic pathway and is generated from CTFα together with a very small (3kDa) peptide p3. γ-secretase is a multiproteic complex composed of anterior pharynx-defective 1 (Aph-1), Nicastrine, presenilin enhancer 2 (Pen-2) and one of the presenilins PS1 or PS2, which constitute the catalytic core of the enzyme (Haass, 2004). γ-secretase first cleaves CTFs at an endoprotelytic site termed ε-site releasing AICD and then proceeds to consecutive exoproteolytic cuts in the Aβ sequence allowing generation of Aβ peptides of several lengths ranging between 35 and 50 amino acids, the major form being Aβ40 and the most pathologically relevant, Aβ42 (Tomita, 2014).

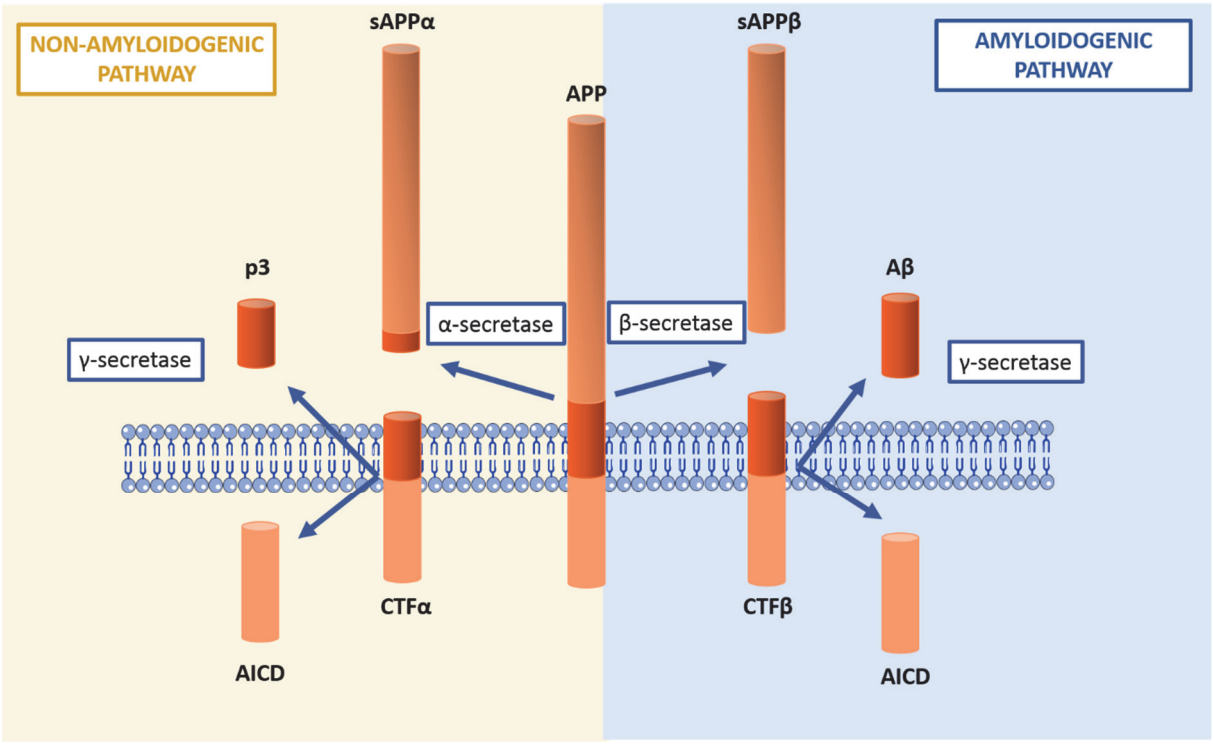



**Fig.6. APP processing.** APP can be processed by 2 pathways: the non-amyloidogenic and amyloidogenic pathway. In the non-amyloidogenic pathway, APP is cleaved by α-secretase to generate sAPPα and CTFα. CTFα can be further processed by γ-secretase yielding p3 peptide and AICD fragment. In the amyloidogenic pathway, APP is first cleaved by β-secretase to generate sAPPβ and CTFβ. CTFβ can be further processed by γ-secretase releasing Aβ peptide and AICD fragment.

Recently, several different processing pathways have been identified for APP, yielding additional N-terminal fragments: δ-secretase, η-secretase and meprin pathway (Andrew et al., 2016). The cleavage products of these pathways have been detected in rodent brains and were decreased after knock-out of corresponding secretases highlighting their physiological relevance. Nevertheless, little is known about their functions except for the cleavage product of η-secretase, Aη-α, that was shown to attenuate activity of hippocampal neurons *in vivo* (Andrew et al., 2016).

### 1.7.3 Trafficking

In neurons, APP can be found in the somatodendritic compartment as well as in the axons and synapses. Given that it undergoes maturation through the secretory pathway, its trafficking starts in the endoplasmic reticulum where it is synthesized (**Fig.7**). From there, APP can either

be transported via the transgolgi network (TGN) to the cell surface or can be directly translocated to the endosomal network (Jiang et al., 2014). Once on the cell surface, APP is readily internalized by clathrin mediated endocytosis since its C-terminal tail contains the YENPTY internalization signal (Lai et al., 1995; Marquez-Sterling et al., 1997). Endosomes containing APP recirculate through the endocytic and recycling pathways to reach the TGN or the cell surface. A measurable fraction of APP can be degraded in late endosomes (Haass et al., 1992). APP has a half-life of around 30-60min (Storey et al., 1996; Perez et al., 1999) and TGN is the major site of resident APP in neurons (Jiang et al., 2014). The cellular distribution of APP has an impact on the way it is processed. Indeed, while α-secretase cleavage occurs at the cell surface, β-secretase operates in the acidic compartments such as early endosomes and the TGN. Secretases also circulate through the secretory pathway. For instance, APP and β-secretase are localized in different types of vesicles in the dendrites but neuronal activity induces their convergence in recycling endosomes therefore favoring Aβ production (Das et al., 2013; Muller et al., 2017). It has been also suggested that the initial cleavage by α- or β-secretase could occur in synaptic vesicles where both BACE1 and ADAM10 were found to co-localize (Lundgren et al., 2015). In addition, it appears that Aβ secretion can be activity-dependent since several independent studies have shown that increasing and decreasing neuronal activity could increase and decrease secreted Aβ levels, respectively (Kamenetz et al., 2003; Cirrito et al., 2005; Tampellini et al., 2009). In this regard, it was shown that Aβ can be secreted from both types of neuronal processes: dendrites and axons, and additionally from synapses as well (DeBoer et al., 2014; Tampellini et al., 2009). It is noteworthy that Aβ release was shown to be independent of glutamate release in stimulated isolated nerve terminals outlining the complexity of Aβ secretion mechanisms (Lundgren et al., 2014). Furthermore, the localization of γ-secretase is important for the type of Aβ species, 40 or 42, which will be preferentially produced from APP as well as its secretion. Indeed, it has been shown that γ-secretase containing PS2 is preferentially located to late endosomes and lysosomes as opposed to PS1 containing γ-secretase, which is more broadly distributed within the cell. Furthermore, γ-secretase located to late endosomes and lysosomes was shown to contribute to longer form of Aβ, Aβ42, generation and buildup of intracellular Aβ pool (Sannerud et al., 2016).

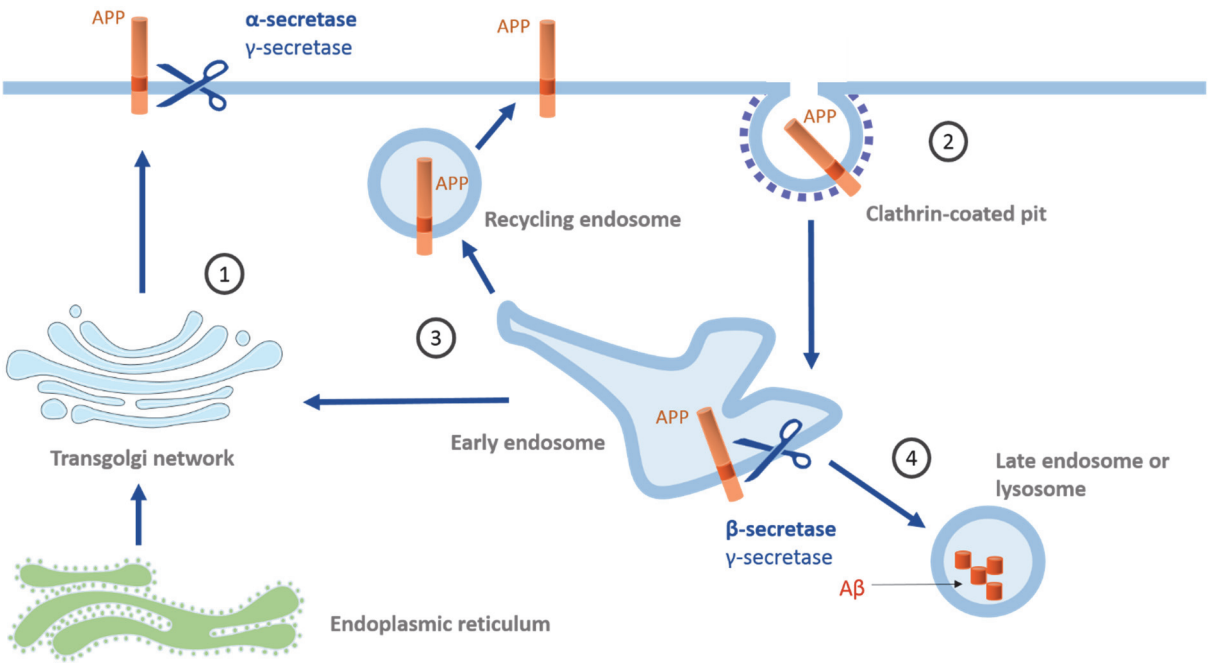



**Fig.7. APP trafficking.** APP is transported from the endoplasmic reticulum to the transgolgi network (TGN) and further to the cell surface (**1**). Once at the membrane, it can be processed by α-secretase or internalized (**2**). Endosomes containing internalized APP can then either recirculate between the TGN and the cell surface (**3**) or mature into late endosomes where APP can be degraded upon fusion with lysososmes (**4**). β-secretase cleavage of APP appears in the acidic compartments such as early endosomes and the TGN.

### 1.7.4 Functions

A lot of work has been done on trying to elucidate APP cellular function. Several evidences indicated that APP could be an essential physiological player. First, *APP* gene family is very well evolutionary conserved and its appearance coincides with the emergence of the earliest nervous system with operational synapses (Shariati and De, 2013). Second, its expression is ubiquitous. Third, in the CNS, APP expression can be detected starting from very early on during embryonic development.

In order to investigate what functions APP family members could exert, different transgenic knock-out models have been generated. Mutants lacking only one member of the APP family show a normal phenotype with only subtle defects. By far the most studied, *App* $^{-/-}$ mice show reduced brain and body weight (Zheng et al., 1995; Li et al., 1996), decreased grip strength (Zheng et al., 1995; Ring et al., 2007) and age-dependent impairments in neuronal morphology, synaptic plasticity and behavior when compared to wild-type animals (Ring et

al., 2007; Dawson et al., 1999a; Seabrook et al., 1999; Tyan et al., 2012; Zou et al., 2016; Lee et al., 2010b). In contrast to these subtle phenotypes, *App*$^{-/-}$ *Aplp2*$^{-/-}$, *Aplp1*$^{-/-}$ *Aplp2*$^{-/-}$ and triple knock-outs (*App*$^{-/-}$ *Aplp2*$^{-/-}$ *Aplp1*$^{-/-}$) die shortly after birth (Heber et al., 2000; Dinet et al., 2016) (von Koch et al., 1997; Herms et al., 2004). Given that single knock-out mice are perfectly viable, this suggests that APP family members have probably redundant roles and could compensate for each other.

Despite the limitations of these models, after more than 20 years of research using various approaches, it appeared that APP would be able to regulate numerous cellular processes like, for example, neuronal development and neurite outgrowth, gene transcription, synaptic activity and plasticity, axonal transport, neuromuscular junction formation and activity, cholesterol, glucose and calcium homeostasis (Shariati and De, 2013; Van Der Kant and Goldstein, 2015; Muller et al., 2017; Pierrot et al., 2013; Santos et al., 2009). Based on these findings, it turns out that APP is somehow an "**A**ll **P**urpose **P**rotein" that is required for many essential cellular mechanisms (Shariati and De, 2013). Below, I will focus on a few of these functions, which are of particular interest for this work.

#### 1.7.4.1 APP and nervous system development

During the embryonic development of the CNS, neuronal precursor cells are born and multiplied in two main proliferative niches: ventricular zone (VZ) and the adjacent subventricular zone (SVZ) (**Fig.8**). These precursor cells can give rise to both neurons and glia. Once the divisions have completed, post-mitotic young neurons that originate from the VZ or the SVZ migrate towards the cortical plate using the fibers provided by radial glia cells, which are anchored in the VZ. When the young neurons reach their final destination located at the surface of the brain, they begin to mature by growing processes (axons and dendrites) that will eventually connect to form functional synapses.

It was long considered that neurogenesis occurs only during embryonic and perinatal stages. However, it is now known that neurogenesis also takes place in the mature adult brain. Newly generated neurons in adults were first evidenced decades ago in the dentate gyrus of the postnatal rat hippocampus (Altman and Das, 1965). It took however many years of research

to confirm these results and convince the scientific community of the accuracy of these observations. For instance, functional integration of these newly born neurons in the adult brain networks was demonstrated only 20 years later in songbirds (Paton and Nottebohm, 1984). There are two main adult neurogenic regions: the subgranular zone (SGZ) in the dentate gyrus of the hippocampus where new dentate granule cells are generated, and the SVZ of the lateral ventricles where new neurons are born and then migrate to the olfactory bulb to become inhibitory neurons (Gage, 2000). From a functional point of view, adult neurogenesis was suggested to play a role in synaptic plasticity and therefore likely contributes to memory formation (Ming and Song, 2011).

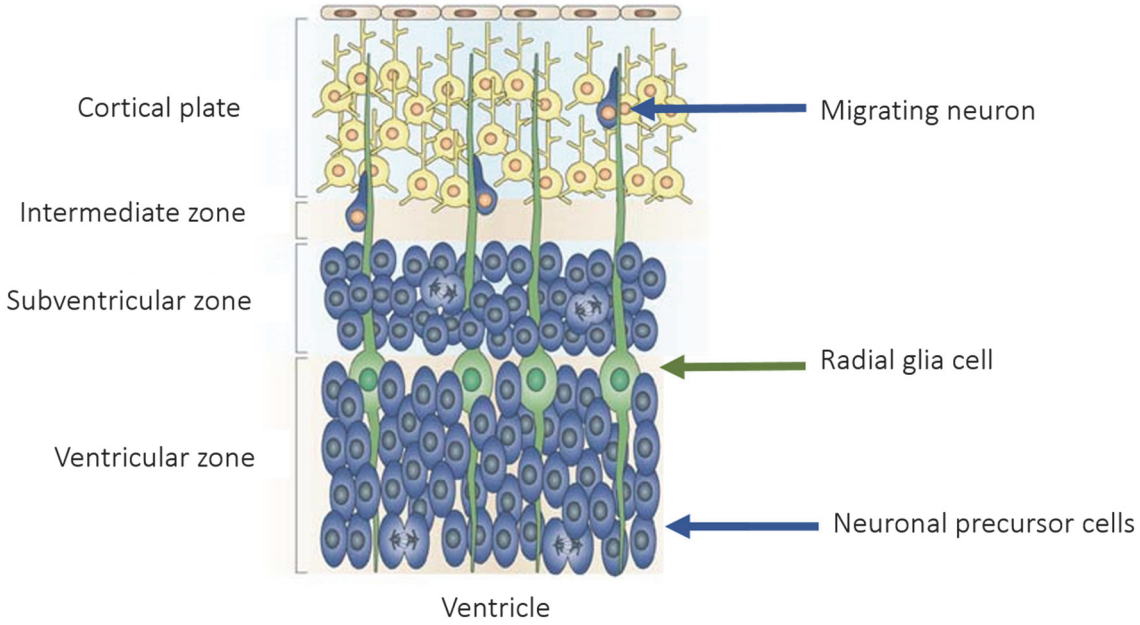



**Fig.8. Cortical development.** Neuronal precursor cells are born and multiplied in two main proliferative zones: ventricular zone and the subventricular zone. Post-mitotic young neurons that originate from the ventricular zone and the subventricular zone migrate towards the cortical plate using the fibers provided by radial glia cells, which are anchored in the ventricular zone. (Adapted from (Herrup and Yang, 2007)).

In mice, APP is expressed in both VZ and SVZ of the developing cortex (Lopez-Sanchez et al., 2005) and APP mRNA can be detected starting from embryonic day 9.5 prior to the peak of neural differentiation and neurite outgrowth (Salbaum and Ruddle, 1994). Interestingly, APP expression levels peak in the second postnatal week in rodents coinciding with the timing of brain maturation and completion of synaptic connections (Loffler and Huber, 1992). Taken

together, these observations highlight a possible involvement of APP in regulation of early and late CNS development. Indeed, it has been evidenced that APP plays a role in different mechanisms of neuronal development including cell proliferation, cell migration, cell differentiation and neurite outgrowth and maintenance:

- **APP and cell proliferation**: A lot of beneficial and important physiological functions have been attributed to sAPPα, the α-secretase soluble cleavage product of APP. In the context of neuronal development, sAPPα was shown to act like a growth factor since it could stimulate the proliferation of neuron stem cells isolated from embryonic rat wild-type neocortex (Hayashi et al., 1994; Ohsawa et al., 1999). Additionally, a role of APP in adult neurogenesis has been reported. Indeed, sAPPα was shown to be able to bind a certain type of progenitor cells expressing early growth factor receptor (EGFR) in the lateral ventricle of mice and to promote adult neurogenesis in these animals (Caille et al., 2004). More recently, in conditional adult *App*$^{-/-}$ mice, increased proliferation of neural progenitor cells was evidenced when compared to wild-type animals (Wang et al., 2014a) indicating that *APP* gene dosage would be important for regulating progenitor proliferation during development.
- **APP and cell migration:** In *App*$^{-/-}$ animals no change in neuronal migration was detected when compared to non-transgenic animals probably due to the existence of compensatory mechanisms by APLPs. Indeed, in triple knock-out mice *App*$^{-/-}$ *Aplp2*$^{-/-}$ *Aplp1*$^{-/-}$, neuronal migration abnormalities have been evidenced: neuronal precursor cells migrated beyond their normal positions (Herms et al., 2004). In contrast, Young-Pearse et al. have shown that acute elimination of APP expression using in utero electroporation of shRNAs in rodent neuronal precursor cells prevented them from entering the cortical plate (Young-Pearse et al., 2007). The divergence in these results might indicate different roles for APPs and APLPs in regulating distinct processes occurring during development.
- **APP and cell differentiation:** Since the earliest time point of APP expression coincides with the timing of neural differentiation, it was also suggested that APP and/or its metabolites might play a role in this process. Indeed, in human embryonic stem cells, the overexpression of APP or its soluble forms causes differentiation towards a neuronal phenotype (Freude et al., 2011).

- **APP and neurite outgrowth and maintenance:** Several studies point to an involvement of APP in shaping the complexity of the dendritic arbor and in the maintenance of spine density and dynamics. Nevertheless these results are mostly inconsistent probably due to differences in experimental settings like for example use of *in vitro* versus *in vivo* models, cell type and anatomical regions analyzed, age of the animals studied. For instance, it was shown that spine density was increased in the cortex of *App*$^{-/-}$ mice and at the same time that γ-secretase inhibition decreased spine density in wild-type mice (Bittner et al., 2009). In contrast, Lee at al. demonstrated that down-regulation of APP reduces the number of spines while upregulation of APP increases their number in the CA1 region of the hippocampus (Lee et al., 2010b). In two more recent studies, decreased spine density was reported in organotypic hippocampal cultures (Weyer et al., 2014) and primary hippocampal cultures from *App*$^{-/-}$ mice (Tyan et al., 2012). Taken together, one could speculate that APP would be able to regulate neurite outgrowth and maintenance differentially dependent on the age and the specific cell type concerned.

#### 1.7.4.2 APP and intracellular signaling

Due to similarities with Notch, a transmembrane protein that is also cleaved by γ-secretase to release an intracellular fragment NICD that is a transcriptional regulator, it was thought that APP could regulate transcription through its intracellular cleavage product AICD. In the early 2000s, AICD was shown to be able to translocate to the nucleus (Cupers et al., 2001; Gao and Pimplikar, 2001; Kimberly et al., 2001). In addition, the formation of a transcriptionally active complex including AICD, the adaptor protein Fe65 and the chromatin-binding protein Tip60 has been shortly pointed out (Cao and Sudhof, 2001; Gao and Pimplikar, 2001). The next step was to try to identify downstream targets of this transcriptional complex. A non-exhaustive list of described AICD target genes includes: tetraspanin family gene *KAI1* (Baek et al., 2002), Glycogen synthase kinase 3 beta (*GSK3β*) (Kim et al., 2003; Ryan and Pimplikar, 2005), neprilysin (Pardossi-Piquard et al., 2005), Early growth factor receptor (*EGFR*) (Zhang et al., 2007), Aquaporin 1 (*AQP1*) (Huysseune et al., 2009), *p53* (Checler et al., 2007) and *APP* itself (von Rotz et al., 2004). Furthermore, it has been suggested that the potentially transcriptionally active AICD fragment would be generated from the amyloidogenic cleavage pathway of APP and not the non-amyloidogenic one (Goodger et al., 2009). Despite the

immense efforts that have been made in this research field, the validity of the proposed AICD targets has been questioned many times and the mechanisms involved in APP-dependent gene regulation still aren't well characterized.

### 1.7.4.3 APP and/or its catabolites in plasticity, learning and memory

Given that in AD synaptic dysfunction correlates with cognitive decline and that Aβ oligomers recovered from patients can alter synaptic plasticity and memory, a potential involvement of APP in plasticity regulation has been suggested.

In the well-described *App*$^{-/-}$ mouse model, an age dependent deficit in synaptic plasticity reflected by a decrease in long term potentiation (LTP) has been described when compared to non-transgenic animals (Seabrook et al., 1999). LTP is a sustained increase in synaptic strength at the postsynaptic site that can be evoked by stimulating the presynaptic site with a train of high frequency pulses. LTP is thought to be a mechanism underlying memory formation and consolidation, and is usually measured in the hippocampus. Accordingly, in *App*$^{-/-}$ transgenic mice memory impairments were also observed (Seabrook et al., 1999; Senechal et al., 2008).

More recently, it was shown that APP could influence network activity patterns necessary for memory formation. Indeed, theta-gamma coupling, which is known to correlate with performance in learning tasks, was shown to be strongly decreased in the parietal cortex and hippocampus of *App*$^{-/-}$ mouse model (Zhang et al., 2016a). In addition, the duration of the UP state (slow depolarizing oscillations in the neocortex), which is a key feature of cortical synaptic integration essentially occurring during slow-wave sleep, was shown to be increased in the prefrontal cortex of *App*$^{-/-}$ mice (Huo et al., 2016).

Among APP processing products, sAPPα has been shown to be able to influence neuronal activity, plasticity and memory. Exogenous sAPPα facilitated LTP induction in rat slices (Ishida et al., 1997) and enhanced memory in chickens, rats and mice (Meziane et al., 1998; Mileusnic et al., 2000; Mileusnic et al., 2004; Roch et al., 1994). Conversely, sAPPα-antibodies or pharmacological inhibition of α-secretase impaired granule cell LTP in adult rats (Taylor et al., 2008).

Despite its supposedly deleterious effects, it appears that Aβ would also be able to contribute to APP-dependent neuronal plasticity regulation. Even though most studies have shown that Aβ impairs LTP (Walsh et al., 2002; Cleary et al., 2005; Hsieh et al., 2006; Wei et al., 2010), some have found evidence for Aβ-mediated facilitation of LTP (Wu et al., 1995; Kim et al., 2001; Trubetskaya et al., 2003; Puzzo et al., 2012; Puzzo et al., 2008). Furthermore, *Psen1* knock-out mice that produce lowered amounts of Aβ show decreased LTP (Morton et al., 2002). In addition, antibodies against rodent Aβ or knock-down of rodent APP by siRNAs resulted in a decrease of LTP in mice associated with a worsening of contextual fear memory performance when compared to untreated wild-type animals (Puzzo et al., 2011). This was further supported by experiments performed in rats and chicks using anti-APP antibodies where blocking APP resulted in impaired cognitive performance (Huber et al., 1993; Mileusnic et al., 2000).

Taken together these evidences clearly outline an involvement of APP and/or its cleavage products in synaptic plasticity regulation.

## 1.8 Synaptic dysfunction and neuronal activity alterations in Alzheimer's disease

### 1.8.1 Synaptic dysfunction in AD

Although senile plaques have been so far considered as essential contributors to cognitive decline in AD patients, therapeutic approaches aiming to reduce brain amyloid load failed to rescue memory deficits in these individuals (for review see (Canter et al., 2016)). Therefore, a lot of research has been done on trying to elucidate different physiopathological mechanisms that could contribute to AD symptoms. A direct correlate between synaptic deficits and cognitive decline has been established a long time ago and led researches to investigate the underlying reasons and consequences (Terry et al., 1991; DeKosky and Scheff, 1990). Interestingly, a lot of observations point out to an altered synaptic functioning in AD. For instance, atypical neuronal synchronicity and oscillations appear long before senile plaques and could be associated with cognitive impairment (Martin et al., 1994; Berendse et al., 2000). Furthermore, in AD transgenic mouse models, learning and synaptic alterations also appear

before the formation of plaques (Massimini et al., 2005; Cleary et al., 2005; Zhang et al., 2016b). Moreover, several categories of people that are at risk for AD (e.g. ApoE4 carriers, FAD family members, MCI patients) display an abnormal activation pattern of certain brain regions during performance of cognitive tasks. For instance, increased activation of the hippocampus and reduced deactivation of default mode network (DMN), brain regions that are normally deactivated during attention-demanding cognitive efforts and are active during inwardly oriented mental activity (e.i introspection, daydreaming), was observed in these individuals (Filippini et al., 2009; Quiroz et al., 2010; Sepulveda-Falla et al., 2012; Bakker et al., 2012; Dickerson et al., 2005; Celone et al., 2006).

Another evidence for synaptic dysfunction in AD came from studies that showed a lack of functional connectivity between brain areas in AD patients by using several different methodological approaches like functional magnetic resonance imaging (Canter et al., 2016) (Martin et al., 1994), magnetic- and electro- encephalography (MEG (Berendse et al., 2000) and EEG (Leuchter et al., 1992), respectively). Furthermore, by combining transcranial magnetic stimulation (TMS) with EEG to characterize changes in functional connectivity and cortical excitability, Casarotto et al. showed that cortical responses to TMS pulses delivered over the frontal lobes are substantially altered in mild AD patients as compared to healthy age-matched controls (Casarotto et al., 2011; Julkunen et al., 2008). Interestingly, recent evidence indicates that deep-brain stimulation of the basal forebrain and hypothalamus in AD patients could slow down the progression of the disease (Laxton et al., 2010; Kuhn et al., 2010). Taken together, these findings point out to an existence of a network dysfunction in AD, which could contribute to cognitive decline.

### 1.8.2 Neuronal activity alterations in AD

A lot of evidences point to neuronal activity alterations in AD. There are both arguments in favor of neuronal hypoactivity and hyperexcitability in AD, indicating that both phenomena might coexist and/or appear in different areas of the brain as well as at different stages of the disease. For instance, it was shown that AD patients have decreased activation of hippocampal formation during memory consolidation (Sperling, 2007). In contrast, it was shown that people at risk for AD like ApoE4 carriers or individuals with MCI are susceptible to task-dependent hippocampal hyperactivity (Filippini et al., 2009). AD-related cortical hyperexcitability

following magnetic stimulation of the cortex has also been reported in AD patients (Di, V et al., 2004). Additionally, PET experiments measuring regional cerebral blood flow, which reflects neuronal activity, have evidenced increased neuronal activity in prefrontal areas of AD patients during performance of memory tasks (Grady et al., 2003). However, there is also evidence for hypoactivity in certain cortical areas of AD patients. Indeed, PET using $^{18}$F-fluorodeoxy-glucose ($^{18}$F-FDG) compound that estimates glucose metabolism, revealed hypometabolism in parietotemporal associative areas and in posterior cingulate areas of AD patients, which could be related to reduced neuronal activity in these regions (Mosconi, 2005; Mosconi et al., 2007).

In addition, it is suggested that AD patients are at greater risk for epileptic seizures. Epidemiological studies have shown that unprovoked seizures could occur in 10-22% of AD patients (Mendez et al., 1994; Sjorgen et al., 1952; Forstl et al., 1992; Amatniek et al., 2006; Imfeld et al., 2013). Interestingly, people with early-onset AD (50-65 years old) experience seizures at much higher rate than late onset AD individuals (>81 years old) (Sherzai et al., 2014). Epileptic activity is even more frequent in pedigrees of early onset autosomal dominant AD. For instance, in a recent study, 48% of individuals carrying FAD mutations out of 132 examined experienced seizures (Zarea et al., 2016). Seizure risk is even more striking in patients harboring APP locus duplication – between 58% and 81% (Cabrejo et al., 2006; McNaughton et al., 2012; Zarea et al., 2016). Furthermore, 84% of Down's syndrome patients who progress to dementia are also prone to experience epileptiform activity (Lai and Williams, 1989). Importantly, seizures can exacerbate cognitive decline in AD patients (Vossel et al., 2017). For example, in a case-control study of ten patients with advanced AD, those with seizures had faster decline in language assessment score as compared to patients without seizures (Volicer et al., 1995). Furthermore, amnesic episodes in AD individuals have been associated with seizures as recorded by EEG indicating that seizures could contribute to symptoms of dementia (Rabinowicz et al., 2000). Conversely, epileptiform activity in patients suffering from temporal lobe epilepsy can also lead to transient amnesia and even AD-like memory impairments (Sinforiani et al., 2003).

Classical EEG-based seizure detection methods are not sensible enough to detect subtle non-convulsive epileptiform episodes in AD patients. Therefore, it is considered that subclinical epileptiform discharges could be present in a much wider percentage of AD cases than clinically detectable seizures. Indeed recently, subclinical epileptiform activity was detected in

14 (42%) of 33 AD patients with 90% of discharges appearing during sleep (Cretin et al., 2016). Furthermore, in this study, patients with subclinical seizure episodes showed faster cognitive decline over time. In line with these results, a very recent study has detected clinically silent hippocampal seizures and epileptiform spikes during sleep in two AD individuals without a history or EEG evidence of seizures by using intracranial implanted electrodes (Lam et al., 2017).

An interesting explanatory hypothesis concerning the dual nature of activity alterations in AD concerns mechanisms of so called "synaptic scaling". Neuronal homeostatic scaling is a local feedback mechanism that allows the neuron to sense levels of activity-dependent calcium entry and accordingly adjust neuronal firing rates (Turrigiano, 2008). It is postulated that in AD, where synaptic function is disrupted, there would be long periods of hypoactivity, which would cause the remaining neurons to increase their sensitivity to excitatory signals so that normal activity levels could be restored. This would result in a much easier transition to high-excitation bursting states and therefore hyperexcitability could occur (Frohlich et al., 2008). Taken together, these findings clearly indicate that neuronal activity is altered in AD and could lead to cognitive dysfunction.

### 1.8.3 APP and neuronal activity

Numerous transgenic AD mouse models that express mutant APP with familial mutations combined or not with mutant PS exhibit hyperexcitability and are susceptible to seizures (Palop and Mucke, 2016). An important feature of these models is increased production of Aβ that results in high brain amyloid load. Given this, it was suggested that senile plaques and excessive Aβ release would be responsible for seizure appearance in these mice. Although bath application of exogenous Aβ often decreases synaptic plasticity (Palop and Mucke, 2010), it was also shown that Aβ could increase electrical activity of excitatory neurons (Minkeviciene et al., 2009) and could be a positive modulator of release probability in hippocampal neurons (Abramov et al., 2009). Another argument in favor of an involvement of Aβ in the regulation of neuronal activity came from the observation that its secretion is activity dependent (Kamenetz et al., 2003; Cirrito et al., 2005; Tampellini et al., 2009).

However, several evidences point out that susceptibility to seizures in AD mouse models might not be only due to Aβ. Indeed, it was shown that these mice display abnormal neuronal activity

well before plaque appearance (Busche et al., 2012). Furthermore, apart from high amyloid production, these models overexpress APP that could by itself modulate neuronal activity. For instance, by using an inducible APP transgenic model, it was recently shown that full length APP or one of its non-Aβ fragments could be responsible for the appearance of seizures in these mice (Born et al., 2014).

Interestingly, impact of APP on neuronal activity has been as well highlighted in a non-AD related transgenic mouse model, *Fmr1*$^{-/-}$ mice, a mouse model of Fragile X Syndrome in which APP metabolism and expression are altered. In these mice, overexpression of APP exacerbated audiogenic induced seizures, while a reduction of APP expression in *Fmr1*$^{-/-}$/*App*$^{+/-}$ partially decreased evoked seizure activity (Westmark et al., 2011; Westmark et al., 2010). Taken together, these results indicate that APP or its metabolites could be able to regulate levels of neuronal activity.

# II. Glutamatergic neurotransmission

## 2.1 General overview

Balanced levels of neuronal activity are a prerequisite for normal brain functioning and are essential contributors to cognition and memory consolidation. Fine tuning of neuronal activity is dependent on a balance between excitatory and inhibitory neurotransmission. In the brain, the main excitatory neurotransmitter is glutamate while the main inhibitory neurotransmitter is γ-aminobutyric acid (GABA).

Glutamate drives about 70% of synaptic neurotransmission in the mammalian CNS and is abundantly present in the brain in a µM (resting state) to mM (during synaptic activity) concentration range (Meldrum, 2000). It is synthesized in neurons from glutamine and can be loaded into synaptic vesicles via vesicular glutamate transporters (VGLUT). Once released in the synaptic cleft by vesicular release, it can be uptaken by the presynaptic neuron or the surrounding glial cells via amino acid transporters (EAATs). After that, it can then be stored or converted to glutamine, which can be recycled back into neurons and transformed again into glutamate. At the postsynaptic terminal, glutamate acts by binding ionotropic and/or

metabotropic glutamate receptors, which results in the appearance of excitatory postsynaptic potential (EPSP) and can eventually contribute to synaptic plasticity mechanisms like formation of LTP (Meldrum, 2000; Smart and Paoletti, 2012).

Glutamatergic neurotransmission in the brain mainly operates through 2 types of ionotropic receptors: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. AMPA receptors are cationic channels that essentially conduct $Na^+$ and $K^+$ ions (in some rare cases $Ca^{2+}$ too). They are considered as the main transducers of fast excitatory neurotransmission in the mammalian brain. From a structural point of view, they mostly form tetramers composed of four glutamate receptor subunits GluA1-GluA4 (previously referred to GluR1–GluR4). AMPA receptor subunit composition depends on the brain region. For instance, at hippocampal excitatory CA3–CA1 synapses, most AMPA receptors are composed of GluA1/GluA2 or GluA3 subunits (Holman et al., 2007).

NMDA receptors conduct $Na^+$ and $K^+$ ions just like AMPA receptors but, conversely, have a very high permeability for $Ca^{2+}$. Besides this, NMDA receptors have several other unique properties like voltage-sensitive block by extracellular $Mg^{2+}$ and unusually slow activation/deactivation kinetics (Cull-Candy et al., 2001). Release of the $Mg^{2+}$ block associated with binding of a co-agonist glycine or D-serine together with glutamate (Erreger et al., 2004) leads to $Ca^{2+}$ influx through the NMDA receptor channel that in turn regulates synaptic strength. NMDA receptors are tetramers composed essentially of two GluN1 subunits assembling with two GluN2 subunits that exist in 4 isoforms GluN2A-D. (Cull-Candy et al., 2001).

When glutamate is released from the presynaptic terminal, it first activates AMPA receptors that mediate fast synaptic transmission (**Fig.9**). Entry of high amounts of $Na^+$ through the AMPA receptor can trigger depolarization great enough to release the $Mg^{2+}$ blockade of NMDA receptor allowing entry of additional $Na^+$ and more importantly $Ca^{2+}$ through its pore. When the frequency of presynaptic stimulation is high, this results in elevated intraneuronal $Ca^{2+}$ concentration in the postsynaptic neuron. $Ca^{2+}$, by activating second messenger cascades, can initiate mechanisms involved in synaptic plasticity and therefore lead to increased synaptic strength. For example, it can induce phosphorylation of AMPA receptors resulting in an

increase in their conductance or it can trigger insertion of additional AMPA receptors into the cell membrane. $Ca^{2+}$ can also activate transcription factors like CREB, which can modulate expression of plasticity related genes necessary for LTP formation and therefore involved in memory consolidation (Meldrum, 2000; Smart and Paoletti, 2012).

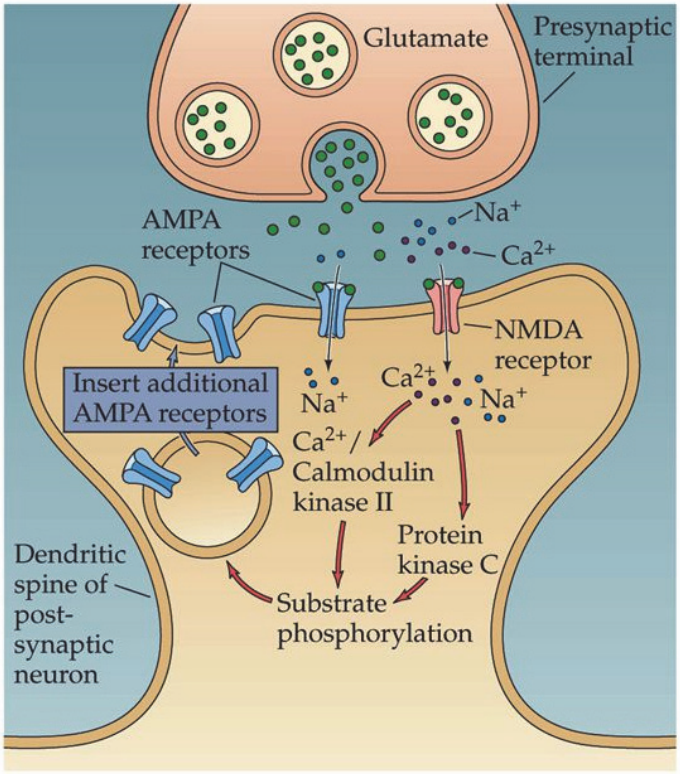



**Fig.9. Glutamatergic neurotransmission.** Once glutamate is released into the synaptic cleft it binds to AMPA and NMDA ionotropic receptors. AMPA receptors are the first to be activated and lead to an important $Na^+$ flux that depolarizes the membrane and lifts the NMDA $Mg^{2+}$ block. NMDA activation causes important $Ca^{2+}$ entry that activates signaling cascades involved in synaptic plasticity aiming to increase glutamatergic synaptic strength, for instance, by triggering AMPA phosphorylation or insertion of additional AMPA receptors into the membrane (Purves D, 2012).

## 2.2 Glutamatergic neurotransmission in AD

Consistent with the direct involvement of glutamatergic neurotransmission in learning and memory, alterations in glutamatergic signaling have been linked with the pathophysiological processes related to AD (Danysz and Parsons, 2012). Sustained and low activation of NMDA receptors can ultimately lead to neuronal death – an effect called 'excitotoxicity'. Indeed, in these conditions the $Mg^{2+}$ blockade of the NMDA receptor can be lifted even by a moderate depolarization, which causes a considerable influx of $Ca^{2+}$ ions into the postsynaptic neuron.

When prolonged, this $Ca^{2+}$ overload can trigger toxic mechanisms resulting in neurodegeneration (Chen et al., 1992; Parsons et al., 1993).

Evidence indicates that glutamate uptake and recycling mechanisms are altered in AD due to modifications in glutamate transporter expression. For instance, it is known that glutamate transporter expression levels and its capacity are decreased in the frontal/temporal cortex of AD patients (Masliah et al., 1996; Li et al., 1997; Tilleux and Hermans, 2007). Additionally, a selective loss of VGLUT has been reported in the brains of AD individuals (Kirvell et al., 2006).

Another factor potentially contributing to NMDA-receptor mediated toxicity in AD is availability of D-serine, the predominant endogenous co-agonist of the NMDA receptor in the forebrain (Danysz and Parsons, 1998). For instance, mRNA levels of serine racemase that generates D-serine from L-serine were reported to be elevated in AD hippocampus (Wu et al., 2004).

As opposed to what could be expected, expression levels of NMDA receptors are decreased rather than increased in AD patients' brains when compared to non-demented control tissues. Although this observation is in contradiction with excitotoxity hypothesis and the increased risk of seizures that AD patients harbor, it could be attributed to compensation mechanisms aiming to reduce neurotoxic NMDA signaling (Danysz and Parsons, 2012).

The location of NMDA receptors, synaptic versus extrasynaptic, is also important in the context of AD pathophysiology. Indeed, it is considered that AD-related excitotoxicity would be mediated by extrasynaptic NMDA receptors rather than synaptic ones. For instance, one of the only drugs available for AD treatment is memantine, an NMDA receptor antagonist. It is considered that memantine has a beneficial effect in AD because it inhibits extrasynaptic NMDA receptors that drive excitotoxicity and spares the synaptic ones, which are essential for neuronal survival and functioning (Hardingham and Bading, 2010).

Taken together, the glutamatergic component appears indeed to be disrupted in AD and most probably contributes to the cognitive deficits observed in this pathology.

## 2.3 APP, Aβ and glutamatergic neurotransmission

Given that APP clearly plays a role in regulation of synaptic plasticity as explained in the previous chapter, and that glutamatergic transmission seems to be impaired in AD, the link between APP and glutamatergic neurotransmission has been investigated.

Increased evoked NMDA and AMPA mediated excitatory postsynaptic currents (EPSCs) were reported in *App*$^{-/-}$ primary hippocampal cultures when compared to wild-type cultures (Priller et al., 2006). In HEK293 (Human Embryonic Kidney Cells 293) cultures transfected with a vector allowing expression of the neuronal isoform of APP, APP695, it was shown that APP could interact with GluN1/GluN2A and GluN1/GluN2B NMDA receptor subtypes and increase their cell surface delivery (Cousins et al., 2009). Additionally, interaction between APP and NMDA receptors and increased surface levels of NMDA receptors subunits GluN1 and GluN2B were also reported in primary neuronal cultures overexpressing APP. In the same study, overexpression of APP in primary hippocampal neurons caused a significant increase in NMDA receptor mediated miniature EPSCs while knockdown of APP with RNAi had the opposite effect (Hoe et al., 2009). Interestingly, it has also been shown that APP could increase cell surface levels of the AMPA receptor subunit GluA2 without altering levels of GluA1 in primary hippocampal neurons overexpressing APP (Lee et al., 2010b).

A lot of work concerning the link between APP and glutamatergic excitatory neurotransmission has focused on Aβ. Indeed, it has been clearly demonstrated that Aβ could reduce glutamatergic synaptic transmission strength and consequently impair synaptic plasticity (Cleary et al., 2005; Walsh et al., 2002). Furthermore, it was recently shown that Aβ-mediated synaptic depression is driven by GluA3-containing AMPA receptors (Reinders et al., 2016). Based on several studies, it seems that Aβ could exert its toxic effect, at least in part, by excessive activation of extrasynaptic NMDA receptors containing GluN2B subunits (Li et al., 2011; Palop and Mucke, 2010). Indeed, increased activation of extra-synaptic GluN2B containing NMDA receptors has been described to trigger excitotoxicity and cell death (Cull-Candy et al., 2001; Hardingham and Bading, 2010). Aβ not only affects the functioning of glutamate receptors but has also been shown to reduce glutamate uptake from the synaptic cleft, therefore again favoring excitotoxity (Fernandez-Tome et al., 2004; Matos et al., 2008).

The physiologically active metabolite of APP, sAPPα also has an impact on glutamatergic synaptic transmission. In this regard, it was reported that sAPPα can facilitate NMDA receptor mediated currents both *in vitro* and *in vivo* (Taylor et al., 2008). sAPPα also seems to have a neuroprotective function since it can mitigate toxic effect of excessive NMDA receptor activation (Ryan et al., 2013).

Based on these evidences, it seems that APP and its cleavage products would be able to regulate glutamatergic neurotransmission. Indeed, while Aβ is mostly described as an inhibitor of excitatory synaptic functioning, sAPPα appears to be neuroprotective.

# III. GABAergic neurotransmission

In the adult central nervous system, γ-amino-butyric acid (GABA) is a major inhibitory neurotransmitter that shapes cortical activity (Isaacson and Scanziani, 2011). It was first identified in the mammalian brain almost seventy years ago (AWAPARA et al., 1950) (ROBERTS and FRANKEL, 1950). GABAergic neurons represent approximately 15-20% of the cortical neuronal population (Meinecke and Peters, 1987) and, in contrast to their counterpart, the glutamatergic excitatory cells, do not generally have long range projections. Therefore, they are also termed interneurons. Given that there exists a tight balance between excitatory and inhibitory neurotransmission, GABAergic neurotransmission just like the glutamatergic one is essential for the induction of LTP (Bliss and Lomo, 1973) and therefore memory formation.

