CHRONIC TRAFFIC NOISE EXPOSURE CONTRIBUTES TO EMERGENCE OF COGNITIVE AND MOTOR FUNCTION IMPAIRMENT AND INCREASES AMYLOID-β DEPOSITION IN MOUSE MODEL OF ALZHEIMER'S DISEASE

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Dedication

This work is dedicated to my husband and my three lovely kids (Dana, Abdulellah, Abdullah). I appreciate your help, understanding, and endless patience through the hard moments of my life. This journey would not have been possible without your support.

Abstract

Traffic noise has become a daily source of stress in the modern societies. living next to high traffic roads has shown to be associated with increased risk of developing dementia. We employed a combination of behavioral, biochemical, and histological techniques to investigate the impact of chronic traffic noise exposure on the development of the Alzheimer's Disease. For that, 21 APP^{NL-G-F} male and female mice (10 males and 11females) were randomly assigned to the traffic noise or control group. Animals were exposed to 8 hours/day of 75 dB for 30 days to model traffic noise, and the effect on corticosterone levels, animals' behavior, and development amyloid-ß (Aβ) plaques were examined at 4 and 6months post-exposure. Noise-exposed animals displayed anxiety-like behavior impaired balance and motor coordination with reduced learning and memory and increased Aβ plaques. These results provide evidence that traffic noise can impact the brain and predispose mice AD development.

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I am incredibly thankful to my supervisor Dr. Majid Mohajerani for his cordial guidance throughout my M. Sc. Journey. He comes across as not only academic supervisor, but also as a mentor who is always open to talk, listen, and help accordingly. It is an enriching experience to be one of his research group. My extreme thanks to Zahra Jafari for training me and answering my questions. Her idea, encouragement, and critiques have immensely developed my research. I also would like to express the most profound appreciation to my committee members Dr. Bryan Kolb and Dr. Robert Sutherland for their valuable feedback about my work. I am particularly grateful for the assistance given by Dr. Ian Wishaw. He taught me how to improve my writing and presentation skills, his feedbacks always boost my confidence and motivate me to work more. A special thanks to Sean Lacoursiere for proofreading my thesis. My thanks extended to all people in CCBN who helped directly and indirectly to accomplish this project work.
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List of Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid-ß
APP	Amyloid precursor protein
HPA	Hypothalamus- pituitary-adrenal axis
MR	Mineralocorticoid receptors(MR)
GR	Glucocorticoid receptors
LTP	Long-term potentiation
LA	Lateral nucleus of the amygdala
PVN	Paraventricular nucleus
CRH	Corticotrophin-releasing hormone
CORT	Corticosterone
PPI	Pre-pulse inhibition
NOR	Novel object recognition
EPM	Elevated plus-maze
RR	Rotarod test
BBT	Balance beam test
MWT	Morris water task
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
TNE	Traffic noise exposure
ASR	Acoustic startle response
FP	Frontal Pole
OA	Olfactory area
IC	Isocortex
ACA	Anterior cingulate area
NA	Nucleus accumbens
HF	Hippocampus formation
PPA	Posterior parietal area
CAA	Cortical amygdala area
EA	Entorhinal area
RA	Retrospienial area
HB	Hind brain
MB	Midbrain

General Introduction

1. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease that accounts for 60-80% of dementia cases. According to the Alzheimer's Association, about 5.5 million Americans currently live with AD, this number is projected to increase to around 13.8 million by 2050. The hours of care provided by caregivers in 2017 are estimated by 18.4 billion hours, which cost over $232 billion, and it is expected to reach $ 1.1 trillion in 2050 (2018 Alzheimer's disease facts and figures, 2018). AD has been classified into two forms: The genetically inheritable early-onset or familiar AD develops before age 60, accounting for about 5% of all cases. The second form is called late-onset AD (sporadic form). Sporadic form is the most common form of AD that targets people aged 65 and older. Although the cause of this form is still not well understood, previous studies suggested that different environmental factors may have a role in developing the sporadic type of AD

(Awada, 2015).

