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GABA-A receptor modulating steroids in acute and chronic stress; relevance for cognition and dementia?

S.K.S. Bengtsson[a], T. Bäckström[a,*], R. Brinton[b], R.W. Irwin[c], M. Johansson[a], J. Sjöstedt[a], M.D. Wang[a]

[a] Umeå Neurosteroid Research Center, Department of Clinical Sciences, University of Umeå, Sweden
[b] Center for Innovation in Brain Science, Professor Departments of Pharmacology and Neurology, College of Medicine, University of Arizona, Tucson, AZ, USA
[c] Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, 90089, USA

ABSTRACT

Cognitive dysfunction, dementia and Alzheimer's disease (AD) are increasing as the population worldwide ages. Therapeutics for these conditions is an unmet need. This review focuses on the role of the positive GABA-A receptor modulating steroid allopregnanolone (APα), it's role in underlying mechanisms for impaired cognition and of AD, and to determine options for therapy of AD. On one hand, APα given intermittently promotes neurogenesis, decreases AD-related pathology and improves cognition. On the other, continuous exposure of APα impairs cognition and deteriorates AD pathology. The disparity between these two outcomes led our groups to analyze the mechanisms underlying the difference. We conclude that the effects of APα depend on administration pattern and that chronic slightly increased APα exposure is harmful to cognitive function and worsens AD pathology whereas single administrations with longer intervals improve cognition and decrease AD pathology. These collaborative assessments provide insights for the therapeutic development of APα and APα antagonists for AD and provide a model for cross laboratory collaborations aimed at generating translatable data for human clinical trials.

1. The object of this review

In this review, steroids modulating the γ-amino-butyric-acid (GABA) receptor type A (GABA-A) receptor are discussed concerning acute and chronic stress and how stress influences learning and memory. Focus is on disturbances in normal individuals, in persons and rodents with non-dementia cognitive impairment and in rodents and humans with dementia. The steroids that enhance/activates the GABA-A receptor are referred to as positive GABA-A receptor modulating steroids (GAMS). Three steroids are mainly discussed: allopregnanolone (APα), and tetrahydrodeoxycorticosterone (THDOC) both increasing at stress (Purdy et al., 1991) and medroxyprogesterone-acetate (MPA) given continuously exogenously to postmenopausal women. Chronically increased concentrations of GAMS, especially APα, are shown to induce memory and learning disturbances (Johansson et al., 2002; Vallee et al., 2001), accelerate dementia development in AD mice models (Bengtsson et al., 2012, 2013) and MPA to double the risk of developing dementia in postmenopausal women (Shumaker et al., 2003). Concurrently intermittent administration of single dosages of APα is shown to increase

the regeneration of neurons and restore learning and memory in a transgenic Alzheimer mice model (Chen et al., 2011; Singh et al., 2012; Brinton, 2013). Taken together, APα can act as both a disturber and an enhancer of memory function in AD models. The purpose of this review is to give a summary of the background to and findings of this seemingly contradiction especially about the hypothesis that two very different mechanisms are operating in parallel giving opposite results in AD animal models. However, before we discuss APα's effect in Alzheimer's disease we give a background of APα related to acute and chronic stress in non-demented and normal individuals. In addition, we discuss GAMS exposure concerning learning and memory disturbances, and take examples from disorders like chronic stress disorders (Johansson et al., 2010; Lupien et al., 2005; Wang et al., 2012a; Yaffe et al., 2010), risk of dementia in Alzheimer's disease (Sindi et al., 2017), Parkinson's dementia (Backstrom et al., 2015, 2018) and hepatic encephalopathy (HE) (Weissenborn et al., 2005; Monfort et al., 2009; Bianchi et al., 2012). We also discuss consequences these findings may have for possible treatments of memory and learning disturbances in non-demented individuals and especially Alzheimer's dementia. From a

Abbreviations

3α/β-HSD	3α/β-hydroxysteroid dehydrogenase
3α5α-adiol	5α-androstane-3α, 20-diol
3xTgAD	The triple transgenic mouse model carries mutant human APPSwe, PS1M146v, and TAUP301L.
17β-HSD10	17β-hydroxysteroid dehydrogenase type 10
ACTH	Adrenocorticotropic hormone
AD	Alzheimer's disease
APP/AβPP	Amyloid precursor protein
APPSwe/Arc	The transgenic mouse model for AD that carries the gene for APP with the Swe and the Arc mutations
APPSwe/PS1	The transgenic mouse model for AD that carries the gene for APP with the Swe mutation, and the gene for PS1 with ΔE9 mutation
APα	Allopregnanolone
Arc	Arctic mutation, see also E693G
Aβ	β-amyloid (exists in various sub-types)
Aβ40	β-amyloid 1–40 – sub-type consisting of amino acids 1-40
Aβ42	β-amyloid 1–42 – sub-type consisting of amino acids 1–42
BrdU	5-bromo-2-deoxyuridine
CAA	Cerebral amyloid angiopathy
CNS	Central nervous system
DHEAS	Dehydroepiandrosterone sulfate
DOC	Deoxycorticosterone
E693G	The arctic mutation – amino acid number 693, glutamate, is replaced by glycine.
fMRI	Functional magnetic resonance imaging
GABA	γ-amino butyric acid
GABA-A	γ-amino butyric acid (receptor) type A
GAMS	GABA-A (receptor) modulating steroid
GAMSA	GABA-A modulating steroid antagonist
HE	Hepatic Encephalopathy
K595N/M596L	The Swedish mutation: Amino acid number 595, lysine, is replaced by asparagine and amino acid number 596, methionine, is replaced by leucine
LTD	Long-term depression
LTP	Long-term potentiation
MPA	Medroxyprogesterone acetate
MWM	Morris water maze
NMDA	N-methyl-D-aspartate receptor
PAM	Positive allosteric modulator
PS	Pregnenolone sulfate
PS1	Presenilin-1
SGZ	Hippocampal dentate gyrus subgranular zone
SVZ	Subventricular zone
StAR	Steroidogenic acute regulatory protein
Swe	The Swedish mutation, see also K595N/M596L
THDOC	Tetrahydrodeoxycorticosterone
TSPO	The cholesterol translocator protein
WHIMS	Women's Health Initiative Memory Study

therapeutic standpoint, the lack of an effective treatment for memory disorders extends beyond neuro-degeneration to a wide range of neuropsychiatric disorders, such as depression, hepatic encephalopathy, schizophrenia and burn out syndrome. While information about the role of GAMS in the mechanisms behind cognitive impairment in these disorders is indeed relevant and urgent, this discussion is only superficially dealt with in this review and lies beyond the scope of the current review.

2. Memory and cognitive function at acute and chronic stress in non-demented individuals

Take-home message: Acute stress can increase strength of memories while chronic stress at many different situations impairs memory and increases the risk for dementia. Both in acute and chronic stress production of GAMS increase in the periphery and also within the brain up to concentrations that affect the GABA-A receptors and with noted effects on behavior and in brain imaging studies. Exogenous positive allosteric modulators e.g. benzodiazepines and medroxyprogesterone-acetate also impairs memory and increases the risk of dementia.

2.1. Effects of acute stress

Acute stress is often thrilling and exciting in small doses. However, a strong acute stress can be overwhelming and develop into acute stress disorder (ASD) or even to post-traumatic stress disorder (PTSD) with cognitive dysfunction (Tiihonen Moller et al., 2014; Lupien et al., 2005; McEwen, 2008). In women, we observed that stress-induced negative mood, relates to premenstrual increases in amygdala volume (Ossewaarde et al., 2013). Our findings show that moderate psychological stress influences emotional circuitry. We also found that a large luteal phase increase in serum APα concentration correlates negatively to the medial prefrontal cortex responses after stress and to a smaller amygdala (Ossewaarde et al., 2010). Repeated episodic stresses can develop into chronic stress and development of allostatic overload with negative impact on health (Fava et al., 2019). While some aspects of steroid production during acute stress will be discussed, acute stress and how it influences long-term memory directly is not the focus of this publication.

2.2. Memory and cognitive function in chronic stress

Chronic stress is a multifaceted condition as it includes many forms, such as post-traumatic stress disorder (PTSD), psychological stress, or psychosocial work related stress, sometimes followed by burnout syndrome or chronic fatigue disorder (Lupien et al., 2005; Fava et al., 2019; Marin et al., 2011; Tiihonen-Möller et al., 2016; Tiihonen Moller et al., 2014). Chronic burnout syndrome in humans affects cognition negatively and high adrenal activity through increased cortisol levels is related to decreased cognition, including memory impairment (Lupien et al., 2005; Sandstrom et al., 2005). Repeated events of psychological stress in midlife and repeated psychosocial stress at work increase the risk for dementia (Johansson et al., 2010; Wang et al., 2012a; Sindi et al., 2017). High job strain, low levels of job control, low social support at work, loss of family support or a parent during adolescence and more stress-related physical symptoms are associated with higher dementia risk later in life (Wang et al., 2012a; Sindi et al., 2017; Wilson et al., 2006; Norton et al., 2011; Vitaliano et al., 2011). In transgenic animal models, stress has been shown to cause impaired memory function (Jeong et al., 2006; Alkadhi et al., 2010; Tran et al., 2010). All these data support the idea that chronic stress should be considered a risk factor for cognitive impairment and AD (Machado et al., 2014). However, the link between chronic stress and dementia or AD is still unknown but steroids, especially GAMS, seem to be one factor of interest to further investigate.

Effectively coping with a stressful situation requires a fast response and its quick termination afterward (de Kloet et al., 2005) and the process underlying the reaction to stress is called "allostasis" (McEwen, 2003). The stress response corresponds to the severity of the stressor, the sensitivity of the recipient and the duration of the stress. Short-term stress may enhance psychological/physiological performance; enhance immuno-protection, as well as mental and physical performance (McEwen, 2008; Tiihonen Moller et al., 2014Dhabhar, 2018). Reactions to chronic or cumulative stressors, however, can be more pronounced

or may have residual effects. The stress response may be insufficient, excessive, or inadequate, and the effort to recapture homeostasis may fail, leading to a condition called "allostatic load" or "allostatic overload" (de Kloet et al., 2005; McEwen, 2003). When allostasis fails, it can lead to long-lasting adaptive changes that become permanent and cannot be relieved by the end of the stressor. This may be one of the biological explanations for the development of several diseases, such as PTSD (Marin et al., 2011; Yehuda, 2002). In women with PTSD and in depressed patients serum APα has been found to be equal to unstressed women or decreased in the cerebrospinal fluid and serum (Rasmusson et al., 2006; van Broekhoven and Verkes, 2003; Moller et al., 2016). Several neurotransmitter systems are involved and have been investigated in relation to stress. Sensitivity to APα and GABA-A receptor sensitivity can be measured using saccadic eye velocity and sedation. Women with PTSD showed decreased sensitivity to diazepam in sedation, and showed decreased sensitivity to APα in saccadic eye velocity (Moller et al., 2016). This is contradictory to women with burnout syndrome who have an increased sensitivity to APα, a positive allosteric effect of the benzodiazepine antagonist flumazenil, but decreased sensitivity to diazepam (a benzodiazepine) all tree findings are suggesting an effect via the α4βδ receptor type, also measured using saccadic eye velocity and sedation (Backstrom et al., 2013). The α4βδ receptor type is known to be hyper sensitive to APα, showing a positive allosteric effect of flumazenil and to be insensitive to diazepam (Belelli et al., 2002; Gangisetty and Reddy, 2010; Smith et al., 1998).

