Impact of amyloid-β peptide overproduction on glucocorticoid-related regulation of glutamatergic receptor function in the hippocampus

By

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Table of Contents

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List of Figures

Abstract

Alzheimer's disease (AD) is a complex disease with unclear etiology. On one hand, pathological hallmarks such as amyloid plaques have been identified. On the other hand, risk factors such as aging, type 2 diabetes, stress and hypercholesterolemia have been associated with AD. The interaction between amyloid pathology and stress may be partly responsible for the etiology of AD, since abnormal activities of hypothalamic-pituitary-adrenal (HPA) axis have been observed in AD patients. In addition, cognitive function, which is impaired in AD, is highly sensitive to stress and stress hormone corticosterone (CORT). The interaction between Aβ peptide and stress could have an impact on the cognitive impairments seen in AD, such as episodic memory deficits that are related to impaired hippocampal function and synaptic plasticity. Hippocampal-dependent synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) depends on glutamatergic receptors activity, such as the N-methyl-D-aspartate receptor (NMDAR) and the α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). These glutamate receptor species are highly sensitive to stress and stress hormone. In this proposal, we examined a hypothesis that under a background of overproduction of Aβ peptide stress-induced modulation of NMDAR is affected. Such alteration of stress-induced regulation of NMDAR in the hippocampus could be related to the impact of stress on the pathogenesis of cognitive impairment in AD.

To test the hypothesis that overproduction of Aβ peptide affects stress-induced regulation of NMDAR in hippocampal synapses, we compared the influences of stress and stress hormone CORT to synaptic NMDAR function in wild type (WT) and McGill-

Thy1-APP mice, an animal model that overexpressed mutated genes of amyloid precursor proteins. At 3 month old, McGill-Thy1-APP mice displayed deficits in hippocampus-dependent memory formation. Using electrophysiological approaches, we found that synaptic NMDAR function in McGill-Thy1-APP mice is significantly lower than WT mice. While CORT decreased synaptic NMDAR function in WT mice, it failed to reduce synaptic NMDAR function in McGill-Thy1-APP mice. We also found that acute restraint stress (30 minutes) reduced synaptic NMDAR function in WT mice but not in McGill-Thy1-APP mice. Finally, we found no changes in the protein expression of corticosteroid receptors between WT and McGill-Thy1-APP mice, suggesting that signaling pathways downstream of these receptors could be responsible for the differences in stress-induced modulation of synaptic NMDAR function between these mice.

We revealed that overproduction of Aβ peptide in McGill-Thy1-APP mice may lead to a reduction in synaptic NMDAR function and the abolishment of stress-induced regulation of synaptic NMDAR function. While the reduction in synaptic NMDAR function in McGill-Thy1-APP mice may underlie the deficits of synaptic plasticity and cognitive deficits of these mice, our findings suggest for the first time that stress-induced modulation of synaptic NMDAR could be impaired during the pathogenesis of AD. Although stress has been suggested to exacerbate the pathogenesis of AD, little is known of the underlying mechanisms. Our findings strongly suggest that synaptic NMDAR function could be a cellular substrate that is influenced by the interaction between Aβ peptide overproduction and stress-induced insults.

Résumé

L'Alzheimer est une maladie très complexe dont l'étiologie est inconnue. D'une part, des caractéristiques pathologiques comme les plaques amyloïdes ont été identifiées, alors que d'une autre part des facteurs de risque comme l'âge, le diabète de type 2, le stress et l'hypercholestérolémie ont étés associés l'Alzheimer. L'interaction entre la pathologie amyloïde et le stress pourrait être en partie responsable de l'étiologie de l'Alzheimer, étant donné qu'il est possible d'observer dans les patients souffrant de l'Alzheimer une activitée anormale du hypothalamic-pituitary-adrenal (HPA) axis. De plus, la fonction cognitive qui est atteinte dans l'Alzheimer, est très sensible au stress et a l'hormone de stress, le corticosterone (CORT). L'interaction entre le peptide Aβ et le stress pourrait avoir un impact sur les déficits observés dans l'Alzheimer, comme les déficits de la mémoire épisodique qui sont liés aux déficits de la fonction de l'hippocampe et de la plasticité synaptique. La plasticité synaptique dépendante de l'hippocampe comme la potentialisation a long-terme (PLT) et la dépression a long-terme (DLT) dépendent de l'activité des récepteurs glutamatergiques comme le récepteur acide N-méthyl-D-aspartique (NMDAR) et le récepteur acide α-Amino-3-hydroxy-5-méthyl-4-isoxazolepropionate (AMPAR). Les récepteurs glutamatergiques de cette espèce sont hautement sensibles au stress et à l'hormone de stress. Dans cette thèse, nous avons examiné l'hypothèse que sous un fond surproduction du peptide Aβ, la modulation des NMDAR par le stress est affectée. Des telles modulations induises par la régulation des NMDAR par le stress dans l'hippocampe pourraient être liées a l'impact du stress sur la pathogénèse des déficits cognitifs observés dans l'Alzheimer.

Pour tester l'hypothèse, que la surproduction du peptide Aβ affecte la régulation des NMDARs par le stress dans les synapses hippocampales, nous avons comparé l'influence du stress et l'hormone du stress CORT, sur la fonction des NMDARs synaptiques des souris de type naturel (TN) et des souris McGill-Thy1-APP, un modèle animal qui surexprime des gènes mutés de la protéine précurseure amyloïde.

À l'âge de trois mois, les souris McGill-Thy1-APP démontrent déjà des déficits de la mémoire liés à la formation de la mémoire dépendante de l'hippocampe. En utilisant des approches d'électrophysiologie, nous avons trouvé que la fonction des NMDARs synaptiques des souris McGill-Thy1-APP est significativement plus basse que celle des souris TN. Alors que CORT a réduit la fonction des NMDARs synaptiques des souris TN, CORT n'a pas réduit la fonction des NMDARs synaptiques des souris McGill-Thy1-APP. Nous avons aussi trouvé que le modèle de mobilisation aigue (30 minutes), a réduit la fonction des NMDARs synaptiques des souris TN mais pas celle des souris McGill-Thy1-APP. Finalement, nous n'avons pas trouvé des changements dans l'expression des protéines des récepteurs corticostéroïdes entre les souris TN et McGill-Thy1-APP, ce qui suggère que des voies de signalisation en aval de ces récepteurs soient responsables des différences observées dans la modulation la fonction des NMDARs synaptiques par le stress entre ces deux types de souris.

Nous avons démontré que la surproduction du peptide Aβ du modèle de souris McGill-Thy1-APP, pourrait amener à la réduction de la fonction des NMDARs synaptiques et a l'abolition de la régulation de fonction des NMDARs synaptiques par le stress. Alors que la réduction de la fonction des NMDARs synaptiques des souris McGill-Thy1-APP pourrait être en partie la cause sous-jacente des déficits cognitifs observés

chez ces souris, nos résultats suggèrent, pour la première fois, que la modulation des NMDARs synaptiques par le stress pourrait être compromise durant la pathogénèse de la maladie d'Alzheimer. Bien que le stress ait été suggéré comme un facteur aggravant la pathogénèse de l'Alzheimer, peu est encore connu des mécanismes sous-jacents. Nos résultats suggèrent fortement que la fonction des NMDARs synaptiques pourrait être un substrat cellulaire qui est influencé par l'interaction entre la surproduction du peptide Aβ et des injures induises pas le stress.

I. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of senile dementia. In Canada alone it is estimated that 500,000 Canadians have AD and this number is expected to double in the next 20 years (Alzheimer Society of Canada, 2012). While understanding the etiology of AD is warranted for developing new therapy, it is increasingly clear that AD is a complex disease. On one hand, biological factors that underlie signature anatomical hallmarks of AD, including amyloid plaques and neurofibrillary tangles (NFTs), have been identified. On the other hand, vulnerability to AD has been related to risk factors such as aging, type 2 diabetes, stress and hypercholesterolemia. While most studies focused on understanding the contribution of each of these factors to the etiology of AD, it is increasingly clear that none of these factors alone is solely responsible for AD etiology.

The complexity of AD etiology can be revealed by studies of amyloid plaque, a pathological hallmark that can be found in all AD patients (Braak & Braak, 1991). Amyloid plaques are composed of amyloid beta (Aβ) peptide, a cleavage product of the amyloid precursor protein (APP) (Selkoe, 2001). Imaging studies have also shown that amyloid load tends to increase as disease progresses for participants with mutations in either *APP*, presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2*) genes (Bateman et al., 2012), all of which could lead to overproduction of Aβ peptide (Cai et al., 1993; Citron et al., 1992, 1994, 1997; Scheuner et al., 1996). In animal studies, rodents that overexpressed these mutated genes exhibited not only amyloid plaques, but also deficits in hippocampal-dependent memory tasks, one of the early symptoms seen in humans (Plancher et al., 2012). These findings strongly suggest that reducing excess Aβ peptide

accumulation could be an effective therapy to ameliorate AD symptoms. However, Aβ peptide targeting therapies failed to meet expectations. For example, immunization of AD patients with synthetic Aβ of AD patients showed no apparent improvements in cognitive function, although both soluble and insoluble forms of Aβ peptide were cleared from the brain (Delrieu et al., 2012; Holmes et al., 2008). In addition, brains of cognitively intact elderly people can have a heavy amyloid plaque load (Nelson et al., 2009).

Alternatively, amyloid pathology may interact with other factors for the etiology of AD symptoms. One of these factors might be stress. Stress, either psychological or physiological threats, activates the hypothalamic-pituitary-adrenal (HPA) axis to release various hormonal signals including glucocorticoids. Hyperactivity of the HPA axis has been found in AD patients (Swaab et al., 1994). AD is commonly comorbid with major depressive disorder (MDD) (Aznar & Knudsen, 2011), a stress-related brain disorder (Swaab et al., 2005). Interaction between stress and amyloid pathology is supported by findings from animal studies, which showed that both stress and the stress hormone glucocorticoid, worsen Aβ deposition as well as memory deficits (Carroll et al., 2011; Cuadrado-Tejedor et al., 2011; Dong et al., 2009; Dong et al., 2011; Dong et al., 2008; Jeong et al., 2006; Kang et al., 2007; Kunimoto et al., 2010; Seo et al., 2011).

Interactions between Aβ peptide deposition and stress could have a significant impact on the genesis of cognitive impairments in AD. One of the early symptoms of AD is memory deficits, with episodic memory appearing to be one of the first affected functions (Convit et al., 2000; Fabrigoule et al., 1998). Episodic memory is part of explicit memory and is the system that deals with the “what”, “where” and “when”

related to personal experiences (Tulving, 2002). Episodic memory is dependent on the hippocampus (Squire et al., 2004) and in turn this structure is one of the first affected in AD (Braak & Braak, 1991). Hippocampal volume is significantly reduced in AD patients (Pievani et al., 2011). Changes in hippocampal structure and function are likely related to Aβ peptide deposition. Indeed, animal models with abnormal Aβ production peptide as well as models using exogenous application of Aβ peptide not only impaired hippocampal-dependent memory, but also altered hippocampal-dependent synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD) (Balducci et al., 2010; Chapman et al., 1999; Hsia et al., 1999; Larson et al., 1999; Li et al., 2009; Moechars et al., 1999; Shankar et al., 2008; Wang et al., 2002), cellular mechanisms that have been highly implicated in hippocampal-dependent memory formation. Likewise, stress and stress hormone glucocorticoids can reduce hippocampal volume (Lupien et al., 1998; Sapolsky et al., 1985). Interestingly, patients suffering from MDD also appear to have smaller hippocampal volume (Campbell et al., 2004). More importantly, stress and glucocorticoids affect hippocampal-dependent plasticity (Diamond et al., 1992; Diamond et al., 1996; Joëls et al., 1989; Krugers et al., 2005; Pavlides et al., 1996; Wiegert et al., 2005). Therefore, the interaction between Aβ peptide overproduction and stress could exacerbate deficits of hippocampal function in AD.

In this thesis, I will test a hypothesis that Aβ peptide overproduction alters glucocorticoid-related regulation of hippocampal function. Hippocampal synaptic plasticity is largely mediated by activation of the N-methyl-D-aspartate receptor (NMDAR). Recent findings from our laboratory strongly suggest that the function of this glutamate receptor is highly sensitive to stress and glucocorticoids. We will examine

whether stress-induced regulation of NMDAR is altered under a background of Aβ peptide overproduction, such as in the McGill-Thy1-APP (APP) mouse model that overexpresses mutated human APP genes. Findings from this study will not only reveal if there is an interaction between Aβ peptide deposition and stress in regulating NMDAR function, but also will shed light on how such an interaction contributes to the pathogenesis of cognitive impairments in AD.

II. Background

II.1. Alzheimer's disease.