## 3.1 GABAergic neuronal populations and AD

GABAergic neuronal population is highly heterogeneous. Indeed, there exists a myriad of GABAergic neuronal subtypes that can be classified according to their physiology, morphology, connectivity, and expression of certain molecular markers such as neuropeptides (somatostatin, cholecystokinin, vasoactive intestinal peptide, and neuropeptide-Y) and $Ca^{2+}$-binding proteins (parvalbumin, calretinin, and calbindin) (Markram et al., 2004; Klausberger and Somogyi, 2008) (**Fig.10**). At least 20 different cortical subtypes and 21 hippocampal

subtypes have been identified (Fishell and Rudy, 2011; Klausberger and Somogyi, 2008). The classification of GABAergic cells is not trivial since interneurons that share the same molecular marker can have divergent morphologies, physiological responses and connectivity patterns. Therefore, categorization of GABAergic neurons based on only one parameter has shown itself inefficient. A future prospect would be to identify subtypes of interneurons by a certain gene expression pattern that would likely explain the diversity in shapes, connections and activity of these cells. Despite this high and complex variability, there are several categories of GABAergic neurons that are abundant in the forebrain and are therefore quite well described.

The two main cortical interneuron subtypes are:

- **Parvalbumin (PV) interneurons:** PV is a calcium binding protein that is expressed in around 40% of neocortical neurons (Rudy et al., 2011). An important subcategory of PV-expressing interneurons are basket cells (Markram et al., 2004). Physiologically, PV-expressing basket cells are often fast-spiking characterized by high-frequency trains of action potentials (Kawaguchi et al., 1987). PV basket neurons are known to innervate the soma and proximal dendrites of excitatory pyramidal neurons (Kawaguchi and Kubota, 1997). Physiologically, they control the phasing and synchronization of neuronal activity (Freund and Katona, 2007).
- **Somatostatin interneurons:** Somatostatin is a peptide expressed in 30% of cortical interneurons. Somatostatin neurons usually target dendrites. Their spiking profile is adaptive and regular (Kawaguchi and Kubota, 1997; Xu et al., 2006). A representative population of somatostatin neurons are Martinotti cells that can be further divided into two subclasses defined by the presence or absence of the calcium-binding protein, calretinin (Xu et al., 2006). Somatostatin interneurons inhibit both pyramidal cortical cells and PV expressing GABAergic neurons.

There is evidence that both of these interneuron subtypes are differentially affected in AD. Indeed, some studies have shown that the number of PV interneurons is decreased in AD cortical tissues (Arai et al., 1987; Satoh et al., 1991). However, in better preserved postmortem samples, no significant change in PV interneurons number was found (Ferrer et al., 1991; Fonseca et al., 1993; Hof et al., 1991). In another study, PV-immunoreactive dystrophic

neurites and aberrant sprouts could be detected in AD cortical sections indicating that even though the number of PV interneurons might remain unchanged in AD, the morphology of these cells could be modified (Ferrer et al., 1993). Furthermore, in the hippocampus of AD patients, PV positive cells were shown to be differentially affected depending on the region concerned: a decrease in the number of PV interneurons was detected in the dentate gyrus/CA4 and CA1–CA2 regions, while PV neurons didn't decline in the CA3 region nor in the subiculum (Brady and Mufson, 1997). From a functional point of view, in the parietal cortex of the hAPPJ20 AD mouse model, a decrease in the interneuron-specific and PV cell-predominant voltage-gated sodium channel subunit Nav1.1 was found (Verret et al., 2012) and was suggested to trigger epileptiform activity. A decrease in Nav1.1 expression in the PV positive cells could also be observed in the parietal cortex tissues of AD patients (Verret et al., 2012). Somatostatin interneurons have been also analysed in AD patients' brains, and decreased numbers of somatostatin positive cells were found in the temporal and frontal cortices as well as in the hippocampus of these individuals (Davies et al., 1980; Rossor et al., 1980; Davies and Terry, 1981; Candy et al., 1985; Dournaud et al., 1994).

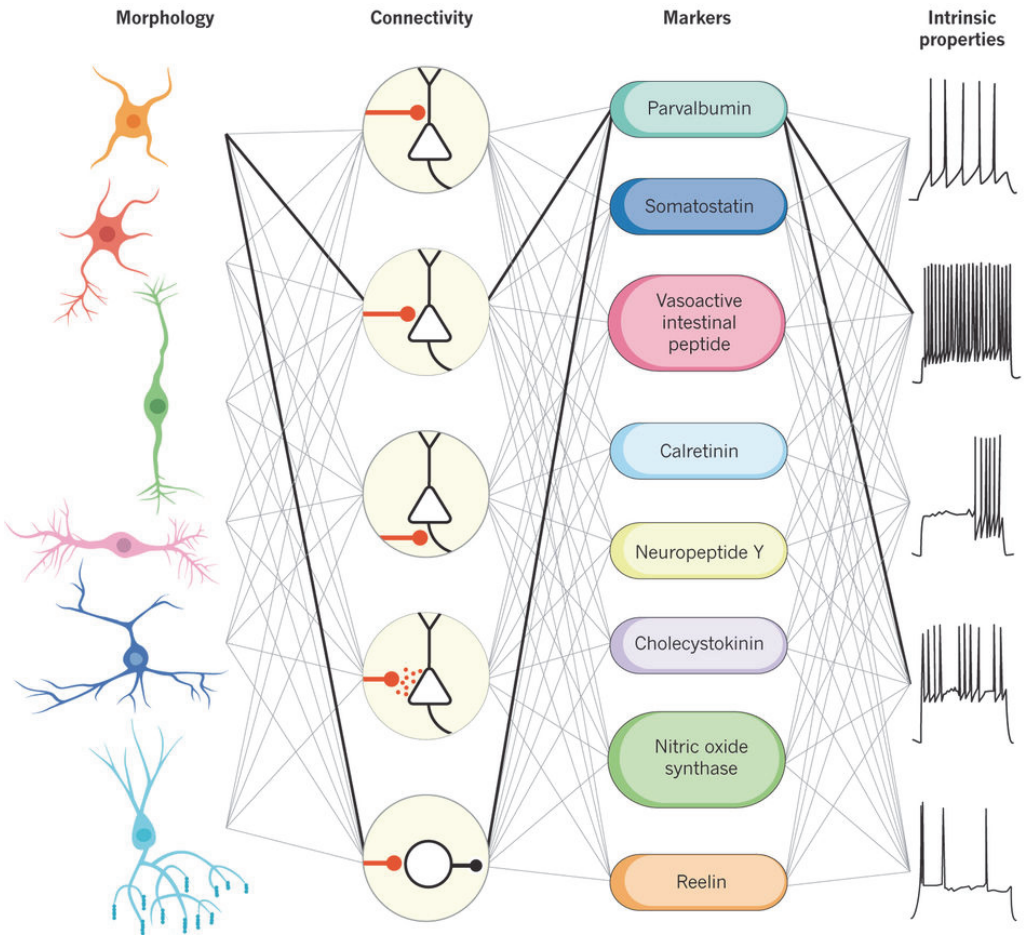



**Fig.10. Diversity in GABAergic neuronal subtypes.** GABAergic neuronal population is highly heterogeneous and somewhat complex to classify. Several classification criteria illustrated here can be used: cell morphology, connectivity, expression of molecular markers and firing profiles. The complexity of classification is due to the fact that a cell of certain morphology can have several types of connections, express several types of markers and have various firing patterns. In the cortex the 2 main interneuron types are parvalbumin and somatostatin expressing cells. Calretinin expressing neurons are a subtype of somatostatin interneurons. Beside these populations, 5HT3aR expressing interneurons can be also found in the cortex. They include vasoactive intestinal peptide positive (VIP) and negative interneurons. The non-VIP neurons can be further subdivided and include Reelin positive neurons. Other cortical interneuronal populations express cholecystokinin, nitric oxide synthase and neuropeptide Y (Kepecs and Fishell, 2014).

## 3.2 GABA synthesis, storage, release and reuptake

GABA is mainly synthesized from glutamate by a pathway termed GABA shunt. In this pathway, glutamate, which can be derived from the Krebs's cycle product α-ketoglutarate, is decarboxylated by the glutamic acid decarboxylase (GAD) to yield GABA (**Fig.11**). There are two main GAD isoforms: GAD65 and GAD67 that are coexpressed in most of the GABA-containing neurons but have a different subcellular distribution (Soghomonian and Martin, 1998). Indeed, GAD67 is essentially present in the cytoplasm and in the cell bodies, whereas GAD65 is highly enriched in axon terminals and is associated with synaptic vesicles (Kaufman et al., 1991). GAD67 knock-out leads to death on the first day after birth due to cleft palate. There is also a >90% reduction in basal GABA levels in the brain of these animals (Asada et al., 1997; Condie et al., 1997). GAD65 knock-out mice have a milder phenotype, but are more prone to seizures (Asada et al., 1996; Kash et al., 1997). It is considered that GAD67 accounts for most GABA synthesis by producing the neuronal activity unrelated GABA pool as well as GABA necessary for basal inhibitory neurotransmission firing, whereas transiently activated GAD65 plays a major role in catalyzing GABA necessary for dampening increased neuronal activity like, for instance, during epileptic seizures (Tian et al., 1999; Patel et al., 2006).

After its synthesis, GABA is loaded into synaptic vesicles by the vesicular neurotransmitter transporter VGAT (Fon and Edwards, 2001). It is then mainly released from the synapses by calcium-dependent exocytosis. GABA synthesis is the rate-limiting step in GABA metabolism (Soghomonian and Martin, 1998) and therefore has a high influence on the cellular and vesicular GABA content (Engel et al., 2001).

Once released, GABA can bind to two main types of receptors: metabotropic $GABA_B$ receptors and ionotropic $GABA_A$ receptors, which can be localized both pre- or postsynaptically. GABA signaling is terminated by reuptake of the neurotransmitter via a special class of plasma-membrane transporters - GATs that are expressed both in synaptic terminals and in surrounding glia (Cherubini and Conti, 2001). Namely, GAT-1 is a transporter essentially expressed in neurons while GAT-3 is an important GABA carrier in astrocytes. Following reuptake, GABA is transaminated by a transaminase termed GABA-T for further reutilization (Kilb, 2012; Owens and Kriegstein, 2002).

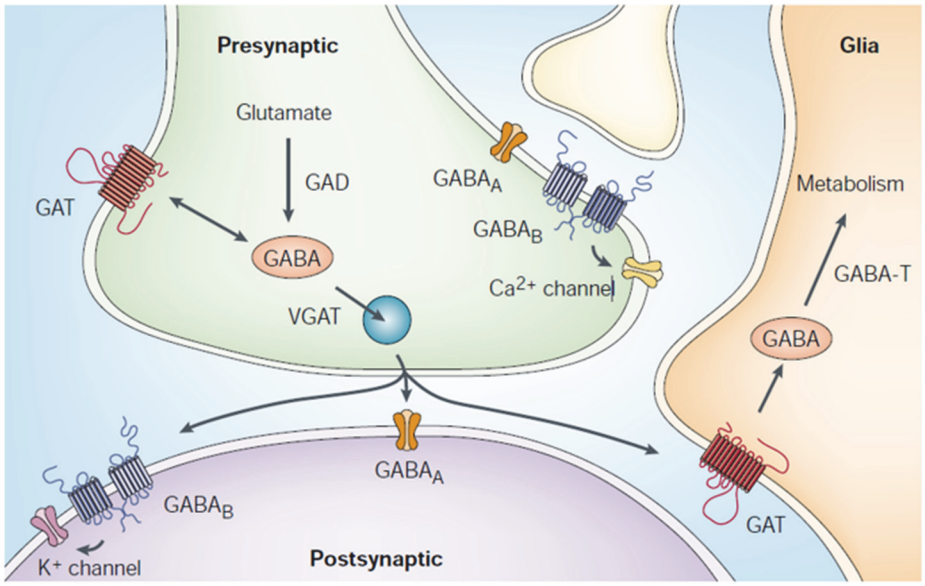



**Fig.11. GABAergic neurotransmission.** GABA is synthetized by the glutamic acid decarboxylase (GAD) and loaded via VGAT transporter into synaptic vesicles. After its release, GABA can bind and activate its ionotropic $GABA_A$ receptors or metabotropic $GABA_B$ receptors. GABA signaling is terminated by reuptake of GABA by GAT transporters, which can be expressed both in neurons and glia. After reuptake, GABA can be further metabolized by glutamate transaminase (GABA-T) for reutilization (Owens and Kriegstein, 2002).

## 3.3 $GABA_B$ receptors

$GABA_B$ receptors are metabotropic receptors that were first identified in the early 1980s (Hill and Bowery, 1981; Bowery et al., 1980). $GABA_B$ receptor signaling occurs through activation of a heterotrimeric G protein that belongs to the $G_{i/o}$ family, which inhibits the production of cAMP from ATP. $GABA_B$ receptor-mediated inhibition has slower kinetics as compared to the $GABA_A$. Indeed, G proteins transduce signals by activating or inhibiting complex signaling cascades involving effectors, second messengers and enzymes. In the case of neurotransmitter metabotropic receptors, activation of these signaling cascades most of the time results in a modulation of channel and/or other receptor activity. $GABA_B$ receptors are expressed both pre- and postsynaptically. They use different mechanisms to regulate cell excitability depending on their localization. Indeed, presynaptic inhibition occurs through a $GABA_B$ receptor-mediated reduction in calcium current at the synaptic terminal triggering a decrease in neurotransmitter release. This type of inhibition can result either in an excitation

or an inhibition of the postsynaptic neuron depending on what type of neurotransmitter is prevented from being released. For instance, if a $GABA_B$ presynaptic autoreceptor inhibits release of GABA this is going to favor depolarization of the postsynaptic cell. This effect is termed disinhibition. Conversely, a $GABA_B$ presynaptic heteroreceptor that blocks glutamate release will trigger inhibition of the postsynaptic neuron. Postsynaptic $GABA_B$ receptor-mediated inhibition is more direct as it activates potassium currents that hyperpolarize and inhibit the postsynaptic cell (Bormann, 1988; Owens and Kriegstein, 2002; Heaney and Kinney, 2016).

Structure-wise, $GABA_B$ receptors form heterodimers consisting of one $GABA_{B1}$ (1a or 1b) and one $GABA_{B2}$ subunit (Emson, 2007). It is generally admitted that the $GABA_{B1a}/GABA_{B2}$ heterodimer localizes to presynaptic neurons whereas the $GABA_{B1b}/GABA_{B2}$ containing receptors are postsynaptic (Kohl and Paulsen, 2010; Heaney and Kinney, 2016).

Solid evidence indicates that $GABA_B$ receptors antagonism enhances synaptic plasticity and LTP (Morrisett et al., 1991; Olpe et al., 1993). Conversely, overexpression of both the $GABA_{B1a}$ (Wu et al., 2007) and the $GABA_{B1b}$ (Stewart et al., 2009) receptor isoforms reduce LTP in the hippocampus of transgenic mice (Heaney and Kinney, 2016). In addition, $GABA_{B1a}^{-/-}$ mice are unable to induce LTP (Vigot et al., 2006). Based on these observations, it is clear that $GABA_B$ receptors are important regulators of neuronal plasticity.

Concerning $GABA_B$ receptors in the context of AD, there is evidence for a decrease in $GABA_B$ receptor content in certain hippocampal regions like in the dentate gyrus and the CA1 region (Chu et al., 1987) as well as in the cerebral cortex (Young, 1987) of AD patients versus control individuals. The distribution and intensity of expression of the $GABA_{B1}$ subunits has been shown to evolve following the progression of NFTs in the hippocampus. For instance, during early stages, its content remained constant or increased while during advanced stages of NFT pathology $GABA_{B1}$ staining decreased in the hippocampus (Iwakiri et al., 2005; Nava-Mesa et al., 2014).

## 3.4 $GABA_A$ receptors

$GABA_A$ receptors are ligand-gated ionotropic receptors with a central pore permeable to anions, essentially to $Cl^-$ and to a lesser extent to $HCO_3^-$. They are members of the Cys loop ligand-gated ion channel superfamily that also includes nicotinic acetylcholine receptors, glycine receptors and ionotropic 5-HT3 serotonin receptors (Sigel and Steinmann, 2012). All these receptors share a motif composed of two cysteine residues separated by 13 amino acid residues. $GABA_A$ receptors are heteropentameric and therefore consist of five subunits among 19 known receptor subunits: α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3. This great number of possible combinations results in a complex heterogeneity of $GABA_A$ receptor subtypes with distinct pharmacological and physiological properties (Mody and Pearce, 2004). In some cases, up to 8 different subunit isoforms were shown to be expressed simultaneously in a single cell (Sigel and Steinmann, 2012). The most common $GABA_A$ receptor in the brain is considered to be composed of α1, β2, and γ2 (McKernan and Whiting, 1996; Sieghart and Sperk, 2002). In a few less abundant subtypes, a δ, ε, or π subunits can be found instead of the γ subunit, and θ subunit can be expressed instead of the β subunit. Distinct receptor subtypes have specific regional, cellular and temporal expression patterns (Olsen and Sieghart, 2008). For instance, α1–3, βn, and γ2 subunit containing $GABA_A$ receptors are mainly synaptic, whereas α4–6 and δ-containing receptors are mainly peri- or extrasynaptic (Belelli et al., 2009; Farrant and Kaila, 2007). While synaptic receptors mediate fast phasic inhibition, extrasynaptic receptors are responsible of persistent tonic inhibition. Phasic inhibition results from classical activation of $GABA_A$ receptors following exposure to activity-dependent synaptic GABA release. Tonic inhibition takes place in a less spatially and temporally restricted manner due to activation of $GABA_A$ receptors at extrasynaptic sites by low concentration of GABA that has escaped from the synaptic cleft or "spilled over" (Farrant and Nusser, 2005). It is admitted that phasic (synaptic) currents play a role in synchronizing network activity whereas tonic currents modulate basal excitability of neuronal networks (Semyanov et al., 2004). $GABA_\rho$ receptors, also known as $GABA_C$ receptors, are now recognized as a subclass of $GABA_A$ receptors, and are highly expressed in the retina (Johnston, 2002). Typically, they are homomeric or heteromeric GABA-responsive ionotropic receptors that differ by their pharmacology from the other $GABA_A$ receptor subtypes (Olsen and Sieghart, 2008).

GABA can exert its inhibitory effects by two $GABA_A$-dependent mechanisms: conductance-based (shunting) and voltage-based (hyperpolarization) inhibition. Shunting inhibition is a short-circuit process explained by an increase in conductance resulting from $GABA_A$ receptor activation that suppresses the spatial and temporal summation of depolarizing membrane responses to excitatory currents or EPSCs (Sjostrom et al., 2008; Gidon and Segev, 2012; Blaesse et al., 2009). Voltage-based inhibition consists of activation of synaptic $GABA_A$ receptors by synaptically released GABA, which causes the flow of $Cl^-$ ions inside the responding neuron, hyperpolarizing the neuronal membrane and causing the appearance of what is termed an inhibitory postsynaptic potential (IPSP) (Farrant and Nusser, 2005; Kaila et al., 2014).

Even though GABA is mainly hyperpolarizing and inhibitory in the mature brain, its actions are primarily depolarizing and sometimes excitatory in the developing brain (Ben-Ari et al., 1989; Cherubini et al., 1991; Ben-Ari, 2002). Depolarizing GABAergic signaling is necessary for promotion of neuronal network development. Once the development is completed, GABA signaling becomes hyperpolarizing and contributes to neuronal activity regulation by preventing excessive excitation. This difference in GABAergic signaling polarity between the immature and mature brain is due to a gradual change in intraneuronal $Cl^-$ concentration that appears during development. Indeed, in immature neurons intraneuronal $Cl^-$ concentration is high while in mature neurons it is low. This influences the direction and the magnitude of the $Cl^-$ flux mediated by the $GABA_A$ receptors and accordingly the polarity of the GABAergic signaling (Kaila et al., 2014; Blaesse et al., 2009).

## 3.5 Neuronal chloride homeostasis

### 3.5.1 Cation-chloride cotransporters

Intraneuronal $Cl^-$ concentration ($[Cl^-]_i$) is regulated by cation chloride cotransporters or CCCs. They were first described and analysed in peripheral tissues. The notion that there must exist a transport system for chloride in neurons dates back to 1970s when it was shown using microelectrodes and the giant neuron of the *Aplysia* abdominal ganglion that the equilibrium potential for $Cl^-$ was less negative than the membrane potential, arguing for an active

extrusion of $Cl^-$ in these cells (Russell and Brown, 1972; Ascher et al., 1976). Later, Thompson et al. have detected lower $[Cl^-]_i$ than could be predicted by a passive distribution of this ion in cortical and hippocampal neurons of vertebrates (Thompson et al., 1988a; Thompson et al., 1988b; Thompson and Gahwiler, 1989a; Thompson and Gahwiler, 1989b). The first CCCs to be characterized at the molecular level were the $Na^+$-$Cl^-$ cotransporter NCC and the $Na^+$-$K^+$-$2Cl^-$ cotransporters NKCC1 and NKCC2 (Gamba et al., 1993; Xu et al., 1994; Gamba et al., 1994). In addition, in these studies it was hypothesized that there should exist a neuron specific $K^+$-driven export of chloride. It was only in the mid-1990s that was discovered a CCC abundantly expressed in neurons and a potential candidate responsible for active $Cl^-$ transport in these cells – KCC2 (Payne et al., 1996).

In mammals, the CCCs family includes 9 members that are encoded by the genes *Slc12a1-9*. CCCs family members are glycoproteins with a molecular weight ranging from 120 to 200 kDa. There are three well characterized subcategories of CCCs: the $Na^+$-$K^+$-$2Cl^-$ cotransporters (NKCC1 and NKCC2), $Na^+$-$Cl^-$ cotransporter (NCC) and $K^+$-$Cl^-$ cotransporters (KCC1, KCC2, KCC3, KCC4). The belonging of the two remaining CCCs, CIP1 (a CCC interacting protein) and CCC9 (a polyamine transporter), to the CCC family was questioned due to their lack of CCC transport function (Hartmann et al., 2014).

CCCs are expressed in all organs, and in addition to their importance for neuronal signaling within the CNS, they are involved in different physiological processes like cell volume regulation, transepithelial ion transport, neuroendrocrine signaling and blood pressure regulation (Kaila et al., 2014). NCC and NKCC2 are preferentially expressed in kidney while all the other CCCs have a very wide distribution in the CNS and some, like NKCC1 and KCC3, are also expressed in the peripheral nervous system (Blaesse et al., 2009; Kaila et al., 2014).

KCC1 is considered to be a “household” CCC expressed in various cell types but its expression is low in the CNS (Payne et al., 1996; Rivera et al., 1999; Rust et al., 2007). KCC3 has largely an unknown function even though it is highly expressed in developing CNS (Le et al., 2006; Pearson et al., 2001; Boettger et al., 2003; Kaila et al., 2014). KCC4 is also highly expressed in the developing brain especially around the ventricular zone but also has an elusive function (Li et al., 2002).

The predicted molecular structure of CCCs consists of 12 membrane spanning segments flanked by large intracellular N- and C-terminal domains (Payne, 2012; Blaesse et al., 2009)

(Kaila et al., 2014). Hetero- and homodimers formation has been described for nearly all CCCs (Blaesse et al., 2006; Pedersen et al., 2008; Simard et al., 2007; Uvarov et al., 2009). Some studies suggest that this oligomerization has an impact on CCCs transport function especially in the case of KCC2. Indeed, it was first shown that the quantity of higher molecular weight KCC2 complexes paralleled the activation state of the KCC2 transporter in the auditory brainstem (Blaesse et al., 2006). Second, disrupting KCC2 oligomerization affected its function without changing its expression levels in cultured GT1-7 cells (immortalized gonadotropin-releasing hormone neurons) (Watanabe et al., 2009). Third, it was suggested that KCC2 can form macromolecular complexes with kainate-type glutamate receptors and that disrupting this interaction decreased KCC2 activity in hippocampal neurons (Mahadevan et al., 2014). Regarding the other CCCs, the requirement of oligomerization for their transport activity is currently unknown (Hartmann and Nothwang, 2014). In the CNS, the two CCCs that have been largely studied and have been shown to influence GABAergic signaling are NKCC1 and KCC2.

### 3.5.2 NKCC1

NKCC1 is the main cotransporter that plays a role in $Cl^-$ uptake as well as in depolarizing $GABA_A$ signaling in immature neuronal networks. The cotransporter uses the $Na^+$ gradient generated by the $Na^+$-$K^+$-ATPase, to uptake 2 $Cl^-$ ions in an electroneutral fashion (in sum this transporter carries 1 $K^+$ and 1 $Na^+$ for 2$Cl^-$), so that activity of the transporter neither generates a current nor is affected by membrane potential (**Fig.12**). NKCC1 is encoded by the gene *Slc12a2.* In the CNS it is expressed in neurons but also in astrocytes (Hubner et al., 2001a; Price et al., 2006). NKCC1 has 2 splice variants NKCC1a and NKCC1b. NKCC1b lacks exon 21 and is more abundantly expressed than NKCC1a in the adult human brain (Morita et al., 2014; Vibat et al., 2001). NKCC1 expression has been suggested to undergo a developmental downregulation in rat and human brain (Hubner et al., 2001a; Yamada et al., 2004; Plotkin et al., 1997). However, other studies have shown an increase in NKCC1 mRNA levels during development in human (**Fig.13**) and rodent CNS (Yan et al., 2001; Mikawa et al., 2002; Clayton et al., 1998; Wang et al., 2002). The downregulation of NKCC1 mRNA levels observed has been considered irrelevant and could be explained by primers used in these studies that would target exon 21 and would thus detect NKCC1a and not NKCC1b (Kaila et al., 2014). Knock-out of the *Slc12a2* gene encoding NKCC1 results in inner ear functional defects, but no overt neurological

phenotype (Payne et al., 2003). Furthermore, discrepant results were obtained concerning the impact of NKCC1 on excitability with one study finding decreased excitability in mice lacking NKCC1 (Dzhala et al., 2005) and others showing that NKCC1 prevents hyperexcitability (Zhu et al., 2008).

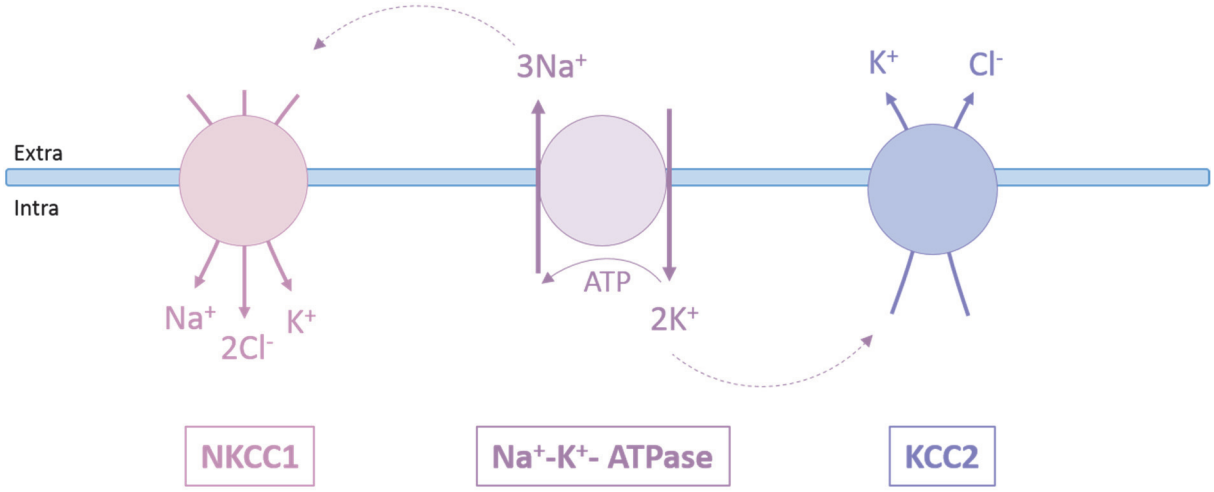



**Fig.12. NKCC1 and KCC2: the two main neuronal chloride cotransporters.** NKCC1 uses the $Na^+$ and $K^+$ gradients generated by the $Na^+$-$K^+$-ATPase, to uptake 2 $Cl^-$ ions together with 1 $Na^+$ and 1$K^+$. KCC2 uses the $K^+$ gradient generated by the $Na^+$-$K^+$-ATPase to extrude 1$Cl^-$ together with 1$K^+$. Both of these transporters are electroneutral meaning that neither the activity of the transporters generates current nor is regulated by membrane potential.

NKCC1 is highly sensitive to $[Cl^-]_i$ and can be regulated by phosphorylation. Indeed, a decrease in $[Cl^-]_i$ below a certain physiological 'set point' triggers phosphorylation of NKCC1 on several threonine residues located in the N-terminus of the protein thereby stimulating its activity and restoring $[Cl^-]_i$ (Lytle and Forbush, III, 1996; Haas et al., 1995; Darman and Forbush, 2002; Vitari et al., 2006). Furthermore, it was suggested that NKCC1 and KCC2, which exert opposite functions, would be regulated by phosphorylation and $[Cl^-]_i$ in a reciprocal manner (Kaila et al., 2014; Kahle et al., 2005).

### 3.5.3 KCC2

KCC2 is by far one of the most well studied CCC in the CNS. It is an electroneutral transporter that carries $K^+$ and $Cl^-$ ions in 1:1 stoichiometry (Payne, 1997) (**Fig.12**). The direction of the ion transport depends on the chemical gradients of $K^+$ and $Cl^-$. In normal physiological conditions,

it is a potent chloride extruder. An important and unique characteristic of KCC2 is that it is a constitutively active KCC transporter. Indeed, while KCC1, KCC3 and KCC4 only become active after an osmotic stress, KCC2 is active in isotonic conditions (Medina et al., 2014).

KCC2 is encoded by the gene *Slc12a5.* In the CNS, its expression is restricted to neurons and it is the only KCC isoform that is not expressed in glia (Payne et al., 1996; Gagnon et al., 2007). There are 10 putative transcription binding sites in the promoter of *Slc12a5* gene (Uvarov et al., 2006). Among these 10 sites, an E-box has been identified, and it was shown that it can be bound by upstream stimulating factors USF1 and USF2 that are able to regulate KCC2 expression (Markkanen et al., 2008). Another transcription factor described to be able to regulate the *Slc12a5* gene expression is early growth response 4 (Egr4) (Ludwig et al., 2011; Aguado et al., 2003). Alternative splicing of *Slc12a5* results in two KCC2 isoforms KCC2a and KCC2b that differ by their N-terminus (Uvarov et al., 2007). While mRNA levels of these two isoforms are more or less comparable in neonatal mouse brain, during postnatal development KCC2b isoform undergoes an important upregulation and therefore constitutes up to 90% of total KCC2 mRNA pool in the adult murine cortex (Uvarov et al., 2007; Uvarov et al., 2009). Analysis of mice lacking KCC2 has allowed to uncover some specific functions of the two isoforms a and b. Complete *Slc12a5* knock-out mice die immediately after birth due to severe motor deficits and respiratory failure. In contrast, mice lacking KCC2b are viable but show abnormal posture and gait as well as frequent seizures starting from a couple of days after birth that probably trigger the premature death of these animals that occurs at around the 2nd postnatal week (Woo et al., 2002). These results indicate that KCC2a would be important for some basic brain function that would allow animals to survive for at least 2 weeks.

In the rodent cortex, robust upregulation of KCC2 starts around the time of birth and KCC2 expression continues to gradually increase during the first postnatal month (Wang et al., 2002; Rivera et al., 1999; Li et al., 2002) (for evolution of KCC2 expression in human see **Fig.13**). Interestingly, low levels of KCC2 protein can be also detected at early embryonic stages (Li et al., 2002; Horn et al., 2010), and overexpression and knock-out studies indicate that KCC2 would play a critical structural role in the maturation of neurons appearing during fetal development (Hubner et al., 2001b; Khalilov et al., 2011). Indeed, several evidences point out that KCC2 could exert a morphogenic role in the development of synapses that would be

independent of its ion transport function and could be possibly mediated by interactions with the spine cytoskeleton (Li et al., 2007; Fiumelli et al., 2013; Horn et al., 2010; Gauvain et al., 2011). These finding are furthermore supported by the fact that dendritic levels of KCC2 are very high (Gulyas et al., 2001). Intriguingly, an involvement of KCC2 in excitatory neurotransmission fine-tuning was also shown. By decreasing expression of KCC2, Gauvain et al. observed an increase in the lateral diffusion of GluA1, leading to reduced postsynaptic clusters of GluA1-containing AMPA receptors, and finally to a reduced efficacy of excitatory neurotransmission (Gauvain et al., 2011). In light of these findings, it was suggested that KCC2 could be able to regulate both excitatory and inhibitory synaptic neurotransmission not only during maturation but probably also in mature networks where the aforementioned structural functions of KCC2 could contribute to synaptic plasticity and memory (Blaesse and Schmidt, 2015).

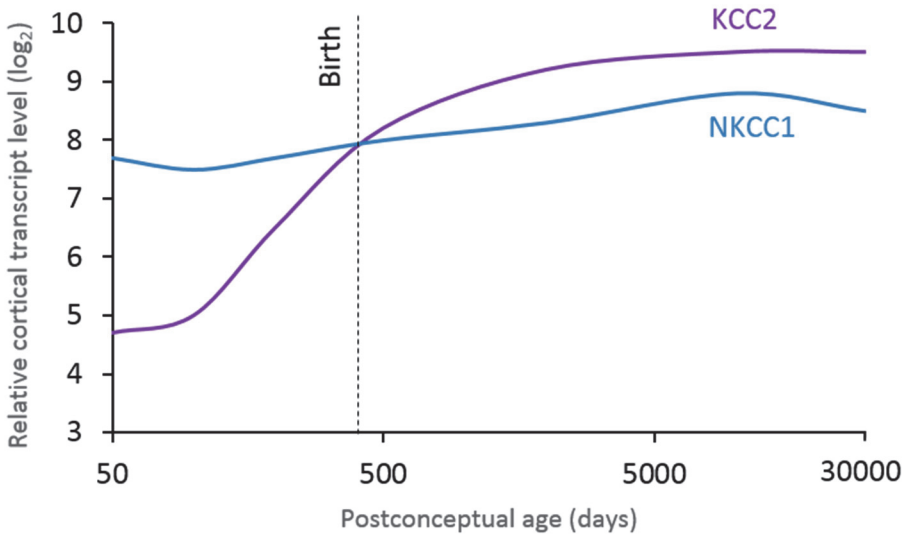



**Fig.13. Evolution of KCC2 and NKCC1 expression levels in the human neocortex during life.** Line plots representing the evolution of transcripts of KCC2 and NKCC1 in the human neocortex from early fetal period to late adulthood (Adapted from: http://hbatlas.org/ and (Kaila et al., 2014)).

As KCC2 protein expression shows great concordance with mRNA levels (Lu et al., 1999; Li et al., 2002; Stein et al., 2004), it was suggested that control of KCC2 mRNA abundance is an efficient way to regulate long-term activity of this cotransporter (Medina et al., 2014). Several mechanisms have been shown to modulate KCC2 expression. For instance, its neuron-specific expression pattern is ensured by two neuron-restrictive silencing elements (NRSE) in its gene

that can be recognized by a transcriptional silencer - neuron-restrictive silencing factor (NRSF) (Karadsheh and Delpire, 2001; Uvarov et al., 2005; Yeo et al., 2009). An important regulatory role in KCC2 expression was described to be played by brain-derived neurotrophic factor (BDNF). BDNF is thought to be able to promote the developmental increase in KCC2 mRNA levels by binding to its tropomyosin-related kinase B (TrkB) receptor and activating a signaling cascade involving extracellular signal-regulated kinases 1 and 2 (ERK1-2). The final effector of this cascade is the Egr4 transcription factor (Ludwig et al., 2011; Aguado et al., 2003). It is noteworthy that, while application of BDNF to immature neurons increases KCC2 expression (Ludwig et al., 2011), exposure of mature neurons to BNDF causes a decrease in KCC2 mRNA and protein levels as well as a decrease in tyrosine-phosphorylation and membrane insertion of this cotransporter (Rivera et al., 2002; Shulga et al., 2008; Rivera et al., 2004; Wake et al., 2007; Boulenguez et al., 2010).

Phosphorylation mechanisms are also highly involved in fine-tuning KCC2 activity. KCC2 residue serine 940 (S940) can be phosphorylated by PKC and this post-translational modification can enhance KCC2 activity (Lee et al., 2007; Lee et al., 2011). There are also two threonine residues, which are conserved in other KCC members, T906 and T1007, whose phosphorylation inhibits KCC2 transport activity (Rinehart et al., 2009). Interestingly, T906 site could play a role in functional up-regulation of KCC2 activity during development since it was shown to be partially phosphorylated in murine neonatal brain and dephosphorylated in adults (Medina et al., 2014). Tyrosine phosphorylation of KCC2 has also been shown in a few studies but its physiological significance remains elusive (Stein et al., 2004; Wake et al., 2007) (Watanabe et al., 2009; Lee et al., 2010a). So far, 2 tyrosine residues have been identified as phosphorylation targets in KCC2, Y903 and Y1087 (Lee et al., 2010a). While the importance of Y903 phosphorylation site is not very well characterized, it has been evidenced that phosphorylation of Y1087 residue decreases KCC2 activity (Chudotvorova et al., 2005; Akerman and Cline, 2006; Pellegrino et al., 2011).

Interestingly, KCC2 can be also regulated by $Zn^{2+}$ in a bidirectional manner. For instance, micromolar concentration of intracellular $Zn^{2+}$ has been shown to inhibit KCC2 activity (Hershfinkel et al., 2009). In contrast, extracellular $Zn^{2+}$ released from mossy fibers strongly increases KCC2 surface expression and therefore activates it (Chorin et al., 2011).

Nevertheless, the underlying causes and consequences of this regulation require further investigation.

KCC2 has also been shown to be able to interact with various proteins. For example, it was shown to interact with brain-type creatine kinase (Inoue et al., 2004), $Na^+$-$K^+$-ATPase (Ikeda et al., 2004), protein associated with Myc (PAM) (Garbarini and Delpire, 2008). Not much is known about the functional significance of these interactions. In addition, it was shown that KCC2 could interact with cytoskeleton-associated protein 4.1N and that interrupting this interaction caused enhanced lateral diffusion of KCC2 away from excitatory synapses indicating that 4.1N would be important for local stabilization of KCC2 at the plasma membrane (Li et al., 2007; Chamma et al., 2013). Other proteins interacting with KCC2 are neuropilin and tolloid like-2 (Neto2) (Ivakine et al., 2013). Loss of Neto2:KCC2 interaction was shown to reduce the activity of this cotransporter. Furthermore, Neto2 would be required for maintenance of stable levels of KCC2 (Medina et al., 2014).

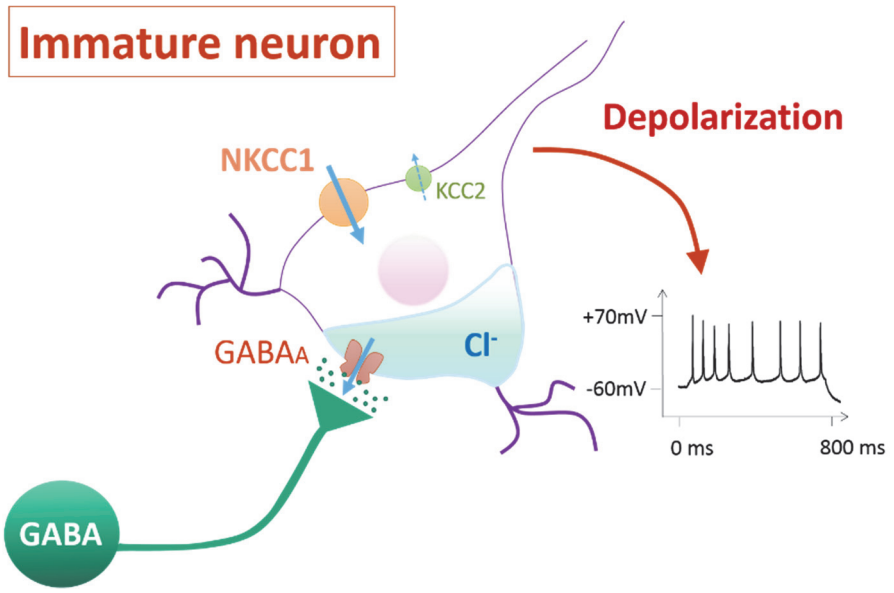
It is worth mentioning that KCC2 is also a target of the plasticity-associated protease calpain. Indeed, activation of NMDA receptors in hippocampal or spinal cord neurons was shown to induce KCC2 cleavage by calpain (Puskarjov et al., 2012; Zhou et al., 2012),. As an add-on, increased neuronal activity also leads to calpain-mediated cleavage of KCC2, triggering dispersal of KCC2 clusters within the plasma membrane (Chamma et al., 2013). These findings are of particular interest since calpain is highly involved in synaptic plasticity and learning regulation and could highlight the importance of KCC2 in these mechanisms.

### 3.5.4 GABAergic signaling polarity

During development there is a shift of GABAergic transmission polarity from depolarizing to hyperpolarizing (Ben-Ari et al., 1989; Cherubini et al., 1991; Ben-Ari, 2002; Valeeva et al., 2016). This is due to a gradual change in $[Cl^-]_i$ from high to low that results from a developmental upregulation of KCC2 expression and activity. This maturation-related decrease in $[Cl^-]_i$ does not appear in other cell types and is very specific to neurons. Thus, although neuroscientists classically regard the immature neurons with their high $[Cl^-]_i$ as aberrant, it is actually the mature neurons with low $[Cl^-]_i$ that are quite exceptional. In

immature neurons $[Cl^-]_i$ is typically in the range of 25-40mM whereas in mature neurons $[Cl^-]_i$ is around 5mM (Blaesse et al., 2009). GABA-dependent depolarization of the neuronal membrane results from an outward flow of $Cl^-$ ions through the $GABA_A$ receptor (**Fig.14**). In the meantime, hyperpolarizing GABA responses are due to an inward flow of $Cl^-$ ions into the cell through the $GABA_A$ receptor. There exists a misleading consideration that depolarizing GABA responses are necessarily excitatory as opposed to hyperpolarizing responses that are always inhibitory. It is important to keep in mind that GABA can also exert its inhibitory action through shunting inhibition that acts by decreasing temporal and spatial summation of excitatory inputs. Therefore, slightly depolarizing GABA responses can still be inhibitory (Blaesse et al., 2009). On the other hand, strongly depolarizing GABA responses can indeed facilitate the triggering of action potentials by glutamatergic neurons (Gao et al., 1998; Jean-Xavier et al., 2007; Leinekugel et al., 1997; Khalilov et al., 1999; Ben-Ari et al., 2007). Furthermore, it is important to keep in mind that depolarizing GABA responses can have a very important physiological impact even without triggering action potentials. Indeed, in particular during development, depolarization caused by GABA is known to activate voltage-gated calcium channels and NMDA receptors. The resulting entry of $Ca^{2+}$ can then trigger numerous signaling cascades and impact neuronal functioning (Ben-Ari et al., 2007).

a.




b.

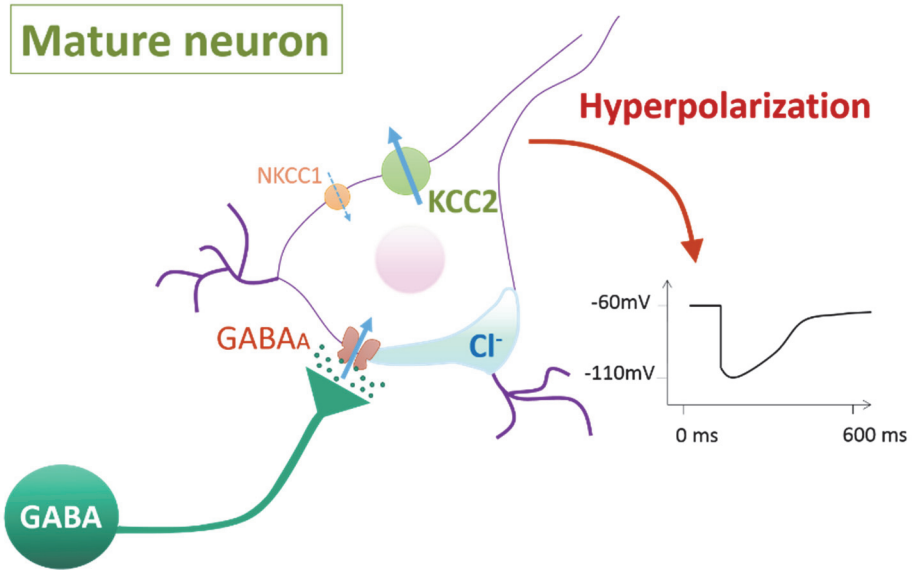



**Fig.14. GABAergic signaling polarity during development.** (**a**) In immature neurons, expression of KCC2 versus NKCC1 is low. Therefore, efflux of $Cl^-$ is decreased and results in higher intraneuronal $Cl^-$ level. In this case, the opening of $GABA_A$ receptors in response to GABA causes efflux of $Cl^-$ through the receptor and a depolarization of the neuronal membrane. In some cases this depolarization is great enough to result in an excitation of the responding neuron. (**b**) In mature neurons, expression of KCC2 versus NKCC1 is increased, which leads to a greater efflux of $Cl^-$ and lower intraneuronal $Cl^-$ level. In this case, the opening of $GABA_A$ in response to GABA causes entrance of $Cl^-$ through the receptor and a hyperpolarzation of the neuronal membrane inhibiting the responding cell.