2.3. Chronic positive GABA-A modulator (PAM) exposure - effects on cognition and risk of AD development

Several exogenous compounds are positive GABA-A receptor modulators, including benzodiazepines, barbiturates, and ethanol. Long-term exposure to these compounds cause permanent cognitive impairments and increased risk for dementia in humans (Barker et al., 2004; Saunders et al., 1991; Penninkilampi and Eslick, 2018), and cognitive decline in rats (Mohammed et al., 1987). Research has found a positive association between the use of anti-epileptic drugs and dementia especially Alzheimer's disease (Carter et al., 2007; Taipale et al., 2018). The mechanism discussed is that anti-epileptic drugs affects cognition by inhibiting neurotransmission and suppressing neuronal excitability in many cases by activating GABAergic mechanisms (Park and Kwon, 2008). Medroxyprogesterone acetate (MPA) is a synthetic progesterone-like compound. Similarly to APα, MPA can induce anesthesia via the GABA-A receptor (Norberg et al., 1987; Meyerson, 1967; Stromberg et al., 2013). MPA is a strong GAMS on the human α5-GABA-A receptor subtype in vitro with similar potency as THDOC, benzodiazepines, and barbiturates (Stromberg et al., 2013). The Women's Health Initiative Memory Study (WHIMS) showed that long-term treatment (four years) with MPA + estrogen doubled the risk for dementia mainly AD (Shumaker et al., 2003). Such an effect was not seen in the estrogen-only treated group (Shumaker et al., 2004). It was also shown that the increase in dementia cases was not a question of vascular dementia due to ischemic events or stroke and it was concluded that the increased dementia frequency was due to cellular-biological effects by MPA (Coker et al., 2009, 2010). Furthermore, it has been shown that positive effects on cognition by estrogen treatment in AD patients are suppressed by MPA (Honjo et al., 2005). MPA given to rats was shown to affect cognition negatively (Braden et al., 2010, 2011). These findings suggest that long-term exogenous positive modulation of the GABA-A receptor increases the risk for cognitive decline and AD. We conclude that chronic long-term exposure to GAMS may have a permanent deteriorating effect on learning and memory and increase the risk for dementia especially AD and is therefore of interest to investigate.

3. Positive GABA-A receptor modulating steroids (GAMS) in acute and chronic stress in non-demented individuals

Take-home message: At acute stress, the neurons are exposed to a strong enhancement of GABAergic action. Some individuals continue to have high steroid concentrations during chronic stress while others have decreased levels, they with high levels have increased risk for impaired memory. GAMS can be formed *de novo* in neurons and glia but the production is regional specific. Hippocampus is a region with GAMS production. Endogenous GABA-A modulating steroid antagonists and negative GABA-A receptor modulators are also produced in the body and can enter the brain. APα is a very potent GABA-A receptor modulator that affects all subtypes of GABA-A receptors. The receptor subtype α5 is found in hippocampus and related to memory and learning. The steroid effect on the GABA-A receptor depends on the type of steroid (agonist, antagonist, or invers agonist), the type of receptor (synaptic or extra-synaptic), and the subunit composition. Giving a 1) GAMS actions, 2) GAMSA action or 3) inhibiting GABAs action on the GABA-A receptor. This is important for possible treatment approaches at memory impairment.

3.1. GAMS in acute short term stress

The adrenals are the main peripheral GAMS production site at acute stress but production occurs in general also in the brain (Purdy et al., 1991). Besides at stress, the gonads produce GAMS and in women large amounts of APα are produced during the luteal phase of the menstrual cycle and during pregnancy (Purdy et al., 1991; Nyberg et al., 2007; Luisi et al., 2000). In humans and rats the production of cortisol, corticosterone, APα, and THDOC increases in parallel during acute stress (Purdy et al., 1991; Droogleever Fortuyn et al., 2004; Serra et al., 2003). The adrenal production of steroids including APα is highly controlled by corticotrophin-releasing hormone (CRH) and adreno-corticotrophic hormone (ACTH) (Camille Melon and Maguire, 2016). CRH neurons integrate information from many different brain regions involving numerous neurotransmitter systems, but the activity of CRH neurons is ultimately regulated by GABAergic inhibition (Herman et al., 2004). Also in humans, the concentration of APα in serum is related to that of the brain (Bixo et al., 1997).

Of the various steroids circulating in blood, mainly cortisol and corticosterone levels are studied at acute psychological and physical stresses. In general, increased cortisol levels return to the basal levels by feedback inhibition mechanisms through the hypothalamus, prefrontal cortex, and the hippocampus (Mizoguchi et al., 2003). However, in certain individuals, the cortisol concentrations remain high during long periods of chronic stress and these individuals are more likely to suffer cognitive disturbances (Lupien et al., 2005). Interestingly, the classical stress hormones cortisol and corticosterone are metabolized to 3α-hydroxy-5α reduced steroids; for example allotetrahydrocortisol (3α-hydroxy-5α-cortisol) (Celotti et al., 1992). Allotetrahydrocortisol enhances the effect of APα inducing increased GABA-A mediated chloride flux (Stromberg et al., 2005). Therefore, one can expect that during acute stress, the neurons are exposed to a strong enhancement of GABAergic action.

In the brain, GAMS can be formed *de novo* in neurons and glia, or generated by metabolism of circulating precursors that originate from peripheral steroidogenic organs, for details care for specific reviews (Compagnone and Mellon, 2000; Mensah-Nyagan et al., 1999; Mellon et al., 2001; Giatti et al., 2012). Briefly, the synthesis of GAMS involves the access of cholesterol to the first steroidogenic enzyme, the Cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase (3β-HSD) (P450scc) within the mitochondria. The rate-limiting step in the GAMS synthesis in the brain is the transport of cholesterol into the mitochondria (Da Pozzo et al., 2012; Papadopoulos et al., 2006a, 2006b). Two proteins among others that are involved in the cholesterol transport are the steroidogenic acute regulatory protein, (StAR) and the

translocator protein, (TSPO). StAR is under regulation of ACTH at least in the adrenal and is important for steroid production (Miller, 2013). Increased activity and expression of TSPO is still considered important for the rate and amount of the steroid produced (Da Pozzo et al., 2012; Miller, 2013) although TSPO is no longer considered to be an absolute requirement in the steroid production (Tu et al., 2014; Stocco et al., 2017). TSPO may be important in Alzheimer's disease as TSPO expression is increased in damaged neural tissue and neuro-inflammation like in AD (Dupont et al., 2017; McNeela et al., 2018). Ammonia is one factor, among others, that increases the activity and expression of TSPO and thereby increases the intra-mitochondrial production of pregnenolone, i.e. the precursor of APα (Itzhak et al., 1995). This is important for GAMS production in hepatic encephalopathy a condition with marked cognitive impairment, as discussed below. Outside the mitochondria, pregnenolone is further metabolized to progesterone, which is further converted to APα by the enzymes 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD) (Mellon et al., 2001). In rats the concentration of APα increases in the brain from 2 ng/g to 5.5 ng/g at acute stress. After adrenalectomy, the APα concentration still increases in the brain at stress but not in serum (Purdy et al., 1991; Cheney et al., 1995; Corpechot et al., 1993). Besides, the baseline endogenous CNS APα concentration decreases from 5.5 pmol/g to 0.5 pmol/g by inhibition of the 5alpha reductase (Puia et al., 2003) indicating a quite high autonomic synthesis of APα in the brain. Enzymes for synthesis in the brain are expressed region- and neuron-specific e.g. 5α-reductase and 3α-HSD are expressed and co-localized in pyramidal neurons and granular cells in the cortex and hippocampus, and in pyramidal-like neurons in the basolateral amygdala (Agis-Balboa et al., 2006, 2007). APα can be inactivated by 3α-HSD back to 5α-dihydroprogesterone (5α-DHP) (Mellon et al., 2001; Mellon and Griffin, 2002; Melcangi and Mensah-Nyagan, 2008). In addition, APα can be inactivated within the mitochondria by an enzyme type 10-17β-hydroxysteroid dehydrogenase (17β-HSD10) (He et al., 2005). 17β-HSD10 also binds proteins and peptides, e.g. amyloid-β (Aβ) that inhibits its metabolic activity and especially decreases the metabolism of APα in hippocampus, hypothalamus, and amygdala (Yang et al., 2011).

3.2. GAMS in long term chronic stress

Glucocorticoid steroids are substantially investigated in relation to chronic stress and a well-established finding is that the marker for adrenal steroid production in humans is cortisol, while that of rodents is corticosterone. APα is less investigated and the data available on chronic stress and APα are mainly from patients and animals at a late stage of chronic stress or dementia. However, findings have shown possible interactive effects between APα and glucocorticoids (Stromberg et al., 2005). The regulation of CRH neurons is altered following stress. Stress results in excitatory actions of GABA on CRH neurons due to a collapse of the chloride gradient (Camille Melon and Maguire, 2016). These excitatory effects of GABA following stress are specific for CRH neurons in the PVN (Sarkar et al., 2011). But during chronic stress, deficits in GABAergic inhibition are observed in extra hypothalamic regions and have been proposed to contribute to increased hippocampal excitability (MacKenzie and Maguire, 2015). These findings demonstrate that the GABAergic regulation and neurosteroid modulation of the HPA axis are state-dependent and that APα and THDOC can, in certain stress situations be excitatory instead of being inhibitory (Pinna et al., 2006). When stressful stimuli are repeated chronically, circulating cortisol is maintained at higher levels over a prolonged period, at least in certain individuals, and these individuals develop cognitive disturbances (Lupien et al., 2005). Chronically elevated steroid levels cause damages to hippocampal and cortical neurons (McEwen et al., 2016). As a result, even when stress stimuli disappear, cortisol levels can be maintained at levels beyond the physiologically normal range due to a vicious cycle caused by the already damaged feedback mechanism. (Lupien et al., 2005; Lee et al.,

2015).

If APα and GAMS also show similar increased and prolonged synthesis in certain individuals during the development of chronic stress is less known and if so to what degree APα is involved in the chronic stress effects on memory and learning will be discussed below. However, APα shows similar diurnal rhythm as cortisol and several other adrenal steroids indicating that a parallel production of APα and cortisol occur (Tiihonen-Möller et al., 2016).

Several studies indicate that APα levels are low in disorders related to a long period of chronic stress. Patients with depression show serum, CSF, and brain reductions of APα and 3α,5α-THDOC levels and/or biosynthesis (Romeo et al., 1998; Agis-Balboa et al., 2014) and in premenopausal women with PTSD the lowest levels of APα are found in the patients with co-morbid depression (Locci and Pinna, 2017). In women with burn-out syndrome, there is no difference in Apα level at baseline but the patients had lower APα concentrations than controls after the same APα dosages given per kg body weight (Bäckström et al., 2013). In rodents, prolonged social isolation gives a down-regulation of neurosteroid production (Agis-Balboa et al., 2007; Vallee, 2014; Serra et al., 2000). However, at acute stress in chronically stressed animals, APα and THDOC concentrations increased more than in controls (Serra et al., 2000). The concentration of GAMS at chronic stress seems thus to be different from glucocorticoids and treatment with exogenous APα has been suggested in these disorders (Schule et al., 2014). In one disorder, postpartum depression, APα clinical trials have been made with positive results (Meltzer-Brody et al., 2018).

3.3. Endogenous GABA-A modulating steroid antagonists and negative GABA-A receptor modulators

Pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS) can inhibit the effect of APα and the GABA-A receptor function and act as negative GABA-A receptor modulators (NAM) (Paul and Purdy, 1992). Isoallopregnanolone (IsoAPα) has an antagonistic effect on APα but does not interact with the effect of GABA itself or barbiturates and benzodiazepines (Stromberg et al., 2006). IsoAPα is thus not a NAM but a GABA-A modulating steroid antagonist (GAMSA) (Stromberg et al., 2006). The 3β-hydroxysteroid dehydrogenase (3β-HSD) is essential for the synthesis of 3β-OH steroids, i.e. pregnanolone (3β–OH–5β-pregnanolone) and isoallopregnanolone (3β–OH–5α-pregnanolone, isoAPα) (Stromstedt et al., 1993). PS and DHAS will not be discussed further in this review as they have been reviewed substantially elsewhere (Mensah-Nyagan et al., 1999; Rustichelli et al., 2013; Dong and Zheng, 2012; Kokate et al., 1999). PS and DHEAS have also effects on other receptors e.g. glutamate receptors (Irwin et al., 1992; Xu et al., 2012).

3.4. GAMS and the GABA-A receptor

APα is one of the most potent GABA-A receptor modulating steroids, to the extent that it can induce anesthesia (Norberg et al., 1987). APα is even more potent than the most potent barbiturate known today (Korkmaz and Wahlstrom, 1997). Activation of the GABA-A receptor will, in most adult situations, lead to hyperpolarization of the neuron carrying the receptor. As a chloride channel, the GABA-A receptor allows chloride flow into the neuron when activated under normal situations in adults (Birzniece et al., 2006a). The GABA-A receptors are usually localized at the postsynaptic neuron within the synaptic cleft (intra-synaptic) or at an extra-synaptic position (Belelli and Lambert, 2005). Intra-synaptic GABA-A receptors, located at the postsynaptic neuron are less sensitive and demand relatively high GABA concentrations to become activated while the extra-synaptic receptors are sensitive to low GABA levels (Belelli et al., 2002; Barnard et al., 1998; Whiting et al., 1999). The different GABA-A receptor subtypes are localized in very specific areas of the brain and therefore the subtype of the receptors is related to the function of that particular brain area. At

least 19 different subunits have been described (6α, 3β, 3γ, δ, ε, θ, π, and 3ρ) (Olsen and Sieghart, 2009; Wisden et al., 1992). A more detailed description of the receptor localization and relation to function is given elsewhere (Birzniece et al., 2006b; Bäckström et al., 2008; Korpi et al., 2002). Still, the receptor type containing the α5 subunit is well-known to be related to memory and learning and is highly expressed in hippocampus and subtypes containing α4,βx,δ is very sensitive to APα and the expression of the receptor is regulated by APα (Belelli et al., 2002; Locci and Pinna, 2017).