AD patients experience various stages of symptoms. AD starts with memory lapses.which manifest into forgetting personal events and experiences (episodic memory). Then, the forgetting extends to include facts and knowledge about the external world (semantic memory). Later, AD progresses to affect other memory forms like implicit memory, which includes skills and procedural learning. Further, anxiety, mood switching, sleep disorders, and hallucinations are symptoms that can adversely impact the patients' daily life (Tarawneh & Holtzman, 2012).

On the molecular level, many neuropathological alterations have been observed in AD; which mainly including the deposition of extracellular amyloid plaques and intercellular neurofibrillary tangles (Wippold, Cairns, Vo, Holtzman, & Morris, 2008). The tangles are twisted fibers of tau protein found inside the neurons and the plaques are abnormal protein called amyloid-β (Aβ) protein deposited outside the neurons (Blennow, Mattsson, Schöll, Hansson, & Zetterberg, 2015; Johnson, Stewart, & Smith, 2010). The exact mechanisms underline AD-associated pathology are unclear but the plaques and tangles are thought to contribute to the degradation of the neurons and the subsequent symptoms of AD (Wippold et al., 2008). 1.2 Alzheimer's disease forms

1.2.1 Early- onset AD (Familial Alzheimer's disease (FAD)):

FAD is a rare form of AD that affects only 5% of people at an age younger than 65 (Dubois et al., 2016). FAD is due to alterations in specific genes that are inherited over many generations. Alterations in amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2) genes have been associated with developing this form. PS1 mutations account for most FAD compare with APP and PS2 genes (Pericak-Vance et al., 1991). Most of AD models carry on or more gene mutations associated with FAD. Such models provided researchers with important insight into AD because both early-onset and late-onset AD are the same disease even though the difference in onset age and genetic cause (Price & Sisodia, 1998).

1.2.2 Late- onset AD (Sporadic Alzheimer's disease (SAD)):

SAD is the most common form of AD that begins after age 60-65. SAD has no specific family link; elderly people might develop this form just as they might develop diabetes,

cancer or other health problem. The late onset of AD is developed due to a complex combination of genes, environmental factors, and lifestyle. Researchers have found many susceptibility genes associated with increase the risk of developing SAD. For example, the ε4 allele of apolipoprotein E (APOE) has been found to play a role in SAD pathogenesis by affecting Aβ aggregation and clearance (J. Kim, Basak, & Holtzman, 2009). Similarly, Lambert et al. and Harold and colleagues found an association between SAD and genetic markers in three genes: clusterin (CLU), complement receptor 1 (CR1), and phosphatidylinositol binding clathrin assembly protein (PICALM). Since an effective treatment for this form has not been reached yet, prevention strategies are suggested to prevent or delay the onset of the disease. Some prevention strategies are regular exercise, healthy diet, quality sleep, stress management, mental stimulation, and social engagement (Solomon et al., 2014).

1.3 Alzheimer's disease-associated pathology

1.3.1 Amyloid-β (Aβ) plaques

One of the hallmarks of Alzheimer's disease is the accumulation of amyloid plaques between the neurons in the brain. These plaques are proteinaceous extracellular deposits consisting of 38-43 amino acid residues, referred to as an Aβ peptide. The peptide appears at first in a soluble form in the interstitial fluid, then aggregating over time into an insoluble form called the senile plaques. These plaques are the key neuropathological hallmark lesions defining AD (Wippold et al., 2008).

The Aβ peptide is formed by abnormal proteolytic processing of the transmembrane amyloid precursor protein (APP). Although the primary function of this protein is not

well known, it has been thought to play a role in synaptic formation and repair (Thinakaran & Koo, 2008). For the most part, APP is processed through the α- secretase pathway, which snips APP and produces a soluble molecule (APPsα) and the carboxyl-terminal C83. The latter is further cleaved by γ- secretase and release non-aggregation fragments that release intracellular and extracellular domains. Under some pathological conditions, APP is processed by an abnormal β- secretase pathway. This process begins when β-secretase slices APP into a soluble fragment (APPsβ) and the membrane-bound fragment C99. Aβ is produced when C99 is further cleaved by γ-secretase, which releases Aβ fragments into the interstitial fluid and APP C-terminal into intercellular domain (Fig1). Aβ fragments aggregate into more complex forms called senile plaques. Both the soluble fragment and insoluble plaque forms of Aβ exhibited neurotoxicity (Braak & Del Trecidi, 2015).