II.1.1. Stages of AD

AD patients are usually categorized as being in mild/early-stage, moderate/mid-stage, and severe/late-stage (Mckhann et al., 2011). These stages are characterized by progressive cognitive impairments, such as memory loss and disorientation, changes in personality and behaviour, as well as loss of autonomy. AD can also be classified according to Braak stages, which are determined post-mortem. The Braak stages range from I-VI and are characterized according to the progression of neuropathological hallmarks, such as amyloid deposits and neurofibrillary tangles (Braak, & Braak, 1991).

II.1.2. Pathological hallmarks of AD

One of these hallmarks is neurofibrillary tangles (NFTs). NFTs are an accumulation of hyperphosphorylated tau (Bancher et al., 1989). Tau is a microtubule-associated protein that plays an important role in the in the cytoskeleton of neurons by stabilizing and assembling microtubules (Avila et al., 2004; Iqbal et al., 2005). Thus, the abnormal hyperphosphorylation seen in AD leads to less stable and collapsing

microtubules, which disturbs normal cell processes (Reddy, 2011). Since hyperphosphorylation decreases the binding of tau with microtubules, tau ends up clustering together inside neurons' axons (Garcia & Cleveland, 2001).

The other major hallmark of AD is amyloid plaques. Amyloid plaques are aggregates of the Aβ peptide (Glenner, & Wong, 1984). Aβ peptide is a cleaved product of APP, which is a transmembrane protein that can serve normal functions in cells, such as maintenance of calcium (Ca^{2+}) homeostasis (Gralle et al., 2007; Kamenetz et al., 2003; Octave et al., 2013; Yanker et al., 1990). There are several APP isoforms with the most common form found in the brain being the 695 amino acids long (König et al., 1992). Through normal processes different enzymes cleave APP consecutively into different sized peptides. The first cleavage is done by α-secretase, producing sAPPα, which is released from the cell surface while an 83 amino acids long C-terminal fragment remains inserted into the membrane (Hardy, 1997). It has been suggested that cleavage of APP by α-secretase is activity-dependent (Nitsch et al., 1993). The cleavage of APP by α-secretase prevents APP from being cleaved into the pathological 42 amino acids-long form of the Aβ peptide. Due to genetic mutations of the *APP*, *PSEN1* and *PSEN2* genes, which will be explained in detail in the following section, the cleavage of APP by α-secretase is by-passed. Instead, APP is first cleaved by β-secretase resulting in a sAPPβ terminal and a transmembrane C-terminal fragment that is 99 amino acids long (C-99) (Hussain et al., 2000). The C-99 fragment is then cleaved by γ-secretase producing a 40 or 42 amino acid-long Aβ peptide and an APP intracellular domain (AICD) (Mattson, 2004). It has been shown that the form of the Aβ peptide that is particularly toxic to neurons is the soluble $A\beta_{42}$ oligomer (Lambert et al., 1998). Aβ peptide is a sticky

molecule that can exist as either monomers or oligomers. Chromy and colleagues (2003) have demonstrated that the most common forms of oligomers are tetramers and pentamers, although other forms also exist. Aβ peptide is considered a major hallmark not only because all AD patients possess amyloid plaques, but also because mutations of genes that could lead to Aβ peptide overproduction (e.g. *APP, PSEN1* or *PSEN2* genes), have been associated with familiar AD (Hardy, 1997). In order to gain a better understanding of this disease, various mutations that can be found in these genes have been transfected into rodents to create models that can express some aspects of the disease, for example the overproduction of the Aβ peptide.

II.1.3. Genetic mutations in transgenic rodent models of AD

The known mutations in the *APP* gene are the Swedish (APP670/671; Mullan et al., 1992), Flemish (A692G; Cras et al., 1998; Levy et al., 1990), Dutch (E693Q; Herzig al., 2004), Florida (I716V; Eckman et al., 1997), and London mutations (APPV717I; Goate et al., 1991; Chartier-Harlin et al., 1991; Suzuki et al., 1994). All these mutations increase the production of the more toxic $A\beta_{42}$ peptide by altering β-secretase and γ-secretase activity (Citron et al., 1992; Hardy, 1997).

The presenilins are homologous transmembrane proteins that are thought to contain eight domains (Doan et al., 1996). More than 150 mutations have already been identified in the *PSEN1* gene while only a little more than ten have been identified in the *PSEN2* gene (Clark et al., 1995; Ertekin-Taner, 2007; Levy-Lahad et al., 1995; Mercken et al., 1996; Sherrington et al., 1995). Mutations in both the *PSEN1* and *PSEN2* genes appear to generate the more toxic form of the Aβ peptide, which is 42 amino acids long (Borchelt et al., 1996; Duff et al., 1996; Lemere et al., 1996; Scheuner et al., 1996).

II.1.4. Transgenic rodent models of AD

One of the most commonly used mouse models of AD is the Tg2576 mouse model developed by Hsiao and colleagues in 1996. It carries the Swedish double mutation. Tg2576 mice were reported to have a 14–fold increase of $A\beta_{42}$ peptide levels compared to wild type mice, with amyloid deposition starting at 11 months of age. Dystrophic neurites were also observed. Another model is the PDAPP mouse model developed by Games and colleagues in 1995. This model possesses the Indiana mutation and transgenic mice appear to develop amyloid plaques as of six months of age as well as dystrophic neurites, astrogliosis and microgliosis. The TgAPP23 mouse model also carries the Swedish double mutation. It was developed in 1997 by Sturchler-Pierrat and colleagues and similar to the PDAPP model deposition of amyloid plaques can be seen as of six months of age. Dystrophic neurites, neuronal loss, astrogliosis, and microgliosis can also be observed. The TgCRND8 model combines both the Swedish double mutation and the Indiana mutation and was developed more recently by Chisti and colleagues (2001). This model shows amyloid plaques as early as three months of age, as well as dystrophic neurites, astrogliosis, and microgliosis. The hAPP-J20 model also combines both the Swedish double mutation and the Indiana mutation but Aβ plaque deposition happens slightly later at around eight months of age (Mucke et al., 2000). Models with double mutations also exist. For example, the TgPSAPP, TgAPP/PS1-A246E, TgAPP/PS1dE9 and the TgTASTPM models contain mutations in the *PSEN1* gene as well as the *APP* gene. Similar to previous models, Aβ plaque deposition can be seen as early as three months of age, as well as dystrophic neurites, astrogliosis, microgliosis and

neuronal cell loss (Borchelt et al., 1997; Holcomb et al., 1998; Howlett et al., 2004; Lee et al., 1997). Oddo and colleagues developed the triple transgenic TgAPP/PS1/Tau line in 2003 (Oddo et al., 2003). This line combines *APP* Swedish double mutation, *PSEN1* and tau mutations in order for the phenotype expressed by these animals to be more closely related to the phenotype seen in humans with AD. This model presents accumulation of intracellular Aβ at around three months of age, followed by depositions of extracellular Aβ at around 12 months of age. NFTs can also be observed as of 12 months of age.

Finally, the McGill-Thy1-APP mouse model was developed at McGill in Dr. Cuello's laboratory (Ferretti et al., 2011). Previously in the same lab a similar model but employing rats was developed. In the rat model, animals were shown to possess intracellular Aβ depositions starting at one week of age, which are well established by three months of age (Leon et al., 2010). Plaque deposition was found to start at around six months of age while by 20 months of age amyloid plaques and diffusible Aβ can be found in almost all areas of the brain. The McGill-Thy1-APP mouse model is similar to the rat model. Authors used the murine thymocyte promoter (Thy-1.2), so the mutations would be primarily expressed in the telencephalon, more specifically in areas with pyramidal neurons. This model expresses the Swedish double mutation and the Indiana mutation, which increases the production of $A\beta_{42}$ peptide. It was found that accumulation of intracellular $A\beta_{42}$ in transgenic mice could already be seen as of 1 week of age. Extracellular amyloid plaques deposition was observed as of 4 months of age and deposition of mature plaques was well established by 6 months of age. Similar to human pathology, plaque deposition started in the hippocampus and entorhinal cortex and slowly extended to other cortical areas. Authors also measured the amount of soluble $A\beta_{42}$ in the

hippocampus of young, pre-plaque mice. This analysis revealed that there is a larger amount of $A\beta_{42}$ produced compared to $A\beta_{40}$. Another landmark associated with AD pathology in humans is the down-regulation of cholinergic markers (Francis et al., 1999). The density of pre-synaptic cholinergic boutons was measured in pre-plaques McGill-Thy1-APP mice. Results show an up-regulation of pre-synaptic cholinergic boutons in pre-plaque animals, while old transgenic animals show a decrease in cholinergic density compared to wildtype littermates.

II.2. Stress.

II.2.1. Mechanisms of stress

The physiological response to stress is mediated by the HPA axis as well as the release of catecholamines from the adrenal cortex. In response to a stressful stimulus the adrenal cortex releases catecholamines while at the same time, the hypothalamus releases corticotropin-releasing hormone (CRH). CRH in turn stimulates the release of adrenocorticotropic hormone (corticotropin; ACTH) from the anterior pituitary. ACTH travels in the bloodstream and finally triggers the release of glucocorticoids from the adrenal cortex (Herman et al., 2003; Sapolsky et al., 2000). In humans the main glucocorticoid is cortisol while in rodents it is corticosterone (CORT). CORT binds to two types of receptors that can be found in the cytosol and function as transcription factors, glucocorticoid receptors (GRs; type II) for which CORT has low affinity, and mineralocorticoid receptors (MRs; type I) for which CORT has 10 times higher affinity (Reul & de Kloet, 1985; Joëls, 2001). It has recently been suggested that fast-acting non-genomic actions of CORT can be mediated by membrane-bound GRs and MRs (Prager &

Johnson, 2009). Thus, at basal levels CORT is believed to mainly bind to MRs while at stress levels CORT binds to both MRs and GRs (de Kloet et al., 1998).

II.2.2. AD and stress

Stress and glucocorticoids could play a significant role in the onset as well as the progression of AD. For example, AD patients have been found to suffer from mild hypercortisolemia (de Bruin et al., 2002; Erkut et al., 2004; Hoogendijk et al., 2006; Murialdo et al., 2000; Rasmuson et al., 2002; Swaab et al., 1994; Umegaki et al., 2000). Salivary and plasma cortisol levels have also been found to correlate with the progression of the disease, that is, as the disease progresses cortisol levels increase (Bemelmans et al., 2007; Giubilei et al., 2001; Weiner et al., 1997). Although expression of glucocorticoid receptors in the hippocampus of AD patients has not been found to be different from that of controls (Seckl et al., 1993; Wetzel et al., 1995). It has also been shown in the literature that there is a positive correlation between experiencing psychological distress and the likelihood of developing AD (Johansson et al., 2010; Wilson et al., 2005, 2006). A positive correlation has also been shown between experiences of psychological distress and the development of mild cognitive impairment (MCI) (Wilson et al., 2007b), a condition that will often progress to AD (Chertkow et al., 2001). Not only does distress increase the chance of developing AD but susceptibility to pre-morbid distress also appears to correlate with the magnitude of episodic memory impairments at the onset of disease (Wilson et al., 2004). On the other hand, pre-morbid distress does not seem to be correlated with pathological hallmarks of progression such as NFTs and amyloid plaques (Wilson et al., 2003, 2006, 2007a). This leads to the hypothesis that stress is more likely to be correlated to the onset of AD rather than its progression (Pardon & Rattray, 2008).

However, since it is difficult to study the effects of stress on disease progression in humans as tissue is usually only obtained post-mortem, or at a single time point during the disease as in a biopsy, it is difficult to conclude that stress does not affect disease progression in humans. Interestingly, in animal models of AD, stress and glucocorticoids have been found to exacerbate disease pathology, such as Aβ production, as well as memory impairments (Carroll et al., 2011; Cuadrado-Tejedor et al., 2011; Dong et al., 2009; Dong et al., 2011; Dong et al., 2008; Goodman et al., 1996; Jeong et al., 2006; Kang et al., 2007; Kunimoto et al., 2010; Sayer et al., 2008; Seo et al., 2011).

Another clue that stress may play a role in AD pathology comes from the high comorbidity rates between AD and major depressive disorder (MDD), a stress related disorder. Studies estimate that between 15-17% of AD patients also suffer from major depressive disorder (MDD) while about 20-63% of MCI patients suffer from MDD (Chen et al., 1999; Panza et al., 2010; Wragg & Jeste, 1989). MDD is in turn believed to be a risk factor in the development of AD (Brommelhoff et al., 2009; Jorm, 2001; Ownby et al., 2006). It has been previously suggested that stress may be a common precipitating factor in both of these diseases (Aznar & Knudssen, 2011). Thus, stress and glucocorticoids may have a great impact on both the disease pathology, such as Aβ peptide production, as well as on cognitive deficits, such as memory impairments.

II.3. Hippocampus.