Interestingly, the subunit composition of the receptor determines its sensitivity to not only GABA but also to GAMS (Belelli et al., 2002) and in addition, the GABA-A receptor can be modulated by a number of therapeutic agents, including benzodiazepines (Macdonald and Olsen, 1994; Sieghart, 1992), barbiturates (Smith and Riskin, 1991), anesthetics, ethanol (Harris et al., 1995), zinc (Smart, 1992) and GAMS (Hawkinson et al., 1994; Puia et al., 1990; Lambert et al., 2001a). Several sex and stress steroids are potent GAMS (Paul and Purdy, 1992; Majewska et al., 1986; Zhu et al., 2001; Crawley et al., 1986; Gasior et al., 1999; Lambert et al., 2001b). For example, both APα and 3α5β pregnanolone (3α5βP), as well as THDOC and 3α5βTHDOC, have significant sedative effects in vivo (Norberg et al., 1987). Also, the A-ring reduced metabolites of testosterone, (3α-hydroxy-5α-androstanediol), acts as a positive GABA-A receptor modulating steroid and is a GAMS (Frye et al., 1996).

The effect of neuroactive steroids on the GABA-A receptor depends on the type of steroids (agonist or antagonist), the type of receptors (synaptic or extrasynaptic), and also the subunit composition. Recent studies indicate at least three neuroactive steroid actions on the GABA-A receptor: (i) a positive GABA-A receptor modulating action by the neuroactive steroid (GAMS effect) (Stromberg et al., 2006; Majewska et al., 1986), (ii) a GABA-A receptor modulating steroid antagonistic (GAMSA) action by e.g. 3β-OH steroids (Stromberg et al., 2006; Wang et al., 2002) and (iii) being negative GABA-A receptor modulators or inverse agonists that closes the GABA-A receptor or hinders GABA's own effect e.g. PS and DHEAS. GAMSA is not acting on the GABA site or the chloride channel but non-competitively inhibits GAMS action (Johansson et al., 2016). Pregnenolone-sulfate (PS) acts as a negative GABA-A receptor modulating steroid or inverse agonist as PS can antagonize the effect of GABA itself (Wang et al., 2006) and has thus different action and channel properties compared to 3β-hydroxysteroids (Wang et al., 1997, 2007). Pregnenolone-sulfate also enhances glutamate receptors by potentiating NMDA receptors in similar concentrations as the effect on the GABA-A receptor (Wu et al., 1991) thus, pregnanolone-sulfate has an increased risk for seizures. (Kokate et al., 1999; Wang et al., 2007; Williamson et al., 2004).

Positive GAMS action can further be divided into (i) an allosteric enhancement of GABA-evoked Cl⁻ current at low concentrations (3 nM) by enhancing GABA's opening of the GABA-A receptor with the main effect being a slower closure of the receptor, investigated in rat hypothalamic cells (Stromberg et al., 2006) or (ii) a direct activation of GABA-A receptors by GAMS without the presence of GABA this occur at lower concentrations in extra synaptic receptors compared to synaptic receptors (Stromberg et al., 2006; Belelli and Lambert, 2005). APα also increases the number of spontaneous Inhibitory Post Synaptic Currents (sIPSC) by a presynaptic action (Haage et al., 2002). A detailed description of the action of GAMS on the GABA-A receptor is out of the scope of this paper but can be found in more specialized papers (Akk et al., 2007).

One of the GABA-A receptor subunits, the α5 subunit, is highly expressed in the hippocampus and considered to be involved in memory and learning processes. Briefly, inhibition of GABA-A receptor with α5 subunit results in increased spatial learning e.g. α5 knockout mice had significantly better performance in the MWM in comparison with wild-type mice (Collinson et al., 2002). Also, the selective blockade of α5 subunit containing GABA-A receptors increased rat learning and memory (Maubach, 2003). For further details on the GABA-A receptor

subunits and memory care for a review on the subject (Birzniece et al., 2006b).

4. Role of GAMS in learning and memory functions in non-demented individuals

Take-home message: Episodic memory is related to hippocampal function. APα inhibits episodic memory and memory retrieval in humans and APα inhibits spatial memory in rodents. In some disorders there are high APα levels and impaired memory. In humans, chronic GAMS, high cortisol and positive GABA-A receptor modulator exposure, are linked to permanent cognitive disturbances and possibly development of dementia, episodic memory is early affected in AD. Treatment with GABA-A receptor modulating steroid antagonist (GAMSA) reduces the memory impairment effect of APα. The α5 GABA-A receptor is important for memory and if α5 GABA-A receptors are dysfunctional a compensatory increase of α4 GABA-A receptors may occur and increases the sensitivity to APα.

4.1. Learning and memory

Memory is the function by which information is encoded, consolidated and retrieved. In both humans and animals, the hippocampus is an essential brain area for learning and memory (Astur et al., 2002; Farr et al., 2000), such as the formation of long-term declarative memories (Teng and Squire, 1999). The hippocampus is affected early in the disease development in for example AD leading to loss of episodic memory, which is reduced ability to consolidate new experiences. The spatial memory function is especially affected in AD, leading to disorientation. An animal test that especially investigates spatial memory and hippocampus related learning in rodents is the Morris Water Maze (MWM) which is a well-known and often used memory and learning test (Morris et al., 1986) and the results from such investigation are discussed below.

4.2. Examples of situations and disorders with high GAMS concentrations and learning and memory disturbances

Hepatic encephalopathy (HE) is an example of a condition with high brain concentration of APα and increased GABAergic tone combined with learning and memory disturbances, for more details of GABAergic tone and APα in HE consult reviews on the topic (Ahboucha et al., 2012; Felipo, 2013). Briefly, APα concentrations are higher in human post mortem cortical brain tissue compared to controls without HE (Ahboucha et al., 2006). Similar results with increases in both APα and THDOC have been obtained in animal models of HE (Ahboucha et al., 2012; Norenberg et al., 1997). Also, the decreased learning and memory function in HE models are counteracted by treatment with GAMSA indicating that GAMSA could be used as a treatment for cognitive disturbances in HE (Johansson et al., 2015). An increased GABAergic tone is one of the main hypotheses of the neuropathology of HE (Sergeeva, 2013; Jones, 2002). The main toxic factor in HE, ammonia, is shown to stimulate the production of GAMS. The initial and rate-limiting step in GAMS synthesis involves the transport of cholesterol into the mitochondria, and ammonia increases the uptake of cholesterol into the mitochondria probably by increasing the activity and expression of TSPO (Lavoie et al., 1990). The intra-mitochondrial production of pregnenolone, the precursor of progesterone and APα, will thus increase (Itzhak et al., 1995; Lavoie et al., 1990; Butterworth, 2010). The increase in TSPO density and capacity leads to an increased steroid production in the brain especially of GAMS like APα and THDOC.

4.3. Consequences of brief or continuous GAMS exposure on cognitive functions in normal and non-demented individuals

Concerning brief GAMS exposure, it is known, that APα in

pharmacological doses decreases the neural activity in the hippo-campus of rats (Landgren et al., 1998). APα can inhibit learning in rats tested in the MWM (Johansson et al., 2002; Vallee et al., 2001; Mayo et al., 1993) and inhibit episodic memory in humans (Kask et al., 2008). Episodic memory is a type of memory that is disturbed early in AD patients (Perry and Hodges, 1996). GAMS also impair LTP formation in rat hippocampus (Dubrovsky et al., 2004) and GAMS hamper memory-related cholinergic action in rat neurons projecting to the hippocampus (George et al., 2006). As GAMS alters LTP, it can be assumed that po-sitive GABA-active steroid compounds may alter memory function (Dubrovsky et al., 2004). Stress has been shown to impair LTP and to enhance LTD in the hippocampus by affecting synaptic plasticity (Howland and Wang, 2008). Functional magnetic resonance imaging (fMRI) studies in women show that progesterone/APα impairs memory by reducing the memory recruitment of those brain regions that support memory formation and retrieval (van Wingen et al., 2007). It is well known that other GABA-A receptor agonists e.g. benzodiazepines (Barker et al., 2004), barbiturates (Mohammed et al., 1987) and alcohol (Saunders et al., 1991; Vincze et al., 2007) also impair memory and learning in humans, and increase the risk for permanent damages, al-though the risk with low and moderate alcohol consumption is under debate (Solfrizzi et al., 2007).

Chronic GAMS exposure or increased sensitivity to GAMS are in humans noted in certain disorders, for example during chronic stress, burn out syndrome, hepatic encephalopathy, and treatment with GABA-A receptor modulating sex-steroids (medroxyprogesterone-acetate, MPA). In these situations/disorders high GAMS tone is linked to cog-nitive disturbances and possibly development of dementia (Shumaker et al., 2003; Lupien et al., 2005; Yaffe et al., 2010; Sandstrom et al., 2005; Bäckström et al., 2013; Inslicht et al., 2006). As mentioned above, in the women's health initiative study (WHIMS), the frequency of probable dementia (mainly AD) doubled after five years of con-tinuous estrogen + MPA treatment (Shumaker et al., 2003). The in-crease in dementia frequency was not due to increase ischemic events but due to a biological factor probably MPA itself (Shumaker et al., 2003; Coker et al., 2009) and estrogen alone did not increase the de-mentia risk (Shumaker et al., 2004; Henderson et al., 1994). MPA acts as a positive GABA-A receptor modulator on the human α5β3γ2 GABA-A receptor with a similar potency as THDOC and MPA can induce an-esthesia in rats (Meyerson, 1967; Stromberg et al., 2013). High levels of cortisol are related to impaired memory (de Quervain et al., 1998). It is shown that the individuals that develop permanent memory and learning disturbances among chronically stressed persons are those individuals with continuing high steroid levels for prolonged times (Lupien et al., 2005).

The doubled dementia frequency of the WHIMS study was seen after a continuous MPA treatment in postmenopausal women and as dis-cussed above the exposure of endogenous GAMS to the GABA-A re-ceptor may also be continuous during chronic stress in vulnerable persons, (Shumaker et al., 2003; Lupien et al., 2005; Ahboucha et al., 2006; Nasman et al., 1991). AD patients show abnormal adrenal reg-ulation and altered response to stress compared to normal controls. This indicates that the production of cortisol and GAMS is distorted in AD patients. Patients with AD have similar cortisol and GAMS response to adrenal stimulation as chronically stressed animals (Nasman et al., 1991, 1996). Patients with mild AD have a high and non-suppressible production of cortisol and probably of GAMS (Nasman et al., 1995).

Using the Morris water maze (MWM) paradigm in rats, acute APα was found to inhibit learning (Johansson et al., 2002). Chronic APα treatment during 5 months at low-stress levels, gives in wild type mice a permanent deterioration in learning and memory especially in female mice (Bengtsson et al., 2016). The MWM testing was made one month after treatment had ended. The deterioration in memory and learning was correlated to a decrease in hippocampal volume (Bengtsson et al., 2016). The GABA-A receptor modulating steroid antagonist (GAMSA), 3β, 20β-dihydroxy-5α-pregnane, reduces the negative effect of APα on

the learning in the MWM (Turkmen et al., 2004). In rat the GAMSA 3β, 20β-dihydroxy-5α-pregnane is shown to be specifically able to block the effect of APα on the GABA-A receptor (Turkmen et al., 2004). Pregnenolone sulfate (PS) also acts as an APα antagonist although with some different channel properties compared to 3β-hydroxy-steroids (Wang et al., 2007). PS infused into rat basal magnocellular nucleus enhanced memory performance, whereas APα disrupted memory (Mayo et al., 1993). Other steroids like pregnenolone, DHEA and DHEAS increased memory performance when injected systemically, centrally or into the amygdala in rats (Flood et al., 1988, 1992; Wolkowitz et al., 1995). DHEA and its sulfate DHEAS are also produced by the adrenal under ACTH control and they have been shown to modulate a variety of neurotransmitter systems, including cholinergic, GABAergic, dopaminergic and glutamatergic systems (Dong and Zheng, 2012; Xu et al., 2012). There is evidence that the concentration of DHEA and DHEAS are decreased in patients suffering from AD (Nasman et al., 1991; Sunderland et al., 1989; Hillen et al., 2000). This can be due to negative feedback effect by GAMS on ACTH production from the pituitary resulting in inhibited adrenal steroid production (Mody and Maguire, 2011).