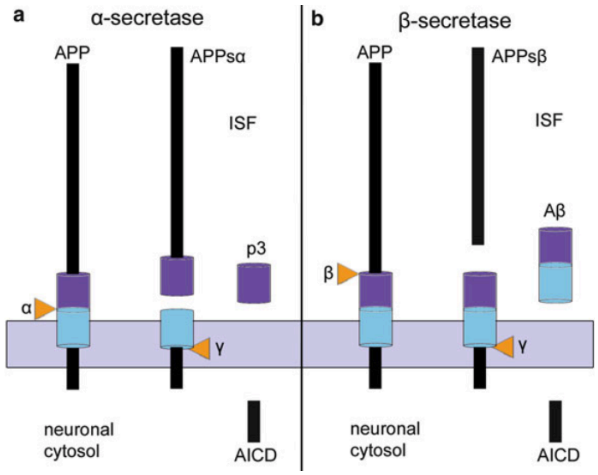

Figure 1. Two processing pathways for the amyloid precursor protein. (a) The normal pathway utilizing α-secretase prevents the formation of Aβ and only produces p3 while, in (b), processing with β-secretase leads to the production of Aβ. The pathway displayed in (b) only occurs in a few vulnerable types of nerve cells. Trafficking and proteolytic processing of APP. Abbreviations: AICD APP intracellular C-terminal domain, APP amyloid precursor protein, APPsα soluble α-remnant of APP, APPsβ soluble β-remnant of APP, ISF interstitial fluid. Reprinted with permission from (Braak & Del Trecidi, 2015), copyright (2015).

The plaques are found in different forms. Diffused plaques are a collection of Aβ peptides that are found in an irregular organization, lacking a dense internal core. These plaques are not surround by dystrophic neurites, and glial reaction. Unlike diffused plaques, neuritic plaques consist of a central compact core of fibrillar Aβ peptide that is neighbored by dystrophic neurites and activated glial cells. As presence of neuritic plaques is associated with synaptic loss and cognitive impairment, they are recognized as one of the neuropathological hallmarks of AD (Wippold et al., 2008).

1.3.2 Neurofibrillary Tangles

Neurofibrillary tangles (NFTs) are another pathologic hallmark associated with AD. Unlike the Aβ plaques, NFTs are formed inside the neurons and consist primarily of twisted fibers called tau protein. Neuron is held together by cytoskeleton that is made of microtubules. The function of microtubules is to give the shape to the cell and transport nutrients and other important substances through the cell. Tau protein acts like railway ties to keep microtubules stabilized. In AD, the microtubule-associated tau protein undergoes hyperphosphorylation (Wippold et al., 2008). Thus, the tau protein is disaggregated into filaments that clump up and form the neurofibrillary tangles. Neurons with tangles and unattached microtubules lose their ability to communicate, which may ultimately result in cell death. Although tangles were found in other neurodegenerative diseases, it is considered to be an essential biomarker of AD (Braak & Del Trecidi, 2015).

1.4 Mouse models of Alzheimer's disease

Many animal models have been generated to study AD mechanisms and evaluation of potential AD therapeutics. These animals should be able to reproduce some of the neuropathological and cognitive changes that characterized the disorder (Gotz, Bodea, & Goedert, 2018). APP mouse models are the commonest models in AD research because of their practical approach for in vivo screening and validation of preventive medications. There are two generations of APP mice. The first generation are a collection created by a random integration of a single or multi mutated genes associated with the familial form of AD; this generation exhibit AD pathology with a limitation due to an overexpression of APP, resulting in an over production of Aβ and other APP fragments. Thus, it would be challenging to differentiate between the impact of Aβ and other overproduced fragments. The second generation was developed using a knock-in technique to overcome the problems of the previous generation. By introducing the FAD mutated gene or genes into a specific locus in the murine APP gene, a natural biological expression of APP will occur. Such a pattern is found in the APP^{NL-G-F} mouse model (Sasaguri et al., 2017).