The timing of the developmental GABAergic polarity shift depends on the species studied as well as on the brain region examined. For instance, rats are born with very low KCC2 expression levels and undergo an important KCC2 upregulation around the 2nd postnatal

week. On the other hand, guinea pigs experience an upregulation of KCC2 before birth (Rivera et al., 1999). In humans, KCC2 mRNA levels rise drastically during the second half of gestation (Vanhatalo et al., 2005; Kaila et al., 2014; Dzhala et al., 2005) (**Fig.13**). From a region point of view, KCC2 expression first appears in the spinal cord, then subcortical brain regions and finally in the cortex (Li et al., 2002; Rivera et al., 1999; Vinay and Jean-Xavier, 2008). There is also sex-specificity for this shift since in various brain regions, hyperpolarizing GABA responses occur earlier in females (Galanopoulou, 2008; Perrot-Sinal et al., 2007).

From a functional point of view, depolarizing GABAergic signaling promotes opening of voltage-gated $Ca^{2+}$ channels and activation of NMDA receptors (Ben-Ari et al., 2007; Fukuda et al., 1998; Hennou et al., 2002; Kandler and Gillespie, 2005; Serafini et al., 1995). These responses lead to activation of downstream $Ca^{2+}$-dependent signaling cascades which play a central role in mediating the trophic effects of GABA during maturation (Ben-Ari et al., 2007; Owens and Kriegstein, 2002).

Depolarizing GABA signaling can be also observed in adult brain in certain brain regions where it probably plays an important functional role. For example, in the suprachiasmatic nucleus, which regulates the sleep-wake cycle, it has been observed that $GABA_A$ responses are hyperpolarizing during the day and depolarizing at night (Gribkoff et al., 1999; De and Pennartz, 2002). In addition, certain cell types may have a different $[Cl^-]_i$ than their surrounding cells and respond to GABA by a depolarization in the mature brain. Indeed, in the cortex and amygdala fast-spiking neurons were found to exhibit depolarizing $GABA_A$ responses compared to regular spiking neurons (Martina et al., 2001; Martina et al., 2001). In the hippocampus, even more specific effects were observed: the same interneurons would cause depolarization of dentate gyrus granule cells but could hyperpolarize the CA1 pyramidal neurons (Sauer et al., 2012).

To make the matter even more complicated, within one cell there exist differences in $Cl^-$ gradient that result in distinct GABA responses whether you are in the soma or in a proximal or distal dendrite. Indeed, differential $GABA_A$ receptor-mediated signaling within a single cell was fist observed already a long time ago (Alger and Nicoll, 1979). More recently, it was confirmed in the neocortex that in the same neuron somatic $GABA_A$ mediated responses were hyperpolarizing while dendritic ones were depolarizing (Gulledge and Stuart, 2003). In addition, increased $[Cl^-]_i$ was found together with consequent depolarizing $GABA_A$ signaling in the initial segment of the axon together with a lack of KCC2 staining and increased presence

of NKCC1 at that site (Khirug et al., 2008). Furthermore, imaging experiments have directly evidenced the existence of $Cl^-$ concentration gradients in retinal "on"-type bipolar cells (Duebel et al., 2006).

Taken together, these arguments point to the complexity and significance of GABAergic neurotransmission polarity fine-tuning.

### 3.5.5 Modifications in NKCC1 and KCC2 expression levels in CNS disorders

The importance of GABAergic signaling polarity has been furthermore supported by the fact that alterations of these mechanisms have been observed in many CNS pathologies. The most well documented of these is epilepsy. Epilepsy is essentially due to an excitation/inhibition imbalance, and given the important role that NKCC1 and KCC2 play in controlling the efficacy of inhibition, it is not surprising that disturbance in their expression and functioning has been observed in epileptic brains.

In 2002, excitatory GABAergic responses were measured in subicular pyramidal cells of hippocampal slices obtained from epileptic patients and were hypothesized to be due to a loss of KCC2 expression (Cohen et al., 2002). This hypothesis was confirmed in a follow-up study that found a decrease in KCC2 mRNA and protein levels in a subset of pyramidal neurons of the subiculum in these patients (Huberfeld et al., 2007). In addition, interictal activity (abnormal activity that appears in between epileptic events) could be blocked by bumetanide, a specific NKCC1 antagonist, in hippocampal slices obtained from epileptic patients, suggesting that a pathological neuronal chloride overload could be at the origin of this activity (Huberfeld et al., 2007). In line with these findings, reduced KCC2 expression was also observed in the hippocampus of temporal lobe epilepsy animal models, triggering a decrease in the inhibition force and neuron excitability enhancement (Li et al., 2008; Pathak et al., 2007). NKCC1 has also been involved in epileptiform activity generation since NKCC1-dependent chloride accumulation is thought to be involved in the appearance of neonatal seizures in rodents (Dzhala et al., 2010; Glykys et al., 2009).

It is not clear whether alteration in chloride homeostasis is a cause or a consequence of seizures. There is some evidence that changes in $Cl^-$ homeostasis would favor the appearance of epileptiform activity. Indeed, in transgenic animals where KCC2 expression level is

decreased, recurrent seizures have been reported (Riekki et al., 2008; Tornberg et al., 2005; Woo et al., 2002; Zhu et al., 2005). In a recent study, it has been shown *in vitro* that decreasing KCC2 expression resulted in an increase of spontaneous epileptiform bursting, while overexpression of KCC2 protected against convulsant-induced epileptiform activity. Furthermore, knock-down of KCC2 in the rat dentate gyrus induced seizure like behaviors and epileptiform EEG suggesting the involvement of KCC2 in seizure triggering (Chen et al., 2017a). Finally, recent identification of rare point variants in KCC2 (KCC2-R952H in an Australian family with early childhood onset of febrile seizures and KCC2-R1049H in a French-Canadian cohort with idiopathic generalized epilepsy) has provided additional evidence in favor of a causative role of KCC2 activity alteration in epilepsy (Puskarjov et al., 2014; Kahle et al., 2014).

In the meantime, some studies have shown that increased neuronal activity induced by glutamate application or repetitive stimulation of excitatory synapses could cause downregulation of KCC2 in an NMDA dependent manner (Lee et al., 2011; Puskarjov et al., 2012; Kitamura et al., 2008). Furthermore, in an elegant study using three independent chambers containing 2 interconnected hippocampi and their commissural fibers that can be perfused separately, kainate treatment of one hippocampus induced generation of secondary epileptic foci in the non-treated hippocampus. In this model, excitatory GABAergic signaling could be measured in the secondary epileptic foci therefore indicating that epileptic seizures themselves could trigger a reversal in GABAergic neurotransmission polarity that could be due to a decrease in KCC2 expression or activity (Khalilov et al., 2003).

GABAergic neurotransmission polarity can also change in the absence of KCC2 expression modifications (Kaila et al., 1997). Epileptic seizures are associated with large extracellular $K^+$ transients (Avoli et al., 2005; Kaila et al., 1997), and a pathological increase in $K^+$ extracellular concentration could lead to a vicious circle that would not only render the membrane potential more depolarized but also favor depolarizing GABAergic responses (Kakazu et al., 2000; Payne et al., 2003). Indeed, as KCC2 uses $K^+$ gradient to extrude $Cl^-$, if extracellular concentration of $K^+$ rises, the extruding capacity of KCC2 will be reduced.

Altered chloride homeostasis has been observed in a number of other pathologies. For instance, in rodent models of chronic pain a decrease in KCC2 expression and activity has been observed in certain regions of the spinal cord and is thought to contribute to the pathophysiology of this disease (Coull et al., 2003; Price et al., 2005). KCC2 expression was also

shown to be decreased following traumatic brain injury (Nabekura et al., 2002; Shulga et al., 2008). Furthermore, imbalanced chloride homeostasis has been also suggested to be involved in certain psychiatric disorders such as schizophrenia (Hyde et al., 2011) and autism (Lemonnier and Ben-Ari, 2010; Ben-Ari, 2015). In the valproate and fragile X rodent models of autism, maternal oxytocin-induced transient shift in GABAergic signaling from depolarizing to hyperpolarizing that occurs in the fetal brain during delivery (Tyzio et al., 2006), was shown to be absent. Furthermore, the autistic phenotype of these animals could be improved by treatment of the mother prior to the delivery with bumetanide (NKCC1 antagonist). In opposition, inhibiting oxytocin signaling in wild-type rats triggered the appearance of autistic-like behaviors in newborns (Tyzio et al., 2014). In the past years, several studies have used bumetanide to treat autistic patients and this treatment resulted in alleviation of certain of their symptoms (Cellot and Cherubini, 2014; Grandgeorge et al., 2014; Hadjikhani et al., 2015; Lemonnier and Ben-Ari, 2010; Lemonnier et al., 2012; Lemonnier et al., 2017). Although the exact mechanism of this beneficial action remains unknown, the results of these studies are promising and require further investigation.

Given the great number of CNS pathologies where altered chloride homeostasis was observed, it is tempting to speculate that this mechanism plays an essential role in normal brain functioning.

## 3.6 GABAergic neurotransmission in AD

In the context of AD, GABAergic neurotransmission remained a lot less studied for a long period of time than its excitatory counterpart. Early studies have shown that GABAergic neurons and receptors were relatively well preserved in the brains of AD patients (Rossor et al., 1982; Reinikainen et al., 1988; Hardy et al., 1987). However, recent evidence seems to indicate that GABAergic neurotransmission also undergoes pathological changes in AD (Govindpani et al., 2017).

A decrease in glutamate and GABA concentrations has been detected in the temporal cortex of AD patients (Gueli and Taibi, 2013). In addition, reduced GABA levels were also previously observed in the CSF of AD patients and healthy aged individuals (Bareggi et al., 1982; Zimmer

et al., 1984; Grouselle et al., 1998; Govindpani et al., 2017). Furthermore, a decrease in GABA concentration could be measured in various cortical areas including the temporal, frontal, parietal and occipital cortices (Govindpani et al., 2017). Although several publications have shown that GABAergic neurons are relatively spared in AD brains (Rossor et al., 1982; Reinikainen et al., 1988; Fuhrer et al., 2017), there is evidence that the number of GABAergic terminals is reduced in the cortex and hippocampus of AD patients versus healthy individuals (Hardy et al., 1987). In addition, immunocytochemistry experiments have shown decreased staining of perisomatic GABAergic terminals especially on cortical neurons in the vicinity of amyloid plaques in AD patients and in AD mouse models (Garcia-Marin et al., 2009; Ramos-Miguel et al., 2015; Bell and Claudio, 2006; Bell et al., 2003; Marttinen et al., 2015; Govindpani et al., 2017).

Alteration in $GABA_A$ receptors composition was also observed in the brains of individuals suffering from AD. While β2/3 subunits appeared to be well preserved (Mizukami et al., 1998), a downregulation of α1 (Mizukami et al., 1998; Rissman et al., 2004) and a possible upregulation of γ subunits were observed in the hippocampus of human AD subjects (Mizukami et al., 1998; Iwakiri et al., 2009). Changes in $GABA_A$ receptors composition have also been observed in the cortex of AD individuals. Indeed, a decrease in α1, α2, α4, β2 and δ was observed in the prefrontal cortex of AD patients (Luchetti et al., 2011). The functional consequence of an AD-related modification of $GABA_A$ receptor composition was demonstrated in an elegant study by Limon et al., where a microtransplantation of $GABA_A$ receptors isolated from temporal cortex of human AD patients was made into Xenopus oocytes (Limon et al., 2012). In this study, a decrease in $GABA_A$ receptor-mediated currents in cells transplanted with receptors recovered from AD brains was shown. Furthermore, reduced protein and mRNA levels of α1 and γ2 subunits were found, whereas α2, β1 and γ1 subunits were upregulated in these cells (Limon et al., 2012).

Based on these data and on GABAergic neurotransmission involvement in memory formation, it is clear that inhibitory circuit dysfunctions must be further characterized in the context of Alzheimer's disease.

## 3.7 APP and GABAergic neurotransmission

Several studies highlight a potential involvement of APP in GABAergic neurotransmission regulation. In *App*$^{-/-}$ mice, decreased paired-pulse inhibition of GABAergic IPSCs was shown to contribute to the impairment of synaptic plasticity illustrated by LTP and behavioral deficits (Seabrook et al., 1999; Dawson et al., 1999b; Senechal et al., 2008; Senechal et al., 2008). Moreover, in the same model, a lowered number of $GABA_A$ receptors and $GABA_B$ receptors has been observed (Fitzjohn et al., 2000). Furthermore, an interaction of APP with $GABA_B$ receptors has been evidenced both *in vitro* and *in vivo* and could have an impact on their function (Norstrom et al., 2010; Schwenk et al., 2016).

The theta-gamma coupling deficit that exists in the parietal cortex and hippocampus of *App*$^{-/-}$ mice is probably due to alterations of local inhibitory networks (Zhang et al., 2016a). It was also shown that deletion of APP in hippocampal neurons causes an increase in L-type voltage gated $Ca^{2+}$ channels expression leading to a disturbance of GABAergic short-term potentiation (a type of synaptic plasticity that is expressed only presynaptically and lasts milliseconds to minutes versus LTP that is expressed postsynaptically and can last 1 to 3 hours) (Yang et al., 2009). Moreover, in the inducible APP overexpressing model of Born et al., increased susceptibility to picrotoxin-evoked ($GABA_A$ antagonist) seizures with a subsequent tonic progression that suggests a GABAergic neurotransmission alteration, was observed in addition to an increase in GABAergic innervation (Born et al., 2014). Finally, in a mouse model of Down syndrome (DS) that carry an extra copy of mouse chromosome 16 syntenic to the long arm of human chromosome 21 (Dierssen, 2012) containing the human *APP* gene (Patterson et al., 1988), an increase in NKCC1 expression leads to excitatory $GABA_A$ receptor signaling (Deidda et al., 2015b).

Taken together these results point to a potential function of APP in GABAergic neurotransmission that requires further investigation.

# Aims

Alzheimer's disease (AD) is the most prevalent cause of dementia characterized by severe memory deficits. Accumulation of amyloid beta peptide (Aβ), derived from the proteolytic cleavage of APP, is one of the main hallmarks of AD. Besides producing Aβ, many physiological functions of APP have been described. Amongst these, APP and/or its catabolites were shown to influence synaptic excitatory and inhibitory neurotransmission.

Cognition and learning are tightly regulated by a balance between excitatory and inhibitory neurotransmission that ensures the stability of cortical activity. Disruption of this balance results in memory impairments such as observed in AD. In the adult central nervous system, γ-amino-butyric acid (GABA) is a major inhibitory neurotransmitter. While in the adult brain GABAergic signaling is hyperpolarizing and inhibitory, in the developing brain, GABA acts as a depolarizing and sometimes even excitatory neurotransmitter. Fast GABAergic inhibition is mediated by activation of ionotropic $GABA_A$ receptors ($GABA_AR$). Since $GABA_AR$ are ligand-gated ion channels that mainly allow the bidirectional flux of $Cl^-$ ions, the nature of GABAergic neurotransmission, depolarizing versus hyperpolarizing, is essentially determined by intracellular concentrations of $Cl^-$ ($[Cl^-]_i$). In the central nervous system (CNS), the two main cation-chloride cotransporters (CCCs) regulating the $[Cl^-]_i$ are $Na^+$-$K^+$-$2Cl^-$ cotransporter 1 (NKCC1) allowing the entrance of $Cl^-$ and $K^+$-$Cl^-$ cotransporter 2 (KCC2), allowing the efflux of chloride.

Several lines of evidence coming from mice lacking or overexpressing APP indicate that APP could play a role in GABAergic neurotransmission. Indeed it has been shown that paired-pulse inhibition of GABAergic IPSCs is significantly decreased in the hippocampus of *App*$^{-/-}$ mice (Dawson et al., 1999b; Senechal et al., 2008). In this same model, the theta-gamma oscillation coupling, involved in inhibitory transmission, was also demonstrated to be impaired (Zhang et al., 2016a). Moreover, in an inducible transgenic model of AD, APP overexpression lead to appearance of seizures and was associated with changes in GABAergic neurotransmission activity and innervation (Born et al., 2014). Despite the significance of these results, the exact relationship between APP and GABA signaling remains not well

understood. Adding up to these findings, our study investigated the link between APP and indirect GABAergic neurotransmission actors - cation-chloride cotransporters NKCC1 and KCC2. We have first assessed whether APP could affect expression levels of NKCC1 and KCC2 in developing neuronal cultures *in vitro*. In this regard, the possible underlying mechanisms of NKCC1 and KCC2 expression regulation by APP were also investigated. Furthermore, as alterations in NKCC1 and KCC2 expression and activity have been observed in many other pathological states where an inhibitory/excitatory imbalance has been described, such as epilepsy, schizophrenia, autism and Down's syndrome, we analyzed whether AD patients could have alterations in NKCC1 and KCC2 expression.

# Materials and Methods

## I. Animals

All animal procedures used in the study were carried out in accordance with institutional and European guidelines as certified by the local Animal Ethics Committee. *App*$^{-/-}$ transgenic mice were kept on C57Bl6/J genetic background and obtained from Jackson Laboratories (strain name: B6.129S7-Apptm1Dbo/J). Animals were housed on a 12 hour light/dark cycle in standard animal care facilities. Both pregnant Wistar rats used for embryonic cortical cell cultures and mice pups of either sex used for intraventricular injections (C57Bl6/J genetic background) were obtained from the Université Catholique de Louvain (UCL, Brussels, Belgium) animal facility.

## II. Culture reagents and antibodies

Unless specified, reagents for cell culture, Western-blotting and calcium imaging were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Antibodies were from the sources indicated: mouse monoclonal WO2 anti-hAPP, rabbit polyclonal anti-KCC2 and rabbit polyclonal anti-APLP1 and APLP2 (EMD Millipore, Billerica, MA, USA, catalog nos. MABN10, 07-432 and Calbiochem catalog nos. 171615 and 171616, respectively); mouse monoclonal anti-NKCC1 (Developmental Studies Hybridoma Bank, Iowa city, IA, USA, catalog no. T4); rabbit polyclonal anti-APP C-terminus and mouse monoclonal α tubulin, (Sigma-Aldrich, St-Louis, MO, USA, catalog nos. A8717 and T6074, respectively); rabbit polyclonal anti-hrGFP (Agilent Technologies, Santa Clara, CA, USA, cat no. 240141) and anti-GFP (Sigma-Aldrich, St-Louis, MO, USA, catalog no. AB10145); rabbit anti-$GABA_A$ receptor subunits α1 and α3 (Alomone Labs, Jerusalem, Israel, catalog n° AGA-001 and AGA-003, respectively); mouse monoclonal anti-CaV1.3 and anti-CACNA1C (Abcam, Cambridge, UK, cat nos; ab85491, and ab84814, respectively) and rabbit anti-USF1 (C-20) (Bio-Connect life sciences, Huissen, The Netherlands, catalog no. sc-229).

# III. Cell culture

17-18 day-old embryos of either sex were taken from Wistar rats or wild type and *App*$^{-/-}$ mice euthanized with CO2. Cortices were isolated and gently disrupted by passing the tissue through a Pasteur pipette with a fire-polished tip. The cells were plated in culture dishes (4 105 cells/cm$^2$) pre-treated with 10 µg/ml poly-L-lysine in phosphate buffered saline (PBS) and cultured for 6 days in vitro in Neurobasal medium supplemented with 2 % (v/v) B-27 medium and 0.5 mM L-glutamine prior to infection with recombinant viruses. The cultures were maintained at 37°C under a 5% CO2 atmosphere.

# IV. Preparation of recombinant viruses and infection of cell cultures

## 4.1 Adenoviruses

Recombinant adenoviruses coding for wild-type human APP695 (AdhAPP), β-cleaved C-terminal fragment of hAPP fused to the APP signal peptide (AdC99) and human recombinant Green Fluorescent Protein-2 (AdhrGFP) were prepared as described previously using the AdEasyTM XL Adenoviral Vector System (Stratagene, La Jolla, CA, USA, catalog no. 240010)77. Briefly, either the pShuttle-CMV-internal ribosomal entry site (IRES)-hrGFP-2 vector (for AdhrGFP) or the same vector containing the cDNA coding for either human APP695 or its C-terminal region (residues 597 to residue 695 for AdC99) was cloned into the pAdEasy-1 vector. Adenoviruses were purified according to the manufacturer's instructions.

The construction and purification of recombinant adenoviruses coding for β-galactosidase (Adβgal) and human APP695 truncated at its C-terminal region from residues 652 to 695 (AdhAPPΔC) were made as described previously (Pierrot et al., 2013). The AdhAPPΔC construct contains a stop codon after the three lysine residues that follow the APP transmembrane domain. Cells infected with AdhrGFP and Adβgal were used as control. After 6 days in vitro (6 DIV), the cells were infected at a multiplicity of infection (MOI) of 10 in a minimal volume of culture medium for 4 h. Infection medium was replaced by fresh culture medium every two days up to analysis (between 13 DIV and 19 DIV).

## 4.2 Lentiviruses

Two different pLKO.1 vectors coding for shRNA targeting APP mRNA were obtained from Sigma-Aldrich, St-Louis, MO, USA, (catalog nos. TRCN0000054874 Clone ID: NM_007471.2-2185s1c1 and TRCN0000054876 Clone ID: NM_007471.2-1583s1c1, respectively) and used for construction of recombinant lentiviruses, as previously described (Salmon and Trono, 2007). Briefly, lentiviruses were produced by calcium phosphate cotransfection of the target plasmid together with lentiviral packaging plasmids (gag-pol, rev, and VsVg plasmids, a kind gift from Pr. T. Michiels (de Duve Institute, Brussels, Belgium)) into HEK293T/17 cells (ATCC®, Manassas, VA, USA, catalog no. CRL-11268TM). Supernatants containing the lentiviruses were harvested 48 h after transfection. Lentiviruses were concentrated and purified with the Lenti-X ™ Concentrator kit (Clontech Laboratories, Mountain View, CA, USA, catalog no. PT4421-2) according to the manufacturer's instructions. Aliquots (30µl) of concentrated lentiviral solution were added to cell cultures on 6 DIV. A recombinant lentivirus coding for the neomycin resistance gene (pTM898neo, a kind gift from Pr. T. Michiels (de Duve Institute, Brussels, Belgium)) was used as a negative control. Cells were analysed on 13 DIV.

## 4.3 Adeno Associated Viruses (AAV)

pAAV-MCS Vector of AAV-DJ/8 Helper Free Expression System was used in order to clone hAPP (Cell Biolabs Inc., San Diego, CA, USA catalog no. VPK-410-DJ-8). This pAAV-hAPP vector contains inverted terminal repeats of an AAV2 sequence bordering a CMV promoter driving expression of hAPP. A control AAV vector pAAV-CMV-GFP was obtained from Connie Cepko (Addgene, Cambridge, MA, USA, catalog no. #67634). pHelper and pRC2-mi342 plasmids used for AAV cross-packaging were obtained from AAVpro Helper Free System (AAV2) (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France, catalog no. #6230). AAV2 particles were generated based on the AAVpro Helper Free System protocol. Briefly, HEK 293T/17 cells (ATCC®, Manassas, VA, USA, catalog no. CRL-11268TM) were transfected with 45µg total of pAAV-hAPP or pAAV-CMV-GFP, pHelper and pRC2-mi342 plasmids (ratio 1:1:1) using the Mirus TransIT 293 (Mirus Bio, Madison, WI, USA, catalog no. MIR 2700) transfection reagent (3µl/1µg of DNA). After 72 hours of transfection, cell pellets were recovered and AAV2 viral

particles were extracted using the AAVpro® Extraction Solution (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France, catalog no. #6235). AAV2 particles dedicated to in vivo usage were purified using the ViraBind™ AAV Purification Mega Kit (Cell Biolabs,inc. San Diego, CA, USA, catalog no. #VPK-141). The genomic titer of the virus was measured using the AAVpro® Titration Kit (for Real Time PCR) Ver.2 (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France, catalog no. #6233). Cultures were transduced on 6 DIV using AAV2-hAPP or AAV2-GFP at an MOI of 3000. A maximum of 100µl microliters of crude viral extract or purified virus was added to each well of a 12 well plate containing approximately 900,000 cells in 1ml.

For AAV2 - hAPP and - GFP transduction in neonatal mouse brain, one or two days after birth (P1-P2), C57Bl6/J neonates were cryoanesthetized and injected using a 10 µl Hamilton glass syringe (Filter Service, Eupen, Belgium, catalog no. HA 7635-01) at an angle of 30°C to a depth of 1.5 mm. Purified concentrated viral vector (2 µl) was slowly injected into each cerebral lateral ventricle. A total of $4.5 \times 10^8$ genomic equivalents (4 µl) was injected into each mouse brain. After injection, pups were allowed to completely recover on a warming blanket and then returned to the home cage for later analysis.

## V. Treatments

Cells were treated for 1 h with bumetanide 10 µM (Sigma-Aldrich, St-Louis, MO, USA, catalog no. B3023) prior to $Ca^{2+}$ imaging. On 12 DIV and for 24 h, cells were treated with (5S)-(t-Butoxycarbonylamino)-6-phenyl-(4R)hydroxy-(2R)benzylhexanoyl)-L-leu-L-phe amide (L-685,458, 1 µM), a γ-secretase inhibitor (Sigma-Aldrich, St-Louis, MO, USA, catalog no. L1790).

## VI. Western-blotting

Cells in culture were washed, scraped off into PBS and centrifuged (9000g x 2 min). The cell pellets were sonicated in lysis buffer (125 mM Tris (pH 6.8), 20% glycerol, and 4% sodium dodecyl sulphate) supplemented with complete Protease Inhibitor Cocktail (Roche, Bâle, Switzerland). For brain protein extraction, samples were homogenized in RIPA buffer (1% (w/v) NP40, 0.5% (w/v) deoxycholic acid, 0.1% (w/v) SDS, 150 mM NaCl, 1 mM EDTA, 50 mM Tris, pH 7.4) containing 1 mM PMSF, 10 mM NaF, 2 mM sodium orthovanadate and 1% (v/v)

protease and phosphatase inhibitor cocktail (Roche, Basel, Switzerland). The samples were clarified by a 2 min centrifugation at 9000g and protein concentrations were determined using a Bicinchoninic Acid Assay (BCA) kit. Samples were heated for 10 min at 70°C in loading buffer (lysis buffer containing 10% (w/v) 2-Mercaptoethanol and 0.004 % (w/v) bromophenol blue). Cell and brain lysates (20 µg and 40 µg of proteins, respectively) were subjected to SDS-PAGE using 4-12 % NupageTM bis-Tris gels for Western-blotting. Nitrocellulose membranes were incubated overnight at 4 °C with the following primary antibodies: human APP-specific WO2 (1:2 000); anti-APP C-terminal (1:2 000); anti-KCC2 (1:1 000); anti-hrGFP and GFP (1:2 000), anti-NKCC1 (1:100); anti-$GABA_A$ receptor subunits α1 and α3 (1:2 000 and 1:1 1000; respectively); anti-CaV1.3 (1:1 000), anti-CACNA1C (1:1 000), anti-α tubulin (1:4 000), anti-βIII tubulin (1:1000), anti-actin (1:1000), anti-APLP1 and APLP2 (1:4 000 and 1:2 000, respectively) and anti-USF1 (1:500). Blots were incubated with horseradish peroxidase-conjugated secondary antibodies, revealed by ECL (Amersham Pharmacia), and quantified using the Quantity OneTM software (Bio-Rad Laboratories, Hercules, CA, USA) with α-tubulin as a loading control.

## VII. RNA extraction and real time PCR

Total RNA was isolated by TriPure Isolation Reagent according to the manufacturer's protocol. RNA samples were resuspended in DEPC-treated water. Reverse transcription was carried out with the iScript cDNA synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA, catalog no. 1708891) using 1 µg of total RNA in a total reaction volume of 20 µL. Controls were performed without reverse transcriptase to rule out amplification of contaminant genomic DNA. Real-time PCR was performed for the amplification of NKCC1, KCC2, Egr4, USF1 and glyceraldehyde phosphate dehydrogenase (GAPDH) cDNAs. Primers were purchased from Sigma-Aldrich (St-Louis, MO); F, Forward primer; R, Reverse primer; E, primer efficiency:

NKCC1 rat

E-107.2%, F-5’ TTCGTGAGAGGAGGAGGAG 3’, R-5’ GATGCCCAGAAGAACCAC 3’

KCC2 rat and mouse

E-102.0%, F-5’ GGTGGAAGTCGTGGAGATG 3’, R-5’ CGAGTGTTGGCTGGATTC 3’

USF1 rat

E-95.7%, F-5’ TGATGATGCTGTTGACGC 3’, R-5’ TGGCTCCCTCCCTGTAAC 3’

Egr4 rat

E-108.2%, F-5’ CGACGAGAAGAAGCGACA 3’, R-5’ CCAGCGAGTAGAAGCCCA 3’

GAPDH rat/mouse

E-106.7%, F-5’CCCCCAATGTATCCGTTGTG3’, R-5’TGATTTCCCGTAGGACCGAT 3’

Real-time PCR was carried out in a total volume of 25 µL containing 8 ng cDNA template, 0.3 µM of the appropriate primers and the IQTM SYBR® Green Supermix 1x (Biorad catalog no. 1708885). The PCR protocol consisted of 40 amplification cycles (95°C for 30 s, 60°C for 45 s and 79°C for 15 s) and was performed using an iCycler IQTM multicolor Real-Time PCR detection system (Bio-Rad). For quantification, a relative standard curve was established for each target gene using four-fold serial dilutions (from 100 to 0.097 ng) of a cDNA template mix prepared using the same conditions. Each sample was normalized to relative expression levels of GAPDH. Calculation of Ct, standard curve preparation and quantification of mRNAs contained in the samples were performed using the "post run data analysis" software provided with the iCycler system (Bio-Rad).

## VIII. Cytosolic free $Ca^{2+}$ measurement in single neurons

For cytosolic free $Ca^{2+}$ measurements, all recordings were carried out at 37°C in Krebs-HEPES buffer (see below) containing 10 µM CNQX (Tocris, Bristol, UK, catalog no. 1045). Acute treatment of neurons with 100 µM GABA (Sigma-Aldrich; St-Louis, MO, USA, catalog no. A2129), 10 µM baclofen (Tocris, catalog no. 0417), 30 µM isoguvacine (Sigma-Aldrich, catalog no. G002) and 10 µM nimodipine (Tocris, catalog no. 0600) was performed in the incubation chamber. Neurons were plated at a density of 1.8 $10^5$ cells/$cm^2$ on pre-coated 15 mm round glass coverslips with 10 µg/ml poly-L-lysine in phosphate buffered saline. Seven to eleven days after adenoviral infection, neurons were incubated in the dark in the presence of the $Ca^{2+}$ indicator fura-2 acetoxymethylester (Fura-2 AM; catalog no. F1225) at a final concentration of 2 µM in Krebs-HEPES buffer (10 mM HEPES, 135 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, pH 7.4) for 30 min at room temperature. Coverslips were then washed and mounted in a heated (37°C) microscope chamber (1 ml). Cells were alternately excited (1 or 2 Hz) at 340 and 380 nm for 100 ms using a Lambda DG-4 Ultra High Speed Wavelength

Switcher (Sutter Instrument, Novato, CA) coupled to a Zeiss Axiovert 200 M inverted microscope (X20 fluorescence objective) (Zeiss Belgium, Zaventem, BE). Images were acquired using a Zeiss Axiocam camera coupled to a 510 nm emission filter and analysed with the Axiovision software. A total of 70-80 neurons was studied in each experiment and non-neuronal cells were excluded from the analysis as previously described by Pickering and coworkers (Pickering et al., 2008). Changes in intracellular $Ca^{2+}$ fluorescence were estimated from fluorescence emission intensity ratios F340/F380 or ΔF obtained after excitation of cells at wavelengths of 340nm and 380nm. These changes were expressed as ΔF/F0 where F0 is the basal fluorescence value corresponding to the mean of ΔF signals measured during a period of 50 s in control conditions (prior to the GABA pulse). The GABA response was defined as a change of ΔF greater than 10 % relative to F0. To estimate the amplitude of the response to GABA, F0 was subtracted from the maximum ΔF value occurring during the 50 s GABA pulse.

## IX. Electrophysiology

In order to preserve intracellular $Cl^-$ concentrations, gramicidin perforated patch recordings were performed to measure evoked action potentials in primary cortical cell cultures expressing hAPP between 13 DIV and 19 DIV. The recording bath solution contained 145mM NaCl, 3mM KCl, 2mM $CaCl_2$, 2mM $MgCl_2$, 15mM HEPES and 10mM glucose (pH adjusted to 7.4 with NaOH, osmolarity: 290mOsm/l). The internal pipette solution contained 140mM KCl, 1mM $MgCl_2$, 4mM MgATP, 0.5mM EGTA and 10mM HEPES (pH adjusted to 7.2 with KOH, osmolarity: 280mOsm/l). A 20mg/ml stock of gramicidin in dimethyl-sulphoxide (DMSO) was diluted freshly to a final concentration of 80µg/ml in the internal pipette solution. To facilitate gigaseal formation, patch pipettes were front filled by dipping the tip into the gramicidin-free solution then back filled with the gramicidin containing solution. All recordings were performed at room temperature. To obtain the frequency-current (f-I) relationship, action potential firing was recorded in response to 10 depolarizing current steps of 20pA using the Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) in presence or absence of added GABA (100µM) (Sigma-Aldrich, St-Louis, MO, USA, catalog no. A2129). Data were filtered at 5 kHz through a lowpass Bessel filter and collected at a frequency of 8 kHz using the Digidata 1322A digitizer (Molecular Devices, Sunnyvale, CA, USA).

Liquid junction potential and capacitance transients were compensated. 20 to 30 min after cell-attached seal formation (seal resistance >3GΩ), series resistance (Rs) typically dropped and stabilized at ~30-40 MΩ. Resting membrane potential measured in current clamp mode (I = 0) was stable. Input and series resistance were monitored during the experiment, and recordings were excluded when any of these parameters changed by >10%. The instantaneous firing rate for each current step was calculated from the inter-spike interval using the pClamp software. Electrodes were pulled from capillary glass (Harvard Apparatus, Les Ulis Cedex, France, catalog no. 30-0053) on a Sutter Instruments P97 puller (Sutter Instruments, Novato, CA, USA) and had a resistance of 3-6MΩ when filled with the internal solution.

## X. Human brain tissues

Frontal cortex brain tissue samples from 9 human control subjects and 9 demented patients were collected with the approval of the Ethical Committee of the Medical School of the Université Libre de Bruxelles or via the rapid autopsy program of the Netherlands Brain Bank (NBB), Amsterdam, which provides postmortem specimens from clinically well documented and neuropathologically confirmed cases. The work of the NBB abides by the ethical code of conduct approved by the ethics committee. Ethical approval and written informed consent from the donors or the next of kin were obtained in all cases. All patients were clinically diagnosed as sporadic cases of Alzheimer's disease (AD). **Table 1** summarizes the clinical data and the neuropathological staging of control subjects and patients with AD according to Braak and Braak staging for neurofibrillary tangles (Braak and Braak, 1991) and the Consortium to Establish a Registry for AD score for senile plaques (Mirra et al., 1991).

## XI. Aβ measurements

Human Aβ1-40 levels were measured in cell culture supernatants using the Multi-Spot Human (6E10) Aβ Triplex Assay and the SECTOR Imager 2400 (MesoScale Discovery, Rockville, MD, USA, catalog no. K15200E-1) according to manufacturer's instructions.

## XII. Cytotoxicity assay

Cell viability was measured by LDH release in the culture medium at 13 DIV using Cytotoxicity Detection kit (Sigma-Aldrich, St-Louis, MO, USA, catalog no. 11644793001) according to the manufacturer's instructions. Relative absorbance was measured at 490 nm using a VICTOR Multilabel Plate Reader (PerkinElmer, Richmond, VA, USA). Background LDH release was determined in non-infected control cultures.

## XIII. Immunofluorescence

Cells were seeded at 105 cells/cm$^2$ on glass coverslips, fixed with 4% v/v formaldehyde at room temperature (RT) then washed in PBS and permeabilized 1 h with 0,4% Triton X100 (v/v) in PBS containing 3% bovine serum albumin (Sigma-Aldrich, St-Louis, MO, USA, catalog no. A7906). After three washes in PBS cells were incubated 1h at RT with primary antibodies: WO2 (1:1 000); anti-KCC2 (1:100), MAP2 (1:1 000). After three PBS washes, cells were incubated for 1h with 5 µg/ml Alexa-labelled secondary antibodies in presence of Hoechst (1:10 000, catalog no. 62249). After three additional PBS washes, preparations were mounted in Fluoprep (Biomérieux, Marcy l'Etoile, FR, catalog no. 75521). Pictures were acquired with an AMG Evos fluorescence digital inverted microscope (Advanced Microscopy Group, Mill Creek, WA, USA). Immunoreactivity was quantified on 20x digital images by using Image J software with thresholds set according to signal intensity (Jensen, 2013) Mouse monoclonal Tuj 1 (Neuron-specific class III β-tubulin; Neuromics, Edina, MN, USA, catalog no. MO 15013). Anti-MAP2 and rabbit polyclonal actin (Sigma-Aldrich, St-Louis, MO, USA, catalog nos. M4403 and A2066, respectively).

## XIV. Statistics

Statistical analyses were performed using GraphPad Prism 7.01. The Shapiro-Wilk test was used to test for the normality of data. Parametric testing procedures (Student's t test or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple-comparison post-test when many subgroups were compared) were applied for normally distributed data, otherwise

nonparametric tests were used (Mann-Whitney or Kruskal-Wallis tests followed by Dunn's multiple-comparison post-test when many subgroups were compared). The total number of samples (n) analysed in all experimental conditions (number of repeated measurements) is indicated in figures legends. Results are presented as mean ± s.e.m. and statistical significance was set at P values (two-tailed tests) > 0.05 (*p > 0.05, **p > 0.01; ***p > 0.001).

# Results

## I. Evolution of KCC2 and NKCC1 expression and GABAergic $Ca^{2+}$ responses during primary cortical culture development.

Since the transition of GABAergic neurotransmission from depolarizing to hyperpolarizing that takes place *in vivo* and *in vitro* is associated with a gradual change in NKCC1 and KCC2 expression levels (Tibau et al., 2013; Ganguly et al., 2001; Soriano et al., 2008), the dynamics and magnitude of these changes were first characterized in our experimental model - primary cortical cultures derived from E18 rat embryos. In these cultures, NKCC1 and KCC2 mRNA levels were analysed between 4 and 17 days *in vitro* (DIV). Significant 3- to 4-fold increase in NKCC1 and KCC2 mRNA levels was observed by 7 DIV (**Fig. 15a**). KCC2 mRNA continued to increase as the culture developed, whereas NKCC1 mRNA gradually decreased after 7 DIV (**Fig. 15a**). These changes were mirrored by modifications in NKCC1 and KCC2 protein levels (**Fig. 15a**). As previously reported, the developmental upregulation of KCC2 mRNA and protein levels is essentially due to the KCC2b isoform (Uvarov et al., 2007; Ludwig et al., 2003). The KCC2 and NKCC1 antibodies, as well as the primers used in qPCR experiments, recognize both isoforms of KCC2 (KCC2a and KCC2b) and NKCC1 (NKCC1a and NKCC1b). In addition, it is worth mentioning that while the KCC2 antibody used in this study is considered to be a very good antibody, the specificity of the NKCC1 monoclonal T4 antibody is a matter of debate (Blaesse et al., 2009). Nevertheless, as we observed a correlation between NKCC1 mRNA levels and protein levels in different conditions analyzed, we tend to consider that our NKCC1 signal is specific.

During the early developmental period, depolarizing GABAergic potentials activate voltage-dependent $Ca^{2+}$ channels (VDCCs) and increase intracellular $Ca^{2+}$ levels ($[Ca^{2+}]_i$) (Connor et al., 1987; Yuste and Katz, 1991; Wang et al., 1994). We monitored GABA-induced $Ca^{2+}$ responses during the maturation of cortical cell cultures by using Fura-2 AM $Ca^{2+}$-sensitive dye (**Fig. 15b**). Measurements of fluorescence intensity changes showed that cells cultured up to 7 DIV

responded to exogenous GABA application (100 µM) with a rapid and reversible increase in $[Ca^{2+}]_i$. Beyond 7 DIV, the percentage of neurons that responded to GABA decreased with the duration of the culture (**Fig. 15b**).

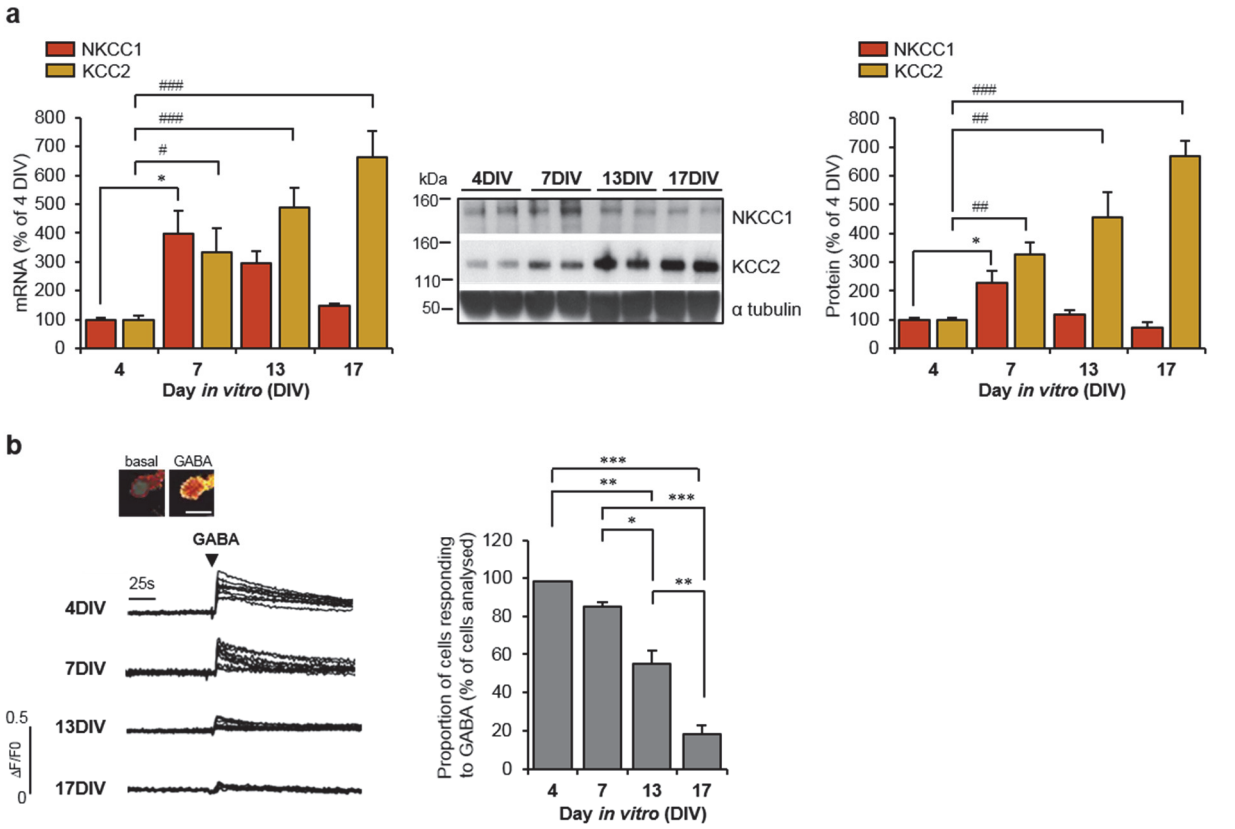



**Fig. 15. Developmental changes in cations $Cl^-$ cotransporters expression and GABA-induced responses in rat cortical cells cultures.** (**a**) Left panel: Time course of *Slc12a2* (coding for NKCC1) and *Slc12a5* (coding for KCC2) mRNA levels analysed between 4 and 17 days *in vitro* (DIV) in primary rat cortical cultures by RT-qPCR (n=7 each analysed in 5 independent experiments). Results were normalized to GAPDH mRNA and expressed as percentage (mean ± s.e.m.) of 4 DIV (∗*P*<0.05, ∗∗*P*<0.01, ∗∗∗*P*<0.001, Kruskal-Wallis test followed by Dunn's multiple-comparison post-test). Middle panel: Representative Western-blot of cell lysates from cortical cultures analysed between 4 and 17 DIV. Expression of NKCC1 and KCC2 was monitored using specific antibodies. Blots were further probed with an anti-α tubulin antibody. Right panel: quantification of NKCC1 and KCC2 / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of 4 DIV (n=11 of each analysed in 6 independent experiments, ∗*P*<0.05, ∗∗*P*<0.01, ∗∗∗*P*<0.001, ANOVA for NKCC1 and Kruskal-Wallis test for KCC2). (**b**) GABA-induced calcium responses in cortical cells during culture development. Top, ratiometric fluorescent dye image in one cell before (basal) and immediately after applying 100 µM GABA. Scale bar, 10 µm. Representative traces of ten cell recordings displaying a $[Ca2^+]_i$ increase in response to GABA. Traces are expressed as ΔF/F0, where ΔF corresponds to a change in F340/F380 ratio and F0 is the basal fluorescence value. Right panel, percentage (mean ± s.e.m.) of cells responding to GABA (n=215 cells for each time point analysed in 4 independent experiments, ∗*P*<0.05, ∗∗*P*<0.01, ∗∗∗*P*<0.001, Kruskal-Wallis test).