The subunit composition of the GABA-A receptor is of major im-portance for the effects on memory. The α5β3γ2 GABA-A receptor subtype in the hippocampus is related to memory and learning. After long-term exposure to GABA agonists, a tolerance/down-regulation of the GABA-system may occur with malfunction as a result (Barnes, 1996; Yu and Ticku, 1995). However, an α5 GABA-A receptor mal function, like at a deletion in chromosome 15 containing the α5 GABA-A receptor gene, gives a 12 fold compensatory increase of a α4 containing GABA-A receptors and we know that α4βδ is hyper sensitive to APα (Belelli et al., 2002; Bittel et al., 2007). In contrast, inflammation with exposure to IL-1β increases α5 GABA-A receptor activity in cultured neurons with decreased ability to induce long term potentiating as a result (Wang et al., 2012b). A change in the GABA-A receptor system may be a factor in the pathogenesis of stress-induced disorders. In women with "burn-out" syndrome, there are indications of an up-regulation of the α4βxδ subunit composition. This is indicated by a) increased APα sensitivity and b) a switch in action of flumazenil from a benzodiazepine an-tagonist or inert compound to become a positive modulator which both suggests presence of α4βxδ-subunit (Bäckström et al., 2013; Whittemore et al., 1996). Exposure to an agonist may cause changes in the GABA-A receptor subunit composition (Smith et al., 1998). It is well known that prolonged exposure to APα changes the expression of the α4 and the α1 subunits (Smith et al., 1998). In fact, in vitro, the α4β2γ2 receptor is less sensitive to GAMS modulation than α1β2γ2 GABA-A receptors (Belelli et al., 2002). However, the α4βδ subunit composition is hypersensitive to APα leading the chloride channel to be affected at much lower APα concentrations than needed for other receptor sub-types (Brown et al., 2002). Thus changes in subunit composition and/or down-regulation of the whole GABA-A receptor could be mechanisms for GAMS induced disorders. The change in subunit composition can be quite rapid. In a rat model for tolerance studies, tolerance against APα anesthesia was developed already after 90 min, and related to changes in GABA-A receptor subunit α4 in thalamus (Birzniece et al., 2006a).

5. Brief background to cognitive deterioration in Alzheimer's disease (AD) related to GAMS effects

Take home message: To understand the effect of GAMS on the de-velopment of AD we will superficially describe some events in AD de-velopment of importance for the GAMS effects. Patients in early AD have high steroid levels while many late-stages AD patients have de-creased levels of APα. Intra synaptic oligomers of amyloid beta (Aβ), especially the 42 amino acids long Aβ polypeptide (Aβ42), are neuro-toxic and cases synaptic dysfunction. Neuronal excitability releases oligomers and Aβ out of the synapse and decreases the risk of oligomer formation. Compounds (e.g. benzodiazepines and GAMS) that decrease

neuronal activity and Aβ release will increase risk for oligomer production and synaptic toxicity.

5.1. GAMS production in AD

Patients with mild to moderate AD show increased concentrations of glucocorticoid and some sex steroids in blood compared to healthy elderly controls (Rasmuson et al., 2002). AD patients also show increased 5α-reduction (Rasmuson et al., 2001). Thus an increased glucocorticoid production is an early feature in AD with an enhanced metabolism leading to 5α reduced metabolites of steroids like APα (Rasmuson et al., 2001). Expression of TSPO and several enzymes in APα synthesis are increased in microglia and astrocytes in the hippocampus of AD patients which may be a reason for higher concentrations of 5α reduced steroids in early AD. (Cosenza-Nashat et al., 2009; Luchetti et al., 2011a). TSPO may be important in Alzheimer's disease as TSPO expression is increased in damaged neural tissue and neuro-inflammation like in AD (Dupont et al., 2017; McNeela et al., 2018). In vivo radioligand studies show increased TSPO in cortical brain regions of AD patients compared to controls the binding is inversely correlated with memory performance (Kreisl et al., 2013).

Late-stage AD patients have decreased levels of 5α reduced steroids, including APα (Luchetti et al., 2011b; Bernardi et al., 2000; Smith et al., 2006). In postmortem analyses, concentrations of APα were considerably reduced in the brains of humans in late-stage AD, and who died of AD, compared to non-AD individuals. The reduction was correlated with the extent of AD pathology (Naylor et al., 2010). Consistent with this finding in humans, a reduced basal level of APα was found in the cerebral cortex of the brain in the triple transgenic mouse model of AD (3 × TgAD), suggesting decreased APα synthesis or increased APα metabolism (Wang et al., 2010). Parallel analyses of serum and cortex levels of APα in 3xTgAD mice indicates a reduced APα level in cerebral cortex due to problem in the brain and not in peripheral sources of APα.

One factor that might be of importance is that APα is metabolized and inactivated by 17β-HSD10 in the mitochondria. However, 17β-HSD10 has a high affinity for several molecules and binds the Aβ peptide. The enzymatic function of the 17β-HSD10 enzyme is inhibited by Aβ1-40 up to 65% (Oppermann et al., 1999), this would increase active APα concentration. Elevated levels of 17β-HSD10 have been found in the brains of AD patients and a mouse AD model (He et al., 2005) (He et al., 2002); (Yang et al., 2005). This may be a natural compensatory mechanism to overcome the inhibition of 17β-HSD10 by intracellular Aβ infiltration. (Yang et al., 2011). The concentration of APα in brain and serum at early stages seems to differ from later stages, however, the results are disparate and the concentrations in brain and serum seem not to correlate at least not in animal models of AD.

5.2. Oligomers of amyloid β-proteins (Aβ) and tau proteins are neurotoxic

AD is characterized by increasing cognitive dysfunction, synaptic loss, and brain atrophy which has a neuro-pathological pattern and particularly affects the hippocampus and the temporal lobe (Dawbarn and Allen, 2007; Goedert, 2015). The most known histopathological feature of AD is the amyloid plaque, first described by Dr. Alois Alzheimer in 1907 (Alzheimer, 1907). Oligomers from soluble pools of Aβ, especially the 42 amino acids long Aβ polypeptide (Aβ42), are neurotoxic (Watson et al., 2005), causing decreased synaptic function, synaptic loss and eventually neuronal death (LaFerla et al., 1995; Selkoe, 2002). Recent studies show that the soluble Aβ form oligomers disturb synaptic functions and correlate with symptom severity (Watson et al., 2005; Lue et al., 1999; McLean et al., 1999; Mucke et al., 2000; Lord et al., 2009). With high enough Aβ concentration, which will occur intra-synaptically, oligomers will be formed (Ding et al., 2012). Intra cellular soluble Aβ, originates from both intraneuronal production and uptake from the extracellular space (Alzheimer, 1907; Watson et al., 2005; Lue et al., 1999; McLean et al., 1999; Naslund et al., 2000; Wirths

et al., 2004). Another pathological accumulated protein is the tau-protein. Tau has a similar propagation and distribution in the brain as Aβ (Goedert, 2015). When the tau protein it gets hyperphosphorylated it oligomerize and intracellular neurofibrillary tangles are made of hyperphosphorylated tau-protein in cortical and limbic areas of the human brain (Tiwari et al., 2019). However, to further discuss the neuropathology of AD is outside the scope of this paper.

5.3. Intrasynaptic Aβ oligomers destroys synaptic function

It seems that the intraneuronal pool of soluble Aβ oligomers is responsible for synapse pathology (Gouras et al., 2000, 2005, 2010, 2014). Interestingly, the levels of intracellular Aβ are determined by synaptic activity (Liu et al., 1999; Tampellini et al., 2009), and Aβ is released at neuronal activity (Nitsch et al., 1993; Cirrito et al., 2005). Aβ is secreted from the synapse at the time of neuronal activity, due to simultaneous secretion of Aβ and neurotransmitters, like glutamate, and increased neuronal activity will thus decrease the intraneuronal Aβ concentration (Nitsch et al., 1993; Cirrito et al., 2005; Iwata et al., 2013; Chang et al., 2019). The capture of Aβ in the cells and plaques the release to CSF decreases and a low Aβ level in CSF is a marker for dementia both in AD (Blennow and Zetterberg, 2018) and Parkinson's disease (Backstrom et al., 2015). By decreasing the level of synaptic activity via decreasing stimulation will increase intraneuronal levels of Aβ e.g. by removal of whiskers in the rat or activation of the GABA-A receptor by using diazepam, the flow in the amyloid cascade was altered in transgenic mouse models for AD (Tampellini et al., 2009, 2010). Chronic treatment with diazepam caused elevated levels of intracellular Aβ, and enhanced formation of neurotoxic oligomers, in turn leading to neuronal dysfunction and cognitive decline (Tampellini et al., 2010). Synaptophysin, a synaptic scaffolding protein, correlates with synaptic function and is used as a marker for synaptic function. Inhibited neuronal activity was shown to decrease the level of synaptophysin (Mucke et al., 2000; Tampellini et al., 2010), which correlated to AD symptoms (Selkoe, 2002). This is to be expected since Aβ is known to disrupt the synaptic function (Gouras et al., 2010, 2014). Treatment with picrotoxin, a GABA-A receptor inhibitor, rescued the memory decline (Yoshiike et al., 2008). GAMS will like benzodiazepines normally decrease neuronal excitability and synaptic activity. Therefore it is not unreasonable to suspect that continuous prolonged exposure to GAMS will increase intra-synaptic oligomer concentration, increase the destruction of the synapses and thereby accelerate memory dysfunction and perhaps ultimately accelerate dementia progress. This will be discussed further below. We hypothesize that endogenous or exogenous GAMS can similarly affect the amyloid cascade as benzodiazepines. This modulation can lead to reduced levels of synaptic activity, which in turn may lead to increased levels of intra-synaptic Aβ, and oligomer formation, resulting in synaptic dysfunction, synaptic and neuronal loss, atrophy, and memory dysfunction.

5.4. GABAergic and synaptic function and cognitive decline in AD

Several neurotransmitter systems are inflicted in AD development, however selectively. Cholinergic neurons are affected and lost early on. This selective loss of neurons disturbs the brain neurochemistry leading to e.g. decline in cholinergic activity, which may underlie both cognitive and psychiatric symptoms. The GABAergic neurons are generally considered relatively spared and only lost in the late stages of disease development (Davies et al., 1998). However, new studies show that both expression of receptor subtypes change and the sensitivity to GABA decrease in GABA-A receptors during the development of AD. Lower expression of GABA-A receptor subunits α1, α2, α4, δ, and β2 mRNAs have been found in the prefrontal cortex in human AD brains (Luchetti et al., 2011b) and of α1, α5, and β3 mRNAs in the AD hippocampus (Luchetti et al., 2011a; Rissman and Mobley, 2011; Mizukami et al., 1998). In temporal cortex from human AD patients and

controls, there is a reduction of α1 and γ2 mRNAs but an increment of α2, β2, and γ1 mRNAs in AD compared to controls (Limon et al., 2012). Thus changes in the expression of GABA-A receptor subunits will probably alter function of the GABA inhibition locally but the net result on cognition, development of AD and implications for APα effects is so far difficult to interpret. GABAergic inhibition may also affect the early degeneration of other neurotransmitter systems and symptoms in AD (Davies et al., 1998; Garcia-Alloza et al., 2006a). For more details on the topic of GABA receptor changes in AD see (Rissman and Mobley, 2011; Rissman et al., 2003, 2007). However, changes in receptor sub-unit expression will lead to altered sensitivity towards APα and which may alter the response to normal APα concentrations (Belelli et al., 2002).