II.3.1. Overview of the hippocampus

The hippocampus is a structure in which two types of synaptic plasticity involved in learning and memory can be found, namely LTP and LTD. LTP is the strengthening of

synaptic transmission between two cells that can last from hours to days (Bliss & Lømo, 1973). Conversely, LTD is a decrease in synaptic strength (Collingridge, et al., 2010). LTP formation depends on activation of glutamatergic receptors, including the NMDAR and the AMPAR. Induction of LTP requires activation of NMDARs. Opening of NMDAR channels allows influx of Ca^{2+} into postsynaptic neurons, which in turn triggers molecular mechanisms to increase functional properties of AMPAR-mediated synaptic transmission. Mechanisms for increasing AMPAR function include an increase in the number of AMPARs in the post-synaptic density (PSD) or the channel conductance of AMPARs. These mechanisms are mediated by activation of kinases such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC) and protein kinase A (PKA) (Adrásfalvy & Magee, 2004; Borgdorff & Choquet, 2002; Derkach et al., 1999; Fukunaga et al., 1993; Makino & Malinow, 2009; Malenka, 2003; Malinow et al., 2000; Park et al., 2004; Sanderson & Dell'Acqua, 2011; Shepherd & Huganir, 2007). On the other hand, LTD induction requires the activation of NMDARs following low frequency stimulation causing an influx of Ca^{2+} into the cell, which is below LTP threshold levels. Ca^{2+} then binds to protein phosphatases, such as calcineurin that once activated will dephosphorylate AMPARs and lead to endocytosis. Thus, the endocytosis of AMPARs as well as the decrease in channel conductance of AMPARs leads to the depression of the post-synaptic cell (Bear & Malenka, 1994; Christie et al., 1994; Dudek & Bear, 1992; Mulkey et al., 1994; Mulkey & Malenka, 1992; Mulkey et al., 1993).

Moser and Moser suggested more than a decade ago that hippocampal function may play different roles in memory and learning depending on which region of the

hippocampus was being activated (Moser & Moser, 1998). Recent evidence from studies looking at differential genetic expression of markers specific to dorsal and ventral cornu ammonis (CA) CA1, CA2, and CA3 neurons showing region specific segregation of these markers lends weight to this hypothesis (Dong et al., 2009; Thompson et al., 2008). Support also comes from connectivity studies showing differential projections from and to specific hippocampal regions (Slomianka et al., 2011). Accordingly, the dorsal or septal hippocampus is believed to play a larger role in spatial-related learning and memory such as, exploration, navigation and locomotion, while the ventral or temporal hippocampus is believed to play a larger role in emotional and motivated-related memory and learning (Fanselow & Dong, 2010).

II.3.2. Glutamatergic receptors

There are two types of glutamatergic receptors, ionotropic and metabotropic. Ionotropic glutamate receptors act as ion channels while metabotropic glutamate receptors act as G-protein coupled receptors. There are three types of ionotropic glutamate receptors, AMPARs, NMDARs, and kainate receptors (Dingledine et al., 1999). Since AMPARs and NMDARs play an important role in hippocampal synaptic plasticity we shall discuss them in greater detail.

II.3.2.1. AMPAR

AMPARs are ligand-gated ion channels that are permeable to Na^+, K^+ and Ca^{2+} depending on which subunits compose the channel (Bettler & Mulle, 1995). The AMPAR has four subunits: GluA1-GluA4 (Seeburg, 1993). AMPARs can either be homomeric or heteromeric compositions of these subunits (Nakanishi, 1992). Channels containing the GluA2 subunit are impermeable to Ca^{2+} (Hollmann et al., 1991). As mentioned in the

previous section, AMPARs play an important role in synaptic plasticity. An increase in AMPAR-mediated synaptic transmission is required for the induction of LTP while a decrease in channel conductance as well as endocytosis of this receptor can lead to the induction of LTD (Adrásfalvy & Magee, 2004; Bear & Malenka, 1994). The activation of AMPARs by glutamate is extremely fast, and it is this characteristic of AMPARs, which are often colocalized in synapses along NMDARs, that allow for the release of the magnesium (Mg^{2+}) block of NMDARs (Dingledine et al., 1999).

II.3.2.2. NMDAR

NMDARs are ion channels that are Ca^{2+} permeable (Lynch & Guttmann, 2002). NMDARs have three subunit types, GluN1-GluN3, which are then further subdivided into GluN2A-D and GluN3A-B (Chatterton et al., 2002; Ciabarra et al., 1995; Hollmann et al., 1993; Ishii et al., 1993; Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992; Sucher et al., 1995; Sugihara et al., 1992). Functional NMDARs are heterotetramers usually composed of GluN1 and GluN2 subunits (Dingledine et al., 1999; Seeburg, 1993). Activation of NMDARs requires binding by glutamate and the co-agonist glycine (Hassel & Dingledine, 2006). Activation of these receptors also requires membrane depolarization, so that the channel's affinity for Mg^{2+} is decreased and the latter is then released thus allowing for Ca^{2+} to flow through, among other ions (Lynch & Guttmann, 2002). Unlike AMPARs, NMDARs are slow in activating (Wyllie et al., 1998). It is the combined action of the rapid depolarization by AMPARs, which allows for the release of the Mg^{2+} block and lead to the slower activation of NMDARs that renders these two receptors essential for synaptic plasticity to occur in the hippocampus.

II.3.3. AD and the hippocampus

The most common symptom amongst AD patients is memory loss. Interestingly, the hippocampus, a key structure for memory formation, is among the first regions to be affected in AD (Braak & Braak, 1991). Deficits in episodic memory are common in AD. Episodic memory is part of explicit memory and is the system that deals with the "what", "where" and "when" related to personal experiences (Tulving, 2002). Lesions at the CA1 region of the hippocampus appear to produce episodic memory impairments in patients (Bartsch, et al., 2011). In fact, studies have shown that deficits in episodic memory are already apparent as early as six years prior to diagnosis of AD (Convit et al., 2000; Fabrigoule et al., 1998). Memory impairments in tasks that are hippocampal dependent, such as the Morris water maze (MWM), have also been seen in animal models of AD. For example, these impairments have been reported for the Tg2576 mice (Lesnè et al., 2006), the PDAPP mice (Dodart et al., 1999), the APP23Tg mice (Kelly et al., 2003; Van Dam et al., 2003), the TgCRND8 mice (Chisti et al., 2001), the hAPP-J20 mice (Chen & Bear, 2007; Palop et al., 2003) and finally in the McGill-Thy1-APP mice (Ferretti et al., 2011). These models all have mutations in the *APP* gene.

II.3.4. Stress and the hippocampus

Hippocampal function is highly sensitive to stress and stress hormone glucocorticoids. The effect of stress and glucocorticoids on hippocampal function depends on their levels, durations and timing of application during a memory task, and the subtypes of corticosteroid receptors (Joëls, 2001; Reul & de Kloet, 1985). In addition, the hippocampus displays regional specific responses to stress and glucocorticoids (Maggio & Segal, 2007).

The relationship between the levels of stress or glucocorticoids and the performance of cognitive tasks follows an inverted-U curve relationship (Okuda et al., 2004; Sandi & Rose, 1994). A similar inverted-U curve relationship between stress/glucocorticoids and LTP has also been shown (Diamond et al., 1992). As such, adrenalectomized animals show memory deficits (Vaher et al., 1994) and impaired LTP (Diamond et al., 1992), pointing towards an important role for basal CORT levels in normal memory function. On the other hand, stress or exposure to CORT at stress levels, can lead to impaired LTP (Alfarez et al., 2002, 2003; Krugers et al., 2005; Pavlides et al., 1996; Wiegert et al., 2005). Impairments in LTP following stress or exposure to CORT at stress levels are believed to be mediated by both MRs and low affinity GRs (Avital et al., 2006; Korz & Frey, 2003; Pavlides et al., 1995, 1996).

The timing and the duration of the stressor or the application of CORT can also differentially regulate hippocampal function. For example, the application of CORT at stress levels has been shown to facilitate LTP when applied immediately before LTP induction (Wiegert et al., 2006). However, LTP induction was impaired at hours following CORT application (Alfarez et al., 2002, 2003; Krugers et al., 2005; Pavlides et al., 1996; Wiegert et al., 2005). Suppression of LTP can also be seen in chronically stressed animals (Alfarez et al., 2003; Gerges et al., 2001; Pavlides et al., 2002).

As mentioned in a previous section, the hippocampus can be divided into two subregions: the dorsal hippocampus and the ventral hippocampus. In turn, these two regions play different roles in memory. Thus, stress and CORT differentially regulate hippocampal function in these two subregions. While CORT and stress suppress LTP in the dorsal hippocampus, they facilitate LTP in the ventral hippocampus (Maggio &

Segal, 2007, 2009). The mechanisms through which CORT and glucocorticoid receptors may regulate these changes in hippocampal-dependent synaptic plasticity however are not fully understood. One of the mechanisms underlying these changes could be through the regulation of glutamatergic receptors by CORT and glucocorticoid receptors.

II.3.5. AD and NMDARs

As mentioned previously, hippocampal-dependent memory appears to be impaired in AD. Since NMDARs mediate the induction of various forms of hippocampal synaptic plasticity, it is possible that Aβ peptide may affect normal NMDAR function. Indeed, *in vivo* studies of NMDAR function in male rats, in response to exogenous Aβ peptide and NMDA application hippocampal neurons showed an increase in the total number of spikes (Molnar et al., 2004; Szegedi et al., 2005). An increase in NMDAR-mediated excitatory postsynaptic currents (EPSCs) has also been demonstrated in *in vitro* settings in the dentate gyrus of male rats following application of exogenous Aβ peptides both intracellularly and extracellularly (Wu et al., 1995). An interesting finding that could explain the underlying mechanism in the increase in function of NMDARs is the apparent inhibition of the sodium-potassium adenosine triphosphate (ATPase Na^+/K^+) pump by $A\beta_{1\text{-}42}$ peptide. This leads to the depolarization of the membrane thus relieving the NMDAR of its Mg^{2+} blockade and increasing tonic activation of NMDARs (Gu et al., 2004). However, other studies performed on cell cultures have found no changes in NMDAR-mediated currents following treatment with exogenous Aβ peptide (Brorson et al., 1995; Mezler et al., 2011). Moreover, studies performed on transgenic AD mice have moreover seen a decrease in synaptic NMDAR function (Dewachter et al., 2009; Duszczyk et al., 2012; Hsia et al., 1999). This is thought to be mediated by a decrease in

the surface expression of GluN2B containing receptors, which in turn is believed to be mediated by calcineurin and striatal enriched tyrosine phosphatase (STEP) (Snyder et al., 2005). The differences seen in the impact of Aβ peptide on NMDAR synaptic function could be due to different methods used to investigate the question.

Interestingly, some studies have shown that Aβ peptide can co-localize with NMDARs in cell cultures (DeFelice et al., 2007) as well as with post-synaptic proteins such as post-synaptic density protein 95 (PSD-95; Lacor et al., 2007). It is possible then that Aβ peptides may be able to either directly or indirectly affect NMDAR function and regulation.

Just as Aβ peptide can affect NMDAR synaptic function, so can NMDAR activity affect Aβ peptide processing. Indeed, studies have shown that increases in neuronal activity can lead to an increase in interstitial fluid (ISF) peptide levels (Cirrito et al., 2005, 2008). Subsequently, other studies demonstrated that activation of synaptic NMDARs increase APP processing by α-secretase (Hoey et al., 2009), while activation of extrasynaptic NMDARs leads to increases in amyloid production (Bordji et al., 2010).

Thus, there is strong evidence for an interaction between NMDARs and Aβ peptide in AD. However, another factor has been shown to regulate glutamatergic receptor function in the hippocampus, that is, stress and glucocorticoids.

II.3.6. Stress and glutamatergic receptors

Stress has been demonstrated to regulate hippocampal synaptic plasticity through the actions of CORT on AMPARs and NMDARs. Indeed, CORT has been shown to influence trafficking of AMPARs by increasing their numbers in synapses (Groc et al.,

2008; Martin et al., 2009). CORT also increases the frequency of AMPAR-mediated miniature EPSCs (Karst & Joëls, 2005).

CORT can also regulate NMDAR function. Previous studies from our lab indicate that acute treatment with stress levels of CORT can potentiate NMDAR-mediated postsynaptic function in adult rat brain slices (Tse et al., 2011). These effects last for at least two hours following treatment. In addition to an increase in NMDAR-mediated synaptic currents, CORT also induced delay-onset changes in subunit composition of NMDARs by increasing the expression of GluN2A subunits in synapses at 1 hour after CORT treatment. Changes in NMDAR function have a significant impact on synaptic plasticity, so that both LTP and LTD were facilitated within 1 hour after CORT treatment. These facilitating effects were short lasting, probably due to the delayed-onset changes in NMDAR subunit composition.

III. Working Hypothesis

Stress and more specifically glucocorticoids have been implicated in the onset of AD in human patients (Johansson et al., 2010; Wilson et al., 2004, 2005, 2006) as well as in the progression of symptoms in several animal models of AD (Carroll et al., 2011; Cuadrado-Tejedor et al., 2011; Dong & Csernansky, 2009; Dong et al., 2008, 2011; Jeong et al., 2006). Under a background of Aβ peptide overproduction, glucocorticoid regulation of glutamatergic receptors may be altered. Since stress and CORT have been shown to increase synaptic NMDAR function (Tse et al., 2011), we hypothesize that this increase in synaptic NMDAR function by stress and CORT will be exacerbated in the McGill-Thy1-APP mice, which is a rodent model of Aβ peptide overproduction.