Given that by 17 DIV the response to GABA was almost completely gone, depolarization with a high concentration of extracellular KCl (50 mM) still induced robust increases in $[Ca^{2+}]_i$, suggesting that decreased responsiveness to GABA was due to a decrease in the extent of GABA-induced depolarization (**Fig. 16a**). These observations are consistent with previous findings in cultured neurons (Ganguly et al., 2001; Soriano et al., 2008). Moreover, GABA responses were blocked by the L-type VDCC antagonist nimodipine (10 µM) (**Fig. 16b**), indicating that the GABA-induced $Ca^{2+}$ increase is mediated by $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels. Furthermore, treatment with the $GABA_B$ agonist baclofen (10 µM) didn't change $[Ca^{2+}]_i$, while the $GABA_A$ receptor specific agonist isoguvacine (30 µM) increased $[Ca^{2+}]_i$, indicating that $GABA_B$ receptors are not involved in GABA-induced changes in $[Ca^{2+}]_i$ (**Fig. 16b**). Taken together, the results indicate that maturation related changes in expression of NKCC1 and KCC2 tightly correlate with the evolution of GABA-induced $Ca^{2+}$ responses in our cortical cell model. Furthermore, a shift in GABAergic signaling is apparent in our cultures starting from 7 DIV and GABA induced depolarization is almost completely absent by 17 DIV.

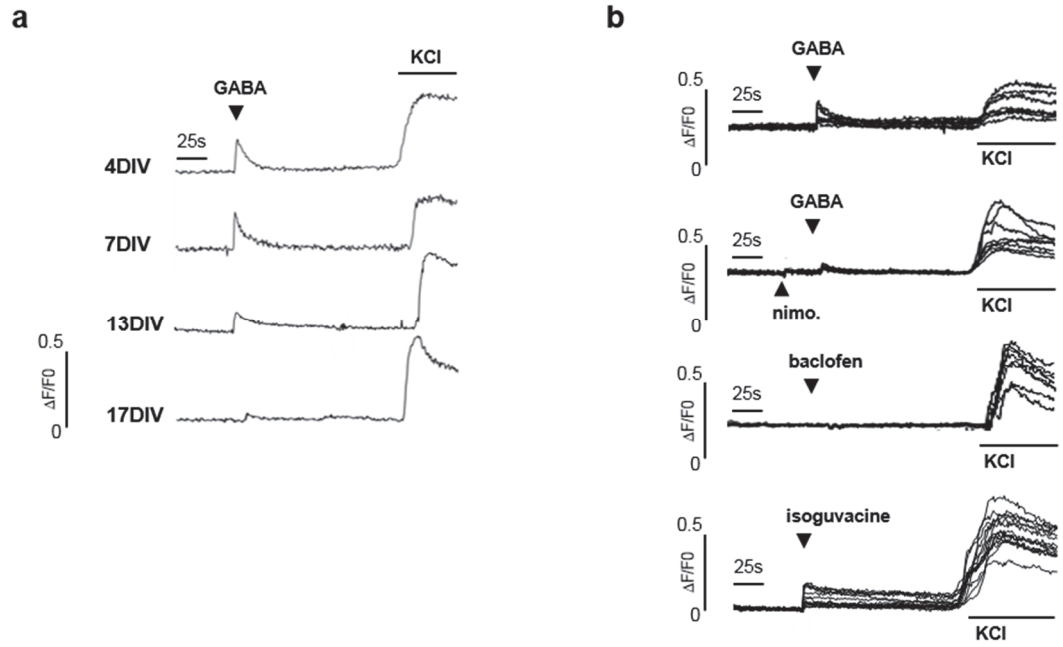



**Fig. 16. Characterization of GABA-induced depolarization in rat cortical cells cultures.** (**a**) Time course of GABA-induced calcium fluorescence in cortical cells cultures between 4 and 17 days in vitro (DIV). One representative recording of a cell baring a [Ca2+]i increase in response to 100 µM GABA at each time point. High concentration of extracellular KCl (50 mM) was applied at the end of the recording. (**b**) Comparative recordings of GABA-induced Ca2+ increase in rat cortical cultures at 13 DIV (GABA 100 µM). Representative traces of ten cells treated with GABA in extracellular solution containing 10 µM L-type VDCC antagonist nimodipine (middle panel), 10 µM $GABA_B$ agonist baclofen or 30 µM GABAA agonist isoguvacine (lower panels). All traces are expressed as ΔF/F0, were ΔF is a change in the F340/F380 ratio and F0 is the basal fluorescence value.

## II. Human APP expression decreases KCC2 expression and renders GABA signaling less hyperpolarizing and less inhibitory in primary cortical cultures.

To address whether APP could modulate GABAergic neurotransmission by affecting expression levels of NKCC1 and KCC2, we expressed human APP (hAPP) in cultured rat cortical cells. Full-length neuronal wild-type hAPP expression was achieved by infecting primary cortical cultures with a recombinant adenoviral vector at 6 DIV, i.e. prior to the occurrence of the GABA shift. At 13 DIV, we probed blots with an antibody, which discriminates between hAPP and endogenous rat APP by recognizing amino-acids 4-10 of human Aβ, as well as using an antibody that recognizes the 19 carboxyl-terminal amino acids of both human and endogenous APP (total APP) (**Fig. 17a**). Seven days after infection, expression of hAPP increased the total content of APP by about 1.6 fold (159.6 ± 33 %) (**Fig. 17a**).

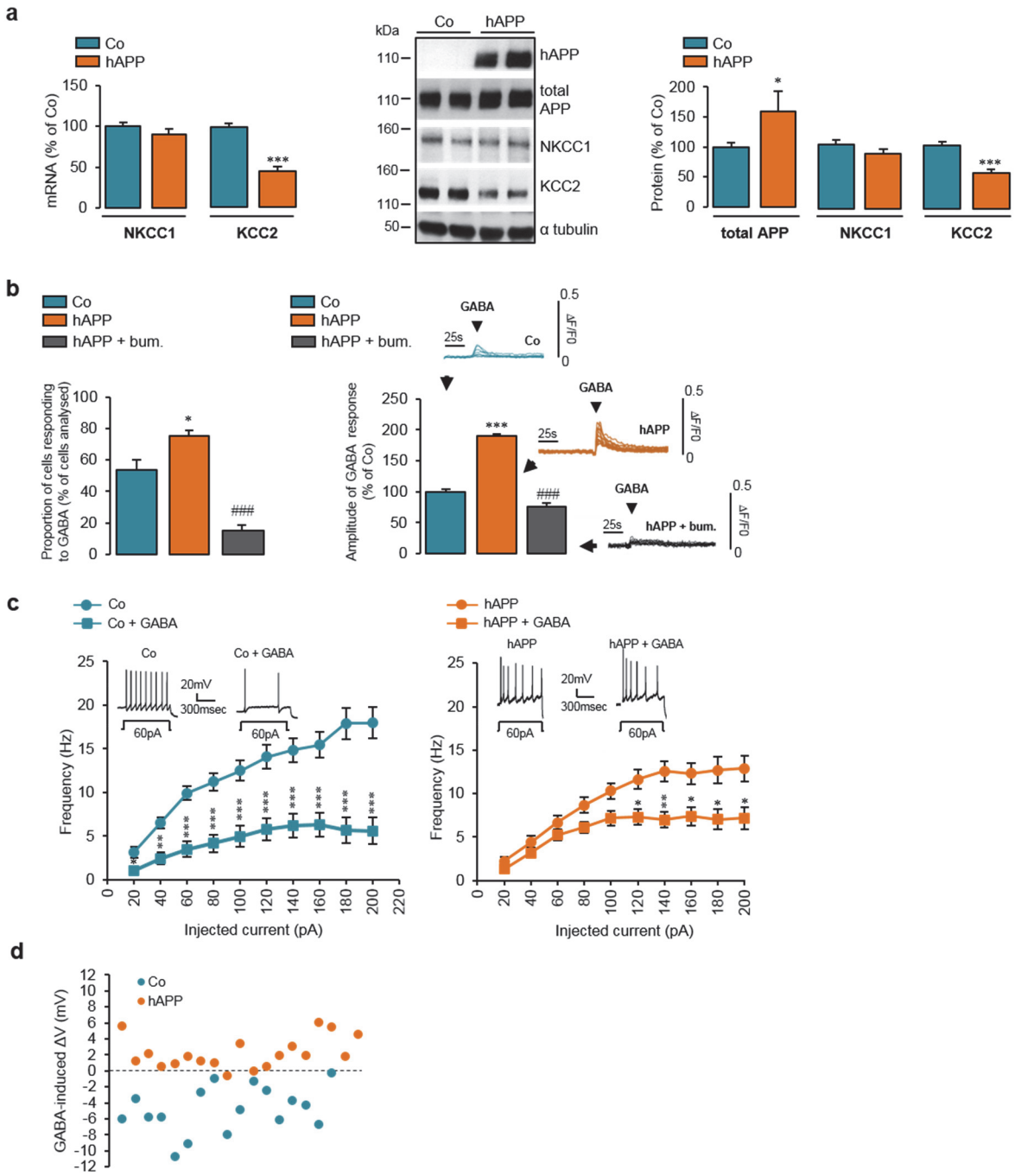



**Fig. 17. Expression of human APP in cortical cultures decreases KCC2 expression and changes GABA-induced responses**. (**a**) Left panel: Comparative RT-qPCR analyses of *Slc12a2* (coding for NKCC1) and *Slc12a5* (coding for KCC2) mRNA levels in primary rat cortical cultures infected or not (control, Co) at 6 DIV with an adenovirus coding for human APP (hAPP). Results were obtained between 13 and 17 DIV (n=20 for each condition in 9 independent experiments), normalized to GAPDH mRNA and expressed as percentage (mean ± s.e.m.) of Co (***$P$<0.001, Student's $t$-test). Middle panel: Representative Western-blot of cell lysates from Co and hAPP primary cortical cultures. Expression of hAPP was monitored between 13 and 17 DIV with the WO2 antibody recognizing hAPP and anti-APP C-terminal antibody recognizing both hAPP and endogenous APP (total APP).

Blots were further probed using anti-NKCC1, -KCC2, and -α tubulin antibodies. Right panel: quantification of total APP, NKCC1 and KCC2/ α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co (Co n=28 and hAPP n=30 in 16 independent experiments, ∗*P*<0.05, ∗∗∗*P*<0.001, Mann-Whitney test (total APP) and Student's *t*-test (NKCC1 and KCC2)). (**b**) GABA (100µM)-induced calcium fluorescence in Co and hAPP expressing cortical cultures measured between 13 and 17 DIV incubated with or without 10 µM bumetanide (bum.) for 1 h. Insets: representative traces of ten cell recordings. Traces are expressed as ΔF/F0, where ΔF corresponds to a change in F340/F380 ratio and F0 is the basal fluorescence value. Percentage (mean ± s.e.m.) of cells responding to 100 µM GABA (left panel) and amplitude of this response (right panel) (Co n=138, hAPP n=122, hAPP+bum. n=208 cells in 4 independent experiments), ANOVA test followed by Bonferroni's multiple-comparison post-test (compared to controls: ∗*P*<0.05, ∗∗∗*P*<0.001; compared to untreated hAPP: ###*P*<0.001). (**c**) Frequency of action potentials depending on the current injected and representative traces of Co (left panel) and hAPP (right panel) expressing cortical neurons before or after a bath application of GABA (100µM) measured between 13 and 19 DIV (Co n=17 and hAPP n=19 cells in 6 independent experiments, ∗*P*<0.05, ∗∗*P*<0.01, ∗∗∗*P*<0.001, Student's *t*-test applied for each current step to compare the effect of GABA on Co or hAPP cultures). (**d**) Difference in membrane potential (ΔV) elicited by a bath application of GABA (100µM) measured in Co and hAPP expressing cortical cells between 13 and 19 DIV (Co n=17 and hAPP n=19 cells in 6 independent experiments).

Transgene expression was homogeneous and didn't affect cell density as assessed by immunocytochemistry (**Fig. 18a**)**.** LDH cytotoxicity test allowed to demonstrate that adenoviral transduction didn't alter cell viability (**Fig. 18b**). In addition, hAPP expression remained stable over time as it was similar after both 7 and 11 days post-infection (13 and 17DIV) (**Fig. 18c**). Expression of hAPP didn't affect NKCC1 expression, but decreased KCC2 mRNA and protein levels by about 50 % (44.8 ± 5.3 and 57.4 ± 5.4 % of control, respectively) (**Fig. 17a**). In some cases, oligomeric forms of KCC2 could be detected and decreased to the same extent as the monomeric protein in hAPP expressing cells (46.8 ± 12 % of control **Fig. 19a**). Moreover, KCC2 immunofluorescent signal was significantly decreased in hAPP expressing neurons (49.6 ± 4.2 % of control, **Fig. 19b**). As control, primary neuronal cultures were infected with an adenovirus coding for human recombinant green fluorescent protein (hrGFP). This infection, was not toxic and didn't modify expression levels of NKCC1 and KCC2 (**Fig. 20a and b**).

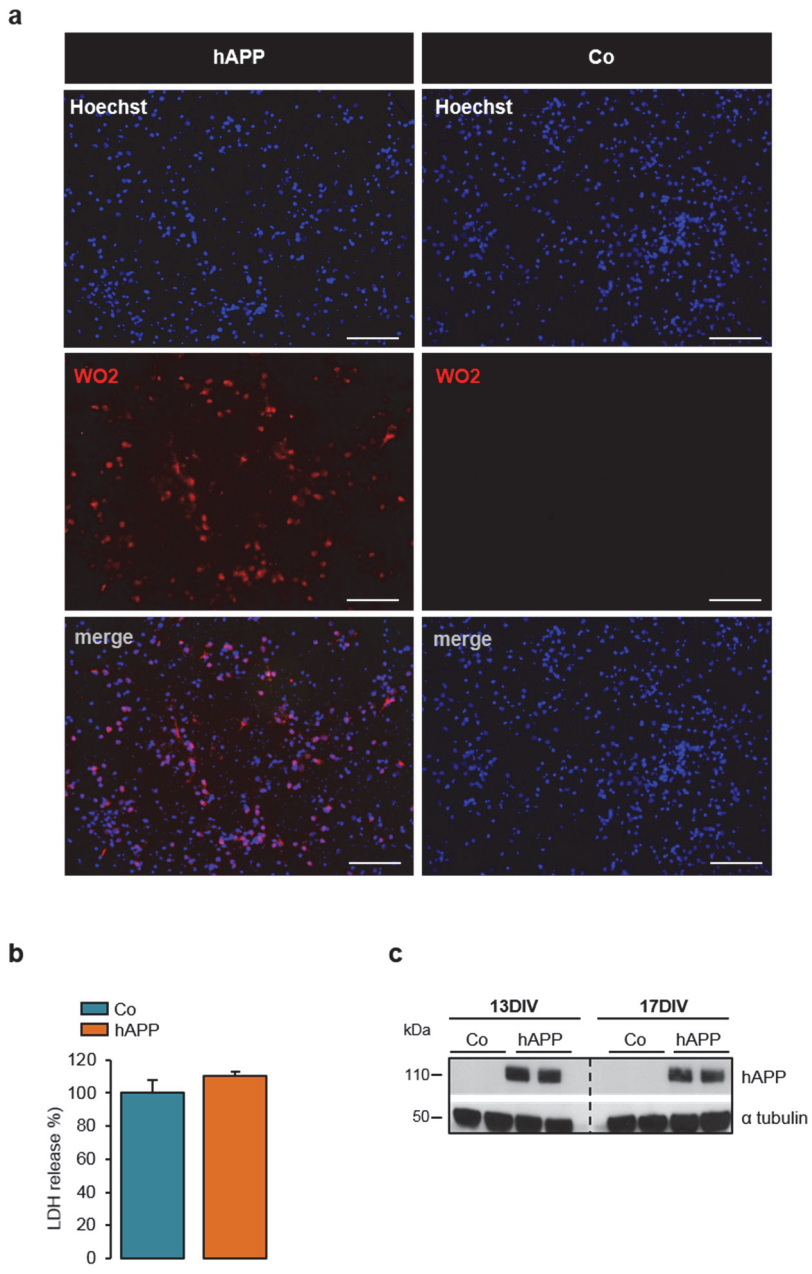



**Fig. 18. Homogeneous, stable and not toxic expression of hAPP.** (**a**) Representative immunofluorescent staining of human APP (hAPP) expression in primary rat cortical cultures infected or not (control, Co) at 6 DIV with an adenovirus coding for hAPP. At 13 DIV, hAPP was labelled with the specific WO2 antibody recognizing the 4-10 amino-acids of the human Aβ sequence. Scale bar, 60 µm. (**b**) Measurement of LDH activity released after hAPP-adenoviral transduction at 13 DIV in primary rat cortical cultures. Background LDH release was determined in non-infected control cultures (Co). Results were expressed as percentage of total LDH release in non-infected control cultures (Co), (Co=12, hAPP=14 analysed in 2 independent experiments, Co vs hAPP $P < 0.05$, Student's t-test, non-significant (n.s.). (**c**) Representative Western-blot of cell lysates from Co and hAPP expressing cortical cultures analysed at 13 and 17 DIV.

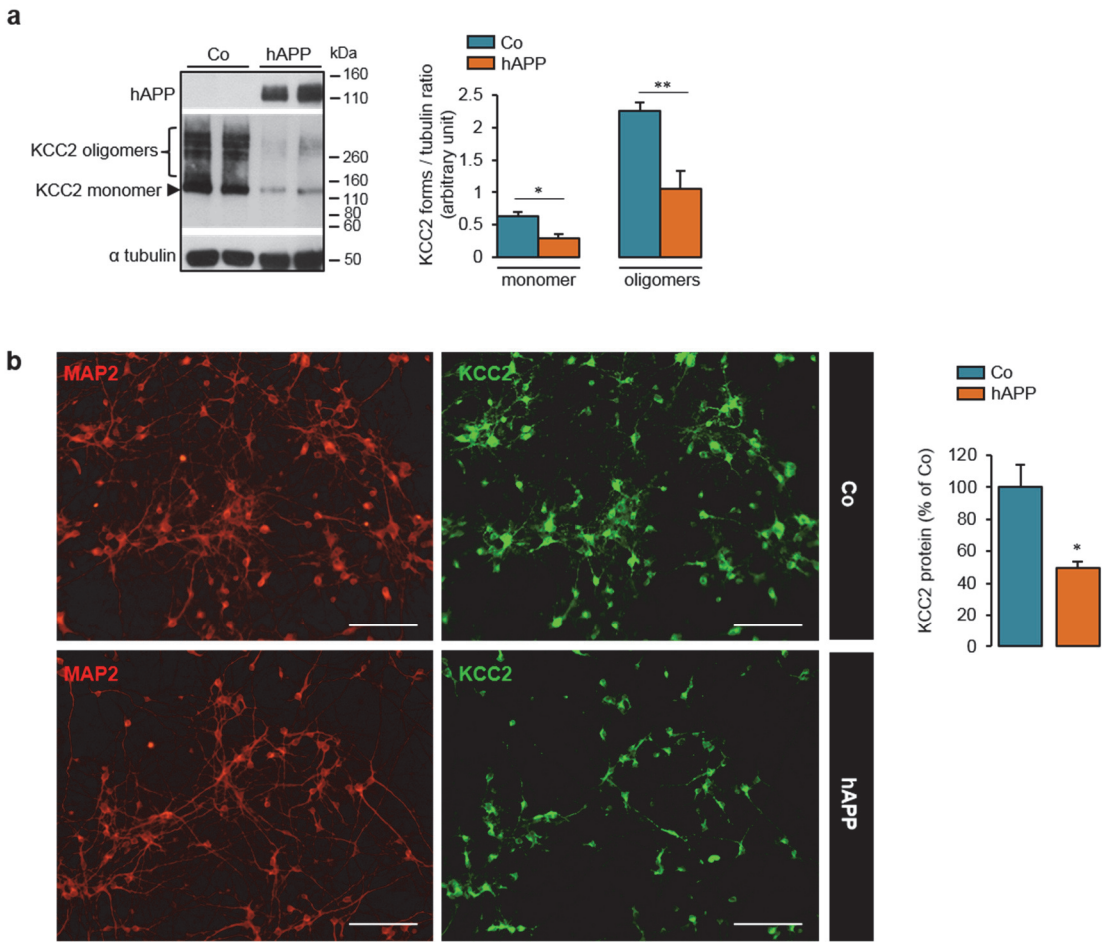



**Fig. 19. hAPP expression decreases KCC2 oligomers and KCC2 immunofluorescent signal.** (**a**) Representative Western-blot of cell lysates from primary rat cortical cultures infected or not (control, Co) at 6 DIV with an adenovirus coding for human APP (hAPP) showing monomeric and oligomeric forms of KCC2 at 17 DIV. Blots were probed using anti-KCC2 and -α tubulin antibodies. Right panel: quantification of monomeric or oligomeric KCC2 (mean ± s.e.m.) / α-tubulin ratios (n = 8 of each analysed in 3 independent experiments, ∗*P*<0.05, ∗∗*P*<0.01, Mann-Whitney test). (**b**) Representative immunofluorescent staining and quantification of KCC2 expression in Co and hAPP expressing cortical cultures. Bottom panel: quantification of KCC2 immunofluorescent signal. Data are expressed as percentage (mean ± s.e.m.) of Co (n=6 of each analysed in 3 independent experiments, ∗*P*<0.05, Student's *t*-test). Scale bar, 60 µm.

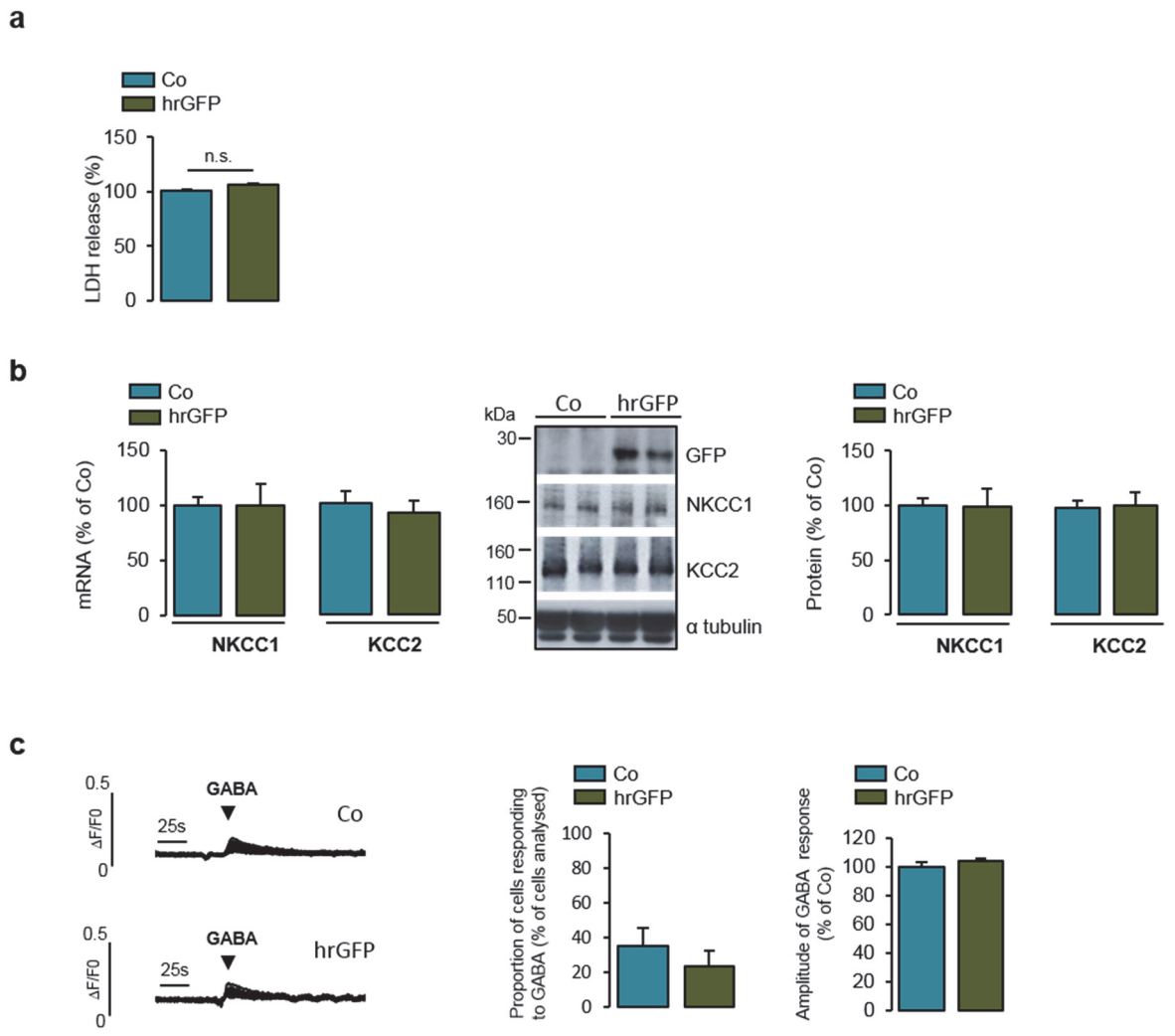



**Fig. 20. Unaffected KCC2 expression and GABA responses in hrGFP expressing cultures.** (**a**) Measurement of LDH activity released from primary rat cortical cultures infected or not (control, Co) at 6 DIV with an adenovirus coding for human recombinant GFP (hrGFP). Results were obtained at 13 DIV and expressed as percentage of total LDH release in Co cultures. Co=12, hrGFP=14 in 2 independent experiments, Co vs hrGFP $P < 0.05$, Student's t-test, non-significant (n.s.). (**b**) Left panel: Comparative RT-qPCR analyses of Slc12a2 (coding for NKCC1) and Slc12a5 (coding for KCC2) mRNA levels from Co and hrGFP cortical cultures. Results were obtained at 13 DIV, normalized to GAPDH mRNA and expressed as percentage (mean ± s.e.m.) of Co (Co n=10 and hrGFP n=10 in 4 independent experiments, Student's t-test). Middle panel: Representative Western-blot of 13 DIV cell lysates from Co and hrGFP expressing cortical cultures. Expression of hrGFP was monitored and blots were further probed using anti-NKCC1, -KCC2, and -α tubulin antibodies. Right panel: quantification of NKCC1 and KCC2 / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co (Co n=7 and hrGFP n=4 analysed in 4 independent experiments, $P < 0.05$, Student's t-test). (**c**) GABA (100µM)-induced calcium fluorescence in Co and hrGFP expressing cortical cultures analysed at 17 DIV. Representative traces of ten cell recordings per condition (total of 213 hrGFP cells analysed in 5 independent experiments). Traces are expressed as ΔF/F0, were ΔF is a change in the F340/F380 ratio and F0 is the basal fluorescence value. Right panel: percentage (mean ± s.e.m.) of cells responding to 100 µM GABA and amplitude of this response (right panels) (Co n=205, hrGFP n=155, cells in 3 independent experiments, $P < 0.05$, Student's t-test).

Given that NKCC1 and KCC2 are regulators of neuronal chloride gradients and that these gradients are important for GABAergic transmission polarity, the decrease in KCC2 levels suggested that hAPP expression could have an impact on GABAergic transmission polarity. To test this hypothesis, we monitored GABA-induced depolarization between 13 and 17 DIV by loading control or hAPP expressing cells with Fura-2AM calcium sensitive dye and treating them with GABA (100 µM). As shown in **Fig. 17b**, hAPP expressing cells displayed increased GABA-induced response as compared to controls. Indeed, the proportion of cells responding to GABA as well as the amplitude of this response were increased in hAPP expressing neurons (75.15 ± 3.9 of total cells analysed and 190.4 ± 2.76 % of control, respectively). It is noteworthy that GABA-induced fluorescence was unaffected in hrGFP infected cells (**Fig. 20c**). Furthermore, the increase in $[Ca^{2+}]_i$ observed in hAPP expressing cells was not due to higher expression of the α1 and α3 $GABA_A$ receptor subunits, predominantly expressed in the adult and developing cortex, respectively (Mehta and Ticku, 1999; Laurie et al., 1992) (**Fig. 21a**). Since expression of the α1C pore-forming subunit of L-type VDCC ($Ca_v1.2$) was previously shown to be modified by APP (Yang et al., 2009) , we measured $Ca_v1.2$ expression as well as another pore forming subunit of L-type channels, α1D ($Ca_v1.3$) in hAPP expressing primary cortical cells. $Ca_v1.2$ and $Ca_v1.3$ expression remained unchanged in hAPP expressing cells (**Fig. 21b**).

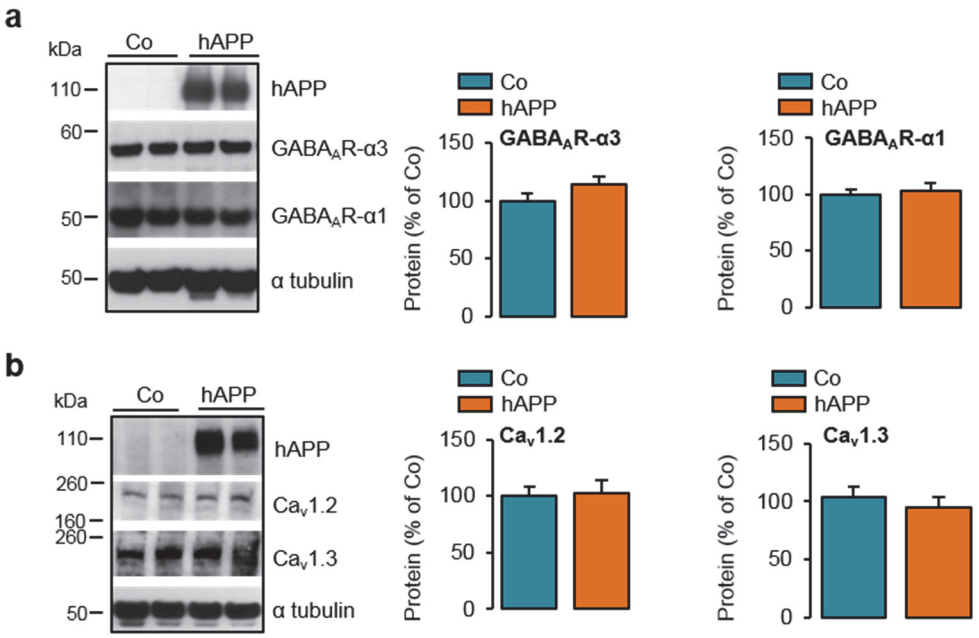


**Fig. 21. hAPP expression does not modify $GABA_AR$ α3/α1 and L-type VDCC α1C/D pore-forming subunits expression**. (**a** and **b**) Representative Western-blots of cell lysates from primary rat cortical cultures infected or not (control, Co) at 6 DIV by an adenovirus coding for human APP (hAPP). Results were obtained between 13 and 17 DIV. Expression of hAPP was monitored with the specific WO2 antibody. Anti-$GABA_AR$ antibodies were used to detect $GABA_AR$-α1 and α3 subunits and anti-CaV1.3 and -CACNA1C to detect α1D and α1C pore-forming subunits of L-type VDCC ($Ca_v1.2$ and $Ca_v1.3$, respectively). Blots were further probed using anti-α tubulin antibody. Right panels: quantification of $GABA_AR$-α3, -α1, $Ca_v1.2$ and $Ca_v1.3$ / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co ((**a**) n=18 of each analysed in 8 independent experiments and (**b**) n=11 of each analysed in 5 independent experiments, $P>0.05$, Student's *t*-test).

In order to confirm that the GABA-induced rise in $[Ca^{2+}]_i$ observed in hAPP expressing neurons was sensitive to changes in chloride concentration, we incubated primary cortical neurons with the NKCC1 inhibitor bumetanide (Payne, 1997). It was assumed that by decreasing $[Cl^-]_i$, bumetanide would render GABA more hyperpolarizing which in turn would result in a reduced GABA-induced $Ca^{2+}$ response. As expected, in hAPP expressing neurons treated with 10 µM bumetanide the proportion of cells responding to GABA as well as the amplitude of the GABA response were decreased compared to untreated hAPP expressing cells (19.63 ± 5.7 % of total cells analysed and 50.3 ± 4.6 % of untreated hAPP) (**Fig. 17b**), indicating that changes in $[Cl^-]_i$ may contribute to GABA-induced increase in $[Ca^{2+}]_i$ observed in hAPP expressing neurons. Taken together the results suggest that GABA is more depolarizing in hAPP expressing neurons, which could be due to a modification in $[Cl^-]_i$ levels resulting from a decrease in KCC2 expression in these cells.

Given the increased depolarization evoked by GABA in hAPP expressing neurons observed by calcium imaging, gramicidin perforated patch recordings in hAPP expressing neurons were next performed in order to evaluate the effect of GABA (100µM) on evoked action potential (AP) frequency and resting membrane potential between 13 DIV and 19 DIV. As expected, addition of GABA reduced AP frequency in control neurons by about 65% (**Fig. 17c**). On the other hand in hAPP expressing neurons, AP frequency was reduced only by about 35% indicating that GABA was less inhibitory in these cells (**Fig. 17c**). Furthermore, while application of GABA elicited a significant hyperpolarization in most control cells analysed (ΔVm -4.83mV ± -0.55), hAPP expressing neurons either slightly depolarized or didn't display a

change in membrane potential in response to GABA (ΔVm 2.25mV ± 0.36) (**Fig. 17d** and **Fig. 22a**). The effect of GABA measured by patch clamp in neurons infected with hrGFP was not significantly different compared to controls (**Fig. 22b** and **c**). These results support the idea that GABA is more depolarizing in hAPP expressing neurons compared to controls. However, the more depolarizing effect of GABA in hAPP expressing cells is not sufficient to elicit excitation. Indeed, GABA remained inhibitory in hAPP expressing neurons, but to a lesser extent than in controls.

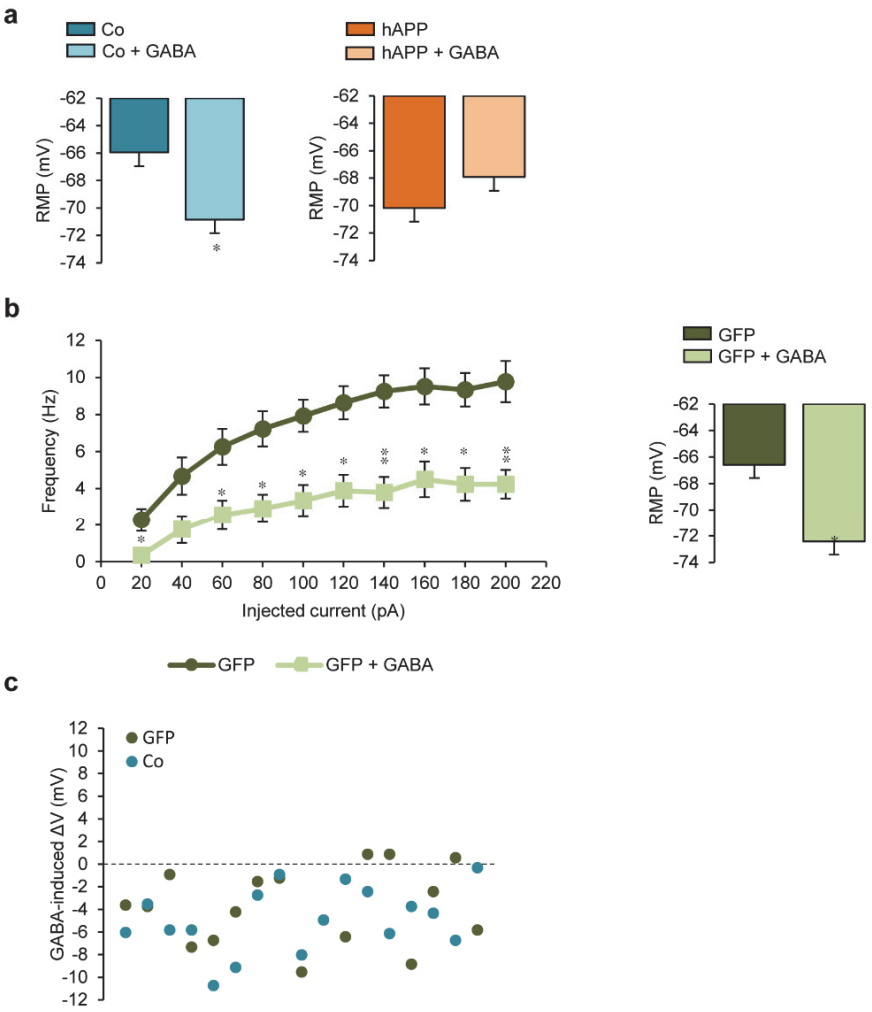



**Fig. 22. Electrophysiological responses to GABA are not affected in hrGFP expressing cultures.** (**a**) Resting membrane potential (RMP) measured between 13 and 19 DIV after a bath application of GABA (100µM) in primary rat cortical cultures infected or not (control, Co) at 6 DIV by an adenovirus coding for human APP (hAPP) (Co n=17 and hAPP n=19 cells analysed in 6 independent experiments, **P*<0.05, Student's *t*-test). (**b**) Frequency of action potentials depending on the current injected in human recombinant GFP (hrGFP) expressing cortical neurons before or after a bath application of GABA (100µM) measured between 13 and 19 DIV (**P*<0.05, ***P*<0.01 Student's *t*-test applied for each current step to compare the effect of GABA on hrGFP cultures) Right panel: RMP measured between 13 and 19 DIV in Co and hrGFP expressing cortical cultures. (**c**) Difference in membrane potential (ΔV) elicited by a bath application of GABA (100µM) measured in hrGFP expressing cortical cultures between 13 and 19 DIV (hrGFP n=18 cells analysed in 4 independent experiments).

## III. APP dependent KCC2 downregulation in primary cortical cultures involves the juxta-/transmembrane domain of APP and could rely on USF1.

We next investigated whether KCC2 downregulation was related to APP expression level. NKCC1 and KCC2 expression were first measured in cultured cortical cells from wild type (*App*$^{+/+}$) and APP deficient (*App*$^{-/-}$) mice. At 13 DIV, the absence of APP and/or its cleavage products didn't modify NKCC1 and KCC2 mRNA and protein levels (**Fig. 23a**). Nevertheless, tendency towards increased KCC2 expression was observed. Given that APP belongs to a multi-gene family of paralogs including amyloid precursor-like proteins (APLPs) 1 and 2 (Wasco et al., 1992), APLP1 and APLP2 could functionally compensate for the loss of APP (von Koch et al., 1997; Heber et al., 2000). Indeed, we observed a non-significant increase in APLP1 expression in *App*$^{-/-}$ compared to *App*$^{+/+}$ cells, suggesting partial compensation of APP deletion by APLP1 (**Fig. 23b**).

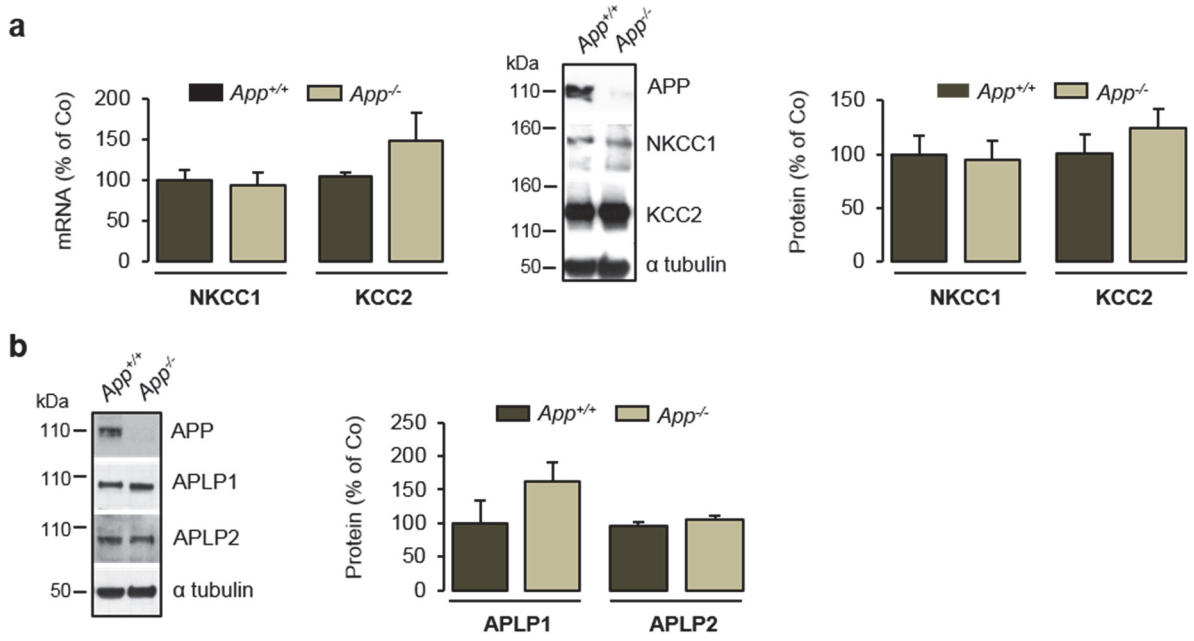


**Fig. 23**. **Unaffected expression of NKCC1 and KCC2 in *App* knock-out cells and APLP1 and 2 expression**. (**a**) Comparative RT-qPCR analyses of *Slc12a2* (coding for NKCC1) and *Slc12a5* (coding for KCC2) mRNA levels from primary cultures of mouse cortical cells of wild type ($App^{+/+}$) and *App* knockout mice ($App^{-/-}$). Results were obtained at 13 DIV, normalized to GAPDH mRNA and expressed as percentage (mean ± s.e.m.) of $App^{+/+}$ (n=10 of each in 8 independent experiments, $P>0.05$, NKCC1: Student's *t*-test and KCC2: Mann-Whitney test). (**a** (Middle panel) and **b**) Representative Western-blots of cell lysates from $App^{+/+}$ and $App^{-/-}$ cortical cultures at 13 DIV. Expression of endogenous APP (APP) was monitored with the anti-APP C-terminal antibody. Blots were further probed with anti-APLP1 and 2, anti-NKCC1, anti-KCC2 and anti-α tubulin antibodies. Right panels: quantification of NKCC1 and KCC2 / α tubulin ratios (**a**) or APLP1 and APLP2 / α tubulin ratios (**b**). Results were expressed as percentage (mean ± s.e.m.) of $App^{+/+}$ ($App^{+/+}$ and $App^{-/-}$ n=3 of each in 3 independent experiments), $P>0.05$, Student's *t*-test.

To bypass compensatory mechanisms that might have masked APP function, APP expression was decreased in cortical cells using an shRNA construct designed to target endogenous APP (APP shRNA). The knock-down of endogenous rat APP was achieved by infecting cells on 6 DIV with recombinant lentiviruses coding for APP shRNA. At 13 DIV, blots were probed with an antibody that recognizes the 19 carboxyl-terminal amino acids of endogenous APP (**Fig. 24a**). One week after APPshRNA lentiviral infection, a 68.8 ± 8.6 % decrease in endogenous APP expression was observed, while APLP1 and APLP2 protein levels remained unchanged (**Fig. 24a**). Using this knock-down approach, NKCC1 expression was not modified after APPshRNA lentiviral infection (**Fig. 25a**). By contrast, a significant increase in KCC2 mRNA and protein levels (219.3 ± 24.2 and 268.2 ± 28.1 % of control, respectively), was observed (**Fig. 25a**). Moreover, infection of cortical cells with a recombinant lentivirus coding for neomycin resistance (neo) didn't change KCC2 protein levels (**Fig. 24b**). The results thus indicate that KCC2 expression depends on APP expression.