The early loss of neurons that is seen in AD most severely affects the hippocampus and the neocortex. A central question is what role Aβ play in synaptic dysfunction. Synapses are considered the earliest site of pathology, and synaptic loss is the best pathological correlate of cognitive impairment in AD both in animal models and humans (Selkoe, 2002; Tampellini and Gouras, 2010; Terry et al., 1991; Hamos et al., 1989; DeKosky and Scheff, 1990; Coleman and Yao, 2003). The amyloid precursor protein (APP) is normally transported down the axons and dendrites to the synapses (Gouras et al., 2010, 2014; Haass et al., 2012), and is preferentially processed to Aβ in the synapses as the proteases (presenilin-1, PS1) that generate Aβ is localized in synapses (Lah et al., 1997). Synapses are sites of early Aβ accumulation and aberrant tau phosphorylation in AD, and the synaptic content of Aβ increases at the early stages of the disease. Evidence from cultured neurons of AD transgenic mouse models and human postmortem AD brains highlights that accumulation of Aβ in synapses is involved in early synapse dysfunction (Gouras et al., 2010). Studies using immunoelectron microscopy and high-resolution immunofluorescence microscopy show that this early subcellular Aβ accumulation leads to Aβ aggregation, oligomer formation, and induction of dysfunctional synapses (Gouras et al., 2014).

6. Regeneration of neurons in degenerative disease

Take-home message: Short and high exposure with long intervals of

APα can increase growth of progenitor cells in hippocampal dentate gyrus. These cells can develop into neurons and may replace dead neurons.

To regenerate neurons or to prevent degeneration of functional neurons would be a great achievement for the treatment of degenerative CNS disorders. By stimulating endogenous regenerative systems or by neural stem cell transplantation it may be possible to prevent, delay and treat neurodegenerative diseases (Wang et al., 2008). In the adult brain, neural stem-cell proliferation zones exist in the hippocampal dentate gyrus subgranular zone (SGZ) and the subventricular zone (SVZ) of the lateral ventricle (Cameron et al., 1993; Altman, 1969; Altman and Das, 1965; Luskin, 1993; Liu et al., 2010). The regenerative ability declines with age but remains the whole life (Cameron and McKay, 1999; Kuhn et al., 1996; Eriksson et al., 1998). Growing stem cells are possible to mark with 5-bromo-2-deoxyuridine (BrdU) and by that possible to identify. In dentate gurus 50–60% of BrdU-labeled cells are neurons identified by neuron markers and 3% are replaced every month (Cameron and McKay, 1999; Liu LaB, 2010). New neurons show granule cell morphology, spine density, and glutamatergic connections (Morgenstern et al., 2008). New granule cells connect to neural circuitries (van Praag et al., 2002) and function in memory and learning situations in the hippocampus (Clelland et al., 2009). The proliferation of granule cell precursors declines with age (Kuhn et al., 1996) and the decline is higher at high corticosteroid concentration (Cameron and McKay, 2001).

As in embryonic cells, GABA is excitatory in adult progenitor cells in SGZ instead of being inhibitory. That is progenitor cells have depolarizing GABA-A receptors not hyperpolarizing GABA-A receptors (Tozuka et al., 2005). In adult neurons, GABA is usually an inhibitory neurotransmitter, but in neural progenitor cells, GABA is *excitatory*. One possible mechanism to the different effects of GABA-A receptor activation is that in adults the intracellular Cl^- concentration of neurons is relatively low and activation of GABA-A receptors gives an influx of Cl^- giving hyper polarization and inhibition of neuronal activity (Backstrom et al., 2011). In contrast, during fetal development the intracellular Cl^- concentration is comparably high, and at activation of GABA-A-receptors an outflow of Cl^- cause's excitation (Kahle et al., 2008). The intracellular Cl^- concentration is determined by the
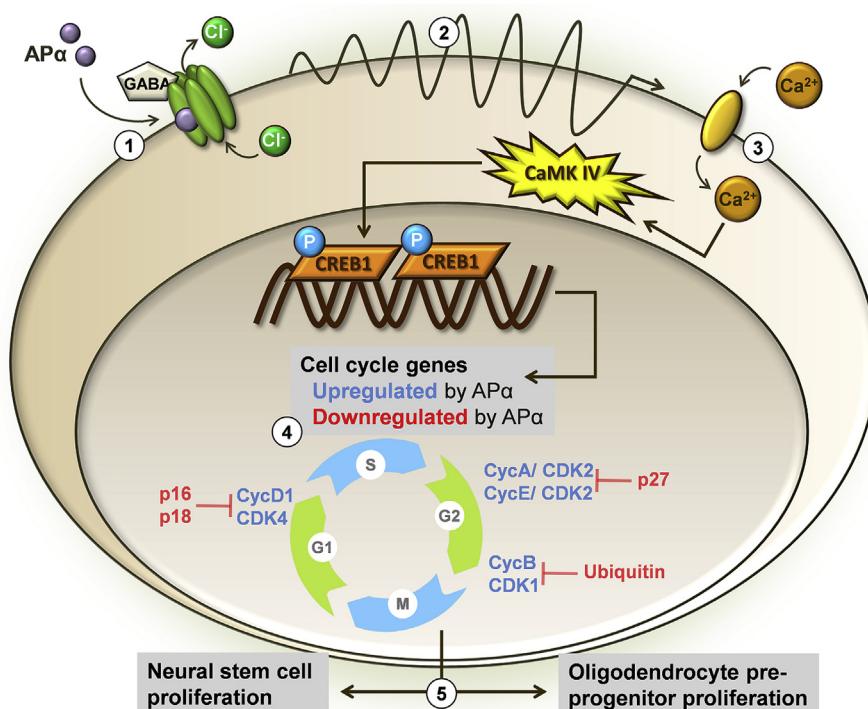

Fig. 1. Mechanism of GABA and APα -induced neural stem cell and oligodendrocyte precursor progenitor mitosis. APα (APα) binds to the GABAA receptor potentiates GABA and in higher concentrations directly activates the GABAA receptor (Chen et al., 2011). In stem cells, the intracellular Cl– is elevated relative to extracellular Cl–. An activation of the GABAA receptor leads to an efflux of Cl– that gives a membrane depolarization (Singh et al., 2012). Membrane depolarization activates voltage-dependent L-type calcium (Ca2+) channels giving an influx of Ca2+ (Purdy et al., 1991). The intracellular Ca2+ rise activates a Ca2+-dependent kinase, which phosphorylates and activates the transcription factor cyclic AMP-responsive element-binding protein 1 (CREB1). The CREB1 activation up-regulates the expression of cell cycle genes that initiates the cell cycle (Johansson et al., 2002). Genes and proteins that repress cell division are down-regulated (Johansson et al., 2002). The end is that successful transition through the cell cycle leads to neural stem cell proliferation in the subgranular zone of the dentate gyrus and oligodendrocyte precursor progenitors in white matter (Vallee et al., 2001). Abbreviations: Ca2+, calcium; CDK, cyclin-dependent kinase; Cl–, chloride; Cyc, cyclin; Ub, ubiquitin.
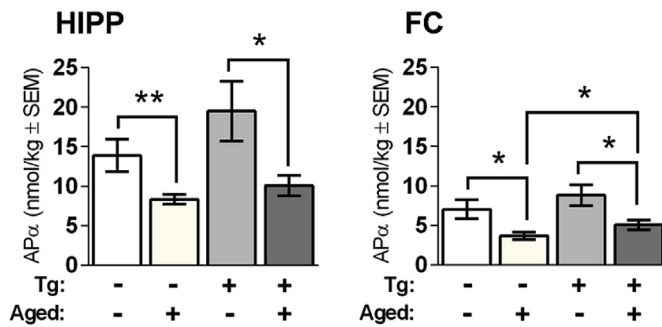
HIPP

FC

Fig. 2. Endogenous APα levels in young and middle-aged mice. The figures show endogenous APα tissue levels in hippocampus (HIPP) and frontal cortex (FC) in young (20 weeks) and middle-aged (36 weeks), wild-type and APPSwe/PS1 mice (Tg) respectively. Group compositions from the left: Young wild-type (nHIPP = 15, nFC = 14), middle-aged wild-type (nHIPP = 13, nFC = 12), young APPSwe/PS1 (nHIPP = 11, nFC = 11), and middle-aged APPSwe/PS1 (nHIPP = 12, nFC = 22). **p < 0.01, *p < 0.05.

activity of inward and outward directed transmembrane Cl^- pumps, where the major inward pump is NKCC1 and the major outward pump is KCC2 (De Koninck, 2007; Price et al., 2009). In adult animals and probably also in humans, the outward directed pump KCC2 dominates, keeping the intracellular Cl^- concentration low in adult neurons (Owens and Kriegstein, 2002).

The depolarization of the cell membrane opens membrane bound calcium channels results in calcium influx into adult neuroprogenitor cells and this starts a cascade of intracellular events that eventually will start the cell cycle and mitosis (Fig. 1, molecular details are given in the legend to figure). APα stimulates this process and thus increases the regeneration of new neurons in the hippocampus (Liu LaB, 2010). A detailed description of the intracellular events at an APα stimulation is out of the scope of this paper but is discussed in detail in (Brinton, 2013).

GABA and GABA-A receptors play an important role in the activity of immature dentate granule cells (Owens and Kriegstein, 2002; Sipila et al., 2004; Overstreet Wadiche et al., 2005) and GABA-A receptors are expressed in growing adult SGZ and SVZ progenitor cells (Mayo et al., 2005; Liu et al., 2005). The results of GABAergic induced activation of progenitor cells, obtained with APα enhancement, was enhanced learning and memory (Chen et al., 2011; Singh et al., 2012; Wang et al., 2010). The reversed membrane potential is essential for APα to activate the cell cycle and induce proliferation (Fig. 1, details are given in the legend to figure). This series of events is achieved by enhanced GABAergic signaling with the optimal frequency of APα administration (Chen et al., 2011). Thus APα in high concentrations with long intervals (weeks) in between would thus recruit progenitor cells that develop to neurons replacing lost neurons in the hippocampus. This issue will be further discussed below.

7. Studies of long-term APα exposure in transgenic AD-mice

Take-home message: In TgAD-mice continuous APα exposure at low stress concentrations for one or 3 months gives permanent deterioration of memory and learning compared to vehicle/placebo treatment. Chronic APα exposure increases soluble Aβ concentration and in wild type mice the hippocampal volume and memory deteriorated after 5 months APα exposure.

It can be concluded from the above review of the literature that continuous exposure to positive GABA-A receptor modulating steroids overtime may permanently deteriorate learning and memory. However, in the above-discussed disorders, several factors can be involved and influence learning, memory, and development of dementia. Therefore, we have specifically studied the effect of APα on memory and learning and some biological markers of dementia. APα was given continuously

for one or three months in doses resembling the baseline levels at mild chronic stress. Below, we report some of the results obtained in these studies using transgenic AD and wild type mice (Bengtsson et al., 2012, 2013, 2016).

After the treatment period and one month with no treatment, the mice were tested for learning and memory performance in the Morris water maze and the brain tissue was analyzed for pathological markers, i.e. soluble and insoluble Aβ40 and Aβ42, and amyloid plaques. We also report some novel characterizations regarding endogenous APα exposure in wild-type and APPSwe/PS1 mice, at acute and chronic stress, respectively.

7.1. Transgenic animal models used in the presentations below

The continuous low-stress level effects of APα on disease development was investigated using the transgenic APPSwe/PS1 (see (Garcia-Alloza et al., 2006b; Savonenko et al., 2005) for animal characteristics), and the APPSwe/Arc see (Knobloch et al., 2007) for animal characteristics) mouse models for AD. Further for testing of daily parenteral APα dosing the 3xTgAD mice model was used. The three AD mice models discussed below are thoroughly described in a review by van Dam and de Deyn (Van Dam and De Deyn, 2006) The APPSwe/PS1 mouse forms more Aβ42 than Aβ40. Aβ42 is more toxic than Aβ40 since it is more prone to form oligomers. The progression of angiopathy is slower than in e.g. the Swe/Arc mouse model. The Swe/Arc mouse model is primarily exposed to Aβ40 and only low levels of Aβ42. The triple transgenic mouse (3xTg), 3xTgAD, carries mutant human APPSwe, PS1M146v, and TAUP301L and the model is described in detail in Oddo et al. (2003). This model develops apart from the amyloid plaques also neurofibrillary tangles (Wang et al., 2010).