IV. Research Project Objectives

In the McGill-Thy1-APP mouse model, we will examine the impact of Aβ peptide overproduction on CORT-induced modulation of NMDARs in the hippocampal CA1 region.

V. Specific Aims

V.1. Aim 1: Determine the effect of Aβ peptide overproduction on CORT-induced regulation of NMDARs and AMPARs in McGill-Thy1-APP mice.

McGill-Thy1-APP mice show cognitive deficits starting at three months of age (Ferretti et al., 2011). We asked if synaptic NMDAR and AMPAR function is altered in this animal model when cognitive impairment appears. In addition, we investigated whether the modulation of NMDARs and AMPARs by CORT is affected in this animal model of amyloidosis. Specific experiments include:

1) Investigate basal NMDAR-mediated synaptic function in control and McGill-Thy1-APP mice in the CA1 region of the hippocampus. Both field recording (population responses) and whole cell patch-clamp recording techniques (single cell) were used.
2) Investigate the impact of stress levels of CORT on basal NMDAR-mediated synaptic function in control and McGill-Thy1-APP mice
3) Using field recording field recording , determine whether the interaction between CORT and Aβ peptide affects the synaptic function of AMPARs, another glutamate receptor subtype that is highly expressed in the hippocampus.

V.2. Aim 2: Determine the effect of Aβ peptide overproduction on stress-induced regulation of NMDARs in McGill-Thy1-APP mice.

We next investigated whether the modulation of NMDARs by stress is also affected in McGill-Thy1-APP mice. Investigate basal NMDAR-mediated synaptic function in stressed control and McGill-Thy1-APP mice in the CA1 region of the hippocampus. Mice were stressed by restraint for 30 minutes before slice preparation. Field recording (population responses) was used.

V.3. Aim 3: To determine if there are changes in HPA axis function due to overproduction of Aβ peptide.

Considering that both GRs and MRs are highly expressed in pyramidal cells of the hippocampal CA1 layer, the potential effects of increased Aβ peptide levels on CORT regulation of NMDARs might be mediated by these receptors. Thus, GR and MR basal levels in the hippocampus will be quantified for both transgenic and control mice through western blot. In addition, the impact of increased Aβ peptide on plasma levels of CORT will be examined with an enzyme-linked immunosorbent assay (ELISA).

Collectively, these experiments will allow for a better understanding of the potential interaction between excess Aβ peptide and CORT on NMDAR function in the McGill-Thy1-APP mouse model at an age when cognitive deficits can already be seen.

VI. Materials and Methods

VI.1. Mice.

McGill-Thy1-APP mice as well as C57Bl/6 control mice (generations F1-F2) are used. The McGill-Thy1-APP mice carry the human APP751 transgene with both the Swedish double mutation (K670N/M671L) and the Indiana mutation (V717F), whose expression is driven by aThy1.2 promoter (Ferretti et al., 2011). Mice are housed in a 12-hour light/dark cycle with lights on at 8:00 am. Food and water are provided *ad libitum.* All animals were anesthetized with isoflurane prior to decapitation, except for animals that underwent restraint stress. Stressed animals were not anesthetized prior to decapitation. This study was approved by the Facility Animal Care Committee of the Douglas Institute, McGill University.

VI.2. Slice preparation.

Slices are prepared as shown in Tse et al., 2011. Briefly, mice were anesthetized by isoflurane and decapitated in order to collect brain tissue. Brains were cut using a *Vibratome* into coronal slices (300-μm thick) in hyperosmotic, ice-cold and carbogenated (5% CO_2, 95% O_2) slice cutting solution (in mM: 252 sucrose, 2.5 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 1.25 KH_2PO_4, 26 $NaHCO_3$ and 10 glucose, ~360 mOsmol/L). Freshly cut slices were first incubated with carbogenated artificial cerebrospinal fluid (aCSF in mM: 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 25 glucose, ~310 mOsmol/L) for 1 hour at 32°C after which they were left at room temperature. CORT was prepared by first dissolving it in ethanol, with the final concentration of ethanol in the CORT-containing aCSF being 0.0007%. $GABA_A$ receptor-mediated inhibitory synaptic transmission was blocked with bicuculline methobromide (5 mM) in all recordings. The Schaffer collateral-commissural pathway was stimulated (constant

current pulses (0.08 ms) through a tungsten bipolar electrode (FHC)) and evoked excitatory postsynaptic responses were recorded in hippocampal CA1 stratum radiatum. Amplification and digitization of the signal was done by a Multiclamp 700B and a Digidata 1400 respectively (Axon). Data were stored in a PC for later analysis using Clampfit (Axon). For electrophysiology experiments under CORT condition, slices were incubated for 30 minutes at room temperature in aCSF solution containing CORT (200mM). All recordings were performed at room temperature.

VI.3. Whole-cell recording.

EPSCs were recorded from individual CA1 pyramidal neurons in whole-cell mode. Intracellular solution in patch pipettes contains (mM) 110 Cs-gluconate, 17.5 CsCl, 2 $MgCl_2$, 0.5 EGTA, 10 HEPES, 4 ATP, and 5 QX-314 (Alomone Labs), with pH adjusted to 7.2 by CsOH (≈290 mOsmol/L). To isolate AMPAR- and NMDAR-mediated EPSCs, pyramidal neurons were voltage-clamped at -60 mV and +40 mV, respectively.

VI.4. Field recording.

Field excitatory postsynaptic potentials (fEPSPs) were recorded extracellularly using an aCSF-filled glass electrode. The relationship between fiber volley (FV) amplitude, which represents depolarization of presynaptic fibers, and fEPSP slope (initial 10-60% slope) was examined. Low Mg^{2+} (0.05 mM) containing aCSF and the AMPAR antagonist DNQX (20 mM) were used during field recordings in order to isolate NMDAR-mediated fEPSPs.

VI.5. GR and MR western blot.

Hippocampal tissue was snap frozen in isopentane that was chilled on dry ice. Tissue was sonicated in lysis buffer (in mM: 50 Tris base, 150 NaCl, 1 EDTA, 1% NP-40, 0.1% SDS, 50 NaF, 100 Na_3VO_4). The homogenate was incubated on ice while shaking at 4°C. Samples were then centrifuged at 13000rpm for 10 minutes at 4°C. Finally, samples were immediately frozen and stored at -80°C. The Bradford (Bio-Rad) method was used to determine the protein concentration. The western blot method was used to quantify protein expression. GR (1:500, abcam, Toronto, Ontario) and MR (1:200, Santa Cruz biotechnology, Dallas, Texas) antibodies were used to estimate the expression of glucocorticoid receptors protein in the hippocampus. Optical density (OD) measurements were normalized with actin (1:1000, Sigma) to control for the amount of protein loaded on the gel.

VI.6. ELISA protocol.

CORT plasma levels were verified using a competitive ELISA kit (Enzo life sciences, Farmingdale, NY, USA) as per the manufacturer's instructions. Blood was collected following decapitation into 15 ml tubes containing 100µL of EDTA. Plasma was separated by centrifugation (2500 rpm, 20 minutes, 4°C) and stored at -80°C until use.

VI.7. Restraint stress protocol.

Mice were restrained with a plastic cone (Braintree scientific, MA, USA) for 30 minutes before being decapitated. Since a 30 minutes acute stress protocol was used for

incubating brain slices in CORT before electrophysiology experiments, the same amount of time was chosen for the restraint stress protocol. Brains were then sliced as previously described. Slices were used to perform electrophysiological experiments.

VI.8. Statistical analyses.

All whole-cell patch clamp results were analyzed using 2-tailed, independent Student's t-tests. Parameters measured for whole-cell patch clamp were the peak of the EPSP at -60mV and the peak of the EPSP at +40mV once the AMPAR component was back to baseline. Field-recordings were analyzed using repeated measures 2-way ANOVAs. Parameters measured for field recordings were the slope of the EPSP and the peak of the FV. Western blots and ELISA results were both analyzed using 2-tailed, independent Student's t-tests. All data were presented as mean ± SEM. Outliers were removed by excluding all data points that were two standard deviations away from the mean once all data were gathered.

VII. Results

VII.1. CORT-induced regulation of NMDARs and AMPARs in McGill-Thy1-APP mice.

VII.1.1. Input/output relationship of the synaptic NMDAR function in McGill-Thy1-APP and WT mice

In order to determine if CORT-induced regulation of NMDARs is altered under an Aβ peptide overproduction background, we first had to determine basal synaptic function of NMDARs for both McGill-Thy1-APP and WT mice. This was accomplished

by recording fEPSPs slope (i.e., output) changes as FV amplitude (i.e., input) increases in both the dorsal and the ventral hippocampus of animals around three months of age. In the dorsal hippocampus under the control condition where no CORT was added, synaptic NMDARs function showed a main effect of group (2-way repeated measures ANOVA, *group effect*, $F_{(3, 31)} = 4.63$, $p = 0.009$) with the four groups being WT control, WT CORT, APP control and APP CORT. An interaction between these four groups and the FV was also observed (2-way repeated measures ANOVA, *group effect*, $F_{(12, 124)} = 2.01$, $p = 0.029$). In order to reveal which groups' FVs were significantly different from one another, a 1-way repeated measures ANOVA was used to compare the WT control and APP control groups. A main effect of group was observed (Figure 1; 1-way repeated measures ANOVA, *group effect*, $F_{(1, 10)} = 14.64$, $p = 0.003$) showing that McGill-Thy1-APP mice have significantly lower synaptic NMDARs function than WT mice in the dorsal hippocampus. In the ventral hippocampus there is no main effect between the four groups (Figures 2; 2-way repeated measures ANOVA, *group effect*, $F_{(3, 28)} = 2.34$, $p = 0.09$).

After establishing basal synaptic NMDAR function under control conditions, we sought to determine the effects of CORT on synaptic NMDAR function. In the dorsal hippocampus of WT animals, a 1-way repeated measures ANOVA shows that there is a main effect of group, with basal synaptic NMDAR function of WT mice being significantly reduced following CORT application (Figure 3; 2-way repeated measures ANOVA, *group effect*, $F_{(1, 10)} = 5.72$, $p = 0.04$).

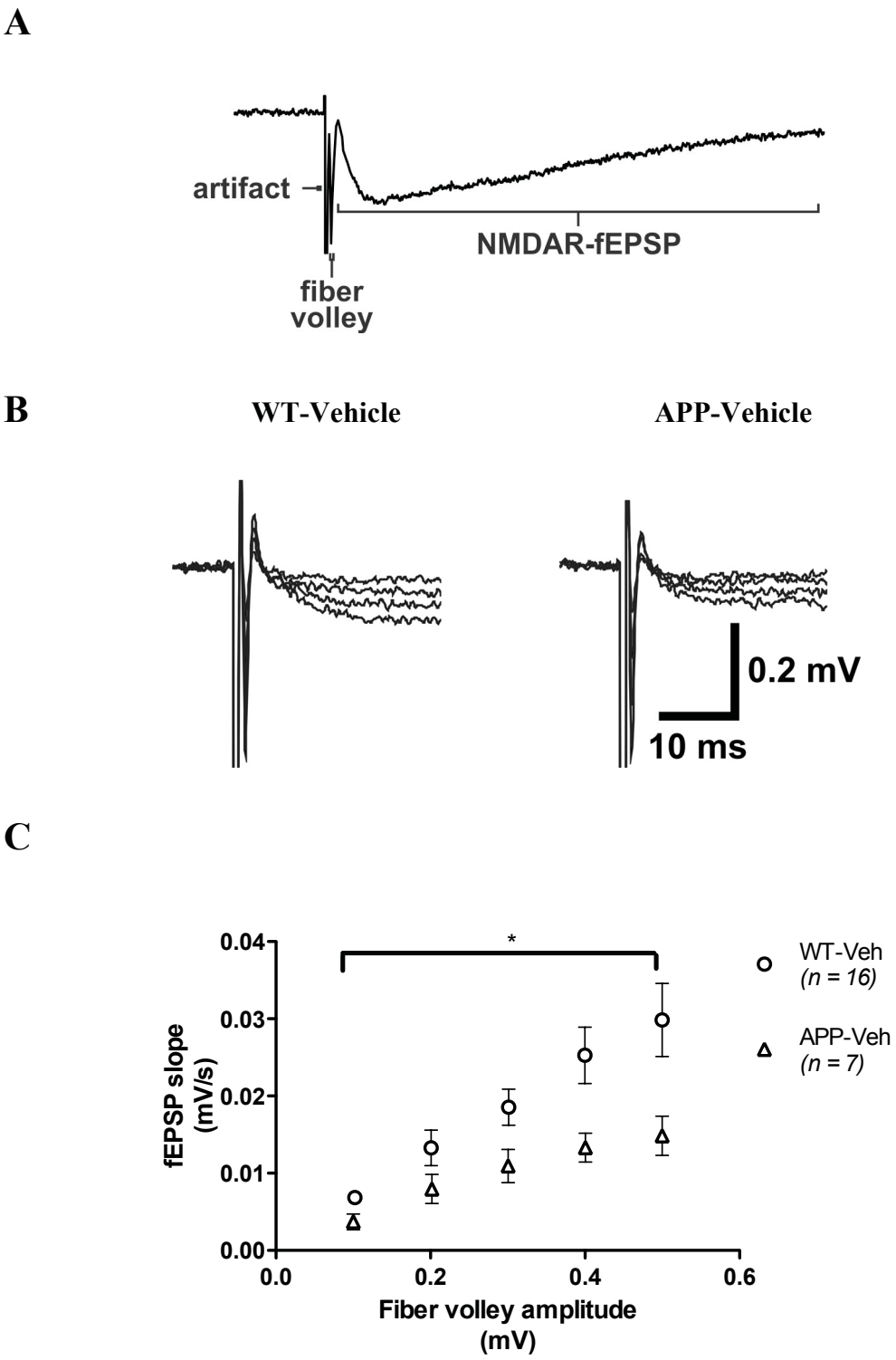


Figure 1. Basal synaptic NMDAR function in the dorsal hippocampus of McGill-Thy1-APP mice is lower than that of WT mice. (A) An example trace of NMDAR-

mediated field excitatory postsynaptic potential (fEPSP). Note the fiber volley immediately after the stimulation artifact, Fiber volley (FV) represents depolarization of presynaptic fibers. (**B)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce fiber volley sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. (**C**) Plot of the relationship between fiber volley size and fEPSP slope for both WT vehicle (n= 16) and McGill-Thy1-APP vehicle (n= 7). Basal synaptic NMDAR function of McGill-Thy1-APP mice was significantly lower than that of WT mice ($F_{(1, 10)} = 14.64$, $p = 0.003$). * $p < 0.05$.