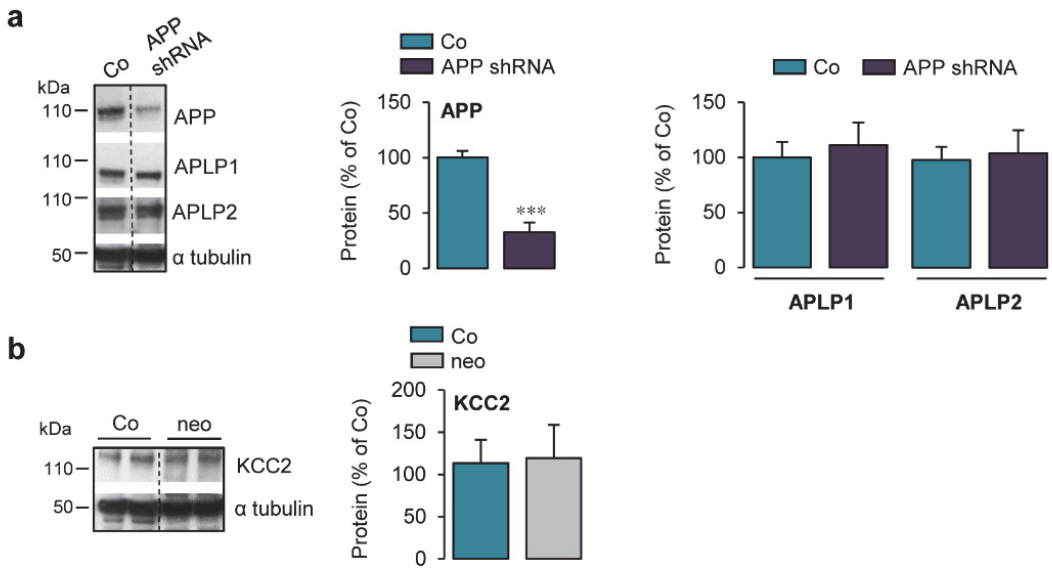



**Fig. 24**. **Unaffected expression of APLP1 and APLP2 in APP knock-down cultures and KCC2 expression in neomycin infected control cultures**. (**a** and **b**) Left panels: Representative Western-blots of cell lysates from primary rat cortical cultures infected or not (control, Co) at 6 DIV with recombinant lentiviruses coding for APP shRNA or the neomycin resistance gene (neo) (**a** and **b** respectively). Results were obtained between 13 DIV and 17 DIV. Expression of endogenous APP (APP) was monitored with the anti-APP C-terminal antibody. Blots were further probed with anti-APLP1 and 2, anti-KCC2 and anti-α tubulin antibodies. Right panels: quantification of APP, APLP1, APLP2 and endogenous APP / α tubulin ratios or KCC2 / α tubulin ratios (**a** and **b**, respectively). Results were expressed as percentage (mean ± s.e.m.) of Co (Co and APPshRNA n=6, neo n=3 in 3 independent experiments), $P>0.05$, ***$P<0.001$, Student's *t*-test.

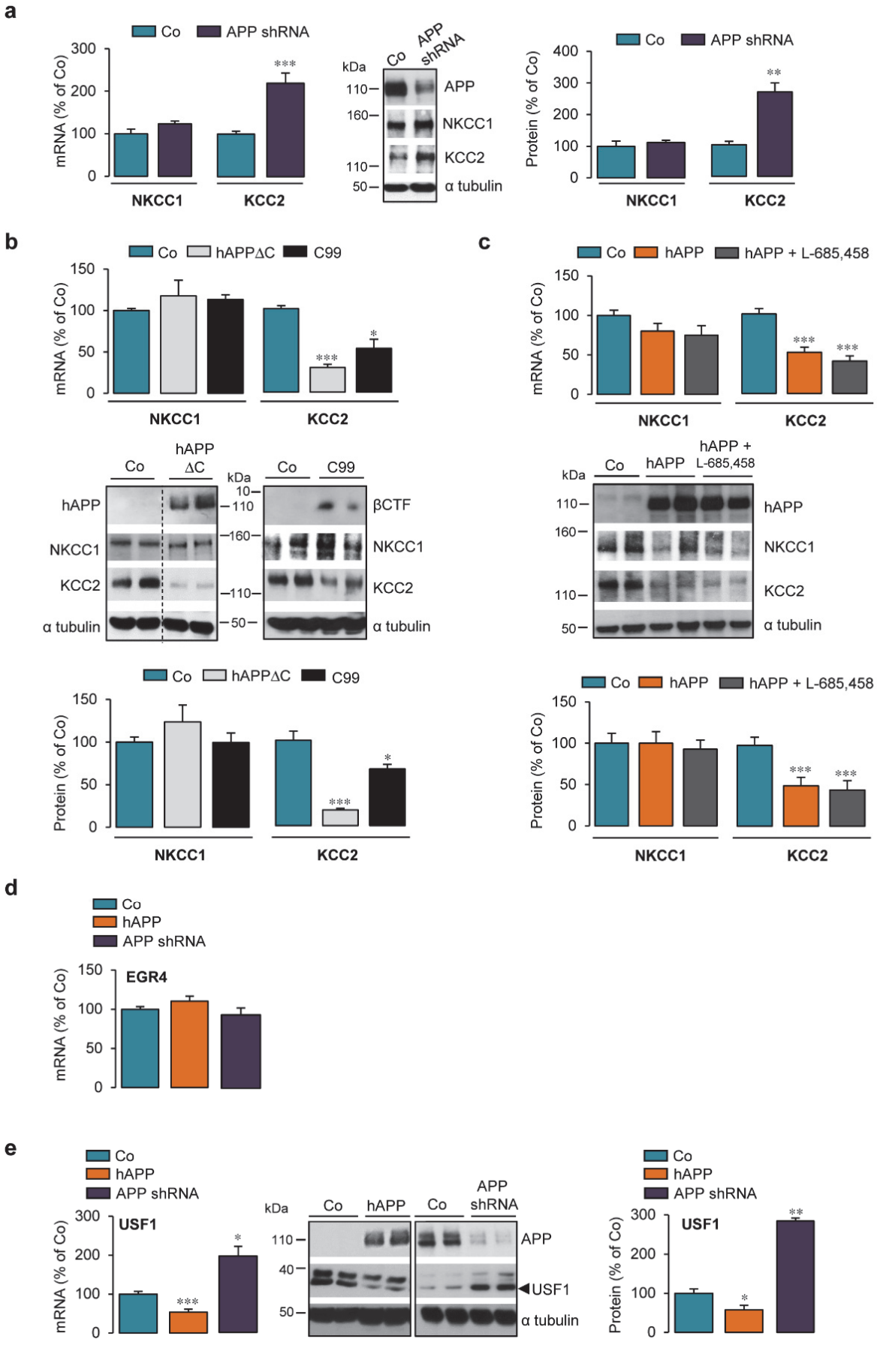

a
Co
APP shRNA
mRNA (% of Co)
NKCC1
KCC2
kDa
APP
NKCC1
KCC2
α tubulin
Protein (% of Co)
b
hAPPΔC
C99
hAPP
ΔC
βCTF
c
hAPP
hAPP + L-685,458
hAPP + L-685,458
d
EGR4
e
USF1

**Fig. 25. APP dependent KCC2 downregulation in primary cortical cultures involves juxta-/transmembrane domain of APP and could rely on USF1.** (**a - e**) Comparative RT-qPCR analyses of *Slc12a2* (coding for NKCC1) and *Slc12a5* (coding for KCC2) (**a**, **b** and **c**) or Egr4 and USF1 (**d** and **e**) mRNA levels in primary rat cortical cultures infected or not (control, Co) at 6 DIV with shRNA APP lentiviruses (**a**, **d** and **e**) or adenoviruses coding for human (hAPP) truncated in its carboxyl- or amino terminal-domain (**b**: hAPPΔC, C99, **c**: hAPP).In (**c**) hAPP expressing cells were treated with 1 µM L-685,458 γ-secretase inhibitor for 24h. Analysis was performed between 13 and 17 DIV. Results were normalized to GAPDH mRNA. (**a**, **b**, **c** and **e**) Representative Western-blots of cell lysates from cortical cultures infected or not (control, Co) at 6 DIV with shRNA APP lentiviruses (**a** and **e**), hAPPΔC, C99 or hAPP adenoviral constructs (**b** and **c**). Between 13 DIV and 17 DIV, expression of hAPP and βCTF was monitored with the specific WO2 antibody and expression of endogenous APP was assessed using the anti-APP C-terminal antibody. Blots were further probed using anti-NKCC1, -KCC2, USF1 and -α tubulin antibodies. NKCC1, KCC2 and USF1 signals were normalized to α tubulin, quantified and expressed as percentage (mean ± s.e.m.) of Co: $*P<0.05$, $**P<0.01$, $***P<0.001$; (**a**) Student's *t*-test for NKCC1 and Mann-Whitney test for KCC2; (**b**) Kruskal-Wallis test; (**c** and **e**): ANOVA (except for USF1 mRNA in (**e**) Kruskal-Wallis test. (**a**) Co n=8 and APPshRNA n=14 (mRNA) and Co n=5 and APPshRNA n=7 (protein) analysed in 5 and 3 independent experiments, respectively; (**b**) Co n=11, APPΔC and C99 n=7 of each in 5 independent experiments; (**c**) hAPP n=7 and hAPP + L-685,458 n=8, each in 3 independent experiments. In **d** and **e**, hAPP and Co n=11 in 5 independent experiments; APPshRNA n=6 (mRNA) and n=11 (protein) in 3 independent experiments.

We next investigated whether cleavage products of APP could be involved in the regulation of KCC2 expression. APP is a type-I transmembrane protein with a large extracellular domain and a short intracellular domain (**Fig. 26**), both contributing to APP function (for review, see (Reinhard et al., 2005)). We first tested whether the APP intracellular domain (AICD), a potential transcriptional regulator (Cao and Sudhof, 2001), could be involved in the downregulation of KCC2. Primary cortical cell cultures were infected with a recombinant adenovirus coding for a hAPP variant containing the APP extracellular and transmembrane domains, but lacking its intracellular domain (APPΔC) (**Fig. 25b**). Expression of APPΔC greatly decreased KCC2 mRNA and protein levels (**Fig. 25b**) (29.4 ± 3.8 and 18.3 ± 1.8 % of control, respectively). Moreover, KCC2 expression was unaffected in cells infected by a control adenovirus coding for β-galactosidase (β-gal, **Fig. 27**). Taken together, the results ruled out involvement of AICD in the hAPP dependent KCC2 decrease.

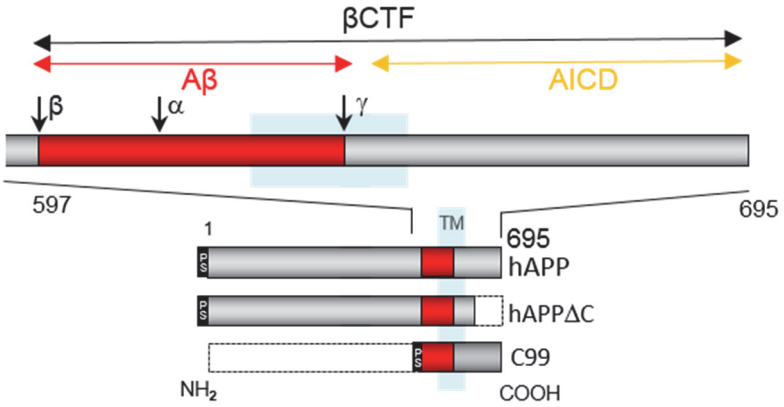



**Fig. 26. APP proteolytic fragments.** Schematic representation of hAPP metabolites and constructs used in this study. hAPP (695 amino acids) is a transmembrane (TM, blue) protein with one membrane spanning domain processed by α-, β- and γ-secretase cleavages to generate β-cleaved carboxyl terminal fragment (βCTF, residue 597 to residue 695 corresponding to the last 99 amino acids of hAPP known as C99), Aβ peptide (red box) and the APP intracellular domain (AICD, yellow). hAPPΔC - hAPP695 truncated in its C-terminal region from residue 652 to residue 695. SP, signal peptide.

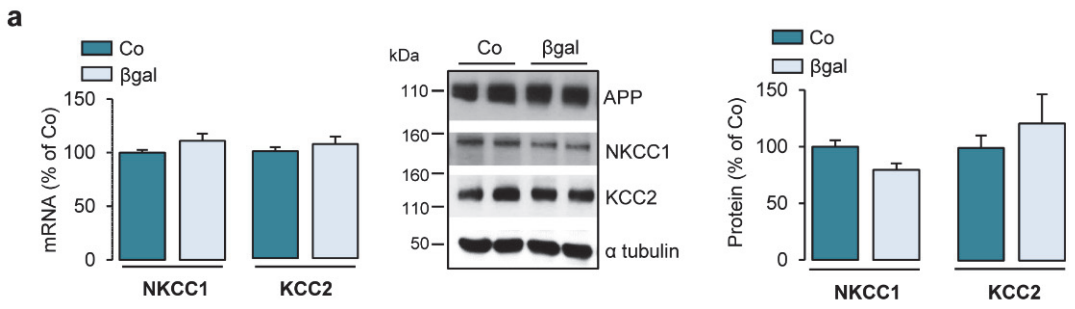



**Fig. 27. Unaffected expression of NKCC1 and KCC2 in βgal infected control cortical cultures**. Left panel: Comparative RT-qPCR analyses of *Slc12a2* (coding for NKCC1) and *Slc12a5* (coding for KCC2) mRNA levels in primary rat cortical cultures infected or not (control, Co) at 6 DIV with an adenovirus coding for β-galactosidase (βgal). Results were obtained between 13 and 17 DIV, normalized to GAPDH mRNA and expressed as percentage (mean ± s.e.m.) of Co (Co n=11 and βgal n=7 analysed in 5 independent experiments); *P*>0.05, Student's *t*-test. Middle panel: Representative Western-blot of cell lysates from βgal cortical cultures. Expression of endogenous APP (APP) was monitored with the anti-APP C-terminal antibody. Blots were further probed with anti-NKCC1, anti-KCC2 and anti-α tubulin antibodies. Right panel: quantification of NKCC1 and KCC2 / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co (Co and βgal n=7 of each analysed in 5 independent experiments), *P*>0.05, Student's *t*-test - except for KCC2 a Mann-Whitney test was applied.

Using a similar strategy, the question of whether the extracellular domain of APP could play a role in regulating KCC2 expression was investigated. An adenoviral vector coding for the transmembrane and intracellular domains of hAPP, i.e. the naturally occurring APP C99 fragment, which begins at the β-secretase cleavage site (CTFβ) (**Fig. 26**), was used. Expression of C99 decreased KCC2 mRNA and protein levels to a similar extent as observed with full-length hAPP (52.3 ± 10.8 and 66.5 ± 5.3 % of control, respectively) (**Fig. 25b**), indicating that the hAPP extracellular domain does not play a role in the hAPP-dependent regulation of KCC2 expression.

γ-secretase processing of APP is physiologically important since it leads to AICD and Aβ production. Involvement of γ-secretase processing in hAPP dependent KCC2 downregulation was studied using a highly potent and selective γ-secretase inhibitor, L-685,458 (1 µM) (Li et al., 2000). Inhibition of γ-secretase activity for 24h in hAPP expressing cells significantly decreased extracellular human Aβ 1-40 levels (**Fig. 28a**) and induced APP carboxyl-terminal fragments (CTFs) accumulation (**Fig. 28b**), but didn't affect NKCC1 and KCC2 mRNA and protein levels (**Fig. 25c**). Together, the results indicate that the hAPP-mediated decrease in KCC2 is AICD and γ-secretase activity independent and does not involve the hAPP extracellular domain.

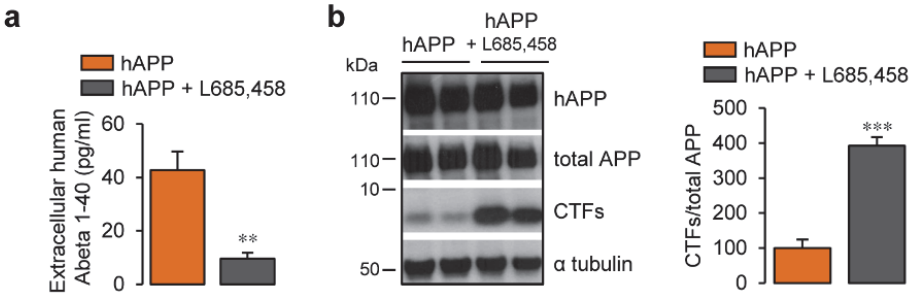



**Fig. 28. Inhibition of γ-secretase activity with L685,458.** (**a**) Effect of 1 µM L685,458 / 24 h, a γ-secretase inhibitor, on human extracellular Aβ1-40 production (pg/ml) at 13 DIV in primary rat cortical cultures infected with an adenovirus coding for human APP (hAPP) (n=9 of each in 3 independent experiments, $**P< 0.01$, Mann-Whitney test). (**b**) Left panel: Representative Western-blot of cells lysates from 13 DIV hAPP expressing cortical cultures treated or not with 10 µM L685,458/24h. Expression of hAPP was monitored with the specific WO2 antibody and expression of total APP and APP carboxyl terminal fragments (CTFs) was monitored with the anti-APP C-terminal antibody. Right panel: quantification CTFs / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of untreated hAPP expressing cells (n=9 of each in 4 independent experiments, $***P< 0.001$, Student's *t*-test).

Given that hAPP is able to decrease KCC2 mRNA levels, expression of two known KCC2 transcriptional regulators was measured in hAPP expressing cells. The KCC2 promoter has 10 putative transcription factor binding sites (Uvarov et al., 2006). Among these, the only two transcription factors clearly described as potent regulators of *Slc12a5* gene coding for KCC2 are Egr4 and USF1 (Uvarov et al., 2006; Markkanen et al., 2008). Although Egr4 can modulate KCC2 expression in developing neurons (Uvarov et al., 2006), Egr4 mRNA level was unchanged in hAPP expressing cells and in cultures infected with APPshRNA lentiviral vector (**Fig. 25d**). By contrast, a 50 % decrease in USF1 mRNA was seen in hAPP expressing cells (53.5 ± 7.7 % of control, **Fig. 25e**). Unfortunately, commercially available Egr4 antibodies didn't detect the Egr4 protein in our cortical cell extracts. On the other hand, USF1 protein levels were decreased in hAPP expressing cells (57.8 ± 11.5 % of control) (**Fig. 25e**). Moreover, mRNA and protein levels of USF1 were increased in cultures infected with an APPshRNA lentiviral vector (201 ± 25.2 and 282 ± 7.7 % of control, respectively) (**Fig. 25e**). Taken together, the results suggest that APP can modulate USF1 and KCC2 expression in a similar manner providing a hint to a putative mechanism of APP dependent KCC2 expression regulation.

## IV. KCC2 expression is decreased *in vivo*.

Since adenoviral hAPP transduction decreased KCC2 expression *in vitro*, we wondered whether hAPP transduction could affect KCC2 and NKCC1 levels *in vivo*. Therefore, serotype 2 associated-adenoviruses coding for full-length neuronal wild-type human APP and green fluorescent protein were produced (AAV-hAPP and AAV-GFP, respectively). AAV constructs were first tested *in vitro* by infecting cultured cortical cells at 6 DIV. At 13 DIV, hAPP and GFP could be detected in AAV-hAPP and AAV-GFP infected cells by Western-blotting, respectively (**Fig. 29a** and **Fig. 30a**, respectively). Moreover, we show that hAPP expression, while not affecting NKCC1 expression, decreased KCC2 protein levels by more than 50 % (43.6 ± 17.1 % of control) (**Fig. 29a**). Furthermore, infection of cortical cells with AAV-GFP didn't modify expression levels of neither NKCC1 nor KCC2 (**Fig. 30a**).

A shift in polarity of $GABA_A$ responses was shown to occur during the first two postnatal weeks in rodent hippocampus (Farrant and Kaila, 2007). Moreover, unlike in the human neocortex (Hyde et al., 2011; Vanhatalo et al., 2005) , KCC2 is robustly upregulated perinatally in rodents and continues to increase during the first postnatal month (Clayton et al., 1998; Kaila et al., 2014). Based on these observations and in order to mimic the timing of adenoviral transductions made *in vitro* (6 DIV), mice pups were intraventricularly injected with an AAV-hAPP or -GFP construct on postnatal day 1 or 2 (P1-P2), i.e. prior to the occurrence of the $GABA_A$ transmission polarity shift (Farrant and Kaila, 2007). Brains of injected mice pups were recovered at P30, the time point when cortical synaptic maturation had been completed (for a review see (Semple et al., 2013). By Western-blotting, hAPP expression in the posterior part of cerebral hemispheres of AAV-hAPP injected mice was examined (**Fig. 29b**). In these samples, KCC2 protein expression decreased while NKCC1 expression remained unchanged (**Fig. 29b**). Furthermore, protein levels of NKCC1 and KCC2 were not affected by AAV-GFP injection (**Fig. 30b**). These results indicate that hAPP was able to decrease KCC2 expression both *in vitro* and *in vivo.*

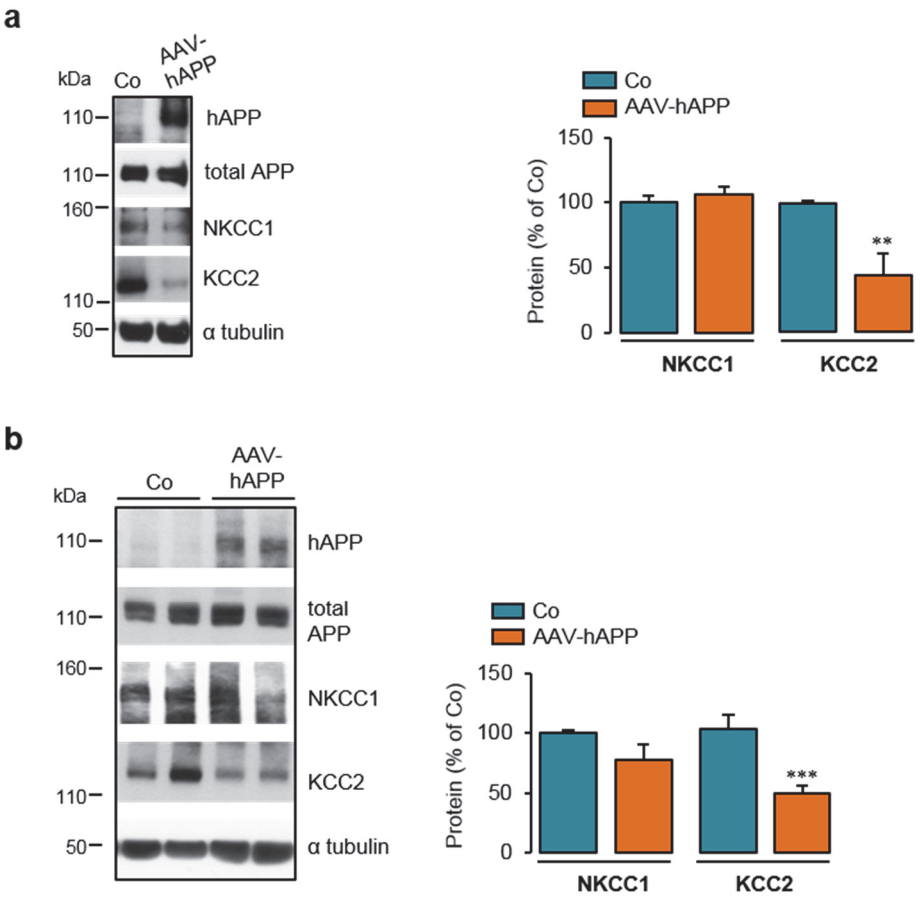



**Fig. 29. NKCC1 and KCC2 expression in AAV-hAPP transduced cortical cells and mouse brains**. (**a**) Representative Western-blot of cell lysates from primary rat cortical cultures infected or not (control, Co) at 6 DIV with serotype 2 associated-adenoviruses coding for wild-type human APP (AAV-hAPP). At 13 DIV, expression of hAPP was monitored with the specific WO2 antibody and expression of both hAPP and endogenous APP (total APP) was analysed using the anti-APP C-terminal antibody. Blots were further probed using anti-NKCC1, -KCC2 and -α tubulin antibodies. Right panels: quantification of NKCC1 and KCC2 / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co (n=3 of each analysed in 3 independent experiments, $**P<0.01$, Student's *t*-test except for KCC2 in (**a**): Mann-Whitney test. (**b**) Representative Western-blot of brain homogenates of mice intraventricularly injected at P1-P2 with AAV-hAPP. 30 days post injection, expression of hAPP was monitored with the specific WO2 antibody and expression of both hAPP and endogenous APP (total APP) with the anti-APP C-terminal antibody. Blots were further probed using anti-NKCC1, -KCC2, and -α tubulin antibodies. NKCC1 and KCC2 / α tubulin ratios were quantified (right panel). Results were expressed as percentage (mean ± s.e.m.) of Co ($***P<0.001$; Mann-Whitney test). Co n=7 mice and AAV-hAPP n=5 mice.

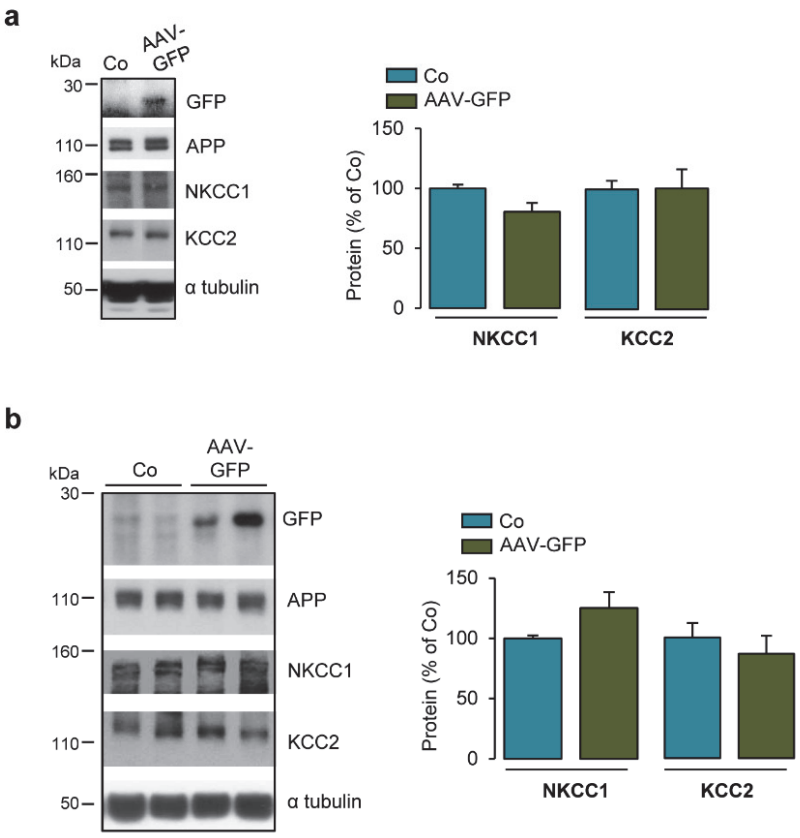



**Fig. 30. AAV-GFP transduction *in vitro* and *in vivo*.** (**a**) Representative Western-blot of cell lysates from primary rat cortical cultures infected or not (control, Co) at 6 DIV with serotype 2 associated-adenoviruses coding for green fluorescent protein (AAV-GFP). Results were obtained at 13 DIV. Expression of endogenous APP (APP) was monitored with the anti-APP C-terminal antibody. Blots were further probed using anti-NKCC1, -KCC2, GFP and -α tubulin antibodies. Right panel: quantification of NKCC1 and KCC2 / α tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co (n=3 of each analysed in 3 independent experiments, $P>0.05$, Student's *t*-test except for KCC2 a Mann-Whitney test was applied). (**b**) Representative Western-blot of brain homogenates from mice intraventricularly injected at P1-P2 with AAV-GFP. 30 days post injection, expression of GFP was monitored with the anti-GFP antibody and endogenous APP (APP) with the anti-APP C-terminal antibody. Blots were further probed using anti-NKCC1, -KCC2, and -α tubulin antibodies. Right panel: NKCC1 and KCC2 / α tubulin ratios were quantified. Results were expressed as percentage (mean ± s.e.m.) of Co (NKCC1, Mann-Whitney test and KCC2 Student's *t*-test. Co n=7 mice and AAV-GFP n=8 mice).

# V. KCC2 expression is decreased in AD patients' brain.

Although APP is not overexpressed in sporadic AD, it is an important player in this pathology where its metabolism and/or function are highly impaired. Therefore, we wondered whether KCC2 and NKCC1 expression could be modified in sporadic AD. We extended our analysis by examining KCC2 and NKCC1 protein levels by Western-blotting in the frontal cortex brain tissues from human control subjects and patients suffering from dementia clinically diagnosed with late onset AD (**Table 1**). While NKCC1 expression remained unchanged, we detected a significant decrease in KCC2 expression in the brains of AD patients (**Fig. 31**). These results indicate that chloride homeostasis could be impaired in the brains of individuals suffering from AD.

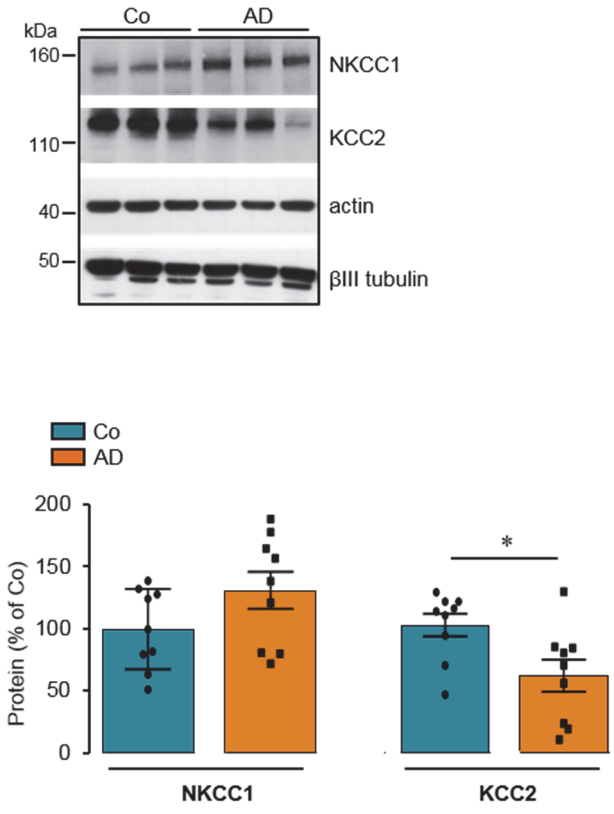



**Fig. 31. Expression of NKCC1 and KCC2 in late onset AD brains.** Representative Western-blot of NKCC1 and KCC2 expression in frontal cortex of postmortem human brain tissues from control subjects (Co, n=9) and patients with Alzheimer's disease (AD, n=9). Blots were probed using anti-NKCC1, -KCC2, -actin and -βIII tubulin antibodies. Bottom panel: quantification of NKCC1 / actin and KCC2 / βIII tubulin ratios. Results were expressed as percentage (mean ± s.e.m.) of Co, ∗$P$<0.05, Student's $t$-test.

| | Case number | Age at death (years) | Sex | Post-mortem interval (h) | Braak stageing (NFT) | CERAD Plaque score (amyloid) |
|---|---|---|---|---|---|---|
| **Control subjects** | | | | | | |
| | 1 | 78 | M | 23 | 0 | 0 |
| | 2 | 69 | M | 6 | 0 | 0 |
| | 3 | 58 | M | 5.5 | I-II | 0 |
| | 4 | 92 | F | NA | 0 | 0 |
| | 5 | 60 | F | 28 | 0 | 0 |
| | 6 | 79 | M | 6.5 | I | A |
| | 7 | 70 | F | 7.5 | II | A |
| | 8 | 79 | M | 6 | II | A |
| | 9 | 78 | F | 7 | I | A |
| **AD patients** | | | | | | |
| | 1 | 72 | M | 44 | VI | C |
| | 2 | 76 | M | 10 | VI | C |
| | 3 | 60 | M | 37 | VI | C |
| | 4 | 78 | F | 24 | VI | C |
| | 5 | 79 | F | 25 | VI | C |
| | 6 | 83 | F | 23 | VI | C |
| | 7 | 73 | F | 6 | VI | C |
| | 8 | 77 | M | 6 | VI | C |
| | 9 | 74 | M | 7.5 | VI | C |

| | Post-mortem interval (h) Mean ± SEM | Age at death (years) Mean ± SEM | Sex F/M |
|---|---|---|---|
| | | | |
| **Control subjects** | 11.19 ± 3.16 | 73.67 ± 3.53 | 4/5 |
| **AD patients** | 20.28 ± 4.65[a] | 74.67 ± 2.14[b] | 4/5 |

**Table 1**. **Summary of the characteristics of control subjects and AD patients**. ([a] and [b]) Mean post-mortem interval ([a]) and age ([b]) were not significantly different between control subjects (n=9) and Alzheimer patients (n=9) (*P* = 0.1368 and *P* = 0.8119, respectively; Student's *t* test). The neuropathological staging of AD patients is determined according to the Braak and Braak staging and the CERAD plaque score. NA, not-available; NFT, neurofibrillary tangles.

# Discussion

In this study, the influence of APP expression on NKCC1 and KCC2 expression levels with an emphasis on GABAergic neurotransmission was investigated. We show that hAPP was able to decrease KCC2 expression both *in vitro* and *in vivo*. Furthermore, the decrease in KCC2 expression observed *in vitro* was associated with a less inhibitory and less hyperpolarizing GABA signaling. In addition, our study unveiled an involvement of the juxta-/transmembrane domain of APP in this regulation. Expression of KCC2 was also shown to be decreased in the cortex of sporadic AD patients.

## I. APP and chloride cotransporters

Several lines of evidence coming from mice lacking or overexpressing APP indicate that APP could play a role in GABAergic neurotransmission. Indeed it has been shown that paired-pulse inhibition of GABAergic IPSCs is decreased in the hippocampus of *App*$^{-/-}$ mice (Seabrook et al., 1999). In this same model, the theta-gamma oscillation coupling, involved in inhibitory transmission, was also demonstrated to be impaired (Zhang et al., 2016a). Moreover, in an inducible transgenic model of AD, APP overexpression, but not a subsequent Aβ increase as established by using γ-secretase inhibitors, leads to appearance of seizures and is associated with changes in GABAergic neurotransmission activity and innervation (Born et al., 2014). Despite the significance of these results, the exact relationship between APP and GABA signaling remains elusive. As an add-on to the previous work, our study investigated the link between APP and indirect GABAergic neurotransmission actors - cation-chloride cotransporters NKCC1 and KCC2. Our results show that expression of human APP in primary cortical cultures was able to decrease KCC2 expression without modifying NKCC1 leading to a less hyperpolarizing and less inhibitory GABA signaling. In contrast, in a mouse model of Down syndrome where APP is overexpressed due to a triplication of mouse chromosome 16 carrying *App* gene (Deidda et al., 2015a; Seo and Isacson, 2005), an increase in NKCC1 expression has been observed without any significant change in KCC2 expression. This discrepancy could be explained by the fact that the genomic region triplicated in this model codes for a whole range of genes besides APP that could be able to influence CCCs expression.

Since we have shown that overexpression of APP decreased KCC2 expression thereby affecting GABAergic neurotransmission and it is known that GABAergic signaling impairments can be found in both mice lacking or overexpressing APP, we were interested to see whether expression of KCC2 could rely on *App* gene dosage. In this regard, we have observed that decreasing APP expression with shRNAs induced an upregulation of KCC2 in our primary cortical cultures. However, KCC2 expression analysed in $App^{-/-}$ primary cortical cultures was not significantly modified. This could be probably due to the existence of compensatory mechanisms by APPs paralogs, APLP1 and APLP2, in this model (von Koch et al., 1997; Heber et al., 2000). Moreover and in opposition to our results, Chen and al. have very recently shown that KCC2 protein levels were decreased in $App^{-/-}$ mouse brains (Chen et al., 2017b). More precisely, the authors have shown that APP and KCC2 can interact and that APP can prevent tyrosine-phosphorylation and ubiquitination of KCC2 thereby stabilizing its content. While in our study we have analyzed the impact of APP on KCC2 expression around the timing of the GABA shift occurrence, we were also interested to see whether APP could influence KCC2 levels in adult mice. Therefore, we have analyzed expression levels of KCC2 in the cortices of $App^{-/-}$ mice at several ages. At the ages of 8-9 months and 12 months, we observed indeed a decrease in KCC2 protein level in $App^{-/-}$ mice (**Fig.32**), confirming the results of Chen et al. In contrast, at the age of 3 months, the absence of APP induced a slight increase in KCC2 expression, in particular at the mRNA level (**Fig.32**), indicating that APP can regulate KCC2 protein level by different mechanisms, depending on the age studied. Based on these results, it appears that in developing systems APP would be able to regulate KCC2 expression by adjusting its mRNA levels, while in adult animals this regulation would mostly rely on post-translation modifications that could modify KCC2 protein content. Moreover, the net effect of these regulations seems to be opposite between developing and adult systems. Indeed, in developing neurons, APP decreases KCC2 expression, while in adult neurons, APP increases KCC2 expression. This notion of differing effects between immature and mature networks is not new, since the same was already shown for BDNF. Indeed, while BDNF increases KCC2 expression in immature neurons (Ludwig et al., 2011), exposure of mature neurons to BNDF causes a decrease in KCC2 mRNA and protein levels (Rivera et al., 2002; Shulga et al., 2008; Rivera et al., 2004; Wake et al., 2007; Boulenguez et al., 2010).

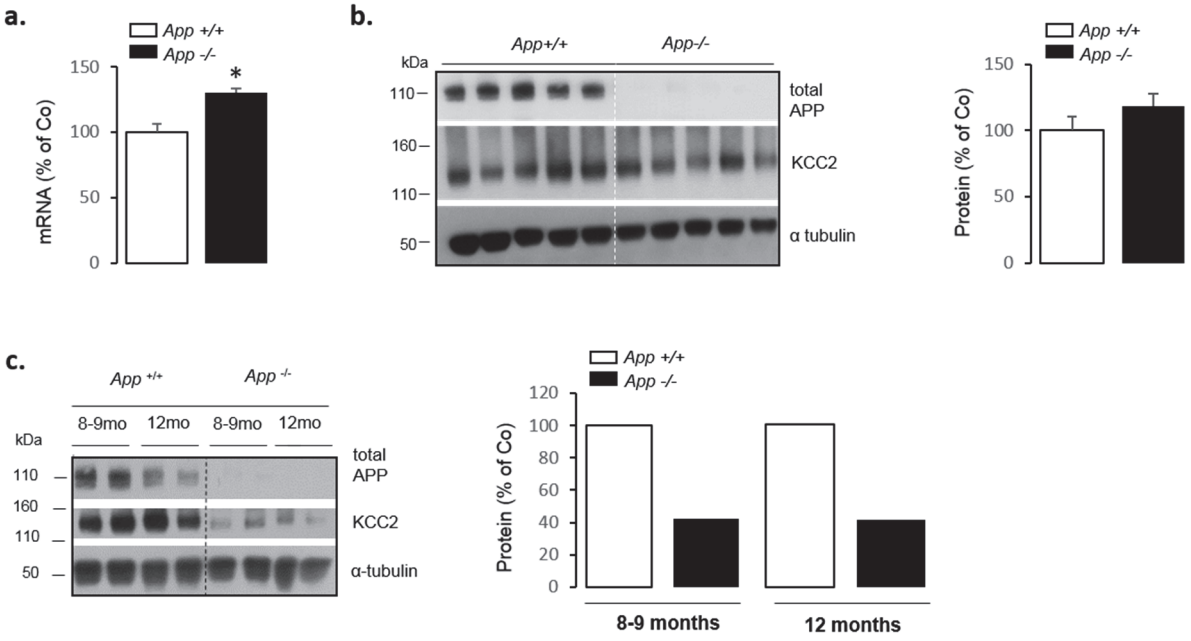



**Fig. 32. KCC2 expression in *App*$^{-/-}$ cortex at 3, 8-9 and 12 months.** (**a**) Comparative RT-qPCR analyses of KCC2 mRNA levels in cortices of *App*$^{+/+}$ and *App*$^{-/-}$ 3 months old mice. Results were normalized by GAPDH mRNA. (**b**)(**c**) Western blots of *App*$^{+/+}$ and App$^{-/-}$ cortices from 3 months old mice (**b**) and from 8-9 months old and 12 months old mice (**c**). Endogenous APP and KCC2 levels were monitored. Blots were further probed using α tubulin antibody. KCC2 / α tubulin ratios were quantified and results were expressed as percentage (mean ± s.e.m.) of *App*$^{+/+}$; ∗P<0.05 (**a**) Student's t-test; *App*$^{+/+}$n=5 and *App*$^{-/-}$ n=5 (**b**) Student's t-test; *App*$^{+/+}$ n=5 and *App*$^{-/-}$ n=5 (**c**) *App*$^{+/+}$ n=2 and *App*$^{-/-}$ n=2.

## II. APP and GABAergic neurotransmission polarity

As illustrated by evoked action potential measurements in hAPP expressing primary cortical cultures, GABA dependent inhibition as well as GABA-induced hyperpolarization were decreased. Nevertheless, we didn't see a shift of GABAergic signaling polarity from inhibitory to excitatory in hAPP expressing neurons despite that lowered expression of KCC2 is often associated with excitatory GABA responses in immature neurons (Ben-Ari et al., 2007), and that decreased KCC2 expression in adult animals can result in excitatory GABA signaling after axonal injury (Nabekura et al., 2002) or favor the appearance of spontaneous and evoked epileptiform activity (Tornberg et al., 2005; Woo et al., 2002). In this regard, it is noteworthy that we recorded spiking activity using cell-attached mode from the cell bodies of hAPP expressing neurons and therefore addressed changes only in their global excitability. Given this, we cannot exclude that specific cell compartments like certain neuronal processes may

respond to GABA differently as KCC2 expression pattern could be modified by hAPP (Jean-Xavier et al., 2007; Doyon et al., 2016). Furthermore, we can't rule out that reduced KCC2 expression could be compensated by an increase in its activity. Indeed, though it was not assessed in this study, it is known that KCC2 activity can be regulated by PKC-dependent phosphorylation of S940 (Lee et al., 2007; Lee et al., 2011) or by interaction with certain proteins like Neto2 (Ivakine et al., 2013). In addition, Chen et al. have recently shown that APP and KCC2 can interact and that APP functions to limit tyrosine-phosphorylation and ubiquitination and thus subsequent degradation of KCC2, furthermore emphasizing the potential importance of these mechanisms for the results that we obtained (Chen et al., 2017b). Surface availability of KCC2 can also influence its activity (Kaila et al., 2014; Medina et al., 2014). Since quantification of KCC2 at the plasma membrane wasn't performed in hAPP expressing neurons we can't exclude that KCC2 localization might influence the GABAergic activity differences that we observed. Moreover, although we measured no change in expression levels of $GABA_AR$ receptor subunits $\alpha3$ and $\alpha1$, the function or availability of theses receptors could be modified by hAPP. Indeed, it was shown that exogenous Aβ application could increase $GABA_AR$ internalization and therefore decrease GABA-dependent inhibition in slices of rat somatosensory cortex (Ulrich, 2015). Finally, it is important to mention that our electrophysiology results establish an association but do not demonstrate the existence of a direct link between KCC2 expression changes and GABAergic activity modifications. Indeed, a more compelling evidence for this link could be provided by measuring the reversal potential of $GABA_AR$ (EGABA) in hAPP expressing cultures that would be expected to shift to more positive values if $[Cl^-]_i$ is indeed increased in these neurons.

With regards to our results, it would be also very interesting to assess whether the extent of KCC2 expression decrease observed could vary depending on the type of neuron concerned e.g. excitatory vs inhibitory, cholinergic vs glutamatergic. Our main working model, primary cortical culture encompasses an important mix of neuronal cell types and also astrocytes. Therefore, a more prominent decrease of KCC2 expression that could appear following hAPP expression in certain types of cells might have a differential impact on the activity of the whole network.

## III. Mechanisms of APP-dependent KCC2 expression regulation

Numerous physiological roles have been assigned to cleavage products of APP. Therefore, we studied which part of hAPP could be responsible for KCC2 expression decrease. Constructs lacking the APP intracellular domain (APPΔC) and extracellular domain (C99) were expressed in primary cortical cells and both constructs decreased KCC2 expression. However, APPΔC expression decreased KCC2 expression to a greater extent than full-length hAPP. This difference could be due to a trafficking disparity between APPΔC and full-length APP (Santos et al., 2011). For example, the APP C-terminal domain contains the YENPTY internalization motif, which is required for APP endocytosis necessary for APP processing (Reinhard et al., 2005). Experiments using APPΔC and C99 supported the idea that the juxta-/transmembrane domain of APP containing Aβ sequence would be important for APP dependent KCC2 regulation. This domain not only contains Aβ sequence, but also contributes to APP dimerization (for review see (Khalifa et al., 2010). Indeed, APP shares several structural similarities with certain transmembrane receptors like Notch (Brou et al., 2000) and the EGFR (Schlessinger, 2002). These receptors are known to form homodimers at the plasma membrane necessary for cellular signaling pathways activation. Similarly to these receptors, APP can form homodimers (Scheuermann et al., 2001). In addition, it is also known that APP can form heterodimers with several proteins including, for example, Notch (Chen et al., 2006), British precursor protein (BRI2) (Fotinopoulou et al., 2005) and sterol regulatory binding protein (SREBP) (Pierrot et al., 2013). Therefore, one could speculate that APP homo- or heterodimerization with identified or yet unidentified partners might be needed to initiate signaling pathways leading to KCC2 expression regulation.