7.2. Endogenous APα brain levels in wild-type and APPSwe/PS1 mice

Below, we report on APα concentrations in the hippocampus and frontal cortex in transgenic APPSwe/PS1 mice compared to wild-type mice, and in middle-aged (36 weeks) mice compared to young (20 weeks), respectively.

7.2.1. Baseline APα levels in mice

We found that aged mice have lower levels of APα, regardless of genotype in both males and females respectively (Fig. 2). (Yang et al., 2011; Corpéchot et al., 1997; Ford et al., 2008a, 2008b; Frye and Walf, 2008). The levels of APα were higher in the hippocampus compared to the frontal cortex in all groups. Interestingly, aged APPSwe/PS1 mice had higher levels compared to aged wild-type mice in the frontal cortex (Fig. 2). This would indicate that the APPSwe/PS1 mice have higher levels of APα in cortex than wild-type mice at a stage of fully developed AD. This does not parallel to what has been found in late-stage AD patients (Bernardi et al., 2000; Smith et al., 2006), but rather that of patients with moderate AD and indicates an endogenous dysregulation of APα production in late AD.

7.2.2. Response to acute and chronic stress

Previous reports in rats show that APα levels are increased at acute stress (Purdy et al., 1991; Vallée et al., 2000). Furthermore, it was shown in rats that chronic stress (social isolation) decreases APα baseline levels but increases the response to acute stress (Serra et al., 2000). The stress response in transgenic AD mice has not been completely investigated regarding APα levels. In our studies, we found that both wild-type and APPSwe/PS1 mice had increased levels of APα after a period of mild chronic stress using a daily resident/intruder stress model for 1 h/day in four weeks (Fig. 3) (Bartolomucci et al., 2005). The day after the last intruder interaction, an acute stress situation was introduced using a forced swim test (see legend to Fig. 3) (Newson et al., 2013). In the acute test, the chronically stressed APPSwe/PS1 mice responded with even higher levels of APα while the wild-type
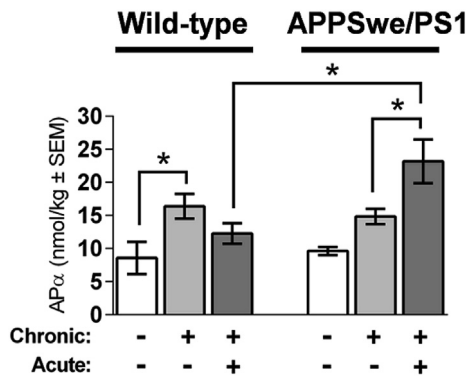
Fig. 3. Endogenous APα tissue levels at chronic mild stress followed by an acute stress. The chronic stress was obtained by daily resident intruder confrontations for 1 h/day in 4 weeks. The stress was confirmed by corticosterone measurements. The acute stress was obtained by a swim in a pool for 60 s the day after the last resident intruder confrontation. The mice were then allowed to rest for 30 min before decapitation to catch the peak allopregnanolone level in the brain after acute stress (Purdy et al., 1991). The figures show the endogenous APα levels in the whole brain hemisphere in young, male wild-type (WT) and APPSwe/PS1 (AD) mice respectively. Group compositions from the left: unstressed (n = 6), chronically stressed (n = 23), chronically and acutely stressed (n = 13). *p < 0.05.

mice had unchanged levels (Fig. 3). The corticosterone concentration in chronically stressed APPSwe/PS1 mice was higher than in the wild type stressed mice but similar to what have been found by others in AD mice (Dong et al., 2008; Arranz et al., 2011). Interestingly, in humans, AD patients were more sensitive to an HPA (hypothalamic-pituitary-adrenocortical axis) stimulation test compared to controls (Nasman et al., 1995, 1996; Bernardi et al., 2000). The presented data suggest that transgenic AD mice have an affected stress-response system, similar to that of AD patients.

7.3. Study paradigm for chronic APα elevation

To further investigate the effect of chronically elevated low-stress levels of APα on the development of AD and cognition, we investigated APPSwe/PS1 and APPSwe/Arc mouse models. A treatment with APα started at 10 weeks of age and the length of the treatment period was selected to correspond to a substantial period of a mouse's life (one or three months of approximately 2 years estimated lifetime). After the end of the treatment, a wash-out period without treatment of four weeks was allowed. The behavioral testing started four weeks after the exogenous APα was removed from the body. This was done to ensure that the long-term, indirect permanent effects of the chronically elevated levels of APα were studied and not the direct effects of the APα (Van Dam and De Deyn, 2006). In the APPSwe/Arc mice, the treatment continued for one month and in APPSwe/PS1 mice treatment duration was three months. Tissue sampling was done when the behavioral testing was finished at 20 weeks for APPSwe/Arc mice and 28 Weeks for the APPSwe/PS1 mice (Bengtsson et al., 2012, 2013). *The achieved APα levels matched the endogenous levels of APα during mild stress, which was confirmed in a pharmacokinetic satellite study* (Figs. 3 and 4).

7.4. Effects of chronic APα exposure on learning and memory

Both one month and three months of chronic APα elevation caused impaired learning and memory performance in transgenic AD mouse models (Bengtsson et al., 2012, 2013). The effects are depicted in Fig. 6 which reveals firstly a major effect on learning ability among the APPSwe/PS1 mice after three months of elevated APα. Curiously, this effect was most evident in the male APPSwe/PS1 mice and less clear in females. Secondly, the APPSwe/Arc had also impaired learning after

only one month of APα treatment, here both males and females were similarly affected. The largest effect in the APPSwe/Arc was seen in the MWM probe trial, where the number of mice with intact memory decreased (Fig. 5). Impaired memory performance was also seen in the APPSwe/PS1 mice but male mice only. The learning impairment was not unexpected, as chronic treatment with other positive GABA-A receptor modulating compounds has led to cognitive decline in other AD mouse models (Tampellini et al., 2010; Yoshiike et al., 2008). However, it has not previously been shown that APα can give rise to similar deteriorating effects. The wild-type mice treated one or three months were unaffected by chronic APα treatment in terms of learning and memory but continues treatment in 5 moths did give a permanent deterioration also in wild type mice especially females (Bengtsson et al., 2016). Permanent negative effects on cognition due to chronic exposure of other positive GABA-A receptor modulators have been seen in wild-type animals (Mohammed et al., 1987; Braden et al., 2010, 2011; Bengtsson et al., 2016), and in humans (Barker et al., 2004; Le Melledo and Baker, 2004; Crowe and Stranks, 2018). The results suggest that a longer period of APα exposure is needed in wild type mice compared to AD-mice to obtain cognitive impairment. The achieved levels of APα were mildly elevated and well within the physiological range. The transgenic AD mice seem to be more sensitive to a chronic APα elevation than their wild-type siblings. The cause of the increased sensitivity is likely due to the presence of Aβ, as this is what separates the transgenic mice from their wild-type siblings. However, it is unknown if and in what manner Aβ leads to heightened sensitivity towards GAMS or if and in what manner elevated presence of APα leads to increased levels of Aβ.

In studies using the 3xTgAD mice model and giving frequent APα administration three times per week also impairs learning and memory (Chen et al., 2011). The paradigm of three subcutaneous injections per week leads to chronic elevation of APα, with similar effects of the treatment as described above with continuous exposure of APα from osmotic pumps. However, if injections are given with longer intervals (once/week or longer) the treatment results in increased learning and memory as will be described below (Chen et al., 2011).

7.5. APα effects on soluble Aβ levels

The level of soluble Aβ was previously shown to negatively correlate with cognitive performance in transgenic mouse models for AD (Savonenko et al., 2005). A summary of our findings on Aβ levels are presented in Fig. 6. We found that chronic APα elevation led to increased levels of soluble Aβ in the APPSwe/PS1 mice (Bengtsson et al., 2012), which suggests that the disease development was accelerated in the APα-treated mice. Compared to in-soluble amyloid plaques, soluble Aβ is an accurate predictor for disease severity, both in animal models (Mucke et al., 2000), and in humans (Lue et al., 1999; McLean et al., 1999). Increased soluble Aβ, and especially increased intra-synaptic
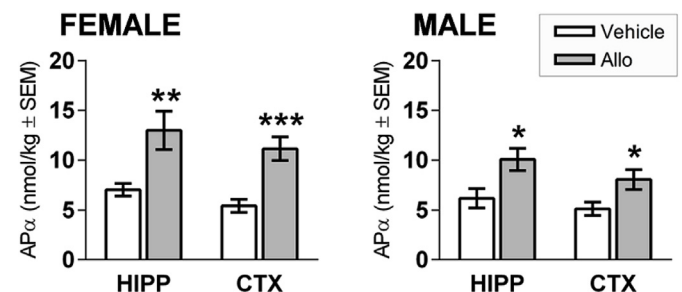

Fig. 4. APα tissue levels during chronic treatment. The figures show the APα levels in the hippocampus (HIPP) and cortex (CTX) in wild-type mice. The mice were treated continuously using ALZET osmotic pumps with APα (n = 20 females + 20 males) or vehicle (n = 10 + 10) for 4 weeks from the age of 10 weeks. Tissues were collected at the end of treatment before pumps were removed. ***p < 0.001, **p < 0.01 * p < 0.05, vs. vehicle.

A Learning curve, mean(SEM) latency

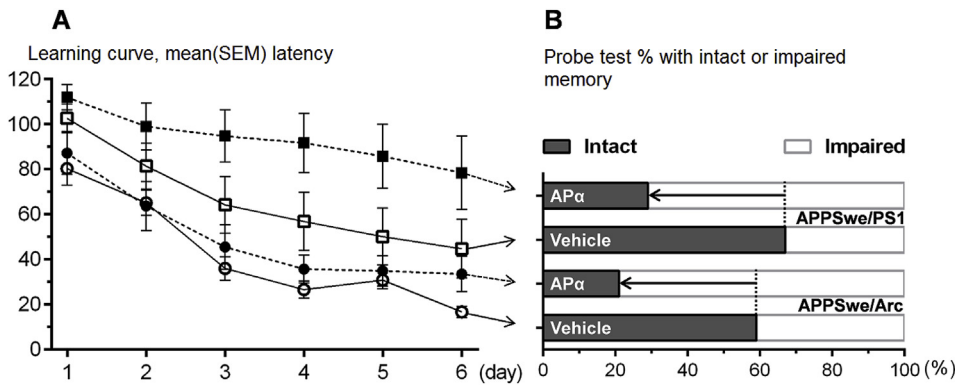**B** Probe test % with intact or impaired memory

Fig. 5. The figure shows A) mean ± SEM learning curves of latency to find the platform in the MWM at test day 1–6. Each animal made 4 MWM tests per day and the mean/animal and day were used in the calculations. B) Shows the percentage of mice with intact and impaired memory performance in the MWM probe trial. The arrow shows the % impaired AD mice relative to the vehicle-treated mice. The mice were treated chronically with allopregnanolone (APα) or vehicle during 4 weeks (Swe/Acr) or 12 weeks (Swe/PS1), from the age of 10 weeks followed by one month without treatment before the MWM testing. APα was given 24 h/day s.c. using ALZET pumps. The pumps were removed one month before MWM testing. In both AD mice types this procedure led to increased learning and memory disturbance at one month after the end of APα treatment.
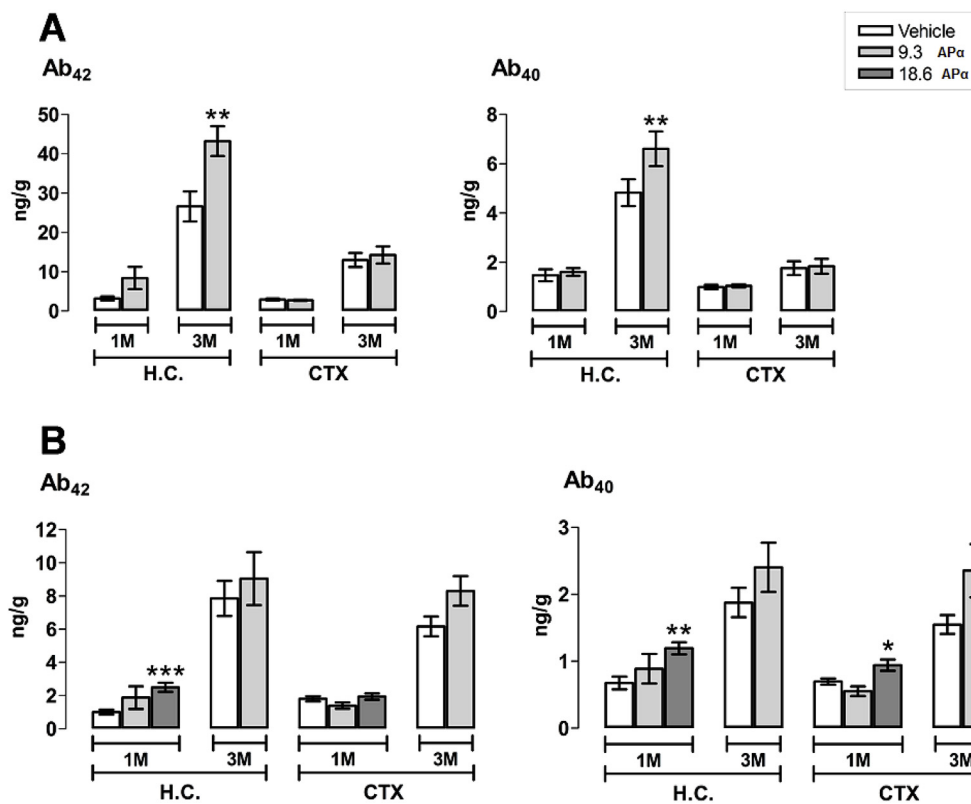