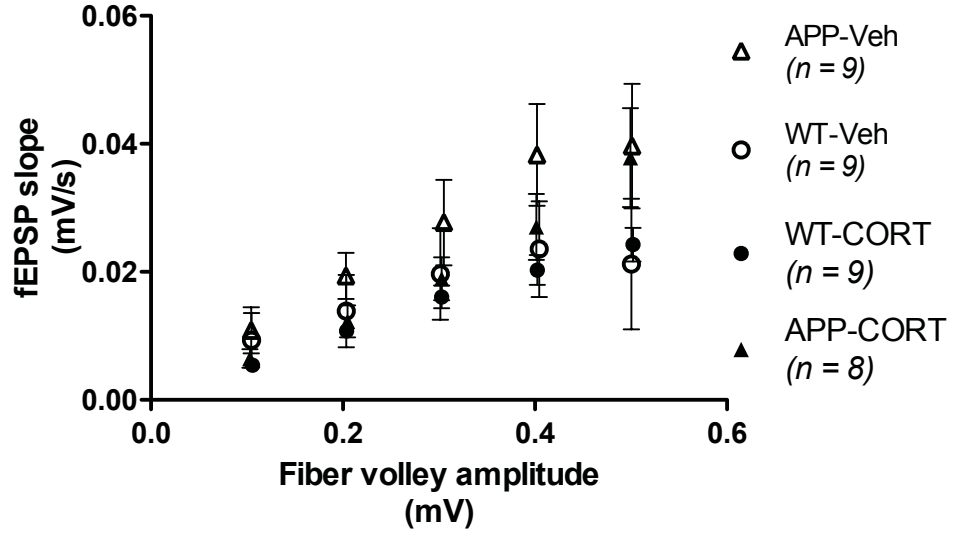


Figure 2. There is not change in basal synaptic NMDAR function in the ventral hippocampus of McGill-Thy1-APP mice compared to WT mice. Plot of the relationship between fiber volley size and fEPSP slope for WT vehicle (n= 9), WT CORT (n= 10), McGill-Thy1-APP vehicle (n= 9), and McGill-Thy1-APP vehicle (n= 8). There were no differences in basal synaptic AMPAR function between McGill-Thy1-APP and WT mice ($F_{(3, 28)} = 2.34$, $p = 0.09$).

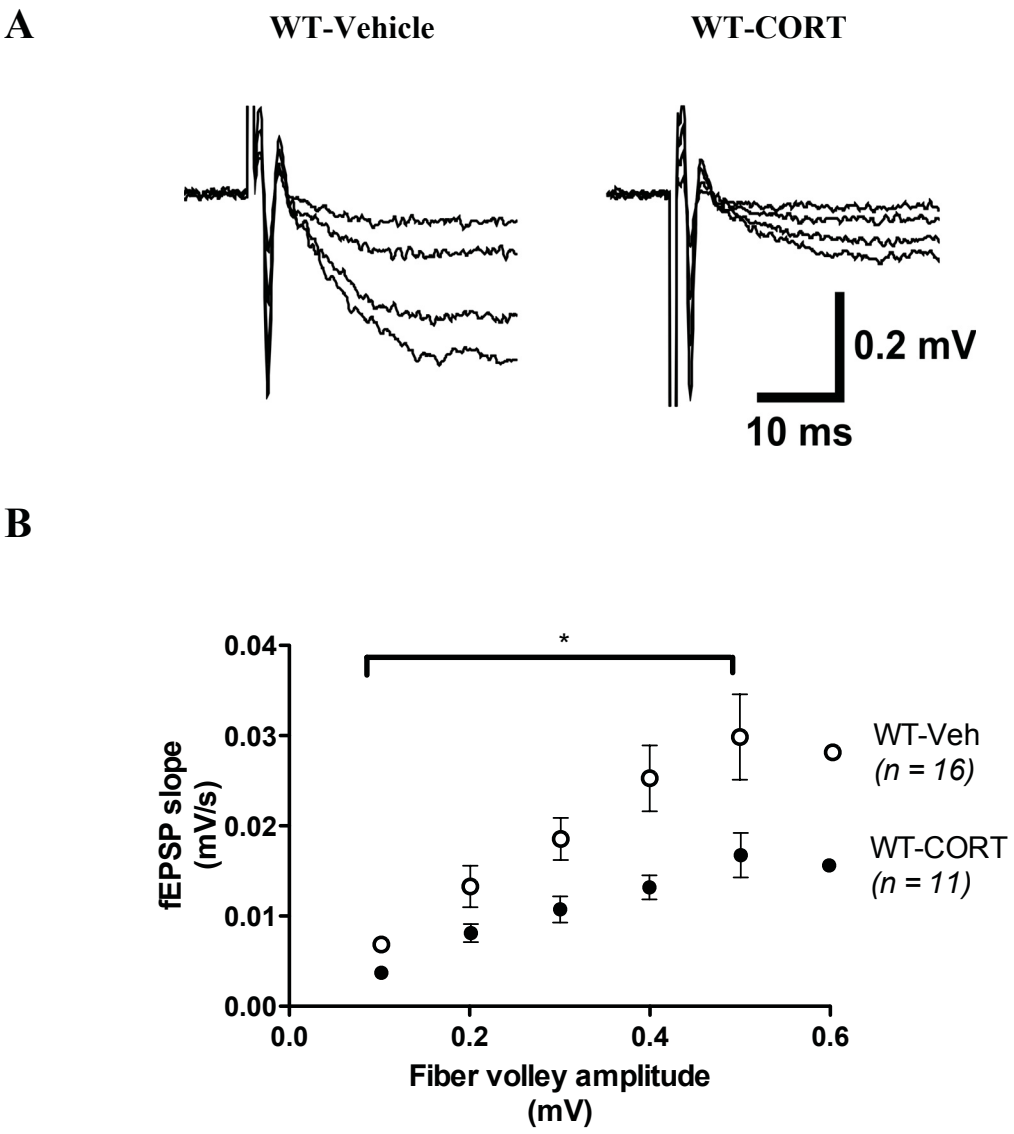


Figure 3. Synaptic NMDAR function in the dorsal hippocampus of WT mice is significantly reduced under CORT condition. (**A)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce fiber volley sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. **(B)** Plot of the relationship between fiber volley size and fEPSP slope for both vehicle (n= 15) and CORT-treated slices (n= 11) for WT mice. CORT significantly reduced fEPSP of WT mice ($F_{(1, 10)} = 5.72$, $p = 0.04$).* $p < 0.05$.

On the other hand, there was no main effect of group in the dorsal hippocampus of McGill-Thy1-APP mice following CORT application (Figure 4; 2-way repeated measures ANOVA, *group effect*, $F_{(1, 10)} = 0.84$, $p = 0.38$) revealing that CORT did not appear to have any effects on synaptic NMDAR function of McGill-Thy1-APP mice.

VII.1.2. NMDAR/AMPAR ratio of the McGill-Thy1-APP and WT mice

The ratio of synaptic current mediated by NMDAR vs. AMPAR (NMDAR/AMPAR ratio) of WT mice at 3 months of age was determined by whole-cell patch clamp by holding the cell at -60mV and +40mV respectively and recording evoked EPSCs under both control and CORT treatment condition. Since significant differences following CORT application were only observed in WT animals in the dorsal hippocampus, NMDAR/AMPAR ratio was performed only for WT animals in the dorsal hippocampus. In the dorsal hippocampus of 3-month-old WT mice, NMDAR/AMPAR ratio under control condition (34.38% ±2.82%) was not significantly changed under 100nM CORT treatment (29.29% ±8.53%)(Figure 5; Student's independent t-test, 2-tailed, $t_{(9)} = -0.61$, $p = 0.55$) or 200nM CORT treatment (32.58% ±6.36%)(Figure 5; Student's independent t-test, 2-tailed, $t_{(10)} = -0.26$, $p = 0.8$).

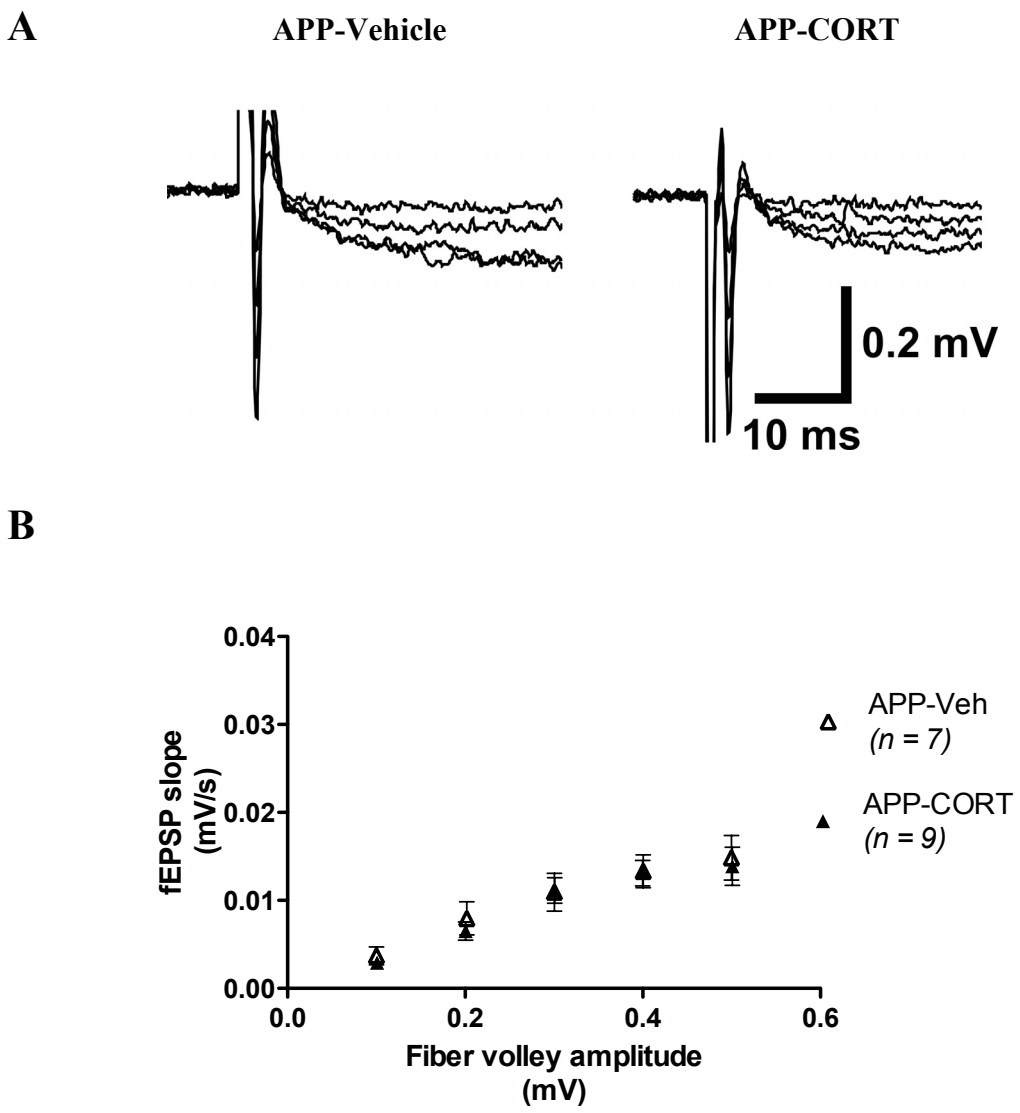
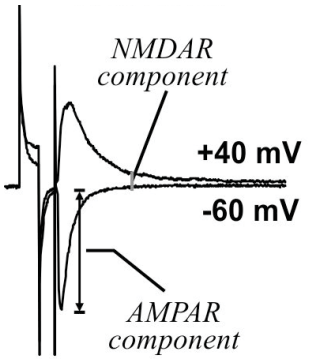


Figure 4. Synaptic NMDAR function in the dorsal hippocampus of McGill-Thy1-APP mice is irresponsive to stress level CORT. (**A)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce fiber volley sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. **(B)** Plot of the relationship between fiber volley size and fEPSP slope for both vehicle (n= 7) and CORT-treated slices (n= 9) in McGill-Thy1-APP mice. There are no significant differences following CORT application ($F_{(1, 10)} = 0.84$, $p = 0.38$).