The juxta-/transmembrane domain of APP also contains the sequence of Aβ peptide that can be released following β- and γ-secretase cleavages of APP. We have used a γ-secretase inhibitor, L-685,458, to treat hAPP expressing primary neuronal cultures in order to investigate whether the γ-secretase cleavage of APP would be important for APP-dependent KCC2 expression regulation. We observed that γ-secretase inhibitor L-685,458 didn't affect KCC2 expression indicating that γ-secretase cleavage is not involved in this regulation. However, we

cannot exclude that the γ-secretase cleavage product, Aβ, could modify KCC2 expression. Indeed, after L-685,458 treatment, residual Aβ was still detectable and could therefore still be able to induce a decrease in KCC2 expression. In line with this, in a special model of AD with no mutations in *App* and *tau* genes that nevertheless harbors AD-like hallmarks including amyloid deposits containing Aβ and neurofibrillary extracellular deposits, decreased KCC2 expression has been observed (Lagostena et al., 2010). One could speculate that the KCC2 decrease observed in these animals could be due to the appearance of Aβ deposits. Based on this, it would be interesting to investigate whether KCC2 expression changes could be influenced by Aβ and its various types of aggregates.

It is important to mention that both use of

Given that hAPP altered KCC2 expression at mRNA level, we investigated whether expression of potent transcriptional regulators of KCC2 (Egr4 and USF1) could be modified by APP (Uvarov et al., 2006; Markkanen et al., 2008). Egr4 mRNA levels did not change in hAPP expressing neurons, whereas a correlation between USF1 and KCC2 expression was observed in these cells. Nevertheless, as Egr4 protein was undetectable, we cannot assess whether its expression level remains unchanged in hAPP expressing cells. Moreover, even if the correlation we observed could involve USF1 in APP dependent KCC2 regulation, there are nine other transcription factor binding sites located in the KCC2 promoter (Uvarov et al., 2006). In this regard, recently it has been shown that the transcriptional regulator methyl CpG binding protein (MeCP2) involved in Rett's syndrome can regulate KCC2 expression by inhibiting KCC2 expression repressor NRSF (Tang et al., 2016). Therefore, it would be interesting to analyse whether MeCP2 and NRSF might be involved in APP-dependent KCC2 expression regulation.

## IV. KCC2 and Alzheimer's disease

Although neuronal APP is not overexpressed in sporadic AD, APP is an important player in this pathology where its metabolism and/or function are highly impaired. Therefore, we examined KCC2 and NKCC1 expression in the frontal cortex of sporadic AD patients and control subjects. While we detected no change in NKCC1 expression, a significant decrease in KCC2 protein was observed in the brains of AD individuals. A potential involvement of Aβ in APP

dependent KCC2 regulation cannot be excluded and one could speculate that increased levels of Aβ and/or an alteration in the physiological function of APP could influence KCC2 expression in AD. In addition, even though there is no global increase in *APP* expression levels in sporadic AD, it was shown that certain neurons could contain multiple copies of *APP* gene with a 4 fold increase of neurons with more than 2 *APP* copies in AD versus non-diseased cortex (Bushman et al., 2015), therefore arguing for a potential involvement of *APP* gene dosage phenomena in KCC2 expression regulation in the context of sporadic AD. Furthermore, there is evidence that even though the expression of the predominant neuronal APP isoform, APP695, is not increased in AD brains, expression of longer APP isoforms APP751 and APP770 could be increased (Moir et al., 1998; Matsui et al., 2007; Preece et al., 2004) and maybe influence KCC2 expression.

Several evidences point to the importance of KCC2 in normal cognitive functioning. First, decreased KCC2 expression was detected in hippocampal slices of young rats after induction of LTP, an important mechanism underlying memory and cognition, suggesting an involvement of KCC2 in weakening GABAergic inhibition and helping to establish LTP (Wang et al., 2006). Furthermore, a recent study by Ferando et al. has explored the relationship between senescence, KCC2 and LTP (Ferando et al., 2016). In particular, young and aged mice that have normal and impaired LTP, respectively, were compared, and more decreased protein levels of KCC2 were found after LTP induction in hippocampal slices from old mice versus young mice. In the same study, it was shown that an LTP impairment could be induced in slices from young animals preincubated with a KCC2 inhibitor VU0240551, while treatment with KCC2 enhancer CLP257 of slices from old mice could restore LTP deficits, indicating that KCC2 function would be important for LTP induction and therefore memory formation as well as its dosage (Ferando et al., 2016). Taken together, these two studies suggest that a slight decrease in KCC2 expression would be able to favor LTP induction, while an important KCC2 decrease could impair LTP and memory formation. In line with this, it was shown that KCC2 hypomorphic mice that retain only 15–20% of normal KCC2 protein levels in the brain have an impaired performance in a water-maze learning task (Tornberg et al., 2005). In addition, in another study, *in vivo* knock-down of KCC2 expression in mice by an shRNA lentivirus induced LTP deficits as compared to mice transduced with a non-target shRNA vector (Chevy et al., 2015). Taken together, all these observations indicate that the KCC2 expression impairment

that we observed in AD brains could contribute to AD-related cognitive dysfunction. Given this, it would be of particular interest to see whether KCC2 could be a potential therapeutic target in the context of AD. In this regard, KCC2 activity enhancers, like for instance CLP257, (Gagnon et al., 2013) or NKCC1 activity blockers, like bumetanide (Ben-Ari, 2017), could be found effective in improving cognitive performance in AD patients. Nevertheless, it is important to mention that just like in our *in vitro* experiments, KCC2 expression decrease observed in AD brains would not necessarily impact GABAergic neurotransmission functioning. Indeed, there could be compensatory changes in KCC2 or NKCC1 post-translational modifications, or a change in KCC2 surface availability. In this regard, it would be of particular interest to see whether GABAergic neurotransmission responses are impaired in individuals harboring AD. To assess this, transcranial magnetic stimulation (TMS) coupled to multi-channel electroencephalography could be performed in AD patients after administration of $GABA_AR$ activating drugs, benzodiazepines.

Benzodiazepines are often prescribed to AD patients to treat their anxiety and sleep disturbance issues but were however shown to have little beneficial effects on these symptoms (Defrancesco et al., 2015). Furthermore, certain studies have pointed out that prolonged use of benzodiazepines (more than 3 months) could even increase AD risk (Billioti de et al., 2014; Lagnaoui et al., 2002). Given that KCC2 is decreased in AD patients, this would most probably influence the functional response triggered by benzodiazepines since the potency of inhibition induced by $GABA_AR$ activation might be decreased in AD patients just like in our hAPP expressing primary cultures. Therefore, it is possible that benzodiazepines could trigger a different behavioral response in AD brains as compared to healthy individuals. In this regard, administration of $GABA_A$ activating drugs to AD patients should probably be revised.

Besides APP and its cleavage products, AD encompasses numerous factors that could contribute to KCC2 expression decrease that we observed in these patients. For instance, it is known that in AD, there is a massive loss of excitatory cholinergic neurons associated with a cholinergic neurotransmission deficit. Interestingly, it was shown that KCC2 expression can be increased by an activation of ionotropic nicotinic receptors that mediate cholinergic neurotransmission (Liu et al., 2006). Therefore, on could consider that the KCC2 expression

decrease observed might be due to a lack of cholinergic neurotransmission signaling in AD brains.

In addition to amyloid plaques, another important hallmark of AD are neurofibrillary tangles that contain hyperphosphorylated microtubule associated protein tau. Not much is known concerning tau's involvement in GABAergic neurotransmission (Li et al., 2016; Govindpani et al., 2017), besides that $GABA_AR$ activation could influence tau phosphorylation (Nykanen et al., 2012) and that in a tau transgenic mouse model, GABA levels are increased (Nilsen et al., 2013). Nevertheless, we cannot rule out that tau and/or its phosphorylation might influence KCC2 expression levels.

It is well established that KCC2 activity and expression are decreased in epileptic brains (Kaila et al., 2014) and it is known that at least 20% of AD patients develop clinically detectable epileptic seizures (Amatniek et al., 2006; Vossel et al., 2017) with even a greater number of AD patients showing subclinical epileptiform activity during sleep (Cretin et al., 2016; Lam et al., 2017). Although, the cause-consequence relationship between decreased KCC2 expression and epileptiform discharges is not yet very well identified, it is tempting to speculate that decreased KCC2 expression could trigger epileptic seizures in the brains of AD patients or at least contribute to their appearance. Furthermore, evidence exists that abnormal epileptiform activity detected in AD patients could be linked to a more severe and rapid impairment of cognitive function (Volicer et al., 1995; Rabinowicz et al., 2000; Cretin et al., 2016). Based on this, one could hypothesize that decreased KCC2 expression observed in AD patients could contribute to the memory deficits observed in these patients by favoring the appearance of seizures. In addition, it would highly interesting to analyze KCC2 expression levels in patients harboring early-onset autosomal dominant familial forms of AD or a microduplication of the *APP* locus that are known to have a greater risk for seizures than sporadic AD individuals (Sherzai et al., 2014).

Another important feature of AD is astrocytic gliosis, a phenomena that refers to morphological and biochemical changes that occur in astrocytes in response to stress caused by an injury or a disease. These changes include both an increase in volume and in number of astrocytes as well abundant astrocytic secretion of cytokines and chemokines aiming to

counter the stress (Acosta et al., 2017). It has been shown that in a mouse model lacking β1-integrin that harbors widespread astrocytic gliosis, KCC2 expression is decreased (Robel et al., 2015). Furthermore, in glioma (form of cancer triggered by astrocytic proliferation) mouse model and in acute tissues from glioma patients, an important decrease in KCC2 expression has also been observed (Pallud et al., 2014; Campbell et al., 2015). Taken together, these evidences suggest that there could be a potential link between astrocytic gliosis and KCC2 expression alteration observed in AD brains.

Finally, it is know that KCC2 mRNA levels are controlled by a miRNA termed miRNA-92 (Barbato et al., 2010) and it has been shown that miRNA-92 levels are increased in the prefrontal cortex and hippocampus of AD patients (Lau et al., 2013). Based on this, one could speculate that KCC2 expression decrease detected in AD cortices could be due to an increase in miRNA-92 levels.

## V. APP and KCC2 in other pathologies

Chloride homeostasis imbalance, and in particular KCC2 expression and function modifications, have been observed in a great number of CNS pathologies like epilepsy, autism, traumatic brain injury, chronic pain and schizophrenia. Interestingly, an APP expression/ processing dysregulation has been described in several of these diseases (Westmark, 2013; Gentleman et al., 1993; Westmark et al., 2016). For instance, increased APP expression levels have been measured in temporal lobe (Sheng et al., 1994) and refractory (Sima et al., 2014) epilepsy patients where decreased KCC2 expression and activity have been documented (Huberfeld et al., 2007; Kaila et al., 2014; Blaesse et al., 2009). Moreover, it was shown that Aβ-positive senile plaques could be found in 10% of temporal lobe epilepsy cases (Mackenzie and Miller, 1994). It is also known that chloride homeostasis is disturbed in autism where bumetanide (NKCC1 antagonist) treatment was found to positively affect symptomology (Lemonnier and Ben-Ari, 2010; Ben-Ari, 2015). Up to 5% of autism cases are due to Fragile X syndrome, which is a common form of inherited intellectual disability caused by a mutation in the fragile X mental retardation 1 (*Fmr1)* gene (Westmark et al., 2016). It was shown that APP expression is increased in *Fmr1*$^{-/-}$ mice, a mouse model of Fragile X syndrome, and that FMR1

can regulate APP mRNA translation (Westmark et al., 2016; Westmark and Malter, 2007). There is also solid evidence that KCC2 expression is downregulated in *Fmr1*$^{-/-}$ mice (Tyzio et al., 2014). Finally, traumatic brain injury is known to be associated with an increase in APP expression levels (Gentleman et al., 1993; Itoh et al., 2009) as well as with a decrease in KCC2 expression levels (Shulga et al., 2008; Nabekura et al., 2002). Taken together, these studies outline an interesting correlation between APP expression/metabolism changes and modifications in KCC2 levels suggesting a possible link between these observations.

# VI. APP and KCC2: going beyond GABAergic neurotransmission

Our study highlights the involvement of APP in the regulation of KCC2 expression, which could influence the polarity of GABAergic neurotransmission. However, it should be mentioned that such regulation could also be extended to the excitatory system. Indeed, KCC2 plays an important structural role in dendrites independent of its $Cl^-$ transport function making it a synchronizing factor in the development of both inhibitory and excitatory neurotransmission (Li et al., 2007). KCC2 is involved in the functioning and development of synapses and could help to stabilize them (Li et al., 2007; Fiumelli et al., 2013; Horn et al., 2010; Gauvain et al., 2011). Given that APP is known to modulate neurite outgrowth and synapse development (Lee et al., 2010b; Tyan et al., 2012), one might speculate that APP-dependent KCC2 regulation plays a role in these mechanisms. In addition, it was shown that decreased KCC2 expression was associated with reduced postsynaptic clusters of GluA1-containing AMPA receptors and impaired excitatory neurontransmission (Gauvain et al., 2011). In this regard, it would be of particular interest to analyse whether APP could affect glutamatergic neurotransmission by regulating KCC2 content.

Furthermore, our study introduces a potential developmental aspect for APP dependent KCC2 regulation. Indeed, hAPP adenoviral infection was performed at 6 DIV, prior to the appearance of the GABA shift. Given this, one might speculate that hAPP expression might block the developmental upregulation of KCC2 in primary cortical cultures. Moreover, it is known that in rodents APP expression peaks at the 2$^{nd}$ postnatal week (Loffler and Huber, 1992), a timing when the GABA “shift” appears and KCC2 expression undergoes an important decrease

(Farrant and Kaila, 2007). Based on these evidences, it would be interesting to study the involvement of APP in inhibitory and excitatory synapse development with a focus on KCC2.

# VII. Concluding remarks

In conclusion, our results point to a new important role of APP in the regulation of KCC2 expression, which could influence GABAergic neurotransmission. This information advances our understanding of the physiological function of APP and sheds light on new mechanisms that could contribute to AD pathology. Future research would be needed to elucidate APP dependent mechanisms of KCC2 regulation and their impact on GABAergic neurotransmission as well as on cognitive processes, in particular in animal models. Influence of APP on the morphogenic role of KCC2 in inhibitory and excitatory synapse development would also be worth investigating. In addition, an in depth insight into APP-dependent KCC2 regulation in the context of other CNS pathologies could contribute to broaden our knowledge about APP and its physiological and pathophysiological function. Finally, it would be interesting to see whether KCC2 could be a therapeutic target in AD since its expression was shown to be affected in the cortex of sporadic AD patients.

# Bibliography


Abramov,E., Dolev,I., Fogel,H., Ciccotosto,G.D., Ruff,E., and Slutsky,I. (2009). Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. Nat. Neurosci. *12*, 1567-1576.

Acosta,C., Anderson,H.D., and Anderson,C.M. (2017). Astrocyte dysfunction in Alzheimer disease. J. Neurosci. Res.

Aguado,F., Carmona,M.A., Pozas,E., Aguilo,A., Martinez-Guijarro,F.J., Alcantara,S., Borrell,V., Yuste,R., Ibanez,C.F., and Soriano,E. (2003). BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2. Development *130*, 1267-1280.

Akerman,C.J. and Cline,H.T. (2006). Depolarizing GABAergic conductances regulate the balance of excitation to inhibition in the developing retinotectal circuit in vivo. J. Neurosci. *26*, 5117-5130.

Alger,B.E. and Nicoll,R.A. (1979). GABA-mediated biphasic inhibitory responses in hippocampus. Nature *281*, 315-317.

Alonso,A.C., Grundke-Iqbal,I., and Iqbal,K. (1996). Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. Nat. Med. *2*, 783-787.

Altman,J. and Das,G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp Neurol. *124*, 319-335.

Alzheimer's Disease International. World Alzheimer Report. 2015.
Ref Type: Generic

Alzheimer,A., Stelzmann,R.A., Schnitzlein,H.N., and Murtagh,F.R. (1995). An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". Clin. Anat. *8*, 429-431.

Amatniek,J.C., Hauser,W.A., DelCastillo-Castaneda,C., Jacobs,D.M., Marder,K., Bell,K., Albert,M., Brandt,J., and Stern,Y. (2006). Incidence and predictors of seizures in patients with Alzheimer's disease. Epilepsia *47*, 867-872.

Andrew,R.J., Kellett,K.A., Thinakaran,G., and Hooper,N.M. (2016). A Greek Tragedy: The Growing Complexity of Alzheimer Amyloid Precursor Protein Proteolysis. J. Biol. Chem. *291*, 19235-19244.

Arai,H., Emson,P.C., Mountjoy,C.Q., Carassco,L.H., and Heizmann,C.W. (1987). Loss of parvalbumin-immunoreactive neurones from cortex in Alzheimer-type dementia. Brain Res. *418*, 164-169.

Arriagada,P.V., Growdon,J.H., Hedley-Whyte,E.T., and Hyman,B.T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology *42*, 631-639.

Asada,H., Kawamura,Y., Maruyama,K., Kume,H., Ding,R., Ji,F.Y., Kanbara,N., Kuzume,H., Sanbo,M., Yagi,T., and Obata,K. (1996). Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. Biochem. Biophys. Res. Commun. *229*, 891-895.

Asada,H., Kawamura,Y., Maruyama,K., Kume,H., Ding,R.G., Kanbara,N., Kuzume,H., Sanbo,M., Yagi,T., and Obata,K. (1997). Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. Proc. Natl. Acad. Sci. U. S. A *94*, 6496-6499.

Ascher,P., Kunze,D., and Neild,T.O. (1976). Chloride distribution in Aplysia neurones. J. Physiol *256*, 441-464.

Ashford,J.W. (2004). APOE genotype effects on Alzheimer's disease onset and epidemiology. J. Mol. Neurosci. *23*, 157-165.

Association Alzheimer. Alzheimer's disease facts and figures. 12 4. 2016. Alzheimer's Dementia. Ref Type: Generic

Atwood,C.S., Martins,R.N., Smith,M.A., and Perry,G. (2002). Senile plaque composition and posttranslational modification of amyloid-beta peptide and associated proteins. Peptides *23*, 1343-1350.

Avoli,M., Louvel,J., Pumain,R., and Kohling,R. (2005). Cellular and molecular mechanisms of epilepsy in the human brain. Prog. Neurobiol. *77*, 166-200.

AWAPARA,J., LANDUA,A.J., FUERST,R., and SEALE,B. (1950). Free gamma-aminobutyric acid in brain. J. Biol. Chem. *187*, 35-39.

Baek,S.H., Ohgi,K.A., Rose,D.W., Koo,E.H., Glass,C.K., and Rosenfeld,M.G. (2002). Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein. Cell *110*, 55-67.

Bakker,A., Krauss,G.L., Albert,M.S., Speck,C.L., Jones,L.R., Stark,C.E., Yassa,M.A., Bassett,S.S., Shelton,A.L., and Gallagher,M. (2012). Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron *74*, 467-474.

Ballatore,C., Lee,V.M., and Trojanowski,J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat. Rev. Neurosci. *8*, 663-672.

Barbato,C., Ruberti,F., Pieri,M., Vilardo,E., Costanzo,M., Ciotti,M.T., Zona,C., and Cogoni,C. (2010). MicroRNA-92 modulates K(+) Cl(-) co-transporter KCC2 expression in cerebellar granule neurons. J. Neurochem. *113*, 591-600.

Bareggi,S.R., Franceschi,M., Bonini,L., Zecca,L., and Smirne,S. (1982). Decreased CSF concentrations of homovanillic acid and gamma-aminobutyric acid in Alzheimer's disease. Age- or disease-related modifications? Arch. Neurol. *39*, 709-712.

Baumgart,M., Snyder,H.M., Carrillo,M.C., Fazio,S., Kim,H., and Johns,H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. Alzheimers. Dement. *11*, 718-726.

Bayer,T.A., Cappai,R., Masters,C.L., Beyreuther,K., and Multhaup,G. (1999). It all sticks together--the APP-related family of proteins and Alzheimer's disease. Mol. Psychiatry *4*, 524-528.

Belelli,D., Harrison,N.L., Maguire,J., Macdonald,R.L., Walker,M.C., and Cope,D.W. (2009). Extrasynaptic GABAA receptors: form, pharmacology, and function. J. Neurosci. *29*, 12757-12763.

Bell,K.F. and Claudio,C.A. (2006). Altered synaptic function in Alzheimer's disease. Eur. J. Pharmacol. *545*, 11-21.

Bell,K.F., de Kort,G.J., Steggerda,S., Shigemoto,R., Ribeiro-da-Silva,A., and Cuello,A.C. (2003). Structural involvement of the glutamatergic presynaptic boutons in a transgenic mouse model expressing early onset amyloid pathology. Neurosci. Lett. *353*, 143-147.

Ben-Ari,Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. Nat. Rev. Neurosci. *3*, 728-739.

Ben-Ari,Y. (2015). Is birth a critical period in the pathogenesis of autism spectrum disorders? Nat. Rev. Neurosci. *16*, 498-505.

Ben-Ari,Y. (2017). NKCC1 Chloride Importer Antagonists Attenuate Many Neurological and Psychiatric Disorders. Trends Neurosci. *40*, 536-554.

Ben-Ari,Y., Cherubini,E., Corradetti,R., and Gaiarsa,J.L. (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. J. Physiol *416*, 303-325.

Ben-Ari,Y., Gaiarsa,J.L., Tyzio,R., and Khazipov,R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev. *87*, 1215-1284.

Berendse,H.W., Verbunt,J.P., Scheltens,P., van Dijk,B.W., and Jonkman,E.J. (2000). Magnetoencephalographic analysis of cortical activity in Alzheimer's disease: a pilot study. Clin. Neurophysiol. *111*, 604-612.

Billings,L.M., Oddo,S., Green,K.N., McGaugh,J.L., and LaFerla,F.M. (2005). Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron *45*, 675-688.

Billioti de,G.S., Moride,Y., Ducruet,T., Kurth,T., Verdoux,H., Tournier,M., Pariente,A., and Begaud,B. (2014). Benzodiazepine use and risk of Alzheimer's disease: case-control study. BMJ *349*, g5205.

Bittner,T., Fuhrmann,M., Burgold,S., Jung,C.K., Volbracht,C., Steiner,H., Mitteregger,G., Kretzschmar,H.A., Haass,C., and Herms,J. (2009). Gamma-secretase inhibition reduces spine density in vivo via an amyloid precursor protein-dependent pathway. J. Neurosci. *29*, 10405-10409.

Blaesse,P., Airaksinen,M.S., Rivera,C., and Kaila,K. (2009). Cation-chloride cotransporters and neuronal function. Neuron *61*, 820-838.

Blaesse,P., Guillemin,I., Schindler,J., Schweizer,M., Delpire,E., Khiroug,L., Friauf,E., and Nothwang,H.G. (2006). Oligomerization of KCC2 correlates with development of inhibitory neurotransmission. J. Neurosci. *26*, 10407-10419.

Blaesse,P. and Schmidt,T. (2015). K-Cl cotransporter KCC2--a moonlighting protein in excitatory and inhibitory synapse development and function. Pflugers Arch. *467*, 615-624.

Bliss,T.V. and Lomo,T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol *232*, 331-356.

Boettger,T., Rust,M.B., Maier,H., Seidenbecher,T., Schweizer,M., Keating,D.J., Faulhaber,J., Ehmke,H., Pfeffer,C., Scheel,O., Lemcke,B., Horst,J., Leuwer,R., Pape,H.C., Volkl,H., Hubner,C.A.,

and Jentsch,T.J. (2003). Loss of K-Cl co-transporter KCC3 causes deafness, neurodegeneration and reduced seizure threshold. EMBO J. *22*, 5422-5434.

Bormann,J. (1988). Electrophysiology of GABAA and GABAB receptor subtypes. Trends Neurosci. *11*, 112-116.

Born,H.A., Kim,J.Y., Savjani,R.R., Das,P., Dabaghian,Y.A., Guo,Q., Yoo,J.W., Schuler,D.R., Cirrito,J.R., Zheng,H., Golde,T.E., Noebels,J.L., and Jankowsky,J.L. (2014). Genetic suppression of transgenic APP rescues Hypersynchronous network activity in a mouse model of Alzeimer's disease. J. Neurosci. *34*, 3826-3840.

Boulenguez,P., Liabeuf,S., Bos,R., Bras,H., Jean-Xavier,C., Brocard,C., Stil,A., Darbon,P., Cattaert,D., Delpire,E., Marsala,M., and Vinay,L. (2010). Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. Nat. Med. *16*, 302-307.

Bowery,N.G., Hill,D.R., Hudson,A.L., Doble,A., Middlemiss,D.N., Shaw,J., and Turnbull,M. (1980). (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature *283*, 92-94.

Braak,H. and Braak,E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. (Berl) *82*, 239-259.

Braak,H. and Braak,E. (1996). Evolution of the neuropathology of Alzheimer's disease. Acta Neurol. Scand. Suppl *165*, 3-12.

Braak,H. and Braak,E. (1997). Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol. Aging *18*, 351-357.

Brady,D.R. and Mufson,E.J. (1997). Parvalbumin-immunoreactive neurons in the hippocampal formation of Alzheimer's diseased brain. Neuroscience *80*, 1113-1125.

Brandt,R. and Lee,G. (1993). Functional organization of microtubule-associated protein tau. Identification of regions which affect microtubule growth, nucleation, and bundle formation in vitro. J. Biol. Chem. *268*, 3414-3419.

Brion,J.P., Couck,A.M., Passareiro,E., and Flament-Durand,J. (1985). Neurofibrillary tangles of Alzheimer's disease: an immunohistochemical study. J. Submicrosc. Cytol. *17*, 89-96.

Brou,C., Logeat,F., Gupta,N., Bessia,C., LeBail,O., Doedens,J.R., Cumano,A., Roux,P., Black,R.A., and Israel,A. (2000). A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol. Cell *5*, 207-216.

Buee,L., Bussiere,T., Buee-Scherrer,V., Delacourte,A., and Hof,P.R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Brain Res. Rev. *33*, 95-130.

Busche,M.A., Chen,X., Henning,H.A., Reichwald,J., Staufenbiel,M., Sakmann,B., and Konnerth,A. (2012). Critical role of soluble amyloid-beta for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A *109*, 8740-8745.

Bushman,D.M., Kaeser,G.E., Siddoway,B., Westra,J.W., Rivera,R.R., Rehen,S.K., Yung,Y.C., and Chun,J. (2015). Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer's disease brains. Elife. *4*.

Cabrejo,L., Guyant-Marechal,L., Laquerriere,A., Vercelletto,M., De la Fourniere,F., Thomas-Anterion,C., Verny,C., Letournel,F., Pasquier,F., Vital,A., Checler,F., Frebourg,T., Campion,D., and Hannequin,D. (2006). Phenotype associated with APP duplication in five families. Brain *129*, 2966-2976.

Caille,I., Allinquant,B., Dupont,E., Bouillot,C., Langer,A., Muller,U., and Prochiantz,A. (2004). Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. Development *131*, 2173-2181.

Campbell,S.L., Robel,S., Cuddapah,V.A., Robert,S., Buckingham,S.C., Kahle,K.T., and Sontheimer,H. (2015). GABAergic disinhibition and impaired KCC2 cotransporter activity underlie tumor-associated epilepsy. Glia *63*, 23-36.

Candy,J.M., Gascoigne,A.D., Biggins,J.A., Smith,A.I., Perry,R.H., Perry,E.K., McDermott,J.R., and Edwardson,J.A. (1985). Somatostatin immunoreactivity in cortical and some subcortical regions in Alzheimer's disease. J. Neurol. Sci. *71*, 315-323.

Canter,R.G., Penney,J., and Tsai,L.H. (2016). The road to restoring neural circuits for the treatment of Alzheimer's disease. Nature *539*, 187-196.

Cao,X. and Sudhof,T.C. (2001). A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. Science *293*, 115-120.

Casarotto,S., Maatta,S., Herukka,S.K., Pigorini,A., Napolitani,M., Gosseries,O., Niskanen,E., Kononen,M., Mervaala,E., Rosanova,M., Soininen,H., and Massimini,M. (2011). Transcranial magnetic stimulation-evoked EEG/cortical potentials in physiological and pathological aging. Neuroreport *22*, 592-597.

Cellot,G. and Cherubini,E. (2014). GABAergic signaling as therapeutic target for autism spectrum disorders. Front Pediatr. *2*, 70.

Celone,K.A., Calhoun,V.D., Dickerson,B.C., Atri,A., Chua,E.F., Miller,S.L., DePeau,K., Rentz,D.M., Selkoe,D.J., Blacker,D., Albert,M.S., and Sperling,R.A. (2006). Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. J. Neurosci. *26*, 10222-10231.

Chamma,I., Heubl,M., Chevy,Q., Renner,M., Moutkine,I., Eugene,E., Poncer,J.C., and Levi,S. (2013). Activity-dependent regulation of the K/Cl transporter KCC2 membrane diffusion, clustering, and function in hippocampal neurons. J. Neurosci. *33*, 15488-15503.

Checler,F., Sunyach,C., Pardossi-Piquard,R., Sevalle,J., Vincent,B., Kawarai,T., Girardot,N., St George-Hyslop,P., and da Costa,C.A. (2007). The gamma/epsilon-secretase-derived APP intracellular domain fragments regulate p53. Curr. Alzheimer Res. *4*, 423-426.

Chen,C.D., Oh,S.Y., Hinman,J.D., and Abraham,C.R. (2006). Visualization of APP dimerization and APP-Notch2 heterodimerization in living cells using bimolecular fluorescence complementation. J. Neurochem. *97*, 30-43.

Chen,H.S., Pellegrini,J.W., Aggarwal,S.K., Lei,S.Z., Warach,S., Jensen,F.E., and Lipton,S.A. (1992). Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. J. Neurosci. *12*, 4427-4436.

Chen,L., Wan,L., Wu,Z., Ren,W., Huang,Y., Qian,B., and Wang,Y. (2017a). KCC2 downregulation facilitates epileptic seizures. Sci. Rep. *7*, 156.

Chen,M., Wang,J., Jiang,J., Zheng,X., Justice,N.J., Wang,K., Ran,X., Li,Y., Huo,Q., Zhang,J., Li,H., Lu,N., Wang,Y., Zheng,H., Long,C., and Yang,L. (2017b). APP modulates KCC2 expression and function in hippocampal GABAergic inhibition. Elife. *6*.

Cherubini,E. and Conti,F. (2001). Generating diversity at GABAergic synapses. Trends Neurosci. *24*, 155-162.

Cherubini,E., Gaiarsa,J.L., and Ben-Ari,Y. (1991). GABA: an excitatory transmitter in early postnatal life. Trends Neurosci. *14*, 515-519.

Chevy,Q., Heubl,M., Goutierre,M., Backer,S., Moutkine,I., Eugene,E., Bloch-Gallego,E., Levi,S., and Poncer,J.C. (2015). KCC2 Gates Activity-Driven AMPA Receptor Traffic through Cofilin Phosphorylation. J. Neurosci. *35*, 15772-15786.

Chorin,E., Vinograd,O., Fleidervish,I., Gilad,D., Herrmann,S., Sekler,I., Aizenman,E., and Hershfinkel,M. (2011). Upregulation of KCC2 activity by zinc-mediated neurotransmission via the mZnR/GPR39 receptor. J. Neurosci. *31*, 12916-12926.

Chu,D.C., Penney,J.B., Jr., and Young,A.B. (1987). Quantitative autoradiography of hippocampal GABAB and GABAA receptor changes in Alzheimer's disease. Neurosci. Lett. *82*, 246-252.

Chudotvorova,I., Ivanov,A., Rama,S., Hubner,C.A., Pellegrino,C., Ben-Ari,Y., and Medina,I. (2005). Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. J. Physiol *566*, 671-679.

Cirrito,J.R., Yamada,K.A., Finn,M.B., Sloviter,R.S., Bales,K.R., May,P.C., Schoepp,D.D., Paul,S.M., Mennerick,S., and Holtzman,D.M. (2005). Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. Neuron *48*, 913-922.

Clayton,G.H., Owens,G.C., Wolff,J.S., and Smith,R.L. (1998). Ontogeny of cation-Cl- cotransporter expression in rat neocortex. Brain Res. Dev. Brain Res. *109*, 281-292.

Cleary,J.P., Walsh,D.M., Hofmeister,J.J., Shankar,G.M., Kuskowski,M.A., Selkoe,D.J., and Ashe,K.H. (2005). Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat. Neurosci. *8*, 79-84.

Cohen,I., Navarro,V., Clemenceau,S., Baulac,M., and Miles,R. (2002). On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science *298*, 1418-1421.

Condie,B.G., Bain,G., Gottlieb,D.I., and Capecchi,M.R. (1997). Cleft palate in mice with a targeted mutation in the gamma-aminobutyric acid-producing enzyme glutamic acid decarboxylase 67. Proc. Natl. Acad. Sci. U. S. A *94*, 11451-11455.

Connor,J.A., Tseng,H.Y., and Hockberger,P.E. (1987). Depolarization- and transmitter-induced changes in intracellular Ca2+ of rat cerebellar granule cells in explant cultures. J. Neurosci. *7*, 1384-1400.

Corder,E.H., Saunders,A.M., Risch,N.J., Strittmatter,W.J., Schmechel,D.E., Gaskell,P.C., Jr., Rimmler,J.B., Locke,P.A., Conneally,P.M., Schmader,K.E., and . (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat. Genet. *7*, 180-184.

Coull,J.A., Boudreau,D., Bachand,K., Prescott,S.A., Nault,F., Sik,A., De,K.P., and De,K.Y. (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature *424*, 938-942.

Coulson,E.J., Paliga,K., Beyreuther,K., and Masters,C.L. (2000). What the evolution of the amyloid protein precursor supergene family tells us about its function. Neurochem. Int. *36*, 175-184.

Cousins,S.L., Hoey,S.E., Anne,S.F., and Perkinton,M.S. (2009). Amyloid precursor protein 695 associates with assembled. J. Neurochem. *111*, 1501-1513.

Cretin,B., Sellal,F., Philippi,N., Bousiges,O., Di,B.L., Martin-Hunyadi,C., and Blanc,F. (2016). Epileptic Prodromal Alzheimer's Disease, a Retrospective Study of 13 New Cases: Expanding the Spectrum of Alzheimer's Disease to an Epileptic Variant? J. Alzheimers. Dis. *52*, 1125-1133.

Cull-Candy,S., Brickley,S., and Farrant,M. (2001). NMDA receptor subunits: diversity, development and disease. Curr. Opin. Neurobiol. *11*, 327-335.

Cupers,P., Orlans,I., Craessaerts,K., Annaert,W., and De,S.B. (2001). The amyloid precursor protein (APP)-cytoplasmic fragment generated by gamma-secretase is rapidly degraded but distributes partially in a nuclear fraction of neurones in culture. J. Neurochem. *78*, 1168-1178.

Daigle,I. and Li,C. (1993). apl-1, a Caenorhabditis elegans gene encoding a protein related to the human beta-amyloid protein precursor. Proc. Natl. Acad. Sci. U. S. A *90*, 12045-12049.

Danysz,W. and Parsons,C.G. (1998). Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol. Rev. *50*, 597-664.

Danysz,W. and Parsons,C.G. (2012). Alzheimer's disease, beta-amyloid, glutamate, NMDA receptors and memantine--searching for the connections. Br. J. Pharmacol. *167*, 324-352.

Darman,R.B. and Forbush,B. (2002). A regulatory locus of phosphorylation in the N terminus of the Na-K-Cl cotransporter, NKCC1. J. Biol. Chem. *277*, 37542-37550.

Das,U., Scott,D.A., Ganguly,A., Koo,E.H., Tang,Y., and Roy,S. (2013). Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. Neuron *79*, 447-460.

Davies,P., Katzman,R., and Terry,R.D. (1980). Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. Nature *288*, 279-280.

Davies,P. and Terry,R.D. (1981). Cortical somatostatin-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer type. Neurobiol. Aging *2*, 9-14.

Dawson,G.R., Seabrook,G.R., Zheng,H., Smith,D.W., Graham,S., O'Dowd,G., Bowery,B.J., Boyce,S., Trumbauer,M.E., Chen,H.Y., Van der Ploeg,L.H., and Sirinathsinghji,D.J. (1999a). Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. Neuroscience *90*, 1-13.

Dawson,G.R., Seabrook,G.R., Zheng,H., Smith,D.W., Graham,S., O'Dowd,G., Bowery,B.J., Boyce,S., Trumbauer,M.E., Chen,H.Y., Van der Ploeg,L.H., and Sirinathsinghji,D.J. (1999b). Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. Neuroscience *90*, 1-13.

De,J.M. and Pennartz,C. (2002). Circadian modulation of GABA function in the rat suprachiasmatic nucleus: excitatory effects during the night phase. J. Neurophysiol. *87*, 834-844.

DeBoer,S.R., Dolios,G., Wang,R., and Sisodia,S.S. (2014). Differential release of beta-amyloid from dendrite- versus axon-targeted APP. J. Neurosci. *34*, 12313-12327.

Defrancesco,M., Marksteiner,J., Fleischhacker,W.W., and Blasko,I. (2015). Use of Benzodiazepines in Alzheimer's Disease: A Systematic Review of Literature. Int. J. Neuropsychopharmacol. *18*, yv055.

Deidda,G., Allegra,M., Cerri,C., Naskar,S., Bony,G., Zunino,G., Bozzi,Y., Caleo,M., and Cancedda,L. (2015a). Early depolarizing GABA controls critical-period plasticity in the rat visual cortex. Nat. Neurosci. *18*, 87-96.

Deidda,G., Parrini,M., Naskar,S., Bozarth,I.F., Contestabile,A., and Cancedda,L. (2015b). Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome. Nat. Med. *21*, 318-326.

DeKosky,S.T. and Scheff,S.W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann. Neurol. *27*, 457-464.

Di,L., V, Oliviero,A., Pilato,F., Saturno,E., Dileone,M., Marra,C., Daniele,A., Ghirlanda,S., Gainotti,G., and Tonali,P.A. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry *75*, 555-559.

Dickerson,B.C., Salat,D.H., Greve,D.N., Chua,E.F., Rand-Giovannetti,E., Rentz,D.M., Bertram,L., Mullin,K., Tanzi,R.E., Blacker,D., Albert,M.S., and Sperling,R.A. (2005). Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology *65*, 404-411.

Dierssen,M. (2012). Down syndrome: the brain in trisomic mode. Nat. Rev. Neurosci. *13*, 844-858.

Dinet,V., Ciccotosto,G.D., Delaunay,K., Borras,C., Ranchon-Cole,I., Kostic,C., Savoldelli,M., El,S.M., Jonet,L., Pirou,C., An,N., Abitbol,M., Arsenijevic,Y., Behar-Cohen,F., Cappai,R., and Mascarelli,F. (2016). Amyloid Precursor-Like Protein 2 deletion-induced retinal synaptopathy related to congenital stationary night blindness: structural, functional and molecular characteristics. Mol. Brain *9*, 64.

Dournaud,P., Cervera-Pierot,P., Hirsch,E., Javoy-Agid,F., Kordon,C., Agid,Y., and Epelbaum,J. (1994). Somatostatin messenger RNA-containing neurons in Alzheimer's disease: an in situ hybridization study in hippocampus, parahippocampal cortex and frontal cortex. Neuroscience *61*, 755-764.

Doyon,N., Vinay,L., Prescott,S.A., and De,K.Y. (2016). Chloride Regulation: A Dynamic Equilibrium Crucial for Synaptic Inhibition. Neuron *89*, 1157-1172.

Drechsel,D.N., Hyman,A.A., Cobb,M.H., and Kirschner,M.W. (1992). Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol. Biol. Cell *3*, 1141-1154.

Duebel,J., Haverkamp,S., Schleich,W., Feng,G., Augustine,G.J., Kuner,T., and Euler,T. (2006). Two-photon imaging reveals somatodendritic chloride gradient in retinal ON-type bipolar cells expressing the biosensor Clomeleon. Neuron *49*, 81-94.

Dzhala,V.I., Kuchibhotla,K.V., Glykys,J.C., Kahle,K.T., Swiercz,W.B., Feng,G., Kuner,T., Augustine,G.J., Bacskai,B.J., and Staley,K.J. (2010). Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. J. Neurosci. *30*, 11745-11761.

Dzhala,V.I., Talos,D.M., Sdrulla,D.A., Brumback,A.C., Mathews,G.C., Benke,T.A., Delpire,E., Jensen,F.E., and Staley,K.J. (2005). NKCC1 transporter facilitates seizures in the developing brain. Nat. Med. *11*, 1205-1213.

Emson,P.C. (2007). GABA(B) receptors: structure and function. Prog. Brain Res. *160*, 43-57.

Engel,D., Pahner,I., Schulze,K., Frahm,C., Jarry,H., Ahnert-Hilger,G., and Draguhn,A. (2001). Plasticity of rat central inhibitory synapses through GABA metabolism. J. Physiol *535*, 473-482.

Erreger,K., Chen,P.E., Wyllie,D.J., and Traynelis,S.F. (2004). Glutamate receptor gating. Crit Rev. Neurobiol. *16*, 187-224.

Farrant,M. and Kaila,K. (2007). The cellular, molecular and ionic basis of GABA(A) receptor signalling. Prog. Brain Res. *160*, 59-87.

Farrant,M. and Nusser,Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat. Rev. Neurosci. *6*, 215-229.

Farrer,L.A., Cupples,L.A., Haines,J.L., Hyman,B., Kukull,W.A., Mayeux,R., Myers,R.H., Pericak-Vance,M.A., Risch,N., and van Duijn,C.M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA *278*, 1349-1356.

Fath,T., Eidenmuller,J., and Brandt,R. (2002). Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. J. Neurosci. *22*, 9733-9741.

Ferando,I., Faas,G.C., and Mody,I. (2016). Diminished KCC2 confounds synapse specificity of LTP during senescence. Nat. Neurosci. *19*, 1197-1200.

Fernandez-Tome,P., Brera,B., Arevalo,M.A., and de Ceballos,M.L. (2004). Beta-amyloid25-35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. Neurobiol. Dis. *15*, 580-589.

Ferrer,I., Soriano,E., Tunon,T., Fonseca,M., and Guionnet,N. (1991). Parvalbumin immunoreactive neurons in normal human temporal neocortex and in patients with Alzheimer's disease. J. Neurol. Sci. *106*, 135-141.

Ferrer,I., Zujar,M.J., Rivera,R., Soria,M., Vidal,A., and Casas,R. (1993). Parvalbumin-immunoreactive dystrophic neurites and aberrant sprouts in the cerebral cortex of patients with Alzheimer's disease. Neurosci. Lett. *158*, 163-166.

Filippini,N., MacIntosh,B.J., Hough,M.G., Goodwin,G.M., Frisoni,G.B., Smith,S.M., Matthews,P.M., Beckmann,C.F., and Mackay,C.E. (2009). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc. Natl. Acad. Sci. U. S. A *106*, 7209-7214.

Fishell,G. and Rudy,B. (2011). Mechanisms of inhibition within the telencephalon: "where the wild things are". Annu. Rev. Neurosci. *34*, 535-567.

Fitzjohn,S.M., Morton,R.A., Kuenzi,F., Davies,C.H., Seabrook,G.R., and Collingridge,G.L. (2000). Similar levels of long-term potentiation in amyloid precursor protein -null and wild-type mice in the CA1 region of picrotoxin treated slices. Neurosci. Lett. *288*, 9-12.

Fiumelli,H., Briner,A., Puskarjov,M., Blaesse,P., Belem,B.J., Dayer,A.G., Kaila,K., Martin,J.L., and Vutskits,L. (2013). An ion transport-independent role for the cation-chloride cotransporter KCC2 in dendritic spinogenesis in vivo. Cereb. Cortex *23*, 378-388.

Fon,E.A. and Edwards,R.H. (2001). Molecular mechanisms of neurotransmitter release. Muscle Nerve *24*, 581-601.

Fonseca,M., Soriano,E., Ferrer,I., Martinez,A., and Tunon,T. (1993). Chandelier cell axons identified by parvalbumin-immunoreactivity in the normal human temporal cortex and in Alzheimer's disease. Neuroscience *55*, 1107-1116.

Forstl,H., Burns,A., Levy,R., Cairns,N., Luthert,P., and Lantos,P. (1992). Neurologic signs in Alzheimer's disease. Results of a prospective clinical and neuropathologic study. Arch. Neurol. *49*, 1038-1042.

Fotinopoulou,A., Tsachaki,M., Vlavaki,M., Poulopoulos,A., Rostagno,A., Frangione,B., Ghiso,J., and Efthimiopoulos,S. (2005). BRI2 interacts with amyloid precursor protein (APP) and regulates amyloid beta (Abeta) production. J. Biol. Chem. *280*, 30768-30772.

Freude,K.K., Penjwini,M., Davis,J.L., LaFerla,F.M., and Blurton-Jones,M. (2011). Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. J. Biol. Chem. *286*, 24264-24274.

Freund,T.F. and Katona,I. (2007). Perisomatic inhibition. Neuron *56*, 33-42.

Frohlich,F., Bazhenov,M., and Sejnowski,T.J. (2008). Pathological effect of homeostatic synaptic scaling on network dynamics in diseases of the cortex. J. Neurosci. *28*, 1709-1720.

Fuhrer,T.E., Palpagama,T.H., Waldvogel,H.J., Synek,B.J.L., Turner,C., Faull,R.L., and Kwakowsky,A. (2017). Impaired expression of GABA transporters in the human Alzheimer's disease hippocampus, subiculum, entorhinal cortex and superior temporal gyrus. Neuroscience *351*, 108-118.