A Ab42, Ab40

B Ab42, Ab40

Fig. 6. Increased tissue levels of soluble Aβ in Swe/PS1 mice after chronic allopregnanolone (APα) treatment. The figures show levels of soluble Aβ42 and Aβ40 in the hippocampus (H.C.) and cortex (CTX) in female (A) and male (B) Swe/PS1 mice, respectively. The mice were treated with allopregnanolone (9.3 nmol/h; 9.3 APα or 18.6 nmol/h; 18.6 APα) or vehicle for 4 weeks (1M) and 12 weeks (3M) from the age of 10 weeks. The data are shown as mean per g wet tissue ± S.E.M. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs. Vehicle.

soluble Aβ, is thought to be a starting point for synaptic failure and neuronal degeneration (Gouras et al., 2005). In our study, the increased hippocampal levels of soluble Aβ correlated to poorer memory performance (Bengtsson et al., 2012). The increase in soluble Aβ was most evident in the hippocampus, and the increase was more substantial in female mice than in males. Interestingly, the levels of soluble Aβ were un-affected by chronic APα treatment for one month in the APPSwe/Arc mice (Bengtsson et al., 2013). Taken together, these data reveal that mild chronic APα elevation leads to increased levels of soluble Aβ in animals with a certain amyloid pathologic profile with a parallel decline in memory and learning performance.

7.6. APα effects on hippocampus volume

Data are indicating that the hippocampal volume relates to cognitive function in humans (Astur et al., 2002; Maguire et al., 2000; Tabatabaei-Jafari et al., 2019; Pohlack et al., 2014). Stress changes the

structure of the hippocampus (McEwen et al., 2016). In our study on long term treatment, 5 months with APα in wild type mice, we noted a decrease in hippocampal weight but an increased weight of cerebellum compared to vehicle treatment in female mice. Furthermore, in female mice, relative hippocampus weight was positively, and cerebellum weight was negatively, correlated to spatial memory performance (Bengtsson et al., 2016).

7.7. Hypothesis for the mechanism behind chronic GAMS-accelerated AD development

The main conclusion from our studies described above is that chronic elevation of APα accelerated the disease development in transgenic AD mice. This was concluded as the APPSwe/PS1 mice responded with impaired learning and memory performance and increased levels of soluble Aβ. Furthermore, the learning and memory dysfunctions were also seen in wild-type mice after a longer period of

APα exposure. These findings were not unexpected since APα is a positive GABA-A modulating steroid and since treatment with other positive GABA-A receptor modulators led to congruent effects in other studies (Tampellini et al., 2010; Yoshiike et al., 2008). The leading question is how chronic exposure to GAMS affects amyloid pathology and brain atrophy. The modified amyloid cascade hypothesis (Wirths et al., 2004) and the inside-out amyloid hypothesis (Gouras et al., 2014) focus on the events before plaque formation, i.e. the intraneuronal pool of soluble Aβ monomers and oligomers. Disturbances in the amyloid cascade can be caused by altered levels of neuronal activity (Liu et al., 1999; Tampellini et al., 2009), as intra synaptic Aβ is released to the extracellular space at depolarization (Nitsch et al., 1993; Cirrito et al., 2005). Based on the above, we propose the following hypothesis of the mechanism behind stress-induced AD (as depicted in Fig. 7). In a non-stressed state, the GABAergic activity is set at a balanced level which allows a certain level of neurotransmission. The Aβ is produced in endosomal vesicles (Takahashi et al., 2004) and secreted to the extra-cellular space along with synaptic neurotransmission, especially glutamate release, leading to lower intracellular/synaptic concentrations of Aβ (Cirrito et al., 2005). With lower intracellular concentrations of Aβ the probability for production of Aβ-oligomers decreases. If the levels of APα are increased e.g. at stress, via production in the adrenals or from production directly in the brain, APα will inhibit neurotransmission in neuronal circuits via positive modulation of GABA-A receptors (Iwata et al., 2013; Chang et al., 2019). As the intracellular Aβ concentration depends on the level of neurotransmission, increased APα levels may cause decreased secretion of Aβ out of the synapse. In turn, this leads to increased intra synaptic levels of soluble Aβ. As a consequence, the production of toxic Aβ oligomers is triggered by the high intracellular concentration of Aβ and synaptic dysfunction will follow, as will cognitive symptoms. Other possible hypotheses may exist as wild type mice also show deterioration in learning after prolonged APα exposure.

8. Studies of acute/episodic APα exposure in 3xTgAD mouse model

Take-home message: APα can activate neural progenitor cells in vitro APα promotes proliferation increase in cultures of human neural stem cells compared to controls. A relationship between neurogenesis and hippocampal-dependent memory and learning functions is shown. However, repeated APα administration of three times/week deterio-rated regeneration of progenitor cell survival that is had the opposite effect on cell regeneration.

One of the devastating facts in AD is that neurons die and this will permanently change the possibility to remember and gain new knowledge. A wish would therefore be to be able to replace the dead neurons with new functional neurons to revitalize the learning and memory function. There are indications that this might be possible through activating progenitor cells in the hippocampus. Such activation can be made by APα (see below). APα may thus by using this mechanism be able to improve memory and learning in AD patients and animals. This is a seemingly opposite effect compared to the above-described acceleration of the disease progress under chronic APα exposure. Below is a description of the mechanism.

8.1. APα and hippocampal progenitor cells

There are effects of APα, discussed below, that can be beneficial for AD patients (Irwin et al., 2012). APα can activate neural progenitor cells in vitro (Wang et al., 2005) and it was found that an injection of APα reversed the neurogenic and memory deficits in the 3xTgAD mouse model using a specific memory test (Wang et al., 2008, 2010). It was shown that one weekly injection of APα led to a proliferation of neuronal progenitor cells in the hippocampus (Chen et al., 2011), and improved cognition in the 3xTgAD mice (Singh et al., 2012). This mechanism with intermittent high doses of APα is altogether a different aspect compared to that of chronic elevation of APα discussed above and this mechanism will further be discussed below.

8.2. Effect of APα on neural regeneration

APα is a growth factor for both neural stem cells and preprogenitor oligodendrocytes (Wang et al., 2005, 2010; Schumacher et al., 2012; Sun et al., 2012). APα increases, the number of BrdU containing cells in the SGZ of 3xTgAD mice in a dose-dependent manner. The proliferation is restored in SGZ to the normal magnitude of non-Tg mice (Wang et al., 2010). APα promotes proliferation increase in cultures of human neural stem cells up to a rate of 37–49% compared to controls (Wang et al.,
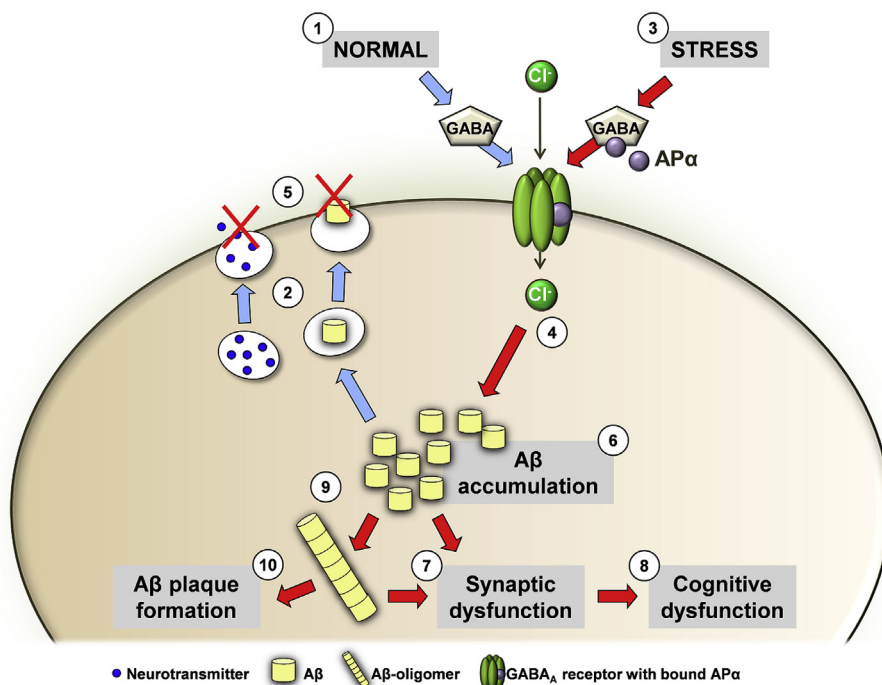

Fig. 7. Model of the proposed mechanism behind GAMS-induced enhancement of AD. Under 1) normal non-stressed conditions, GABA trigger the GABA-A receptor in a manner that ensures excitatory balance in the post-synaptic neuron. 2) Balanced neuronal activity allows sufficient secretion of Aβ out of the synapse, which occurs in parallel to that of neurotransmitters. 3) At stress, there is an increase in APα which modulates the GABAA receptor, and in mature neurons mainly by enhancing the inhibiting effect of GABA. This leads to a 4) larger influx of Cl-which lowers the excitability of the postsynaptic neuron, which 5) decreases the release of neurotransmitters and that of Aβ. Decreased secretion of Aβ leads to 6) intra-neuronal Aβ accumulation and 9) formation of toxic oligomers, which 7) disturbs synaptic function and leads to synaptic failure with 8) cognitive decline as a consequence. Also, the intraneuronal Aβ accumulation eventually leads to 9) increased formation of Aβ-oligomers, which 7) further disturbs synaptic and neuronal function and forms the basis for 10) Aβ plaque formation.
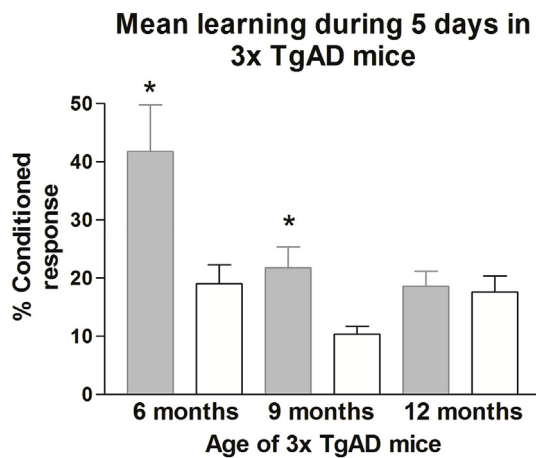
Mean learning during 5 days in 3x TgAD mice

Fig. 8. Mean learning of conditioned response after five days of training in 3xTgAD mice (7–15 mice/group) and one week after a single dosage of 10 mg/kg of APα (filled bars) or vehicle (open bars). New redrawn figure adapted from (Singh et al., 2012).

2005). In stem cells, the intracellular Cl⁻ ion concentration is higher than the extracellular concentration. At GABA-A receptor activation efflux of Cl⁻ ions occur. APα enhances the GABA-A receptor efflux of Cl⁻ ions and starts mitosis via influx of calcium ions as described above (Fig. 1) (Wang et al., 2005) giving a considerable increase in the number of newly generated neurons(2, 202).