A



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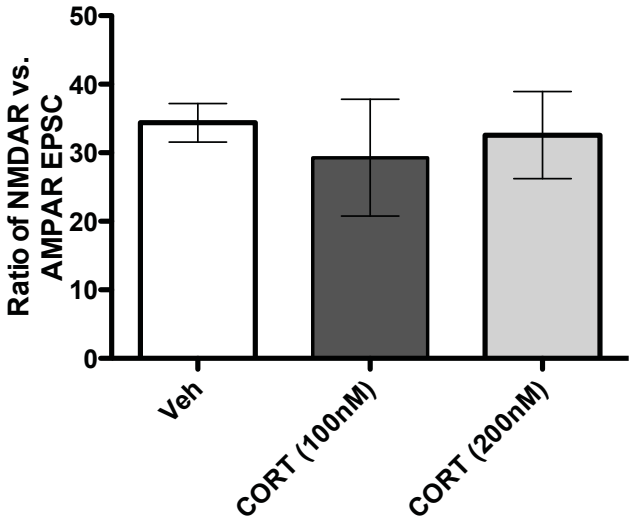


Figure 5. Both 100nM and 200nM CORT have no effect on NMDAR/AMPAR ratio of EPSCs mediated in the dorsal hippocampus of WT mice. (A) Schematic diagram shows the NMDAR and AMPAR components of evoked excitatory postsynaptic current recorded from a CA1 neuron. Note that the NMDAR component was measured at the time window after the disappearance of the AMPAR component. **(B)** No changes were observed in NMDAR/AMPAR ratio under 100 nM CORT condition ($t_{(9)} = -0.61$, $p = 0.55$) or 200 nM CORT condition ($t_{(6)} = -0.64$, $p = 0.55$) in WT mice.

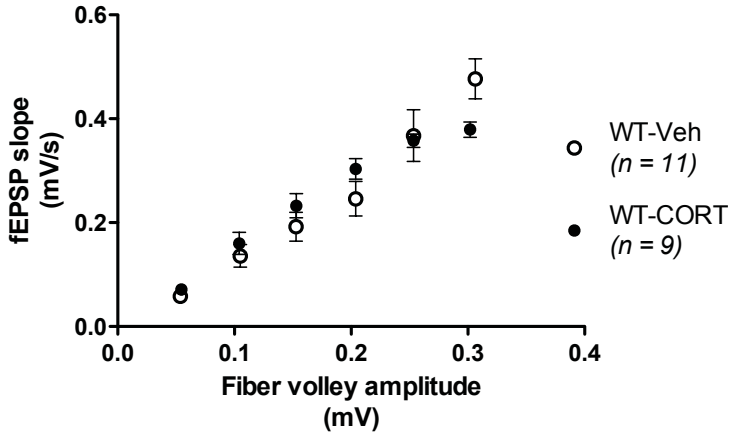
VII.1.3. Input/output relationship of the synaptic AMPAR function in McGill-Thy1-APP and WT mice

Changes in AMPAR synaptic function have been observed following CORT treatment (Groc et al., 2008; Karst & Joels, 2005; Martin et al., 2009), thus synaptic function of AMPARs of 3 month-old WT mice was examined for both control and CORT-treated slices. Once again, fEPSPs slope (i.e., output) change was recorded as FV amplitude increases. Both in the dorsal and ventral hippocampus, no main effect of group was observed (Figures 6; 2-way repeated measures ANOVA, *group effect*, $F_{(1, 14)} = 1.78$, $p = 0.2$; $F_{(1, 12)} = 0.002$, $p = 0.97$, respectively).

VII.2. Restraint stress-induced regulation of NMDARs in McGill-Thy1-APP mice.

Since we have found that CORT appears to reduce synaptic NMDAR function in the dorsal hippocampus of WT mice, we sought to determine if an *in vivo* stress paradigm could induce a similar change in synaptic NMDAR function. To test that, we started by assessing basal synaptic NMDAR function for both McGill-Thy1-APP and WT mice, under control condition by using the input/output method. We found that once again, synaptic NMDAR function of McGill-Thy1-APP mice in the control condition is significantly lower than that of WT mice (Figure 7; 2-way repeated measures ANOVA, *genotype effect*, $F_{(5, 70)} = 2.38$, $p = 0.047$). A significant interaction between group and FV was observed, comparing WT control, WT restraint stress, APP control, and APP restraint stress with the FV (2-way repeated measures ANOVA, *group*FV interaction*, $F_{(15, 135)} = 1.97$, $p = 0.022$).

A



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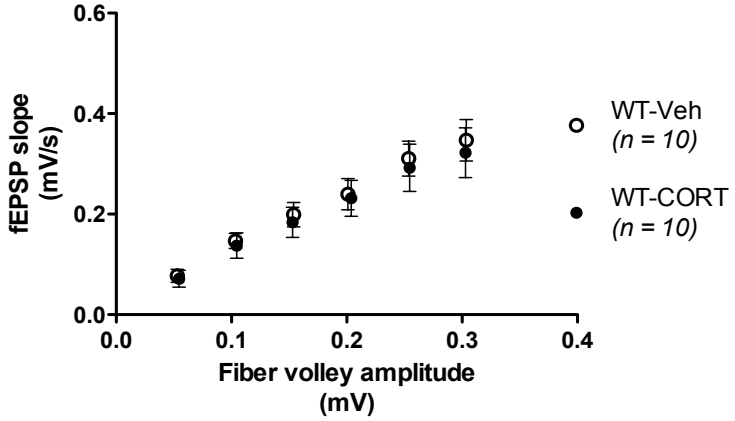
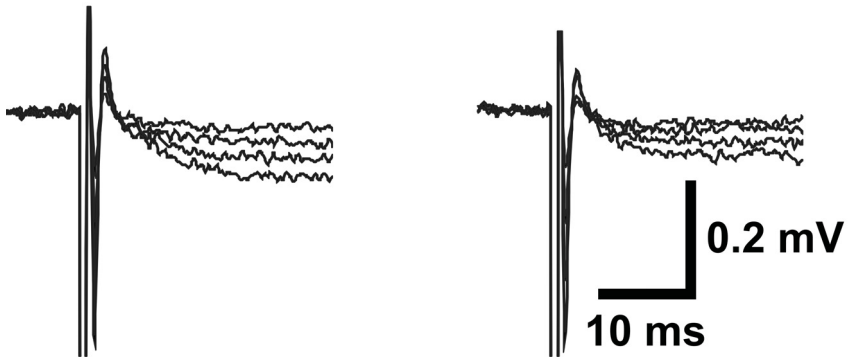


Figure 6. Stress level CORT has no effect on AMPAR-mediated fEPSP of WT mice in the dorsal hippocampus. (A) Plot of the relationship between FV size and fEPSP slope for both vehicle (n= 11) and CORT-treated slices (n= 9) for WT mice. There were no significant differences following CORT application ($F_{(1, 14)} = 1.78$, $p = 0.2$). **(B)** Plot of the relationship between FV size and fEPSP slope for both vehicle (n= 10) and CORT-treated slices (n= 10) for WT mice. CORT did not significantly change fEPSP of AMPARs in WT mice ($F_{(1, 12)} = 0.002$, $p = 0.97$).

A



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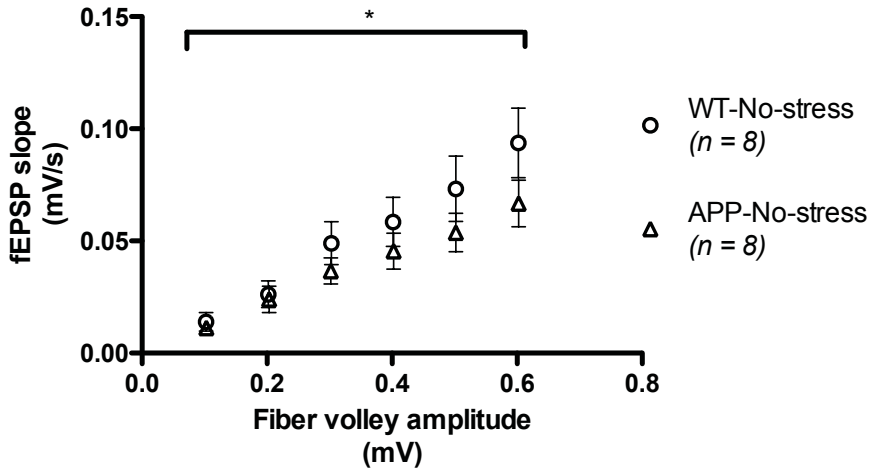

Figure 7. Basal synaptic NMDAR function in the dorsal hippocampus of WT mice is stronger than that of McGill-Thy1-APP mice. (**A)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce FV sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. (**B**) Plot of the relationship between FV size and fEPSP slope for both WT vehicle (n= 8) and McGill-Thy1-APP vehicle (n= 8). Basal synaptic McGill-Thy1-APP function was significantly lower than that of WT mice ($F_{(5, 70)} = 2.38$, $p = 0.047$).* $p < 0.05$.

A 1-way repeated measures ANOVA was used to compare WT synaptic NMDAR function under the control and restraint stress conditions. We found that basal synaptic NMDAR function of WT mice was significantly reduced under the restraint stress condition compared to the control condition (Figure 8; Repeated measures 1-way ANOVA, *treatment effect*, $F_{(5, 60)} = 3.264$, $p = 0.011$) just as seen following CORT treatment. Also as previously seen under CORT condition, restraint stress did not appear to have any significant effects on synaptic NMDAR function of McGill-Thy1-APP mice (Figure 9; Repeated measures 2-way ANOVA, *treatment effect*, $F_{(1, 15)} = 0.4$, $p = 0.53$).

VII.3. Changes in HPA axis function following overproduction of Aβ peptide.

VII.3.1. Changes in MR expression in McGill-Thy1-APP and WT mice

Since the effect observed in NMDAR synaptic function following either CORT application or restraint stress may be due to changes in corticosteroid receptor expression levels, basal levels of MR were assessed. There are no significant differences in basal expression levels of MRs between McGill-Thy1-APP and WT mice (Figure 10; Student's independent t-test, 2-tailed, $t_{(10)} = -0.87$, $p = 0.41$) in the dorsal hippocampus. In the ventral hippocampus, no significant differences in basal MR expression were found either (Figure 10; Student's independent t-test, 2-tailed, $t_{(10)} = -0.25$, $p = 0.8$).

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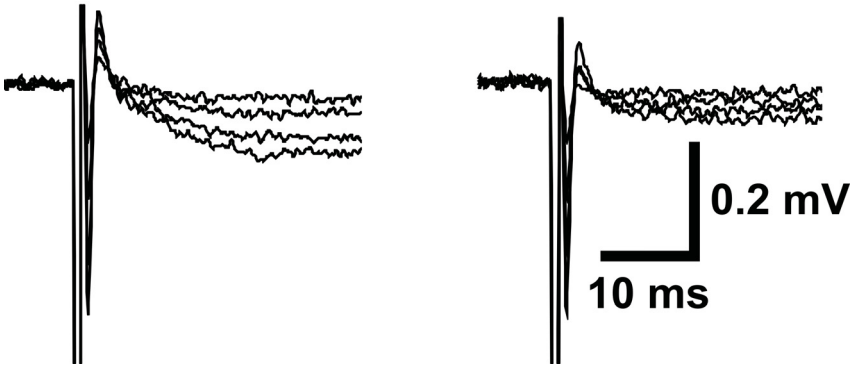

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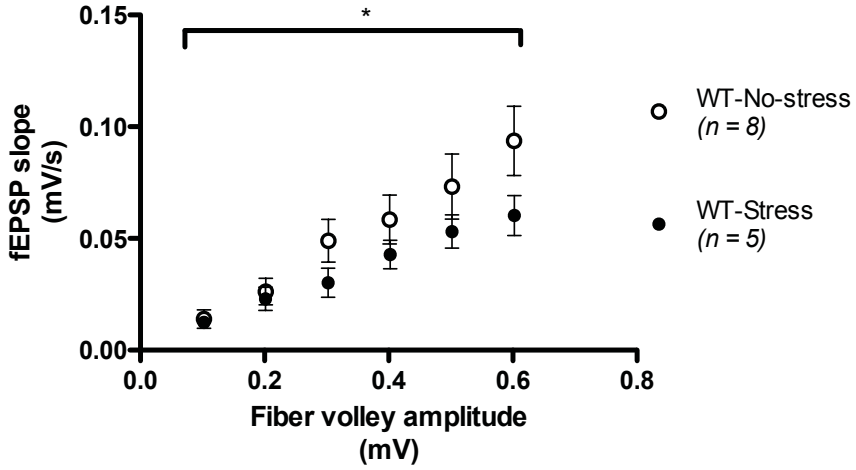

Figure 8. Synaptic NMDAR function in the dorsal hippocampus of WT mice is significantly reduced under restraint stress paradigm. (**A)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce FV sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. **(B)** Plot of the relationship between FV size and fEPSP slope for both vehicle (n= 8) and restraint stress condition (n= 5) for WT mice. WT synaptic NMDAR function was significantly reduced following restraint stress paradigm ($F_{(5, 60)} = 3.264$, $p = 0.011$).* $p < 0.05$.