Fukuda,A., Muramatsu,K., Okabe,A., Shimano,Y., Hida,H., Fujimoto,I., and Nishino,H. (1998). Changes in intracellular Ca2+ induced by GABAA receptor activation and reduction in Cl- gradient in neonatal rat neocortex. J. Neurophysiol. *79*, 439-446.

Gage,F.H. (2000). Mammalian neural stem cells. Science *287*, 1433-1438.

Gagnon,K.B., Adragna,N.C., Fyffe,R.E., and Lauf,P.K. (2007). Characterization of glial cell K-Cl cotransport. Cell Physiol Biochem. *20*, 121-130.

Gagnon,M., Bergeron,M.J., Lavertu,G., Castonguay,A., Tripathy,S., Bonin,R.P., Perez-Sanchez,J., Boudreau,D., Wang,B., Dumas,L., Valade,I., Bachand,K., Jacob-Wagner,M., Tardif,C., Kianicka,I., Isenring,P., Attardo,G., Coull,J.A., and De,K.Y. (2013). Chloride extrusion enhancers as novel therapeutics for neurological diseases. Nat. Med. *19*, 1524-1528.

Galanopoulou,A.S. (2008). Sexually dimorphic expression of KCC2 and GABA function. Epilepsy Res. *80*, 99-113.

Gamba,G., Miyanoshita,A., Lombardi,M., Lytton,J., Lee,W.S., Hediger,M.A., and Hebert,S.C. (1994). Molecular cloning, primary structure, and characterization of two members of the mammalian electroneutral sodium-(potassium)-chloride cotransporter family expressed in kidney. J. Biol. Chem. *269*, 17713-17722.

Gamba,G., Saltzberg,S.N., Lombardi,M., Miyanoshita,A., Lytton,J., Hediger,M.A., Brenner,B.M., and Hebert,S.C. (1993). Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. Proc. Natl. Acad. Sci. U. S. A *90*, 2749-2753.

Ganguly,K., Schinder,A.F., Wong,S.T., and Poo,M. (2001). GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell *105*, 521-532.

Gao,X.B., Chen,G., and van den Pol,A.N. (1998). GABA-dependent firing of glutamate-evoked action potentials at AMPA/kainate receptors in developing hypothalamic neurons. J. Neurophysiol. *79*, 716-726.

Gao,Y. and Pimplikar,S.W. (2001). The gamma -secretase-cleaved C-terminal fragment of amyloid precursor protein mediates signaling to the nucleus. Proc. Natl. Acad. Sci. U. S. A *98*, 14979-14984.

Garbarini,N. and Delpire,E. (2008). The RCC1 domain of protein associated with Myc (PAM) interacts with and regulates KCC2. Cell Physiol Biochem. *22*, 31-44.

Garcia-Marin,V., Blazquez-Llorca,L., Rodriguez,J.R., Boluda,S., Muntane,G., Ferrer,I., and Defelipe,J. (2009). Diminished perisomatic GABAergic terminals on cortical neurons adjacent to amyloid plaques. Front Neuroanat. *3*, 28.

Gauvain,G., Chamma,I., Chevy,Q., Cabezas,C., Irinopoulou,T., Bodrug,N., Carnaud,M., Levi,S., and Poncer,J.C. (2011). The neuronal K-Cl cotransporter KCC2 influences postsynaptic AMPA receptor content and lateral diffusion in dendritic spines. Proc. Natl. Acad. Sci. U. S. A *108*, 15474-15479.

Gentleman,S.M., Nash,M.J., Sweeting,C.J., Graham,D.I., and Roberts,G.W. (1993). Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. Neurosci. Lett. *160*, 139-144.

Gidon,A. and Segev,I. (2012). Principles governing the operation of synaptic inhibition in dendrites. Neuron *75*, 330-341.

Glenner,G.G., Wong,C.W., Quaranta,V., and Eanes,E.D. (1984). The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. Appl. Pathol. *2*, 357-369.

Glykys,J., Dzhala,V.I., Kuchibhotla,K.V., Feng,G., Kuner,T., Augustine,G., Bacskai,B.J., and Staley,K.J. (2009). Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. Neuron *63*, 657-672.

Goodger,Z.V., Rajendran,L., Trutzel,A., Kohli,B.M., Nitsch,R.M., and Konietzko,U. (2009). Nuclear signaling by the APP intracellular domain occurs predominantly through the amyloidogenic processing pathway. J. Cell Sci. *122*, 3703-3714.

Govindpani,K., Calvo-Flores,G.B., Vinnakota,C., Waldvogel,H.J., Faull,R.L., and Kwakowsky,A. (2017). Towards a Better Understanding of GABAergic Remodeling in Alzheimer's Disease. Int. J. Mol. Sci. *18*.

Grady,C.L., McIntosh,A.R., Beig,S., Keightley,M.L., Burian,H., and Black,S.E. (2003). Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. J. Neurosci. *23*, 986-993.

Grandgeorge,M., Lemonnier,E., Degrez,C., and Jallot,N. (2014). The effect of bumetanide treatment on the sensory behaviours of a young girl with Asperger syndrome. BMJ Case. Rep. *2014*.

Gribkoff,V.K., Pieschl,R.L., Wisialowski,T.A., Park,W.K., Strecker,G.J., de Jeu,M.T., Pennartz,C.M., and Dudek,F.E. (1999). A reexamination of the role of GABA in the mammalian suprachiasmatic nucleus. J. Biol. Rhythms *14*, 126-130.

Grouselle,D., Winsky-Sommerer,R., David,J.P., Delacourte,A., Dournaud,P., and Epelbaum,J. (1998). Loss of somatostatin-like immunoreactivity in the frontal cortex of Alzheimer patients carrying the apolipoprotein epsilon 4 allele. Neurosci. Lett. *255*, 21-24.

Gueli,M.C. and Taibi,G. (2013). Alzheimer's disease: amino acid levels and brain metabolic status. Neurol. Sci. *34*, 1575-1579.

Gulledge,A.T. and Stuart,G.J. (2003). Excitatory actions of GABA in the cortex. Neuron *37*, 299-309.

Gulyas,A.I., Sik,A., Payne,J.A., Kaila,K., and Freund,T.F. (2001). The KCl cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in the rat hippocampus. Eur. J. Neurosci. *13*, 2205-2217.

Gustke,N., Trinczek,B., Biernat,J., Mandelkow,E.M., and Mandelkow,E. (1994). Domains of tau protein and interactions with microtubules. Biochemistry *33*, 9511-9522.

Haas,M., McBrayer,D., and Lytle,C. (1995). [Cl-]i-dependent phosphorylation of the Na-K-Cl cotransport protein of dog tracheal epithelial cells. J. Biol. Chem. *270*, 28955-28961.

Haass,C. (2004). Take five--BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. EMBO J. *23*, 483-488.

Haass,C., Schlossmacher,M.G., Hung,A.Y., Vigo-Pelfrey,C., Mellon,A., Ostaszewski,B.L., Lieberburg,I., Koo,E.H., Schenk,D., Teplow,D.B., and . (1992). Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature *359*, 322-325.

Hadjikhani,N., Zurcher,N.R., Rogier,O., Ruest,T., Hippolyte,L., Ben-Ari,Y., and Lemonnier,E. (2015). Improving emotional face perception in autism with diuretic bumetanide: a proof-of-concept behavioral and functional brain imaging pilot study. Autism *19*, 149-157.

Hardingham,G.E. and Bading,H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat. Rev. Neurosci. *11*, 682-696.

Hardy,J., Cowburn,R., Barton,A., Reynolds,G., Dodd,P., Wester,P., O'Carroll,A.M., Lofdahl,E., and Winblad,B. (1987). A disorder of cortical GABAergic innervation in Alzheimer's disease. Neurosci. Lett. *73*, 192-196.

Hardy,J. and Selkoe,D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science *297*, 353-356.

Hardy,J.A. and Higgins,G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science *256*, 184-185.

Hartmann,A.M. and Nothwang,H.G. (2014). Molecular and evolutionary insights into the structural organization of cation chloride cotransporters. Front Cell Neurosci. *8*, 470.

Hartmann,A.M., Tesch,D., Nothwang,H.G., and Bininda-Emonds,O.R. (2014). Evolution of the cation chloride cotransporter family: ancient origins, gene losses, and subfunctionalization through duplication. Mol. Biol. Evol. *31*, 434-447.

Hayashi,Y., Kashiwagi,K., Ohta,J., Nakajima,M., Kawashima,T., and Yoshikawa,K. (1994). Alzheimer amyloid protein precursor enhances proliferation of neural stem cells from fetal rat brain. Biochem. Biophys. Res. Commun. *205*, 936-943.

Heaney,C.F. and Kinney,J.W. (2016). Role of GABA(B) receptors in learning and memory and neurological disorders. Neurosci. Biobehav. Rev. *63*, 1-28.

Heber,S., Herms,J., Gajic,V., Hainfellner,J., Aguzzi,A., Rulicke,T., von,K.H., Von,K.C., Sisodia,S., Tremml,P., Lipp,H.P., Wolfer,D.P., and Muller,U. (2000). Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. J. Neurosci. *20*, 7951-7963.

Hebert,L.E., Weuve,J., Scherr,P.A., and Evans,D.A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology *80*, 1778-1783.

Hennou,S., Khalilov,I., Diabira,D., Ben-Ari,Y., and Gozlan,H. (2002). Early sequential formation of functional GABA(A) and glutamatergic synapses on CA1 interneurons of the rat foetal hippocampus. Eur. J. Neurosci. *16*, 197-208.

Herms,J., Anliker,B., Heber,S., Ring,S., Fuhrmann,M., Kretzschmar,H., Sisodia,S., and Muller,U. (2004). Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. EMBO J. *23*, 4106-4115.

Herrup,K. and Yang,Y. (2007). Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nat. Rev. Neurosci. *8*, 368-378.

Hershfinkel,M., Kandler,K., Knoch,M.E., Dagan-Rabin,M., Aras,M.A., Abramovitch-Dahan,C., Sekler,I., and Aizenman,E. (2009). Intracellular zinc inhibits KCC2 transporter activity. Nat. Neurosci. *12*, 725-727.

Hill,D.R. and Bowery,N.G. (1981). 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. Nature *290*, 149-152.

Hoe,H.S., Fu,Z., Makarova,A., Lee,J.Y., Lu,C., Feng,L., Pajoohesh-Ganji,A., Matsuoka,Y., Hyman,B.T., Ehlers,M.D., Vicini,S., Pak,D.T., and Rebeck,G.W. (2009). The effects of amyloid precursor protein on postsynaptic composition and activity. J. Biol. Chem. *284*, 8495-8506.

Hof,P.R., Cox,K., Young,W.G., Celio,M.R., Rogers,J., and Morrison,J.H. (1991). Parvalbumin-immunoreactive neurons in the neocortex are resistant to degeneration in Alzheimer's disease. J. Neuropathol. Exp. Neurol. *50*, 451-462.

Holman,D., Feligioni,M., and Henley,J.M. (2007). Differential redistribution of native AMPA receptor complexes following LTD induction in acute hippocampal slices. Neuropharmacology *52*, 92-99.

Horn,Z., Ringstedt,T., Blaesse,P., Kaila,K., and Herlenius,E. (2010). Premature expression of KCC2 in embryonic mice perturbs neural development by an ion transport-independent mechanism. Eur. J. Neurosci. *31*, 2142-2155.

Hsieh,H., Boehm,J., Sato,C., Iwatsubo,T., Tomita,T., Sisodia,S., and Malinow,R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron *52*, 831-843.

Huber,G., Martin,J.R., Loffler,J., and Moreau,J.L. (1993). Involvement of amyloid precursor protein in memory formation in the rat: an indirect antibody approach. Brain Res. *603*, 348-352.

Huberfeld,G., Wittner,L., Clemenceau,S., Baulac,M., Kaila,K., Miles,R., and Rivera,C. (2007). Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J. Neurosci. *27*, 9866-9873.

Hubner,C.A., Lorke,D.E., and Hermans-Borgmeyer,I. (2001a). Expression of the Na-K-2Cl-cotransporter NKCC1 during mouse development. Mech. Dev. *102*, 267-269.

Hubner,C.A., Stein,V., Hermans-Borgmeyer,I., Meyer,T., Ballanyi,K., and Jentsch,T.J. (2001b). Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. Neuron *30*, 515-524.

Huo,Q., Chen,M., He,Q., Zhang,J., Li,B., Jin,K., Chen,X., Long,C., and Yang,L. (2016). Prefrontal Cortical GABAergic Dysfunction Contributes to Aberrant UP-State Duration in APP Knockout Mice. Cereb. Cortex.

Huysseune,S., Kienlen-Campard,P., Hebert,S., Tasiaux,B., Leroy,K., Devuyst,O., Brion,J.P., De,S.B., and Octave,J.N. (2009). Epigenetic control of aquaporin 1 expression by the amyloid precursor protein. FASEB J. *23*, 4158-4167.

Hyde,T.M., Lipska,B.K., Ali,T., Mathew,S.V., Law,A.J., Metitiri,O.E., Straub,R.E., Ye,T., Colantuoni,C., Herman,M.M., Bigelow,L.B., Weinberger,D.R., and Kleinman,J.E. (2011). Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J. Neurosci. *31*, 11088-11095.

Ikeda,K., Onimaru,H., Yamada,J., Inoue,K., Ueno,S., Onaka,T., Toyoda,H., Arata,A., Ishikawa,T.O., Taketo,M.M., Fukuda,A., and Kawakami,K. (2004). Malfunction of respiratory-related neuronal activity in Na+, K+-ATPase alpha2 subunit-deficient mice is attributable to abnormal Cl- homeostasis in brainstem neurons. J. Neurosci. *24*, 10693-10701.

Imfeld,P., Bodmer,M., Schuerch,M., Jick,S.S., and Meier,C.R. (2013). Seizures in patients with Alzheimer's disease or vascular dementia: a population-based nested case-control analysis. Epilepsia *54*, 700-707.

Inoue,K., Ueno,S., and Fukuda,A. (2004). Interaction of neuron-specific K+-Cl- cotransporter, KCC2, with brain-type creatine kinase. FEBS Lett. *564*, 131-135.

Isaacson,J.S. and Scanziani,M. (2011). How inhibition shapes cortical activity. Neuron *72*, 231-243.

Ishida,A., Furukawa,K., Keller,J.N., and Mattson,M.P. (1997). Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. Neuroreport *8*, 2133-2137.

Itoh,T., Satou,T., Nishida,S., Tsubaki,M., Hashimoto,S., and Ito,H. (2009). Expression of amyloid precursor protein after rat traumatic brain injury. Neurol. Res. *31*, 103-109.

Ivakine,E.A., Acton,B.A., Mahadevan,V., Ormond,J., Tang,M., Pressey,J.C., Huang,M.Y., Ng,D., Delpire,E., Salter,M.W., Woodin,M.A., and McInnes,R.R. (2013). Neto2 is a KCC2 interacting protein required for neuronal Cl- regulation in hippocampal neurons. Proc. Natl. Acad. Sci. U. S. A *110*, 3561-3566.

Iwakiri,M., Mizukami,K., Ikonomovic,M.D., Ishikawa,M., Abrahamson,E.E., DeKosky,S.T., and Asada,T. (2009). An immunohistochemical study of GABA A receptor gamma subunits in Alzheimer's disease hippocampus: relationship to neurofibrillary tangle progression. Neuropathology. *29*, 263-269.

Iwakiri,M., Mizukami,K., Ikonomovic,M.D., Ishikawa,M., Hidaka,S., Abrahamson,E.E., DeKosky,S.T., and Asada,T. (2005). Changes in hippocampal GABABR1 subunit expression in Alzheimer's patients: association with Braak staging. Acta Neuropathol. *109*, 467-474.

Jean-Xavier,C., Mentis,G.Z., O'Donovan,M.J., Cattaert,D., and Vinay,L. (2007). Dual personality of GABA/glycine-mediated depolarizations in immature spinal cord. Proc. Natl. Acad. Sci. U. S. A *104*, 11477-11482.

Jensen,E.C. (2013). Quantitative analysis of histological staining and fluorescence using ImageJ. Anat. Rec. (Hoboken. ) *296*, 378-381.

Jiang,S., Li,Y., Zhang,X., Bu,G., Xu,H., and Zhang,Y.W. (2014). Trafficking regulation of proteins in Alzheimer's disease. Mol. Neurodegener. *9*, 6.

Johnston,G.A. (2002). Medicinal chemistry and molecular pharmacology of GABA(C) receptors. Curr. Top. Med. Chem. *2*, 903-913.

Julkunen,P., Jauhiainen,A.M., Westeren-Punnonen,S., Pirinen,E., Soininen,H., Kononen,M., Paakkonen,A., Maatta,S., and Karhu,J. (2008). Navigated TMS combined with EEG in mild cognitive impairment and Alzheimer's disease: a pilot study. J. Neurosci. Methods *172*, 270-276.

Kahle,K.T., Merner,N.D., Friedel,P., Silayeva,L., Liang,B., Khanna,A., Shang,Y., Lachance-Touchette,P., Bourassa,C., Levert,A., Dion,P.A., Walcott,B., Spiegelman,D., Dionne-Laporte,A., Hodgkinson,A., Awadalla,P., Nikbakht,H., Majewski,J., Cossette,P., Deeb,T.Z., Moss,S.J., Medina,I., and Rouleau,G.A. (2014). Genetically encoded impairment of neuronal KCC2 cotransporter function in human idiopathic generalized epilepsy. EMBO Rep. *15*, 766-774.

Kahle,K.T., Rinehart,J., de Los,H.P., Louvi,A., Meade,P., Vazquez,N., Hebert,S.C., Gamba,G., Gimenez,I., and Lifton,R.P. (2005). WNK3 modulates transport of Cl- in and out of cells: implications for control of cell volume and neuronal excitability. Proc. Natl. Acad. Sci. U. S. A *102*, 16783-16788.

Kaila,K., Lamsa,K., Smirnov,S., Taira,T., and Voipio,J. (1997). Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K+ transient. J. Neurosci. *17*, 7662-7672.

Kaila,K., Price,T.J., Payne,J.A., Puskarjov,M., and Voipio,J. (2014). Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat. Rev. Neurosci. *15*, 637-654.

Kakazu,Y., Uchida,S., Nakagawa,T., Akaike,N., and Nabekura,J. (2000). Reversibility and cation selectivity of the K(+)-Cl(-) cotransport in rat central neurons. J. Neurophysiol. *84*, 281-288.

Kamenetz,F., Tomita,T., Hsieh,H., Seabrook,G., Borchelt,D., Iwatsubo,T., Sisodia,S., and Malinow,R. (2003). APP processing and synaptic function. Neuron *37*, 925-937.

Kandler,K. and Gillespie,D.C. (2005). Developmental refinement of inhibitory sound-localization circuits. Trends Neurosci. *28*, 290-296.

Kanekiyo,T., Xu,H., and Bu,G. (2014). ApoE and Abeta in Alzheimer's disease: accidental encounters or partners? Neuron *81*, 740-754.

Kang J,L.H.U.A.S.J.M.C.G.K.M.G.B.K.M.-H.B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. 325 *6106*, 733-736.

Karadsheh,M.F. and Delpire,E. (2001). Neuronal restrictive silencing element is found in the KCC2 gene: molecular basis for KCC2-specific expression in neurons. J. Neurophysiol. *85*, 995-997.

Kash,S.F., Johnson,R.S., Tecott,L.H., Noebels,J.L., Mayfield,R.D., Hanahan,D., and Baekkeskov,S. (1997). Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc. Natl. Acad. Sci. U. S. A *94*, 14060-14065.

Kaufman,D.L., Houser,C.R., and Tobin,A.J. (1991). Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J. Neurochem. *56*, 720-723.

Kawaguchi,Y., Katsumaru,H., Kosaka,T., Heizmann,C.W., and Hama,K. (1987). Fast spiking cells in rat hippocampus (CA1 region) contain the calcium-binding protein parvalbumin. Brain Res. *416*, 369-374.

Kawaguchi,Y. and Kubota,Y. (1997). GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb. Cortex *7*, 476-486.

Kepecs,A. and Fishell,G. (2014). Interneuron cell types are fit to function. Nature *505*, 318-326.

Khalifa,N.B., Van,H.J., Tasiaux,B., Huysseune,S., Smith,S.O., Constantinescu,S.N., Octave,J.N., and Kienlen-Campard,P. (2010). What is the role of amyloid precursor protein dimerization? Cell Adh. Migr. *4*, 268-272.

Khalilov,I., Chazal,G., Chudotvorova,I., Pellegrino,C., Corby,S., Ferrand,N., Gubkina,O., Nardou,R., Tyzio,R., Yamamoto,S., Jentsch,T.J., Hubner,C.A., Gaiarsa,J.L., Ben-Ari,Y., and Medina,I. (2011). Enhanced Synaptic Activity and Epileptiform Events in the Embryonic KCC2 Deficient Hippocampus. Front Cell Neurosci. *5*, 23.

Khalilov,I., Dzhala,V., Ben-Ari,Y., and Khazipov,R. (1999). Dual role of GABA in the neonatal rat hippocampus. Dev. Neurosci. *21*, 310-319.

Khalilov,I., Holmes,G.L., and Ben-Ari,Y. (2003). In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nat. Neurosci. *6*, 1079-1085.

Khirug,S., Yamada,J., Afzalov,R., Voipio,J., Khiroug,L., and Kaila,K. (2008). GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. J. Neurosci. *28*, 4635-4639.

Kidd,M. (1964). ALZHEIMER'S DISEASE--AN ELECTRON MICROSCOPICAL STUDY. Brain *87*, 307-320.

Kilb,W. (2012). Development of the GABAergic system from birth to adolescence. Neuroscientist. *18*, 613-630.

Kim,H.S., Kim,E.M., Lee,J.P., Park,C.H., Kim,S., Seo,J.H., Chang,K.A., Yu,E., Jeong,S.J., Chong,Y.H., and Suh,Y.H. (2003). C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression. FASEB J. *17*, 1951-1953.

Kim,J.H., Anwyl,R., Suh,Y.H., Djamgoz,M.B., and Rowan,M.J. (2001). Use-dependent effects of amyloidogenic fragments of (beta)-amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. J. Neurosci. *21*, 1327-1333.

Kimberly,W.T., Zheng,J.B., Guenette,S.Y., and Selkoe,D.J. (2001). The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. J. Biol. Chem. *276*, 40288-40292.

Kirvell,S.L., Esiri,M., and Francis,P.T. (2006). Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. J. Neurochem. *98*, 939-950.

Kitamura,A., Ishibashi,H., Watanabe,M., Takatsuru,Y., Brodwick,M., and Nabekura,J. (2008). Sustained depolarizing shift of the GABA reversal potential by glutamate receptor activation in hippocampal neurons. Neurosci. Res. *62*, 270-277.

Klausberger,T. and Somogyi,P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science *321*, 53-57.

Kohl,M.M. and Paulsen,O. (2010). The roles of GABAB receptors in cortical network activity. Adv. Pharmacol. *58*, 205-229.

Kuhn,P.H., Wang,H., Dislich,B., Colombo,A., Zeitschel,U., Ellwart,J.W., Kremmer,E., Rossner,S., and Lichtenthaler,S.F. (2010). ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J. *29*, 3020-3032.

Lagnaoui,R., Begaud,B., Moore,N., Chaslerie,A., Fourrier,A., Letenneur,L., Dartigues,J.F., and Moride,Y. (2002). Benzodiazepine use and risk of dementia: a nested case-control study. J. Clin. Epidemiol. *55*, 314-318.

Lagostena,L., Rosato-Siri,M., D'Onofrio,M., Brandi,R., Arisi,I., Capsoni,S., Franzot,J., Cattaneo,A., and Cherubini,E. (2010). In the adult hippocampus, chronic nerve growth factor deprivation shifts GABAergic signaling from the hyperpolarizing to the depolarizing direction. J. Neurosci. *30*, 885-893.

Lai,A., Sisodia,S.S., and Trowbridge,I.S. (1995). Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. J. Biol. Chem. *270*, 3565-3573.

Lai,F. and Williams,R.S. (1989). A prospective study of Alzheimer disease in Down syndrome. Arch. Neurol. *46*, 849-853.

Lam,A.D., Deck,G., Goldman,A., Eskandar,E.N., Noebels,J., and Cole,A.J. (2017). Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. Nat. Med. *23*, 678-680.

Lau,P., Bossers,K., Janky,R., Salta,E., Frigerio,C.S., Barbash,S., Rothman,R., Sierksma,A.S., Thathiah,A., Greenberg,D., Papadopoulou,A.S., Achsel,T., Ayoubi,T., Soreq,H., Verhaagen,J., Swaab,D.F., Aerts,S., and De,S.B. (2013). Alteration of the microRNA network during the progression of Alzheimer's disease. EMBO Mol. Med. *5*, 1613-1634.

Laurie,D.J., Wisden,W., and Seeburg,P.H. (1992). The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J. Neurosci. *12*, 4151-4172.

Laxton,A.W., Tang-Wai,D.F., McAndrews,M.P., Zumsteg,D., Wennberg,R., Keren,R., Wherrett,J., Naglie,G., Hamani,C., Smith,G.S., and Lozano,A.M. (2010). A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. Ann. Neurol. *68*, 521-534.

Le,R.P., Ivanov,T.R., Stanley,P.J., Baudoin,F.M., Chan,F., Pinteaux,E., Brown,P.D., and Luckman,S.M. (2006). KCC3 and KCC4 expression in rat adult forebrain. Brain Res. *1110*, 39-45.

Lee,H.H., Deeb,T.Z., Walker,J.A., Davies,P.A., and Moss,S.J. (2011). NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptor-mediated currents. Nat. Neurosci. *14*, 736-743.

Lee,H.H., Jurd,R., and Moss,S.J. (2010a). Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride co-transporter KCC2. Mol. Cell Neurosci. *45*, 173-179.

Lee,H.H., Walker,J.A., Williams,J.R., Goodier,R.J., Payne,J.A., and Moss,S.J. (2007). Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. J. Biol. Chem. *282*, 29777-29784.

Lee,K.J., Moussa,C.E., Lee,Y., Sung,Y., Howell,B.W., Turner,R.S., Pak,D.T., and Hoe,H.S. (2010b). Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines. Neuroscience *169*, 344-356.

Leinekugel,X., Medina,I., Khalilov,I., Ben-Ari,Y., and Khazipov,R. (1997). Ca2+ oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA receptors in the neonatal hippocampus. Neuron *18*, 243-255.

Lemonnier,E. and Ben-Ari,Y. (2010). The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. Acta Paediatr. *99*, 1885-1888.

Lemonnier,E., Degrez,C., Phelep,M., Tyzio,R., Josse,F., Grandgeorge,M., Hadjikhani,N., and Ben-Ari,Y. (2012). A randomised controlled trial of bumetanide in the treatment of autism in children. Transl. Psychiatry *2*, e202.

Lemonnier,E., Villeneuve,N., Sonie,S., Serret,S., Rosier,A., Roue,M., Brosset,P., Viellard,M., Bernoux,D., Rondeau,S., Thummler,S., Ravel,D., and Ben-Ari,Y. (2017). Effects of bumetanide on neurobehavioral function in children and adolescents with autism spectrum disorders. Transl. Psychiatry *7*, e1124.

Leuchter,A.F., Newton,T.F., Cook,I.A., Walter,D.O., Rosenberg-Thompson,S., and Lachenbruch,P.A. (1992). Changes in brain functional connectivity in Alzheimer-type and multi-infarct dementia. Brain *115 ( Pt 5)*, 1543-1561.

Li,H., Khirug,S., Cai,C., Ludwig,A., Blaesse,P., Kolikova,J., Afzalov,R., Coleman,S.K., Lauri,S., Airaksinen,M.S., Keinanen,K., Khiroug,L., Saarma,M., Kaila,K., and Rivera,C. (2007). KCC2 interacts with the dendritic cytoskeleton to promote spine development. Neuron *56*, 1019-1033.

Li,H., Tornberg,J., Kaila,K., Airaksinen,M.S., and Rivera,C. (2002). Patterns of cation-chloride cotransporter expression during embryonic rodent CNS development. Eur. J. Neurosci. *16*, 2358-2370.

Li,S., Jin,M., Koeglsperger,T., Shepardson,N.E., Shankar,G.M., and Selkoe,D.J. (2011). Soluble Abeta oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. J. Neurosci. *31*, 6627-6638.

Li,S., Mallory,M., Alford,M., Tanaka,S., and Masliah,E. (1997). Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. J. Neuropathol. Exp. Neurol. *56*, 901-911.

Li,X., Zhou,J., Chen,Z., Chen,S., Zhu,F., and Zhou,L. (2008). Long-term expressional changes of Na+ -K+ -Cl- co-transporter 1 (NKCC1) and K+ -Cl- co-transporter 2 (KCC2) in CA1 region of hippocampus following lithium-pilocarpine induced status epilepticus (PISE). Brain Res. *1221*, 141-146.

Li,Y., Sun,H., Chen,Z., Xu,H., Bu,G., and Zheng,H. (2016). Implications of GABAergic Neurotransmission in Alzheimer's Disease. Front Aging Neurosci. *8*, 31.

Li,Y.M., Xu,M., Lai,M.T., Huang,Q., Castro,J.L., DiMuzio-Mower,J., Harrison,T., Lellis,C., Nadin,A., Neduvelil,J.G., Register,R.B., Sardana,M.K., Shearman,M.S., Smith,A.L., Shi,X.P., Yin,K.C., Shafer,J.A., and Gardell,S.J. (2000). Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. Nature *405*, 689-694.

Li,Z.W., Stark,G., Gotz,J., Rulicke,T., Gschwind,M., Huber,G., Muller,U., and Weissmann,C. (1996). Generation of mice with a 200-kb amyloid precursor protein gene deletion by Cre recombinase-mediated site-specific recombination in embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A *93*, 6158-6162.

Limon,A., Reyes-Ruiz,J.M., and Miledi,R. (2012). Loss of functional GABA(A) receptors in the Alzheimer diseased brain. Proc. Natl. Acad. Sci. U. S. A *109*, 10071-10076.

Liu,S.J., Zhang,J.Y., Li,H.L., Fang,Z.Y., Wang,Q., Deng,H.M., Gong,C.X., Grundke-Iqbal,I., Iqbal,K., and Wang,J.Z. (2004). Tau becomes a more favorable substrate for GSK-3 when it is prephosphorylated by PKA in rat brain. J. Biol. Chem. *279*, 50078-50088.

Liu,Z., Neff,R.A., and Berg,D.K. (2006). Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. Science *314*, 1610-1613.

Loffler,J. and Huber,G. (1992). Beta-amyloid precursor protein isoforms in various rat brain regions and during brain development. J. Neurochem. *59*, 1316-1324.

Lopez-Sanchez,N., Muller,U., and Frade,J.M. (2005). Lengthening of G2/mitosis in cortical precursors from mice lacking beta-amyloid precursor protein. Neuroscience *130*, 51-60.

Lorent,K., Overbergh,L., Moechars,D., De,S.B., Van,L.F., and Van den Berghe,H. (1995). Expression in mouse embryos and in adult mouse brain of three members of the amyloid precursor protein family, of the alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein and

of its ligands apolipoprotein E, lipoprotein lipase, alpha-2-macroglobulin and the 40,000 molecular weight receptor-associated protein. Neuroscience *65*, 1009-1025.

Lu,J., Karadsheh,M., and Delpire,E. (1999). Developmental regulation of the neuronal-specific isoform of K-Cl cotransporter KCC2 in postnatal rat brains. J. Neurobiol. *39*, 558-568.

Luchetti,S., Bossers,K., Van de Bilt,S., Agrapart,V., Morales,R.R., Frajese,G.V., and Swaab,D.F. (2011). Neurosteroid biosynthetic pathways changes in prefrontal cortex in Alzheimer's disease. Neurobiol. Aging *32*, 1964-1976.

Ludwig,A., Li,H., Saarma,M., Kaila,K., and Rivera,C. (2003). Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. Eur. J. Neurosci. *18*, 3199-3206.

Ludwig,A., Uvarov,P., Soni,S., Thomas-Crusells,J., Airaksinen,M.S., and Rivera,C. (2011). Early growth response 4 mediates BDNF induction of potassium chloride cotransporter 2 transcription. J. Neurosci. *31*, 644-649.

Lundgren,J.L., Ahmed,S., Schedin-Weiss,S., Gouras,G.K., Winblad,B., Tjernberg,L.O., and Frykman,S. (2015). ADAM10 and BACE1 are localized to synaptic vesicles. J. Neurochem. *135*, 606-615.

Lundgren,J.L., Ahmed,S., Winblad,B., Gouras,G.K., Tjernberg,L.O., and Frykman,S. (2014). Activity-independent release of the amyloid beta-peptide from rat brain nerve terminals. Neurosci. Lett. *566*, 125-130.

Luo,L.Q., Martin-Morris,L.E., and White,K. (1990). Identification, secretion, and neural expression of APPL, a Drosophila protein similar to human amyloid protein precursor. J. Neurosci. *10*, 3849-3861.

Lytle,C. and Forbush,B., III (1996). Regulatory phosphorylation of the secretory Na-K-Cl cotransporter: modulation by cytoplasmic Cl. Am. J. Physiol *270*, C437-C448.

Mackenzie,I.R. and Miller,L.A. (1994). Senile plaques in temporal lobe epilepsy. Acta Neuropathol. *87*, 504-510.

Mahadevan,V., Pressey,J.C., Acton,B.A., Uvarov,P., Huang,M.Y., Chevrier,J., Puchalski,A., Li,C.M., Ivakine,E.A., Airaksinen,M.S., Delpire,E., McInnes,R.R., and Woodin,M.A. (2014). Kainate receptors coexist in a functional complex with KCC2 and regulate chloride homeostasis in hippocampal neurons. Cell Rep. *7*, 1762-1770.

Markkanen,M., Uvarov,P., and Airaksinen,M.S. (2008). Role of upstream stimulating factors in the transcriptional regulation of the neuron-specific K-Cl cotransporter KCC2. Brain Res. *1236*, 8-15.

Markram,H., Toledo-Rodriguez,M., Wang,Y., Gupta,A., Silberberg,G., and Wu,C. (2004). Interneurons of the neocortical inhibitory system. Nat. Rev. Neurosci. *5*, 793-807.

Marquez-Sterling,N.R., Lo,A.C., Sisodia,S.S., and Koo,E.H. (1997). Trafficking of cell-surface beta-amyloid precursor protein: evidence that a sorting intermediate participates in synaptic vesicle recycling. J. Neurosci. *17*, 140-151.

Martin,L.J., Pardo,C.A., Cork,L.C., and Price,D.L. (1994). Synaptic pathology and glial responses to neuronal injury precede the formation of senile plaques and amyloid deposits in the aging cerebral cortex. Am. J. Pathol. *145*, 1358-1381.

Martin-Morris,L.E. and White,K. (1990). The Drosophila transcript encoded by the beta-amyloid protein precursor-like gene is restricted to the nervous system. Development *110*, 185-195.

Martina,M., Royer,S., and Pare,D. (2001). Cell-type-specific GABA responses and chloride homeostasis in the cortex and amygdala. J. Neurophysiol. *86*, 2887-2895.

Marttinen,M., Kurkinen,K.M., Soininen,H., Haapasalo,A., and Hiltunen,M. (2015). Synaptic dysfunction and septin protein family members in neurodegenerative diseases. Mol. Neurodegener. *10*, 16.

Masliah,E., Alford,M., DeTeresa,R., Mallory,M., and Hansen,L. (1996). Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann. Neurol. *40*, 759-766.

Massimini,M., Ferrarelli,F., Huber,R., Esser,S.K., Singh,H., and Tononi,G. (2005). Breakdown of cortical effective connectivity during sleep. Science *309*, 2228-2232.

Masters,C.L., Bateman,R., Blennow,K., Rowe,C.C., Sperling,R.A., and Cummings,J.L. (2015). Alzheimer's disease. Nat. Rev. Dis. Primers. *1*, 15056.

Masters,C.L., Multhaup,G., Simms,G., Pottgiesser,J., Martins,R.N., and Beyreuther,K. (1985). Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J. *4*, 2757-2763.

Matos,M., Augusto,E., Oliveira,C.R., and Agostinho,P. (2008). Amyloid-beta peptide decreases glutamate uptake in cultured astrocytes: involvement of oxidative stress and mitogen-activated protein kinase cascades. Neuroscience *156*, 898-910.

Matsui,T., Ingelsson,M., Fukumoto,H., Ramasamy,K., Kowa,H., Frosch,M.P., Irizarry,M.C., and Hyman,B.T. (2007). Expression of APP pathway mRNAs and proteins in Alzheimer's disease. Brain Res. *1161*, 116-123.

McKernan,R.M. and Whiting,P.J. (1996). Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci. *19*, 139-143.

McLean,C.A., Cherny,R.A., Fraser,F.W., Fuller,S.J., Smith,M.J., Beyreuther,K., Bush,A.I., and Masters,C.L. (1999). Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann. Neurol. *46*, 860-866.

McNaughton,D., Knight,W., Guerreiro,R., Ryan,N., Lowe,J., Poulter,M., Nicholl,D.J., Hardy,J., Revesz,T., Lowe,J., Rossor,M., Collinge,J., and Mead,S. (2012). Duplication of amyloid precursor protein (APP), but not prion protein (PRNP) gene is a significant cause of early onset dementia in a large UK series. Neurobiol. Aging *33*, 426-21.

Medina,I., Friedel,P., Rivera,C., Kahle,K.T., Kourdougli,N., Uvarov,P., and Pellegrino,C. (2014). Current view on the functional regulation of the neuronal K(+)-Cl(-) cotransporter KCC2. Front Cell Neurosci. *8*, 27.

Mehta,A.K. and Ticku,M.K. (1999). An update on GABAA receptors. Brain Res. Brain Res. Rev. *29*, 196-217.

Meinecke,D.L. and Peters,A. (1987). GABA immunoreactive neurons in rat visual cortex. J. Comp Neurol. *261*, 388-404.

Meldrum,B.S. (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J. Nutr. *130*, 1007S-1015S.

Mendez,M.F., Catanzaro,P., Doss,R.C., ARguello,R., and Frey,W.H. (1994). Seizures in Alzheimer's disease: clinicopathologic study. J. Geriatr. Psychiatry Neurol. *7*, 230-233.

Meziane,H., Dodart,J.C., Mathis,C., Little,S., Clemens,J., Paul,S.M., and Ungerer,A. (1998). Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. Proc. Natl. Acad. Sci. U. S. A *95*, 12683-12688.

Mikawa,S., Wang,C., Shu,F., Wang,T., Fukuda,A., and Sato,K. (2002). Developmental changes in KCC1, KCC2 and NKCC1 mRNAs in the rat cerebellum. Brain Res. Dev. Brain Res. *136*, 93-100.

Mileusnic,R., Lancashire,C.L., Johnston,A.N., and Rose,S.P. (2000). APP is required during an early phase of memory formation. Eur. J. Neurosci. *12*, 4487-4495.

Mileusnic,R., Lancashire,C.L., and Rose,S.P. (2004). The peptide sequence Arg-Glu-Arg, present in the amyloid precursor protein, protects against memory loss caused by A beta and acts as a cognitive enhancer. Eur. J. Neurosci. *19*, 1933-1938.

Ming,G.L. and Song,H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron *70*, 687-702.

Minkeviciene,R., Rheims,S., Dobszay,M.B., Zilberter,M., Hartikainen,J., Fulop,L., Penke,B., Zilberter,Y., Harkany,T., Pitkanen,A., and Tanila,H. (2009). Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. J. Neurosci. *29*, 3453-3462.

Mirra,S.S., Heyman,A., McKeel,D., Sumi,S.M., Crain,B.J., Brownlee,L.M., Vogel,F.S., Hughes,J.P., van,B.G., and Berg,L. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology *41*, 479-486.

Mizukami,K., Grayson,D.R., Ikonomovic,M.D., Sheffield,R., and Armstrong,D.M. (1998). GABAA receptor beta 2 and beta 3 subunits mRNA in the hippocampal formation of aged human brain with Alzheimer-related neuropathology. Brain Res. Mol. Brain Res. *56*, 268-272.

Mody,I. and Pearce,R.A. (2004). Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci. *27*, 569-575.

Moir,R.D., Lynch,T., Bush,A.I., Whyte,S., Henry,A., Portbury,S., Multhaup,G., Small,D.H., Tanzi,R.E., Beyreuther,K., and Masters,C.L. (1998). Relative increase in Alzheimer's disease of soluble forms of cerebral Abeta amyloid protein precursor containing the Kunitz protease inhibitory domain. J. Biol. Chem. *273*, 5013-5019.

Morita,Y., Callicott,J.H., Testa,L.R., Mighdoll,M.I., Dickinson,D., Chen,Q., Tao,R., Lipska,B.K., Kolachana,B., Law,A.J., Ye,T., Straub,R.E., Weinberger,D.R., Kleinman,J.E., and Hyde,T.M. (2014). Characteristics of the cation cotransporter NKCC1 in human brain: alternate transcripts, expression in development, and potential relationships to brain function and schizophrenia. J. Neurosci. *34*, 4929-4940.

Morrisett,R.A., Mott,D.D., Lewis,D.V., Swartzwelder,H.S., and Wilson,W.A. (1991). GABAB-receptor-mediated inhibition of the N-methyl-D-aspartate component of synaptic transmission in the rat hippocampus. J. Neurosci. *11*, 203-209.

Morton,R.A., Kuenzi,F.M., Fitzjohn,S.M., Rosahl,T.W., Smith,D., Zheng,H., Shearman,M., Collingridge,G.L., and Seabrook,G.R. (2002). Impairment in hippocampal long-term potentiation in mice under-expressing the Alzheimer's disease related gene presenilin-1. Neurosci. Lett. *319*, 37-40.

Mosconi,L. (2005). Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur. J. Nucl. Med. Mol. Imaging *32*, 486-510.

Mosconi,L., Brys,M., Glodzik-Sobanska,L., De,S.S., Rusinek,H., and de Leon,M.J. (2007). Early detection of Alzheimer's disease using neuroimaging. Exp. Gerontol. *42*, 129-138.

Moser,E.I. (2011). The multi-laned hippocampus. Nat. Neurosci. *14*, 407-408.

Mucke,L., Masliah,E., Yu,G.Q., Mallory,M., Rockenstein,E.M., Tatsuno,G., Hu,K., Kholodenko,D., Johnson-Wood,K., and McConlogue,L. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. *20*, 4050-4058.

Muller,U.C., Deller,T., and Korte,M. (2017). Not just amyloid: physiological functions of the amyloid precursor protein family. Nat. Rev. Neurosci. *18*, 281-298.

Muller,U.C. and Zheng,H. (2012). Physiological functions of APP family proteins. Cold Spring Harb. Perspect. Med. *2*, a006288.

Musa,A., Lehrach,H., and Russo,V.A. (2001). Distinct expression patterns of two zebrafish homologues of the human APP gene during embryonic development. Dev. Genes Evol. *211*, 563-567.

Nabekura,J., Ueno,T., Okabe,A., Furuta,A., Iwaki,T., Shimizu-Okabe,C., Fukuda,A., and Akaike,N. (2002). Reduction of KCC2 expression and GABAA receptor-mediated excitation after in vivo axonal injury. J. Neurosci. *22*, 4412-4417.

Nava-Mesa,M.O., Jimenez-Diaz,L., Yajeya,J., and Navarro-Lopez,J.D. (2014). GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic dysfunction in early stages of Alzheimer's disease. Front Cell Neurosci. *8*, 167.

Nilsen,L.H., Rae,C., Ittner,L.M., Gotz,J., and Sonnewald,U. (2013). Glutamate metabolism is impaired in transgenic mice with tau hyperphosphorylation. J. Cereb. Blood Flow Metab *33*, 684-691.

Norstrom,E.M., Zhang,C., Tanzi,R., and Sisodia,S.S. (2010). Identification of NEEP21 as a ss-amyloid precursor protein-interacting protein in vivo that modulates amyloidogenic processing in vitro. J. Neurosci. *30*, 15677-15685.

Nykanen,N.P., Kysenius,K., Sakha,P., Tammela,P., and Huttunen,H.J. (2012). gamma-Aminobutyric acid type A (GABAA) receptor activation modulates tau phosphorylation. J. Biol. Chem. *287*, 6743-6752.

Ohsawa,I., Takamura,C., Morimoto,T., Ishiguro,M., and Kohsaka,S. (1999). Amino-terminal region of secreted form of amyloid precursor protein stimulates proliferation of neural stem cells. Eur. J. Neurosci. *11*, 1907-1913.

Olpe,H.R., Steinmann,M.W., Ferrat,T., Pozza,M.F., Greiner,K., Brugger,F., Froestl,W., Mickel,S.J., and Bittiger,H. (1993). The actions of orally active GABAB receptor antagonists on GABAergic transmission in vivo and in vitro. Eur. J. Pharmacol. *233*, 179-186.

Olsen,R.W. and Sieghart,W. (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol. Rev. *60*, 243-260.

Owens,D.F. and Kriegstein,A.R. (2002). Is there more to GABA than synaptic inhibition? Nat. Rev. Neurosci. *3*, 715-727.