In normal young mice, APα does not influence an already robust, and perhaps maximal, neurogenic system. However, at 12 months of age, wild type mice begin to exhibit a neurogenic response to APα and it is statistically significant at 15 months of age. In aged and 3 × TgAD mice (see below), exhaustion of the regenerative system coincides with loss of response to APα and probably to other factors that promote endogenous neurogenesis (Singh et al., 2012; Rodriguez et al., 2008). This indicates a window of opportunity for promoting endogenous neural stem cells for regeneration. Analyses in normal mice indicates that a threshold for proliferation exists and that the decrease in proliferation in the brain of aging and 3 × TgAD mice is required for APα -induced regeneration.

Deficits in neuronal regeneration at aging are evident in several AD transgenic mouse models, including the 3 × TgAD mice (Singh et al., 2012; Wang et al., 2010; Rodriguez et al., 2008). The proliferation rate of newborn cells has been compared to the behavioral learning of a new task after an APα treatment or vehicle treatment. There was a significant correlation between the number of BrdU-positive cells and learning during APα treatment but no change occurred after vehicle administration regardless of training condition (Singh et al., 2012).

Three features of APα regulation of neurogenesis are important to consider. First, APα restored the regenerative potential of the brain to a normal rate, not to supra-normal (Singh et al., 2012; Wang et al., 2010). Second, the regenerative effect of APα is dose-dependent, with a classic growth factor inverted-U-shaped dose-response curve (Wang et al., 2005, 2010), whereby exceeding the neurogenic dose does not lead to greater response rather the opposite. Both of these characteristics indicate that the regenerative system affected by APα is tightly regulated, with closely guarded thresholds for both activation and magnitude of proliferation. The third feature of APα regulation of neurogenesis is that the regenerative effect of APα co-varies with age and AD pathology burden. APα was neurogenically active in the hippocampus of 3 × TgAD mice at 3, 6 and 9 months of age. At 12 months of age, when the hippocampus is burdened with extraneuronal plaques, APα is no longer effective (Singh et al., 2012). In wild type mice, APα promotion of neurogenesis was initially evident at 12 months of age and was statistically significant at 15 months of age (Singh et al., 2012). Frequent intermittent administration of APα reduces the burden of AD

pathology in the 3 × TgAD mouse brain (Chen et al., 2011).

8.3. Effect on learning and memory of episodic APα administration

There is a strong relationship between neurogenesis and most hippocampal-dependent memory and learning functions (Aimone et al., 2011; Deng et al., 2010; Shors et al., 2002). Especially associative learning and memory across time seem to be dependent on the generation of new neurons in the dentate gyrus (Aimone et al., 2011; Deng et al., 2010; Haughey et al., 2002). A model for testing APα effect on memory and learning is the hippocampus-dependent trace eye-blink conditioning paradigm (Singh et al., 2012). The conditioning learning is accomplished by repeated trials of an auditory tone followed by a mildly aversive shock stimulus. This paired conditioning test is dependent upon the generation of new neurons in the dentate gyrus (Singh et al., 2012; Lee and Kim, 2004). In the experimental design for behavior test and cell survival determination, three-month-old male 3xTgAD and wild type mice were used. They received a single s.c. injection of APα (10 mg/kg) or vehicle 7 days before the onset of the learning trial. The rationale for the 7-day interval was to allow time for the proliferation, migration, and integration of newly generated neurons into the dentate gyrus. Following the learning trial, mice were transferred back to their home cage for 9 days with memory function determined on day 22 of the experiment (Singh et al., 2012).

At 3 months of age, 3xTgAD mice showed worse learning ability compared to wild type mice (Singh et al., 2012). APα restored the learning and memory in 3xTgAD mice to a level similar to wild type mice, but APα did not affect the wild type mice. Vehicle treated 3xTgAD mice showed a memory impairment compared to APα-treated 3xTgAD mice and vehicle-treated wild type mice (Fig. 8) (Singh et al., 2012). APα reversed the learning, memory and neurogenic deficits in 6-month and 9 months-old 3xTgAD mice but not in 12 months old 3xTgAD mice (Singh et al., 2012). In APα treated 3-months old 3xTgAD mice the number of surviving neural progenitor cells correlated significantly with APα-induced memory performance in the eye-blink-test but there was no correlation in the vehicle-treated mice between surviving progenitor cells and memory performance (Wang et al., 2010).

The effect of repeated administration of APα shows that regeneration is achieved with either one administration/month or one administration/week of APα but not with administrations three times/week. Instead, there is a deterioration with the three times/week regime (Chen et al., 2011) (Fig. 9). The optimal APα treatment regime for both regeneration and reduction of pathology was obtained with one administration per week (Chen et al., 2011). A second factor for an efficient APα treatment is that the administration has to start early in the disease development at the stage similar to minimal cognitive impairment or mild AD. APα treatment starting after Aβ plaque occurrence is less efficient. Thus intermittent APα treatment would delay AD disease progress best when brains still have neurogenic capacity.

The treatment regimen of once/week during 6 months has been tried in preclinical trials in 3 × TgAD mice (Chen et al., 2011). Using a treatment regimen of one administration/week, APα increased survival of newly generated neurons, reduced Aβ production in hippocampus, cortex, and amygdala, and reduced microglial activation in opposite to frequent treatments (3times/week) (Fig. 9). This may also happen at continuous infusion of APα with the result we see in Fig. 5 (Bengtsson et al., 2012, 2013). The intermittent treatment regimens seem beneficial while continuous treatment for extended duration has met with adverse outcomes.

9. Importance of interrupting continuous endogenous APα exposure and having intervals between exogenous APα administrations in AD treatments

Take-home message: Chronic continuous exposure to APα endogenously produced or exogenously given is enhancing the disease
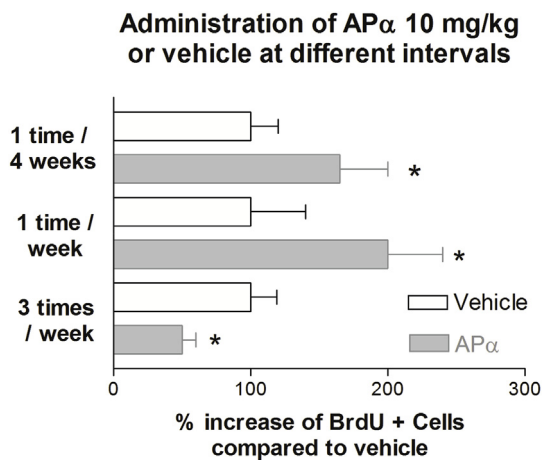
Fig. 9. Optimization of APα treatment regimens for regeneration and repair. APα administered a once per week treatment regimen with a single dose of APα (10 mg/kg) for 6 months was optimal to promote neurogenesis. By contrast, frequent administration 3 times/week deteriorated regeneration of progenitor cell survival. Abbreviation: APα, = Allopregnanolone. Adapted from Chen et al., 2011), PLoS ONE 6(8): e24293.https://doi.org/10.1371/journal.pone.0024293 (Chen et al., 2011).

progress. On the other hand intermittent administration in a high enough dosage activates the progenitor cells and improves cognition.

If a treatment to increase the proliferation and survival of progenitor cells will be successful the treatment should be in harmony with renewal processes in vivo but it should not by itself give negative effects on the AD development. Dose and treatment regimens should therefore be optimized to maximize behavioral improvement, regenerative response, and disease-modification (Chen et al., 2011) and as a neurodegenerative disease is lifelong, the treatment should be lifelong as well.

However, as shown in several studies chronic continuous exposure to APα endogenously produced or exogenously given is enhancing the disease progress, as can be read in sections above. Rather in situations of continuous APα exposure in the brain regardless of origin (endogenous or exogenous), the logical treatment would be to antagonize the APα effect on the GABA-A receptor and by that perhaps delay the AD development as outlined in Fig. 8. To antagonize the APα effect may be possible with GABA-A modulating steroid antagonists (GAMSA), that is compounds shown to be able to block the APα effect on the GABA-A receptor and hinder the APα deterioration effect on learning and memory (Johansson et al., 2015, 2016, 2018; Turkmen et al., 2004).

On the other hand, an optimal AD treatment from the perspective of the GAMS effect in the AD mice would be to be able to both stop the deteriorating effects of continuous APα exposure but also to be able to recruit new progenitor cells that would develop into new neurons replacing the dead. As discussed above to give APα as an intermittent treatment regimen is very important for a possible regeneration.

As with all therapeutics, the dose and administration interval of APα matters. Intermittent administration (once/week) and avoid the 'first pass' effect through the liver (that is, non-oral routes) and a high enough dosage to activate the progenitor cells exert long-term efficacy in the brain. The dosage of APα used for activating progenitor cells in the AD mice is about 8-times higher than the daily dosage used in the continuous APα treatment as described above (Bengtsson et al., 2012). The continuous infusion is also clearly different pharmacokinetically and pharmacodynamically than the single s.c. administration once/week.

The findings point on a biphasic effect of APα on AD disease state. Under certain conditions, APα further exacerbated the AD progression and negatively impacted behavior measured by the MWM swim test. However, when the levels of APα are higher and given in the single

pulse dose, the neurobiological system was able to induce neurogenesis and improvements of learning and memory in the eye-blink test. Comparison of intermittent bolus dosing and continuous release treatment regimens further demonstrate the importance of precise neurosteroid treatment paradigms.

Concerning administration of high dosages of APα. In humans, APα blood levels are highest in late pregnancy up to 157 nmol/l (50 ng/ml) or higher (Luisi et al., 2000). High APα in early pregnancy is linked to sleepiness but not to other severe adverse effects for either mother or fetus (Luisi et al., 2000). Brain APα concentrations in the post-mortem non-pregnant human brain range from 14 to 21 ng/g (Bixo et al., 1997). An IV dosage of 0.09 mg/kg to humans gives a maximal serum concentration of 80 nmol/l, with a distribution half-life of mean 44 min and an elimination half-life of 261 min. That is a bolus dosage is rapidly eliminated from the bloodstream (Timby et al., 2006).

In conclusion, the best option concerning APα exposure in AD would be to block the continued APα exposure and also, make use of the effect by a single dosage of APα to recruit new progenitor cells that develop into neurons replacing the already dead neurons.

9.1. Hypothesis for the treatment regime

In this paper, we have presented two seemingly contradictory scenarios. However, the mechanisms presented are probably working in parallel and with the knowledge presented above a hypothesis for a treatment regime in AD evolves. The optimal treatment based on results in rodents would be to give APα once per week and 12–24 h later start a GABA-A modulating steroid antagonist (GAMSA) treatment for five days. Again 24 h after the last GAMSA administration, depending on the pharmacokinetics of the GAMSA used, give a new APα administration followed by five days with GAMSA and so on in treatment cycles. The optimization of dosages and timing of the regime remains to be investigated. Today human studies using these two drug types APα (GAMS) and GAMSA are on the way or already published (ClinicalTrials.gov Identifier: NCT02221622) and (Johansson et al., 2018; Bixo et al., 2017). In Brinton's studies 10 mg/kg of APα in single s.c. injections were used in mice (Wang et al., 2010) and in the antagonist studies the dosage of the GAMSA UC1011 needed to block the negative APα effect on learning and memory was 10 times higher than the APα dosage (Turkmen et al., 2004) suggesting the serum concentration of UC1011 should be around 10 times higher than the endogenous chronic concentration of APα. There are however several factors that may influence the choice of a dosage and dosage interval that can vary e.g. depending on the administration route resorption, metabolism and target potency of the compound and of course species differences between rodents and man.

10. Conclusions and clinical relevance

Cognitive dysfunction, dementia and Alzheimer's disease (AD) are increasing as the population ages worldwide. A critically unmet need is to develop therapeutics that prevent, delay and treat these conditions. Evidence presented in this review point to an enhanced development of AD in transgenic mice due to chronic continuous exposure of slightly elevated APα levels that correspond to levels at mild chronic stress. Secondly, the effect of APα in higher dosages given in intervals of at least one week can improve stem cell proliferation, survival, and maturation to neurons. Intermittent APα injections improves memory and learning in TgAD mice (Singh et al., 2012). Thirdly, GAMSA treatment improves memory and learning in hepatic encephalopathy models (Johansson et al., 2015).

Together, we conclude in this review that the effect of APα depends on the pattern of administration and doses given. However, negative effects of APα can be blocked with APα antagonists, GAMSA and single APα administrations with longer intervals (more than a week in TgAD-mice) improve memory and learning performance and decrease disease

development.

Declaration of competing interest

Acknowledgments

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