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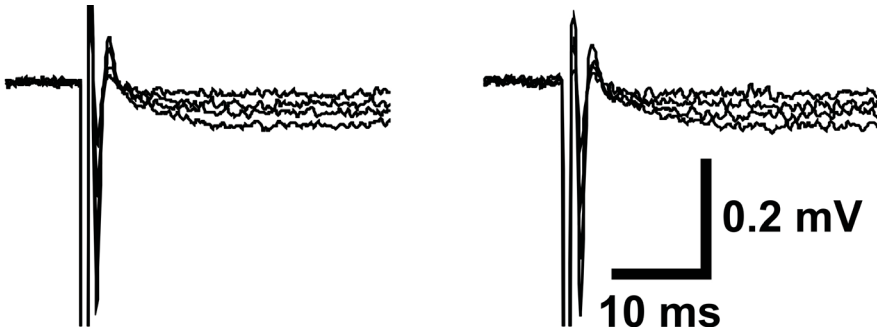

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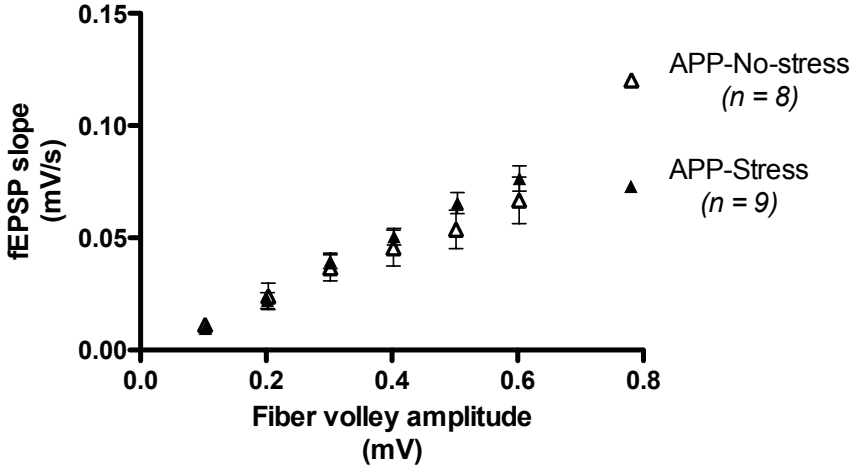

Figure 9. Synaptic NMDAR function in the dorsal hippocampus of McGill-Thy1-APP mice shows no difference following restraint stress paradigm. (**A)** Representative traces of NMDAR-mediated fEPSP in the dorsal hippocampus. In order to produce FV sizes ranging from 0.1 to 0.5 mV strength of stimulation was adjusted accordingly. **(B)** Plot of the relationship between FV size and fEPSP slope for both vehicle (n= 8) and restraint stress condition (n= 9) for McGill-Thy1-APP mice. McGill-Thy1-APP synaptic NMDAR function was not significantly different following restraint stress paradigm ($F_{(1, 15)} = 0.4$, $p = 0.53$).

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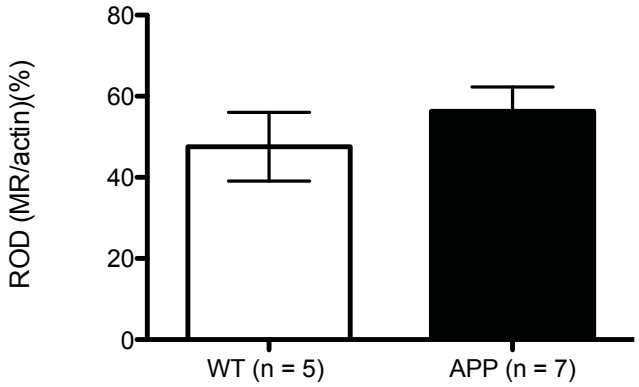

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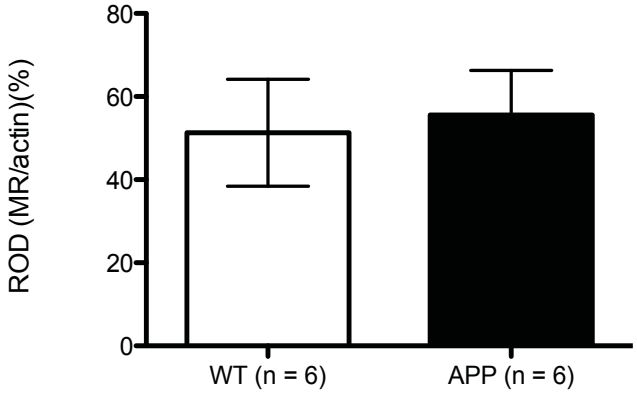

Figure 10. No differences in MR protein expression were observed in the dorsal and ventral hippocampus between McGill-Thy1-APP and WT mice. (A) Histograms summarize the relative optical density (MR/actin) for both McGill-Thy1-APP (n =7) and WT (n = 5) mice. There were no significant differences in MR protein expression levels between McGill-Thy1-APP and WT mice in the dorsal hippocampus ($t_{(10)} = -0.87$, $p = 0.41$). **(B)** Histograms summarize the relative optical density (MR/actin) for both McGill-Thy1-APP (n =6) and WT (n = 6) mice. There were no significant differences in MR expression levels between McGill-Thy1-APP and WT mice in the ventral hippocampus ($t_{(10)} = -0.25$, $p = 0.8$).

VII.3.2. Changes in GR expression in McGill-Thy1-APP and WT mice

Basal levels of GR were also assessed. Just as with MRs, there were no significant differences in basal GR expression levels between McGill-Thy1-APP and WT mice in the dorsal hippocampus (Figure 11; Student's independent t-test, 2-tailed, $t_{(10)} = 0.36$, $p = 0.73$). Although, no significant differences were seen in basal GR expression level in the ventral hippocampus, there is a trend towards a decrease of GR expression levels in McGill-Thy1-APP mice compared to WT mice (Figure 11; Student's independent t-test, 2-tailed, $t_{(9)} = 1.95$, $p = 0.08$).

VII.3.3. Basal plasma CORT levels in McGill-Thy1-APP and WT mice

Finally, since the differences observed in synaptic NMDAR function could be due to altered levels of plasma CORT, basal plasma CORT level was assessed for both McGill-Thy1-APP and WT mice with an ELISA CORT kit. We found that basal McGill-Thy1-APP mice plasma CORT levels were significantly lower than that of WT mice (Figure 12; Student's independent t-test, 2-tailed, $t_{(8)} = 2.77$, $p = 0.024$).

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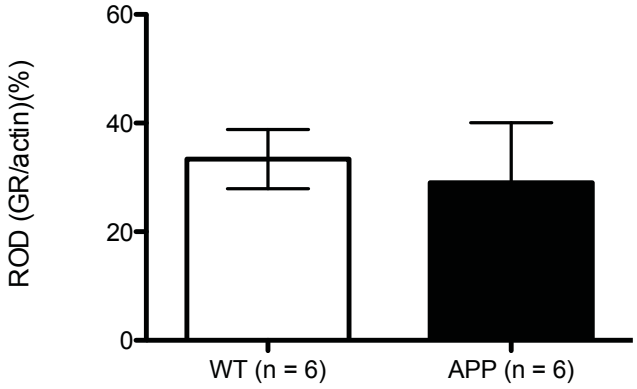

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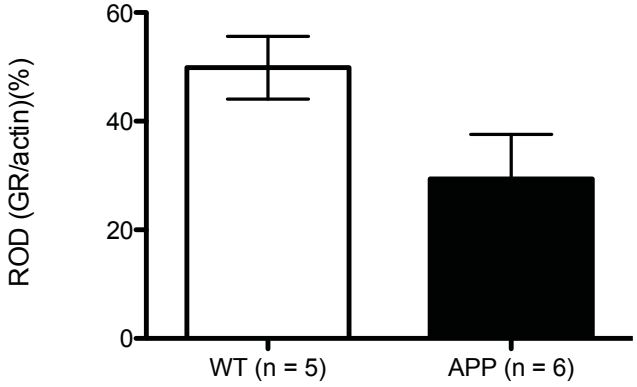

Figure 11. No differences in GR protein expression were observed in the dorsal and ventral hippocampus between McGill-Thy1-APP and WT mice. (A) Histograms summarize the relative optical density (GR/actin) for both McGill-Thy1-APP (n =6) and WT (n = 6) mice. There were no significant differences in GR protein expression levels between McGill-Thy1-APP and WT mice in the dorsal hippocampus ($t_{(10)} = 0.36$, $p = 0.73$). **(B)** Histograms summarize the relative optical density (GR/actin) for both McGill-Thy1-APP (n =6) and WT (n = 5) mice. There were no significant differences in GR protein expression levels between McGill-Thy1-APP and WT mice ($t_{(9)} = 1.95$, $p = 0.08$).

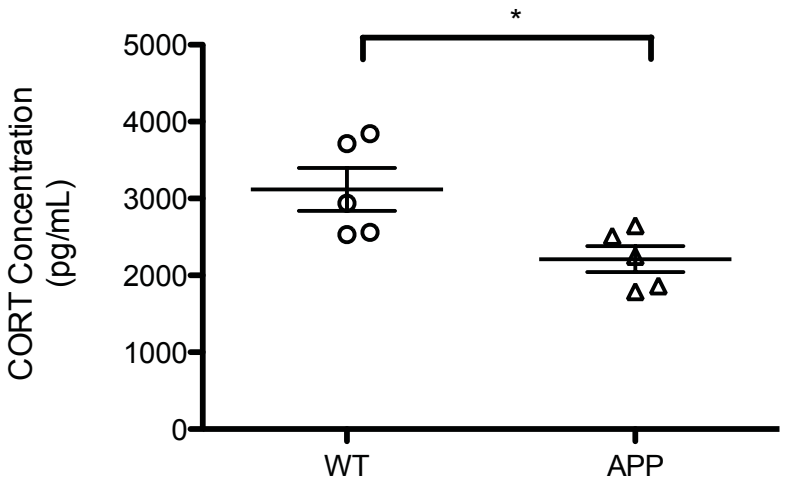

Figure 12. Plasma CORT levels of McGill-Thy1-APP animals were significantly lower than that of WT mice. Scatterplots represent the concentration of CORT (pg/mL) for both McGill-Thy1-APP (n = 5) and WT (n = 5) mice. McGill-Thy1-APP animals had significantly lower blood CORT levels than WT mice ($t_{(8)} = 2.77$, $p = 0.024$). * $p < 0.05$.

VIII. Discussion

VIII. 1. Summary of findings.

In this study we sought to characterize basal synaptic NMDAR function in both the McGill-Thy1-APP mouse as well as in WT mice at both control and stress levels. Thus, we first examined the difference in synaptic NMDAR function between McGill-Thy1-APP and WT mice both in the dorsal and in the ventral hippocampus. We found that in the dorsal hippocampus, synaptic NMDAR function in the McGill-Thy1-APP mice was significantly lower than WT mice, while no differences were seen in the ventral hippocampus (Figures 1, 2).

After establishing basal synaptic NMDAR function under control conditions in McGill-Thy1-APP and WT mice, we sought to determine synaptic NMDAR function under stress conditions. In the dorsal hippocampus of WT mice, we observed a significant decrease in synaptic NMDAR function following CORT application (Figure 3) as well as restraint stress (Figure 8), however no differences in synaptic NMDAR function were observed in the ventral hippocampus (Figure 2). We have also observed that CORT (Figure 4) as well as restraint stress (Figure 9) appear to have no effect on synaptic NMDAR function of McGill-Thy1-APP mice in both the dorsal and the ventral hippocampus.