Pallud,J., Le Van,Q.M., Bielle,F., Pellegrino,C., Varlet,P., Cresto,N., Baulac,M., Duyckaerts,C., Kourdougli,N., Chazal,G., Devaux,B., Rivera,C., Miles,R., Capelle,L., and Huberfeld,G. (2014). Cortical GABAergic excitation contributes to epileptic activities around human glioma. Sci. Transl. Med. *6*, 244ra89.

Palop,J.J. and Mucke,L. (2010). Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat. Neurosci. *13*, 812-818.

Palop,J.J. and Mucke,L. (2016). Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat. Rev. Neurosci. *17*, 777-792.

Pardossi-Piquard,R., Petit,A., Kawarai,T., Sunyach,C., Alves da,C.C., Vincent,B., Ring,S., D'Adamio,L., Shen,J., Muller,U., St George,H.P., and Checler,F. (2005). Presenilin-dependent transcriptional control of the Abeta-degrading enzyme neprilysin by intracellular domains of betaAPP and APLP. Neuron *46*, 541-554.

Parsons,C.G., Gruner,R., Rozental,J., Millar,J., and Lodge,D. (1993). Patch clamp studies on the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). Neuropharmacology *32*, 1337-1350.

Patel,A.B., de Graaf,R.A., Martin,D.L., Battaglioli,G., and Behar,K.L. (2006). Evidence that GAD65 mediates increased GABA synthesis during intense neuronal activity in vivo. J. Neurochem. *97*, 385-396.

Pathak,H.R., Weissinger,F., Terunuma,M., Carlson,G.C., Hsu,F.C., Moss,S.J., and Coulter,D.A. (2007). Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. J. Neurosci. *27*, 14012-14022.

Paton,J.A. and Nottebohm,F.N. (1984). Neurons generated in the adult brain are recruited into functional circuits. Science *225*, 1046-1048.

Patterson,D., Gardiner,K., Kao,F.T., Tanzi,R., Watkins,P., and Gusella,J.F. (1988). Mapping of the gene encoding the beta-amyloid precursor protein and its relationship to the Down syndrome region of chromosome 21. Proc. Natl. Acad. Sci. U. S. A *85*, 8266-8270.

Payne,J.A. (1997). Functional characterization of the neuronal-specific K-Cl cotransporter: implications for [K+]o regulation. Am. J. Physiol *273*, C1516-C1525.

Payne,J.A. (2012). Molecular operation of the cation chloride cotransporters: ion binding and inhibitor interaction. Curr. Top. Membr. *70*, 215-237.

Payne,J.A., Rivera,C., Voipio,J., and Kaila,K. (2003). Cation-chloride co-transporters in neuronal communication, development and trauma. Trends Neurosci. *26*, 199-206.

Payne,J.A., Stevenson,T.J., and Donaldson,L.F. (1996). Molecular characterization of a putative K-Cl cotransporter in rat brain. A neuronal-specific isoform. J. Biol. Chem. *271*, 16245-16252.

Pearson,M.M., Lu,J., Mount,D.B., and Delpire,E. (2001). Localization of the K(+)-Cl(-) cotransporter, KCC3, in the central and peripheral nervous systems: expression in the choroid plexus, large neurons and white matter tracts. Neuroscience *103*, 481-491.

Pedersen,M., Carmosino,M., and Forbush,B. (2008). Intramolecular and intermolecular fluorescence resonance energy transfer in fluorescent protein-tagged Na-K-Cl cotransporter (NKCC1): sensitivity to regulatory conformational change and cell volume. J. Biol. Chem. *283*, 2663-2674.

Pellegrino,C., Gubkina,O., Schaefer,M., Becq,H., Ludwig,A., Mukhtarov,M., Chudotvorova,I., Corby,S., Salyha,Y., Salozhin,S., Bregestovski,P., and Medina,I. (2011). Knocking down of the KCC2 in rat hippocampal neurons increases intracellular chloride concentration and compromises neuronal survival. J. Physiol *589*, 2475-2496.

Perez,R.G., Soriano,S., Hayes,J.D., Ostaszewski,B., Xia,W., Selkoe,D.J., Chen,X., Stokin,G.B., and Koo,E.H. (1999). Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. J. Biol. Chem. *274*, 18851-18856.

Perl,D.P. (2010). Neuropathology of Alzheimer's disease. Mt. Sinai J. Med. *77*, 32-42.

Perrot-Sinal,T.S., Sinal,C.J., Reader,J.C., Speert,D.B., and McCarthy,M.M. (2007). Sex differences in the chloride cotransporters, NKCC1 and KCC2, in the developing hypothalamus. J. Neuroendocrinol. *19*, 302-308.

Pickering,M., Pickering,B.W., Murphy,K.J., and O'Connor,J.J. (2008). Discrimination of cell types in mixed cortical culture using calcium imaging: a comparison to immunocytochemical labeling. J. Neurosci. Methods *173*, 27-33.

Pierrot,N., Tyteca,D., D'auria,L., Dewachter,I., Gailly,P., Hendrickx,A., Tasiaux,B., Haylani,L.E., Muls,N., N'kuli,F., Laquerriere,A., Demoulin,J.B., Campion,D., Brion,J.P., Courtoy,P.J., Kienlen-Campard,P., and Octave,J.N. (2013). Amyloid precursor protein controls cholesterol turnover needed for neuronal activity. EMBO Mol. Med. *5*, 608-625.

Plotkin,M.D., Snyder,E.Y., Hebert,S.C., and Delpire,E. (1997). Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain. J. Neurobiol. *33*, 781-795.

Preece,P., Virley,D.J., Costandi,M., Coombes,R., Moss,S.J., Mudge,A.W., Jazin,E., and Cairns,N.J. (2004). Amyloid precursor protein mRNA levels in Alzheimer's disease brain. Brain Res. Mol. Brain Res. *122*, 1-9.

Price,T.J., Cervero,F., and De,K.Y. (2005). Role of cation-chloride-cotransporters (CCC) in pain and hyperalgesia. Curr. Top. Med. Chem. *5*, 547-555.

Price,T.J., Hargreaves,K.M., and Cervero,F. (2006). Protein expression and mRNA cellular distribution of the NKCC1 cotransporter in the dorsal root and trigeminal ganglia of the rat. Brain Res. *1112*, 146-158.

Priller,C., Bauer,T., Mitteregger,G., Krebs,B., Kretzschmar,H.A., and Herms,J. (2006). Synapse formation and function is modulated by the amyloid precursor protein. J. Neurosci. *26*, 7212-7221.

Prox,J., Bernreuther,C., Altmeppen,H., Grendel,J., Glatzel,M., D'Hooge,R., Stroobants,S., Ahmed,T., Balschun,D., Willem,M., Lammich,S., Isbrandt,D., Schweizer,M., Horre,K., De,S.B., and Saftig,P. (2013). Postnatal disruption of the disintegrin/metalloproteinase ADAM10 in brain causes epileptic seizures, learning deficits, altered spine morphology, and defective synaptic functions. J. Neurosci. *33*, 12915-28, 12928a.

Purves D,A.G.F.D.H.W.L.A.W.L. (2012). Neuroscience. Sinauer Associates).

Puskarjov,M., Ahmad,F., Kaila,K., and Blaesse,P. (2012). Activity-dependent cleavage of the K-Cl cotransporter KCC2 mediated by calcium-activated protease calpain. J. Neurosci. *32*, 11356-11364.

Puskarjov,M., Seja,P., Heron,S.E., Williams,T.C., Ahmad,F., Iona,X., Oliver,K.L., Grinton,B.E., Vutskits,L., Scheffer,I.E., Petrou,S., Blaesse,P., Dibbens,L.M., Berkovic,S.F., and Kaila,K. (2014). A variant of KCC2 from patients with febrile seizures impairs neuronal Cl- extrusion and dendritic spine formation. EMBO Rep. *15*, 723-729.

Puzzo,D., Privitera,L., Fa',M., Staniszewski,A., Hashimoto,G., Aziz,F., Sakurai,M., Ribe,E.M., Troy,C.M., Mercken,M., Jung,S.S., Palmeri,A., and Arancio,O. (2011). Endogenous amyloid-beta is necessary for hippocampal synaptic plasticity and memory. Ann. Neurol. *69*, 819-830.

Puzzo,D., Privitera,L., Leznik,E., Fa,M., Staniszewski,A., Palmeri,A., and Arancio,O. (2008). Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J. Neurosci. *28*, 14537-14545.

Puzzo,D., Privitera,L., and Palmeri,A. (2012). Hormetic effect of amyloid-beta peptide in synaptic plasticity and memory. Neurobiol. Aging *33*, 1484-24.

Querfurth,H.W. and LaFerla,F.M. (2010). Alzheimer's disease. N. Engl. J. Med. *362*, 329-344.

Quiroz,Y.T., Budson,A.E., Celone,K., Ruiz,A., Newmark,R., Castrillon,G., Lopera,F., and Stern,C.E. (2010). Hippocampal hyperactivation in presymptomatic familial Alzheimer's disease. Ann. Neurol. *68*, 865-875.

Rabinowicz,A.L., Starkstein,S.E., Leiguarda,R.C., and Coleman,A.E. (2000). Transient epileptic amnesia in dementia: a treatable unrecognized cause of episodic amnestic wandering. Alzheimer Dis. Assoc. Disord. *14*, 231-233.

Ramos-Miguel,A., Hercher,C., Beasley,C.L., Barr,A.M., Bayer,T.A., Falkai,P., Leurgans,S.E., Schneider,J.A., Bennett,D.A., and Honer,W.G. (2015). Loss of Munc18-1 long splice variant in GABAergic terminals is associated with cognitive decline and increased risk of dementia in a community sample. Mol. Neurodegener. *10*, 65.

Reinders,N.R., Pao,Y., Renner,M.C., da Silva-Matos,C.M., Lodder,T.R., Malinow,R., and Kessels,H.W. (2016). Amyloid-beta effects on synapses and memory require AMPA receptor subunit GluA3. Proc. Natl. Acad. Sci. U. S. A *113*, E6526-E6534.

Reinhard,C., Hebert,S.S., and De,S.B. (2005). The amyloid-beta precursor protein: integrating structure with biological function. EMBO J. *24*, 3996-4006.

Reinikainen,K.J., Paljarvi,L., Huuskonen,M., Soininen,H., Laakso,M., and Riekkinen,P.J. (1988). A post-mortem study of noradrenergic, serotonergic and GABAergic neurons in Alzheimer's disease. J. Neurol. Sci. *84*, 101-116.

Riekki,R., Pavlov,I., Tornberg,J., Lauri,S.E., Airaksinen,M.S., and Taira,T. (2008). Altered synaptic dynamics and hippocampal excitability but normal long-term plasticity in mice lacking hyperpolarizing GABA A receptor-mediated inhibition in CA1 pyramidal neurons. J. Neurophysiol. *99*, 3075-3089.

Rinehart,J., Maksimova,Y.D., Tanis,J.E., Stone,K.L., Hodson,C.A., Zhang,J., Risinger,M., Pan,W., Wu,D., Colangelo,C.M., Forbush,B., Joiner,C.H., Gulcicek,E.E., Gallagher,P.G., and Lifton,R.P. (2009). Sites of regulated phosphorylation that control K-Cl cotransporter activity. Cell *138*, 525-536.

Ring,S., Weyer,S.W., Kilian,S.B., Waldron,E., Pietrzik,C.U., Filippov,M.A., Herms,J., Buchholz,C., Eckman,C.B., Korte,M., Wolfer,D.P., and Muller,U.C. (2007). The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. J. Neurosci. *27*, 7817-7826.

Rissman,R.A., Bennett,D.A., and Armstrong,D.M. (2004). Subregional analysis of GABA(A) receptor subunit mRNAs in the hippocampus of older persons with and without cognitive impairment. J. Chem. Neuroanat. *28*, 17-25.

Rivera,C., Li,H., Thomas-Crusells,J., Lahtinen,H., Viitanen,T., Nanobashvili,A., Kokaia,Z., Airaksinen,M.S., Voipio,J., Kaila,K., and Saarma,M. (2002). BDNF-induced TrkB activation down-regulates the K+-Cl- cotransporter KCC2 and impairs neuronal Cl- extrusion. J. Cell Biol. *159*, 747-752.

Rivera,C., Voipio,J., Payne,J.A., Ruusuvuori,E., Lahtinen,H., Lamsa,K., Pirvola,U., Saarma,M., and Kaila,K. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature *397*, 251-255.

Rivera,C., Voipio,J., Thomas-Crusells,J., Li,H., Emri,Z., Sipila,S., Payne,J.A., Minichiello,L., Saarma,M., and Kaila,K. (2004). Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J. Neurosci. *24*, 4683-4691.

Robel,S., Buckingham,S.C., Boni,J.L., Campbell,S.L., Danbolt,N.C., Riedemann,T., Sutor,B., and Sontheimer,H. (2015). Reactive astrogliosis causes the development of spontaneous seizures. J. Neurosci. *35*, 3330-3345.

ROBERTS,E. and FRANKEL,S. (1950). gamma-Aminobutyric acid in brain: its formation from glutamic acid. J. Biol. Chem. *187*, 55-63.

Roch,J.M., Masliah,E., Roch-Levecq,A.C., Sundsmo,M.P., Otero,D.A., Veinbergs,I., and Saitoh,T. (1994). Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid beta/A4 protein precursor. Proc. Natl. Acad. Sci. U. S. A *91*, 7450-7454.

Rossor,M.N., Emson,P.C., Mountjoy,C.Q., Roth,M., and Iversen,L.L. (1980). Reduced amounts of immunoreactive somatostatin in the temporal cortex in senile dementia of Alzheimer type. Neurosci. Lett. *20*, 373-377.

Rossor,M.N., Garrett,N.J., Johnson,A.L., Mountjoy,C.Q., Roth,M., and Iversen,L.L. (1982). A post-mortem study of the cholinergic and GABA systems in senile dementia. Brain *105*, 313-330.

Rovelet-Lecrux,A., Hannequin,D., Raux,G., Le,M.N., Laquerriere,A., Vital,A., Dumanchin,C., Feuillette,S., Brice,A., Vercelletto,M., Dubas,F., Frebourg,T., and Campion,D. (2006). APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat. Genet. *38*, 24-26.

Rudy,B., Fishell,G., Lee,S., and Hjerling-Leffler,J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev. Neurobiol. *71*, 45-61.

Russell,J.M. and Brown,A.M. (1972). Active transport of chloride by the giant neuron of the Aplysia abdominal ganglion. J. Gen. Physiol *60*, 499-518.

Rust,M.B., Alper,S.L., Rudhard,Y., Shmukler,B.E., Vicente,R., Brugnara,C., Trudel,M., Jentsch,T.J., and Hubner,C.A. (2007). Disruption of erythroid K-Cl cotransporters alters erythrocyte volume and partially rescues erythrocyte dehydration in SAD mice. J. Clin. Invest *117*, 1708-1717.

Ryan,K.A. and Pimplikar,S.W. (2005). Activation of GSK-3 and phosphorylation of CRMP2 in transgenic mice expressing APP intracellular domain. J. Cell Biol. *171*, 327-335.

Ryan,M.M., Morris,G.P., Mockett,B.G., Bourne,K., Abraham,W.C., Tate,W.P., and Williams,J.M. (2013). Time-dependent changes in gene expression induced by secreted amyloid precursor protein-alpha in the rat hippocampus. BMC. Genomics *14*, 376.

Salbaum,J.M. and Ruddle,F.H. (1994). Embryonic expression pattern of amyloid protein precursor suggests a role in differentiation of specific subsets of neurons. J. Exp. Zool. *269*, 116-127.

Salmon,P. and Trono,D. (2007). Production and titration of lentiviral vectors. Curr. Protoc. Hum. Genet. *Chapter 12*, Unit.

Sannerud,R., Esselens,C., Ejsmont,P., Mattera,R., Rochin,L., Tharkeshwar,A.K., De,B.G., De,W., V, Habets,R., Baert,V., Vermeire,W., Michiels,C., Groot,A.J., Wouters,R., Dillen,K., Vints,K., Baatsen,P., Munck,S., Derua,R., Waelkens,E., Basi,G.S., Mercken,M., Vooijs,M., Bollen,M., Schymkowitz,J., Rousseau,F., Bonifacino,J.S., Van,N.G., De,S.B., and Annaert,W. (2016). Restricted Location of PSEN2/gamma-Secretase Determines Substrate Specificity and Generates an Intracellular Abeta Pool. Cell *166*, 193-208.

Santos,S.F., Pierrot,N., Morel,N., Gailly,P., Sindic,C., and Octave,J.N. (2009). Expression of human amyloid precursor protein in rat cortical neurons inhibits calcium oscillations. J. Neurosci. *29*, 4708-4718.

Santos,S.F., Tasiaux,B., Sindic,C., and Octave,J.N. (2011). Inhibition of neuronal calcium oscillations by cell surface APP phosphorylated on T668. Neurobiol. Aging *32*, 2308-2313.

Satoh,J., Tabira,T., Sano,M., Nakayama,H., and Tateishi,J. (1991). Parvalbumin-immunoreactive neurons in the human central nervous system are decreased in Alzheimer's disease. Acta Neuropathol. *81*, 388-395.

Sauer,J.F., Struber,M., and Bartos,M. (2012). Interneurons provide circuit-specific depolarization and hyperpolarization. J. Neurosci. *32*, 4224-4229.

Scheuermann,S., Hambsch,B., Hesse,L., Stumm,J., Schmidt,C., Beher,D., Bayer,T.A., Beyreuther,K., and Multhaup,G. (2001). Homodimerization of amyloid precursor protein and its implication in the amyloidogenic pathway of Alzheimer's disease. J. Biol. Chem. *276*, 33923-33929.

Schlessinger,J. (2002). Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell *110*, 669-672.

Schweers,O., Schonbrunn-Hanebeck,E., Marx,A., and Mandelkow,E. (1994). Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure. J. Biol. Chem. *269*, 24290-24297.

Schwenk,J., Perez-Garci,E., Schneider,A., Kollewe,A., Gauthier-Kemper,A., Fritzius,T., Raveh,A., Dinamarca,M.C., Hanuschkin,A., Bildl,W., Klingauf,J., Gassmann,M., Schulte,U., Bettler,B., and Fakler,B. (2016). Modular composition and dynamics of native GABAB receptors identified by high-resolution proteomics. Nat. Neurosci. *19*, 233-242.

Seabrook,G.R., Smith,D.W., Bowery,B.J., Easter,A., Reynolds,T., Fitzjohn,S.M., Morton,R.A., Zheng,H., Dawson,G.R., Sirinathsinghji,D.J., Davies,C.H., Collingridge,G.L., and Hill,R.G. (1999). Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. Neuropharmacology *38*, 349-359.

Selkoe,D.J. (2008). Biochemistry and molecular biology of amyloid beta-protein and the mechanism of Alzheimer's disease. Handb. Clin. Neurol. *89*, 245-260.

Selkoe,D.J. and Hardy,J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol. Med. *8*, 595-608.

Semple,B.D., Blomgren,K., Gimlin,K., Ferriero,D.M., and Noble-Haeusslein,L.J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog. Neurobiol. *106-107*, 1-16.

Semyanov,A., Walker,M.C., Kullmann,D.M., and Silver,R.A. (2004). Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci. *27*, 262-269.

Senechal,Y., Kelly,P.H., and Dev,K.K. (2008). Amyloid precursor protein knockout mice show age-dependent deficits in passive avoidance learning. Behav. Brain Res. *186*, 126-132.

Seo,H. and Isacson,O. (2005). Abnormal APP, cholinergic and cognitive function in Ts65Dn Down's model mice. Exp. Neurol. *193*, 469-480.

Sepulveda-Falla,D., Glatzel,M., and Lopera,F. (2012). Phenotypic profile of early-onset familial Alzheimer's disease caused by presenilin-1 E280A mutation. J. Alzheimers. Dis. *32*, 1-12.

Serafini,R., Valeyev,A.Y., Barker,J.L., and Poulter,M.O. (1995). Depolarizing GABA-activated Cl-channels in embryonic rat spinal and olfactory bulb cells. J. Physiol *488 ( Pt 2)*, 371-386.

Sergeant,N., Delacourte,A., and Buee,L. (2005). Tau protein as a differential biomarker of tauopathies. Biochim. Biophys. Acta *1739*, 179-197.

Shariati,S.A. and De,S.B. (2013). Redundancy and divergence in the amyloid precursor protein family. FEBS Lett. *587*, 2036-2045.

Sheng,J.G., Boop,F.A., Mrak,R.E., and Griffin,W.S. (1994). Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. J. Neurochem. *63*, 1872-1879.

Sherzai,D., Losey,T., Vega,S., and Sherzai,A. (2014). Seizures and dementia in the elderly: Nationwide Inpatient Sample 1999-2008. Epilepsy Behav. *36*, 53-56.

Shulga,A., Thomas-Crusells,J., Sigl,T., Blaesse,A., Mestres,P., Meyer,M., Yan,Q., Kaila,K., Saarma,M., Rivera,C., and Giehl,K.M. (2008). Posttraumatic GABA(A)-mediated [Ca2+]i increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. J. Neurosci. *28*, 6996-7005.

Sieghart,W. and Sperk,G. (2002). Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr. Top. Med. Chem. *2*, 795-816.

Sigel,E. and Steinmann,M.E. (2012). Structure, function, and modulation of GABA(A) receptors. J. Biol. Chem. *287*, 40224-40231.

Sima,X., Xu,J., Li,J., Zhong,W., and You,C. (2014). Expression of beta-amyloid precursor protein in refractory epilepsy. Mol. Med. Rep. *9*, 1242-1248.

Simard,C.F., Bergeron,M.J., Frenette-Cotton,R., Carpentier,G.A., Pelchat,M.E., Caron,L., and Isenring,P. (2007). Homooligomeric and heterooligomeric associations between K+-Cl- cotransporter isoforms and between K+-Cl- and Na+-K+-Cl- cotransporters. J. Biol. Chem. *282*, 18083-18093.

Simons,M., De,S.B., Multhaup,G., Tienari,P.J., Dotti,C.G., and Beyreuther,K. (1996). Amyloidogenic processing of the human amyloid precursor protein in primary cultures of rat hippocampal neurons. J. Neurosci. *16*, 899-908.

Sinforiani,E., Manni,R., Bernasconi,L., Banchieri,L.M., and Zucchella,C. (2003). Memory disturbances and temporal lobe epilepsy simulating Alzheimer's disease: a case report. Funct. Neurol. *18*, 39-41.

Sjorgen,T., Sjorgen,H., and LINDGREN,A.G. (1952). Morbus Alzheimer and morbus Pick; a genetic, clinical and patho-anatomical study. Acta Psychiatr. Neurol. Scand. Suppl *82*, 1-152.

Sjostrom,P.J., Rancz,E.A., Roth,A., and Hausser,M. (2008). Dendritic excitability and synaptic plasticity. Physiol Rev. *88*, 769-840.

Slunt,H.H., Thinakaran,G., Von,K.C., Lo,A.C., Tanzi,R.E., and Sisodia,S.S. (1994). Expression of a ubiquitous, cross-reactive homologue of the mouse beta-amyloid precursor protein (APP). J. Biol. Chem. *269*, 2637-2644.

Smart,T.G. and Paoletti,P. (2012). Synaptic neurotransmitter-gated receptors. Cold Spring Harb. Perspect. Biol. *4*.

Soghomonian,J.J. and Martin,D.L. (1998). Two isoforms of glutamate decarboxylase: why? Trends Pharmacol. Sci. *19*, 500-505.

Soriano,J., Rodriguez,M.M., Tlusty,T., and Moses,E. (2008). Development of input connections in neural cultures. Proc. Natl. Acad. Sci. U. S. A *105*, 13758-13763.

Sperling,R. (2007). Functional MRI studies of associative encoding in normal aging, mild cognitive impairment, and Alzheimer's disease. Ann. N. Y. Acad. Sci. *1097*, 146-155.

Stein,V., Hermans-Borgmeyer,I., Jentsch,T.J., and Hubner,C.A. (2004). Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. J. Comp Neurol. *468*, 57-64.

Stewart,L.S., Wu,Y., Eubanks,J.H., Han,H., Leschenko,Y., Perez Velazquez,J.L., Cortez,M.A., and Snead,O.C., III (2009). Severity of atypical absence phenotype in GABAB transgenic mice is subunit specific. Epilepsy Behav. *14*, 577-581.

Stoothoff,W.H. and Johnson,G.V. (2005). Tau phosphorylation: physiological and pathological consequences. Biochim. Biophys. Acta *1739*, 280-297.

Storey,E., Spurck,T., Pickett-Heaps,J., Beyreuther,K., and Masters,C.L. (1996). The amyloid precursor protein of Alzheimer's disease is found on the surface of static but not activity motile portions of neurites. Brain Res. *735*, 59-66.

Strittmatter,W.J., Saunders,A.M., Schmechel,D., Pericak-Vance,M., Enghild,J., Salvesen,G.S., and Roses,A.D. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A *90*, 1977-1981.

Tampellini,D., Rahman,N., Gallo,E.F., Huang,Z., Dumont,M., Capetillo-Zarate,E., Ma,T., Zheng,R., Lu,B., Nanus,D.M., Lin,M.T., and Gouras,G.K. (2009). Synaptic activity reduces intraneuronal Abeta, promotes APP transport to synapses, and protects against Abeta-related synaptic alterations. J. Neurosci. *29*, 9704-9713.

Tang,X., Kim,J., Zhou,L., Wengert,E., Zhang,L., Wu,Z., Carromeu,C., Muotri,A.R., Marchetto,M.C., Gage,F.H., and Chen,G. (2016). KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome. Proc. Natl. Acad. Sci. U. S. A *113*, 751-756.

Tanzi,R.E. and Bertram,L. (2005). Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell *120*, 545-555.

Taylor,C.J., Ireland,D.R., Ballagh,I., Bourne,K., Marechal,N.M., Turner,P.R., Bilkey,D.K., Tate,W.P., and Abraham,W.C. (2008). Endogenous secreted amyloid precursor protein-alpha regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. Neurobiol. Dis. *31*, 250-260.

Terry,R.D. (1963). THE FINE STRUCTURE OF NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE. J. Neuropathol. Exp. Neurol. *22*, 629-642.

Terry,R.D., Masliah,E., Salmon,D.P., Butters,N., DeTeresa,R., Hill,R., Hansen,L.A., and Katzman,R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. *30*, 572-580.

Thinakaran,G., Kitt,C.A., Roskams,A.J., Slunt,H.H., Masliah,E., Von,K.C., Ginsberg,S.D., Ronnett,G.V., Reed,R.R., Price,D.L., and . (1995). Distribution of an APP homolog, APLP2, in the mouse olfactory system: a potential role for APLP2 in axogenesis. J. Neurosci. *15*, 6314-6326.

Thinakaran,G. and Koo,E.H. (2008). Amyloid precursor protein trafficking, processing, and function. J. Biol. Chem. *283*, 29615-29619.

Thompson,S.M., Deisz,R.A., and Prince,D.A. (1988a). Outward chloride/cation co-transport in mammalian cortical neurons. Neurosci. Lett. *89*, 49-54.

Thompson,S.M., Deisz,R.A., and Prince,D.A. (1988b). Relative contributions of passive equilibrium and active transport to the distribution of chloride in mammalian cortical neurons. J. Neurophysiol. *60*, 105-124.

Thompson,S.M. and Gahwiler,B.H. (1989a). Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. J. Neurophysiol. *61*, 501-511.

Thompson,S.M. and Gahwiler,B.H. (1989b). Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on ECl- in hippocampal CA3 neurons. J. Neurophysiol. *61*, 512-523.

Tian,N., Petersen,C., Kash,S., Baekkeskov,S., Copenhagen,D., and Nicoll,R. (1999). The role of the synthetic enzyme GAD65 in the control of neuronal gamma-aminobutyric acid release. Proc. Natl. Acad. Sci. U. S. A *96*, 12911-12916.

Tibau,E., Valencia,M., and Soriano,J. (2013). Identification of neuronal network properties from the spectral analysis of calcium imaging signals in neuronal cultures. Front Neural Circuits. *7*, 199.

Tilleux,S. and Hermans,E. (2007). Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J. Neurosci. Res. *85*, 2059-2070.

Tomita,T. (2014). Molecular mechanism of intramembrane proteolysis by gamma-secretase. J. Biochem. *156*, 195-201.

Tornberg,J., Voikar,V., Savilahti,H., Rauvala,H., and Airaksinen,M.S. (2005). Behavioural phenotypes of hypomorphic KCC2-deficient mice. Eur. J. Neurosci. *21*, 1327-1337.

Trubetskaya,V.V., Stepanichev,M.Y., Onufriev,M.V., Lazareva,N.A., Markevich,V.A., and Gulyaeva,N.V. (2003). Administration of aggregated beta-amyloid peptide (25-35) induces changes in long-term potentiation in the hippocampus in vivo. Neurosci. Behav. Physiol *33*, 95-98.

Turrigiano,G.G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. Cell *135*, 422-435.

Tyan,S.H., Shih,A.Y., Walsh,J.J., Maruyama,H., Sarsoza,F., Ku,L., Eggert,S., Hof,P.R., Koo,E.H., and Dickstein,D.L. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. Mol. Cell Neurosci. *51*, 43-52.

Tyzio,R., Cossart,R., Khalilov,I., Minlebaev,M., Hubner,C.A., Represa,A., Ben-Ari,Y., and Khazipov,R. (2006). Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. Science *314*, 1788-1792.

Tyzio,R., Nardou,R., Ferrari,D.C., Tsintsadze,T., Shahrokhi,A., Eftekhari,S., Khalilov,I., Tsintsadze,V., Brouchoud,C., Chazal,G., Lemonnier,E., Lozovaya,N., Burnashev,N., and Ben-Ari,Y. (2014). Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. Science *343*, 675-679.

Ulrich,D. (2015). Amyloid-beta Impairs Synaptic Inhibition via GABA(A) Receptor Endocytosis. J. Neurosci. *35*, 9205-9210.

Uvarov,P., Ludwig,A., Markkanen,M., Pruunsild,P., Kaila,K., Delpire,E., Timmusk,T., Rivera,C., and Airaksinen,M.S. (2007). A novel N-terminal isoform of the neuron-specific K-Cl cotransporter KCC2. J. Biol. Chem. *282*, 30570-30576.

Uvarov,P., Ludwig,A., Markkanen,M., Rivera,C., and Airaksinen,M.S. (2006). Upregulation of the neuron-specific K+/Cl- cotransporter expression by transcription factor early growth response 4. J. Neurosci. *26*, 13463-13473.

Uvarov,P., Ludwig,A., Markkanen,M., Soni,S., Hubner,C.A., Rivera,C., and Airaksinen,M.S. (2009). Coexpression and heteromerization of two neuronal K-Cl cotransporter isoforms in neonatal brain. J. Biol. Chem. *284*, 13696-13704.

Uvarov,P., Pruunsild,P., Timmusk,T., and Airaksinen,M.S. (2005). Neuronal K+/Cl- co-transporter (KCC2) transgenes lacking neurone restrictive silencer element recapitulate CNS neurone-specific expression and developmental up-regulation of endogenous KCC2 gene. J. Neurochem. *95*, 1144-1155.

Valeeva,G., Tressard,T., Mukhtarov,M., Baude,A., and Khazipov,R. (2016). An Optogenetic Approach for Investigation of Excitatory and Inhibitory Network GABA Actions in Mice Expressing Channelrhodopsin-2 in GABAergic Neurons. J. Neurosci. *36*, 5961-5973.

Van Der Kant,R. and Goldstein,L.S. (2015). Cellular functions of the amyloid precursor protein from development to dementia. Dev. Cell *32*, 502-515.

Vanhatalo,S., Palva,J.M., Andersson,S., Rivera,C., Voipio,J., and Kaila,K. (2005). Slow endogenous activity transients and developmental expression of K+-Cl- cotransporter 2 in the immature human cortex. Eur. J. Neurosci. *22*, 2799-2804.

Vassar,R., Kuhn,P.H., Haass,C., Kennedy,M.E., Rajendran,L., Wong,P.C., and Lichtenthaler,S.F. (2014). Function, therapeutic potential and cell biology of BACE proteases: current status and future prospects. J. Neurochem. *130*, 4-28.

Verret,L., Mann,E.O., Hang,G.B., Barth,A.M., Cobos,I., Ho,K., Devidze,N., Masliah,E., Kreitzer,A.C., Mody,I., Mucke,L., and Palop,J.J. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell *149*, 708-721.

Vibat,C.R., Holland,M.J., Kang,J.J., Putney,L.K., and O'Donnell,M.E. (2001). Quantitation of Na+-K+-2Cl- cotransport splice variants in human tissues using kinetic polymerase chain reaction. Anal. Biochem. *298*, 218-230.

Vigot,R., Barbieri,S., Brauner-Osborne,H., Turecek,R., Shigemoto,R., Zhang,Y.P., Lujan,R., Jacobson,L.H., Biermann,B., Fritschy,J.M., Vacher,C.M., Muller,M., Sansig,G., Guetg,N., Cryan,J.F., Kaupmann,K., Gassmann,M., Oertner,T.G., and Bettler,B. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. Neuron *50*, 589-601.

Villemagne,V.L., Pike,K.E., Chetelat,G., Ellis,K.A., Mulligan,R.S., Bourgeat,P., Ackermann,U., Jones,G., Szoeke,C., Salvado,O., Martins,R., O'Keefe,G., Mathis,C.A., Klunk,W.E., Ames,D., Masters,C.L., and Rowe,C.C. (2011). Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Ann. Neurol. *69*, 181-192.

Vinay,L. and Jean-Xavier,C. (2008). Plasticity of spinal cord locomotor networks and contribution of cation-chloride cotransporters. Brain Res. Rev. *57*, 103-110.

Vitari,A.C., Thastrup,J., Rafiqi,F.H., Deak,M., Morrice,N.A., Karlsson,H.K., and Alessi,D.R. (2006). Functional interactions of the SPAK/OSR1 kinases with their upstream activator WNK1 and downstream substrate NKCC1. Biochem. J. *397*, 223-231.

Volicer,L., Smith,S., and Volicer,B.J. (1995). Effect of seizures on progression of dementia of the Alzheimer type. Dementia *6*, 258-263.

von Koch,C.S., Zheng,H., Chen,H., Trumbauer,M., Thinakaran,G., Van der Ploeg,L.H., Price,D.L., and Sisodia,S.S. (1997). Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. Neurobiol. Aging *18*, 661-669.

von Rotz,R.C., Kohli,B.M., Bosset,J., Meier,M., Suzuki,T., Nitsch,R.M., and Konietzko,U. (2004). The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. J. Cell Sci. *117*, 4435-4448.

Vossel,K.A., Tartaglia,M.C., Nygaard,H.B., Zeman,A.Z., and Miller,B.L. (2017). Epileptic activity in Alzheimer's disease: causes and clinical relevance. Lancet Neurol. *16*, 311-322.

Wake,H., Watanabe,M., Moorhouse,A.J., Kanematsu,T., Horibe,S., Matsukawa,N., Asai,K., Ojika,K., Hirata,M., and Nabekura,J. (2007). Early changes in KCC2 phosphorylation in response to neuronal stress result in functional downregulation. J. Neurosci. *27*, 1642-1650.

Walsh,D.M., Klyubin,I., Fadeeva,J.V., Cullen,W.K., Anwyl,R., Wolfe,M.S., Rowan,M.J., and Selkoe,D.J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature *416*, 535-539.

Wang,B., Wang,Z., Sun,L., Yang,L., Li,H., Cole,A.L., Rodriguez-Rivera,J., Lu,H.C., and Zheng,H. (2014a). The amyloid precursor protein controls adult hippocampal neurogenesis through GABAergic interneurons. J. Neurosci. *34*, 13314-13325.

Wang,C., Shimizu-Okabe,C., Watanabe,K., Okabe,A., Matsuzaki,H., Ogawa,T., Mori,N., Fukuda,A., and Sato,K. (2002). Developmental changes in KCC1, KCC2, and NKCC1 mRNA expressions in the rat brain. Brain Res. Dev. Brain Res. *139*, 59-66.

Wang,J., Reichling,D.B., Kyrozis,A., and MacDermott,A.B. (1994). Developmental loss of. Eur. J. Neurosci. *6*, 1275-1280.

Wang,J.Z., Gao,X., and Wang,Z.H. (2014b). The physiology and pathology of microtubule-associated protein tau. Essays Biochem. *56*, 111-123.

Wang,J.Z., Gong,C.X., Zaidi,T., Grundke-Iqbal,I., and Iqbal,K. (1995). Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase-2A and -2B. J. Biol. Chem. *270*, 4854-4860.

Wang,W., Gong,N., and Xu,T.L. (2006). Downregulation of KCC2 following LTP contributes to EPSP-spike potentiation in rat hippocampus. Biochem. Biophys. Res. Commun. *343*, 1209-1215.

Wasco,W., Bupp,K., Magendantz,M., Gusella,J.F., Tanzi,R.E., and Solomon,F. (1992). Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid beta protein precursor. Proc. Natl. Acad. Sci. U. S. A *89*, 10758-10762.

Watanabe,M., Wake,H., Moorhouse,A.J., and Nabekura,J. (2009). Clustering of neuronal K+-Cl- cotransporters in lipid rafts by tyrosine phosphorylation. J. Biol. Chem. *284*, 27980-27988.

Wei,W., Nguyen,L.N., Kessels,H.W., Hagiwara,H., Sisodia,S., and Malinow,R. (2010). Amyloid beta from axons and dendrites reduces local spine number and plasticity. Nat. Neurosci. *13*, 190-196.

Weingarten,M.D., Lockwood,A.H., Hwo,S.Y., and Kirschner,M.W. (1975). A protein factor essential for microtubule assembly. Proc. Natl. Acad. Sci. U. S. A *72*, 1858-1862.

Westmark,C.J. (2013). What's hAPPening at synapses? The role of amyloid beta-protein precursor and beta-amyloid in neurological disorders. Mol. Psychiatry *18*, 425-434.

Westmark,C.J. and Malter,J.S. (2007). FMRP mediates mGluR5-dependent translation of amyloid precursor protein. PLoS. Biol. *5*, e52.

Westmark,C.J., Sokol,D.K., Maloney,B., and Lahiri,D.K. (2016). Novel roles of amyloid-beta precursor protein metabolites in fragile X syndrome and autism. Mol. Psychiatry *21*, 1333-1341.

Westmark,C.J., Westmark,P.R., and Malter,J.S. (2010). Alzheimer's disease and Down syndrome rodent models exhibit audiogenic seizures. J. Alzheimers. Dis. *20*, 1009-1013.

Westmark,C.J., Westmark,P.R., O'Riordan,K.J., Ray,B.C., Hervey,C.M., Salamat,M.S., Abozeid,S.H., Stein,K.M., Stodola,L.A., Tranfaglia,M., Burger,C., Berry-Kravis,E.M., and Malter,J.S. (2011). Reversal of fragile X phenotypes by manipulation of AbetaPP/Abeta levels in Fmr1KO mice. PLoS. One. *6*, e26549.

Weyer,S.W., Zagrebelsky,M., Herrmann,U., Hick,M., Ganss,L., Gobbert,J., Gruber,M., Altmann,C., Korte,M., Deller,T., and Muller,U.C. (2014). Comparative analysis of single and combined APP/APLP knockouts reveals reduced spine density in APP-KO mice that is prevented by APPsalpha expression. Acta Neuropathol. Commun. *2*, 36.

Wisniewski,K.E., Wisniewski,H.M., and Wen,G.Y. (1985). Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann. Neurol. *17*, 278-282.

Woo,N.S., Lu,J., England,R., McClellan,R., Dufour,S., Mount,D.B., Deutch,A.Y., Lovinger,D.M., and Delpire,E. (2002). Hyperexcitability and epilepsy associated with disruption of the mouse neuronal-specific K-Cl cotransporter gene. Hippocampus *12*, 258-268.

Wu,J., Anwyl,R., and Rowan,M.J. (1995). beta-Amyloid-(1-40) increases long-term potentiation in rat hippocampus in vitro. Eur. J. Pharmacol. *284*, R1-R3.

Wu,S.Z., Bodles,A.M., Porter,M.M., Griffin,W.S., Basile,A.S., and Barger,S.W. (2004). Induction of serine racemase expression and D-serine release from microglia by amyloid beta-peptide. J. Neuroinflammation. *1*, 2.

Wu,Y., Chan,K.F., Eubanks,J.H., Guin Ting,W.C., Cortez,M.A., Shen,L., Che,L.C., Perez,V.J., Tian,W.Y., Jia,Z., and Carter,S.O., III (2007). Transgenic mice over-expressing GABA(B)R1a receptors acquire an atypical absence epilepsy-like phenotype. Neurobiol. Dis. *26*, 439-451.

Xu,J.C., Lytle,C., Zhu,T.T., Payne,J.A., Benz E Jr, and Forbush,B., III (1994). Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. Proc. Natl. Acad. Sci. U. S. A *91*, 2201-2205.

Xu,X., Roby,K.D., and Callaway,E.M. (2006). Mouse cortical inhibitory neuron type that coexpresses somatostatin and calretinin. J. Comp Neurol. *499*, 144-160.

Yamada,J., Okabe,A., Toyoda,H., Kilb,W., Luhmann,H.J., and Fukuda,A. (2004). Cl- uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. J. Physiol *557*, 829-841.

Yan,Y., Dempsey,R.J., and Sun,D. (2001). Expression of Na(+)-K(+)-Cl(-) cotransporter in rat brain during development and its localization in mature astrocytes. Brain Res. *911*, 43-55.

Yang,L., Wang,Z., Wang,B., Justice,N.J., and Zheng,H. (2009). Amyloid precursor protein regulates Cav1.2 L-type calcium channel levels and function to influence GABAergic short-term plasticity. J. Neurosci. *29*, 15660-15668.

Yeo,M., Berglund,K., Augustine,G., and Liedtke,W. (2009). Novel repression of Kcc2 transcription by REST-RE-1 controls developmental switch in neuronal chloride. J. Neurosci. *29*, 14652-14662.

Young,A.B. (1987). Cortical amino acidergic pathways in Alzheimer's disease. J. Neural Transm. Suppl *24*, 147-152.

Young-Pearse,T.L., Bai,J., Chang,R., Zheng,J.B., LoTurco,J.J., and Selkoe,D.J. (2007). A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. J. Neurosci. *27*, 14459-14469.

Yuste,R. and Katz,L.C. (1991). Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. Neuron *6*, 333-344.

Zarea,A., Charbonnier,C., Rovelet-Lecrux,A., Nicolas,G., Rousseau,S., Borden,A., Pariente,J., Le,B., I, Pasquier,F., Formaglio,M., Martinaud,O., Rollin-Sillaire,A., Sarazin,M., Croisile,B., Boutoleau-Bretonniere,C., Ceccaldi,M., Gabelle,A., Chamard,L., Blanc,F., Sellal,F., Paquet,C., Campion,D., Hannequin,D., and Wallon,D. (2016). Seizures in dominantly inherited Alzheimer disease. Neurology *87*, 912-919.

Zhang,X., Zhong,W., Brankack,J., Weyer,S.W., Muller,U.C., Tort,A.B., and Draguhn,A. (2016a). Impaired theta-gamma coupling in APP-deficient mice. Sci. Rep. *6*, 21948.

Zhang,Y., Li,P., Feng,J., and Wu,M. (2016b). Dysfunction of NMDA receptors in Alzheimer's disease. Neurol. Sci. *37*, 1039-1047.

Zhang,Y.W., Wang,R., Liu,Q., Zhang,H., Liao,F.F., and Xu,H. (2007). Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. Proc. Natl. Acad. Sci. U. S. A *104*, 10613-10618.

Zheng,H., Jiang,M., Trumbauer,M.E., Sirinathsinghji,D.J., Hopkins,R., Smith,D.W., Heavens,R.P., Dawson,G.R., Boyce,S., Conner,M.W., Stevens,K.A., Slunt,H.H., Sisoda,S.S., Chen,H.Y., and Van der Ploeg,L.H. (1995). beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell *81*, 525-531.

Zhou,H.Y., Chen,S.R., Byun,H.S., Chen,H., Li,L., Han,H.D., Lopez-Berestein,G., Sood,A.K., and Pan,H.L. (2012). N-methyl-D-aspartate receptor- and calpain-mediated proteolytic cleavage of K+-Cl- cotransporter-2 impairs spinal chloride homeostasis in neuropathic pain. J. Biol. Chem. *287*, 33853-33864.

Zhu,L., Lovinger,D., and Delpire,E. (2005). Cortical neurons lacking KCC2 expression show impaired regulation of intracellular chloride. J. Neurophysiol. *93*, 1557-1568.

Zhu,L., Polley,N., Mathews,G.C., and Delpire,E. (2008). NKCC1 and KCC2 prevent hyperexcitability in the mouse hippocampus. Epilepsy Res. *79*, 201-212.

Zimmer,R., Teelken,A.W., Trieling,W.B., Weber,W., Weihmayr,T., and Lauter,H. (1984). Gamma-aminobutyric acid and homovanillic acid concentration in the CSF of patients with senile dementia of Alzheimer's type. Arch. Neurol. *41*, 602-604.

Zou,C., Crux,S., Marinesco,S., Montagna,E., Sgobio,C., Shi,Y., Shi,S., Zhu,K., Dorostkar,M.M., Muller,U.C., and Herms,J. (2016). Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis. EMBO J. *35*, 2213-2222.