To find out whether CORT affects the functional properties of other glutamatergic receptors, we examined the impact of CORT on synaptic AMPAR function in the hippocampus of WT animals. As a difference in synaptic NMDAR function under CORT and stress conditions was only observed in WT animals, we sought to determine synaptic AMPAR function in 3 month-old WT mice only. Both in the dorsal and in the ventral

hippocampus of WT mice, there were no significant differences observed in WT mice following CORT application compared to control conditions (Figure 6).

Since a significant difference in synaptic NMDAR function following CORT application was observed in the dorsal hippocampus of WT mice using field recordings, we used whole-cell patch clamp to confirm the changes observed in synaptic NMDAR function induced by CORT. However, no difference in NMDAR/AMPAR ratio has been observed following CORT application in the dorsal hippocampus of WT mice (Figure 5).

Finally, we investigated whether the changes seen in synaptic NMDAR function between McGill-Thy1-APP and WT mice were due to any differences in HPA axis. We found that there were no significant differences in corticosteroid receptors, either MR (Figure 10) or GR (Figure 11) between non-stressed McGill-Thy1-APP and WT mice. Basal plasma CORT level was also examined. We found that McGill-Thy1-APP mice appear to have significantly lower plasma CORT levels than WT mice (Figure 12).

To our knowledge, this is the first study to look at synaptic NMDAR function under an acute stress condition in a transgenic model of AD. This is also the first study looking at the effect of endogenous Aβ peptide overproduction on synaptic NMDAR function, which may be more relevant in a context of AD since it mimics certain aspects of the disease more closely than exogenously applied Aβ peptide. Another aspect of this study that is highly relevant to cognitive impairments observed both in AD patients and transgenic rodent models is the fact that we looked at synaptic NMDAR function which underlies synaptic plasticity in the hippocampus and potentially underlies the cognitive deficits observed.

VIII.2. Impact of Aβ peptide overproduction on synaptic NMDAR function.

We first observed that basal synaptic NMDAR function of McGill-Thy1-APP mice under control conditions was significantly lower than that of WT mice. Although some previous studies have found an increase in synaptic NMDAR function following application of exogenous Aβ peptide (Gu et al., 2004; Molnar et al., 2004; Szegedi et al., 2005; Wu et al., 1995), studies looking at synaptic NMDAR function in transgenic animals that overexpress Aβ peptide have, in fact, shown a decrease in synaptic function (Dewachter et al., 2009; Duszczyk et al., 2012; Hsia et al., 1999; Snyder et al., 2005). Models presenting endogenous Aβ peptide might be more relevant for the study of AD, especially considering the difficulties that can arise when studying the effects of exogenously applied Aβ peptide. These difficulties could arise from differences in the characteristic, dosage, duration of treatment and method of administration of exogenously applied Aβ peptide, which renders comparison of the results somewhat difficult. For example, Gu and colleagues (2004) performed their study on *xenopus* oocytes and administered two different types of Aβ peptides, $A\beta_{1-42}$ and $A\beta_{25-35}$ at different concentrations, which are 40μM and 590μM respectively. Molnar and colleagues (2004), on the other hand, did *in vivo* recordings in the CA1 hippocampal area of rats and administered both $A\beta_{1-42}$ at 10μM and $A\beta_{25-35}$ at 10μM iontophoretically. Szegedi and colleagues (2005) also performed *in vivo* recordings in the CA1 hippocampal area of rats, while $A\beta_{1-42}$ at 50 μM was delivered by iontophoresis. Finally, Wu and colleagues (1995) also performed experiments on rats, however they used $A\beta_{1-40}$ peptide at concentrations ranging between 100 and 200 nM. The differences seen in the protocol of studies using

exogenously applied Aβ peptides highlight the importance of investigating synaptic NMDAR function in a transgenic model that overexpresses Aβ peptide.

In regard to the decrease in synaptic NMDAR function of McGill-Thy1-APP mice compared to WT mice, the decrease may be due to endocytosis of NMDARs, a mechanism that has been previously demonstrated to happen to NMDARs in primary hippocampal cultures of transgenic mice overexpressing Aβ peptide (Snyder et al., 2005). Interestingly, a decrease in the GluN2B subunit expression of the NMDAR has also been seen in AD patients (Mishizen-Eberz et al., 2004). As the McGill-Thy1-APP mouse already shows impairments in hippocampal-dependent memory task at 3 months of age (Ferretti et al., 2011), the decrease in synaptic NMDAR function we observed in this study may be a putative mechanisms to impair synaptic plasticity and cognitive function of these mice.

VIII.3. Impact of CORT and stress on synaptic NMDAR function in WT and McGill-Thy1-APP mice.

Another important observation seen in this study is the reduction in the synaptic NMDAR function of WT mice shown both in the CORT and restraint stress conditions compared to the control condition. Although a previous study by our lab has shown an increase in synaptic NMDAR function following CORT administration in rats (Tse et al., 2011), we saw the opposite of what we expected. These differences however, may be due to strain and species differences under an acute stress paradigm. For example, acute social defeat does not induce social avoidance in C57BL/6 mice, while the same stress paradigm performed with male Wistar rats could induce social avoidance (Toth &

Neumann, 2013). Thus, different species of animals as well as different strains of the same species (e.g., C57BL/6 *vs.* DBA/2) may display differences in stress susceptibility, which may be reflected at the level of synaptic NMDAR function.

This study is the first to show the effect of stress and stress hormones on synaptic NMDAR function in a transgenic AD model. The lack of change in synaptic NMDAR function of McGill-Thy1-APP mice under stress conditions may represent an occluding effect of Aβ peptide overproduction. As synaptic NMDAR function of McGill-Thy1-APP mice in the control conditions is already significantly lower than that of WT mice, it is possible that there is no more room for a further decrease of the synaptic NMDAR function under stress conditions. Unlike chronic stress that has been associated with various pathological outcomes, acute stress is generally believed to be adaptive. The lack of stress-induced changes in synaptic NMDAR function in McGill-Thy1-APP mice may represent a maladaptive response to stress. For instance, acute stress and acute treatment of CORT has been shown to facilitate spatial memory formation in rodents (Sandi et al., 1997). Future studies may investigate whether these cognitive facilitating effects of stress are abolished in McGill-Thy1-APP mice.

VIII.4. Changes in synaptic AMPAR function of WT mice under CORT condition.

Another finding of the present study is that CORT appears to have no effect on synaptic AMPAR function of WT mice. One of the reasons could be that CORT exerts changes in synaptic NMDARs function only. Our lab has previously shown in hippocampal slices from adult rats that CORT enhanced synaptic NMDAR but not AMPAR function (Tse et al., 2011). However, it is also possible that it takes longer for

the effect of CORT on AMPAR synaptic function to become apparent. Indeed, there is evidence that increases in AMPAR synaptic function take hours to develop (Groc et al., 2008; Karst & Joels, 2005).

In order to confirm the effect of CORT on synaptic NMDAR function seen in the dorsal hippocampus of WT mice, we performed whole-cell patch clamp experiments to compare NMDAR/AMPAR ratio under control conditions with NMDAR/AMPAR ratio under CORT condition. Unlike the field recording results, we did not see any significant differences in NMDAR/AMPAR ratio of WT mice between control and CORT conditions. This discrepancy may be due to the fact that the effect of CORT we observed in the input/output experiment becomes more prominent at higher stimulation strengths (i.e. high fiber volley size). Since only one stimulation strength was used to induce synaptic current in the whole cell experiment, it is possible that the stimulations strength used in this experiment was too low to see any significant differences under CORT condition.

VIII.5. Differences in plasma CORT levels and corticosteroid receptor protein expression in McGill-Thy1-APP.

Finally, we sought to examine properties of the HPA axis in McGill-Thy1-APP mice compared with WT mice by first looking at the protein expression of corticosteroid receptor. We found that there were no significant differences in protein expression of either MRs or GRs in McGill-Thy1-APP mice compared to WT mice. Our finding is similar to previous findings that no differences were found in the immunoreactivity of GRs in the CA1 area between WT mice and Tg2576 mice (Carroll et al., 2011). Note that

not all studies revealed no changes in GR expression. For instance, increases in GR mRNA levels have been found in 3xTgAPP mice, a model that contains the Swedish double mutation, a PS1 mutation and a tau mutation (Hebda-Bauer et al., 2013). Since we observed no differences in the expression of corticosteroid receptors between WT and McGill-Thy1-APP mice, it is possible that the differences in CORT-induced modulation in synaptic NMDAR function between these mice are related to alteration in signalling pathways downstream of corticosteroid receptor activation.

We found that McGill-Thy1-APP mice appear to have significantly lower plasma CORT levels than WT mice. A study on 3xTgAPP mice did not show any significant differences in basal plasma CORT levels between the 3xTgAPP animals and WT animals (Hebda-Bauer et al., 2013). Another study employing Tg2576 mice also shows no apparent differences in basal CORT levels between Tg2576 and WT mice (Carroll et al., 2011). The disparity in results seen between these studies and the present study could be due to differences in transgenic models. First of all, the APP transgene is not the same. The McGill-Thy1-APP model carries the human APP751 transgene while previous studies have used both the Tg2576 and the 3xTg-AD models, which carry the human 695APP transgene (Hsiao et al., 1996; Oddo et al., 2003). Second of all, the number of mutations between the models is also not the same. The McGill-Thy1-APP model carries the Swedish double mutation as well as the Indiana mutation (Ferretti et al., 2011), while the Tg2576 model carries only the Swedish double mutation (Hsiao et al., 1996) and the 3xTg-AD model carries the Swedish double mutation as well as a PS1 and tau mutations (Oddo et al., 2003). The variations seen in these models may account for differences observed in plasma CORT levels. Although, both the Carroll and colleagues (2011) and

Hebda-Bauer and colleagues (2013) studies did not see differences in plasma CORT levels between APP and WT mice, it is possible to observe that there is in fact a disparity between basal CORT levels of animals of both studies. In the Hebda-Bauer and colleagues (2013) study for both WT and APP animals, basal CORT levels were around 1.3μg/dL while in the Carroll and colleagues (2011) study, study for both WT and APP animals, basal CORT levels were around 180pg/mL. It is also possible that the difference observed in basal plasma CORT levels between these studies may stem from different methods used to assess plasma CORT levels. Carroll and colleagues (2011) used an ELISA to estimate plasma CORT levels, while Hebda-Bauer and colleagues (2013) used radioimmunoassay to estimate plasma CORT levels.

VIII.6. Limitations and future directions

A limitation of this study was that different stress paradigms were not tested in this study, for example, the effects of psychological stress (e.g., social isolation) as opposed to physical stress (e.g. footshock, restraint stress). Different stress duration was not assessed either, for example, looking at the difference between acute *versus* chronic stress. However, this is in part due to the small number of animals available at a time. Perhaps due to the presence of the human APP transgene, female mice displayed lower number of pups than controls. Females usually miscarried the first couple of pregnancies. This led to small cohorts as well as extended periods of time with no animals in between cohorts.

An important aspect of this study is that all tests were performed when animals were 3 months-old when cognitive deficits and Aβ peptide pathology are already

established in this model (Ferretti et al., 2011). However, this is also a *caveat*, since this does not allows us to see how basal and stress-induced alteration of NMDAR synaptic function changes as disease progresses. It is important to uncover if, as disease progresses, a dysfunction in stress-induced plastic changes in synaptic NMDAR function is a persistent phenotype. Alternatively, it would be interesting in the future to look at synaptic NMDAR function in the McGill-Thy1-APP model at earlier ages to find out if changes in synaptic NMDAR function is an early onset phenotype of these mice. It would also be important to look at the effect that chronic stress might have on synaptic NMDAR function in this model at different time ages, since chronic stress has been shown to have adverse effects in many mental disorders, such as depression (Checkley, 1996).

VIII.7. Conclusion

In conclusion, we have found that basal synaptic NMDAR function in the dorsal hippocampus of McGill-Thy1-APP mice is significantly lower than that of WT mice. We have also found that while both acute CORT treatment and stress decrease synaptic NMDAR function of WT mice, we found that stress or stress hormone CORT does not affect synaptic NMDAR function of McGill-Thy1-APP mice. The reduction in NMDAR synaptic function of McGill-Thy1-APP mice could be detrimental and potentially underlie, in part, the cognitive deficits seen in this model. It appears that the changes seen in synaptic NMDAR function between McGill-Thy1-APP mice and WT mice are not due to any changes in corticosteroid receptors expression. However, we have observed that McGill-Thy1-APP mice seem to have lower plasma CORT levels than WT mice. Our findings suggest that synaptic NMDAR function in the hippocampus is altered in McGill-

Thy1-APP mice. Alteration of CORT-induced changes in synaptic NMDAR function in this mouse suggest that stress-induced modulation of synaptic function could be a cellular substrate that is affected by amyloidosis. Future studies could examine potential alteration of stress-induced changes in other functional properties of synapse (e.g. synaptic plasticity) and cognitive function. Finally, malfunctioning of stress-induced changes in synaptic function may be related to the influence of stress in the pathogenesis of AD.

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