1.4.1 APP(NL-G-F) mouse model of Alzheimer's

Aβ plaques are a critical biomarker in AD. Thus, using AD models displaying Aβ will allow for the understanding of the underlying mechanisms of AD. Unlike previous generations of AD mice models, the APP(NL-G-F) is a model that exhibits APP at wild-type levels overcoming the artifacts that may affect interpretation of the results because of the APP overexpression paradigm. APP(NL-G-F) model was generated by applying the APP knock-in strategy. Humanized Aβ sequence and three FAD mutations APP

KM670/671NL (Swedish), APP1716F (Iberian), and APP E693G (Arctic) were introduced into the mouse APP gene. Thus, this model develops Aβ pathology and an increased Aβ42/Aβ40 ratio without over expressing APP (Nilsson, Saito, & Saido, 2014). In addition, it recapitulates most of AD pathology in an age depending manner. Cortical deposition of amyloid plaques begins to show up in mouse brain at 2 months and saturate by 6 months, where they were accompanied by activated microglia cells. Synaptic loss and neuroinflammation are also present in both hippocampus and cortex. Beside the molecular pathologies, the APP(NL-G-F) model exhibits various cognitive deficits starting at 6 months; specifically, a spatial memory deficit was detected using the Y-maze. Some limitations are presented in this model. Despite the humanization of Aβ sequence, the identity of the amino acid between human and mouse amyloid precursor protein (APP) is 96.6%, which may lead the mouse APP to behave differently from human APP. For instance, APP variants in KPI domain-containing APP are not expressed in mouse brain unlike in human brain (Sasaguri et al., 2017). The absence of neurofibrillary tangles and neuronal loss also count as a limitation of this model. However, this model is still considered invaluable to understand the disease mechanism and test possible therapeutics (Mehla et al., 2019).

2. Alzheimer's disease and stress

Given that most cases of AD are irreversible and untreatable, it is important to determine the factors that predispose individuals to the disease. There are many theories regarding the etiology of Alzheimer's disease, but no consensus has been reached. For example, amyloid cascade hypothesis suggests that amyloid-β protein, the main former of Amyloid plaques, is a causative factor of AD pathology. It contributes in neurofibrillary tangles formation, cell loss, vascular damage that seen in AD (Hardy & Higgins, 1992). Others believe that interaction of different risk factors, such as age, genetic background, amyloid plaques, neurofibrillary tangles, inflammation, oxidative stress, cholinergic depletion, and stroke may lead to develop AD (McDonald, 2002). Although aging is the greatest risk factor for AD, the lifetime stress experience could be a contributing factor for the development of this disease.

Many studies have examined the association between stress, as a measurement of by high cortisol levels, and AD-like changes. Wilson et al. (2003) reported that, elderly people who were prone to experience psychological distress are more likely to develop AD than their age-equivalent, non-stressed individuals. Another longitudinal study found that higher hypothalamic-pituitary-adrenal (HPA) axis activity, as reflected by high cortisol levels, is associated with more rapid disease progression in subjects with Alzheimer-type dementia (Csernansky et al., 2006).

Stress activates a biological reaction in the HPA axis, in which cortisol is the ultimate result of its activation. Activation of the HPA axis is started by the release of the corticotrophin releasing hormone (CRH) which induces the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. ACTH travels down into

the adrenal cortex on the top of the kidney to stimulate the release of the glucocorticoid (GCs) hormones. It has been shown that long term exposure to stress causes an increase in overall cortisol output, which travels through bloodstream back to the brain, and may lead to the impairment of cognitive and neuronal functions (McDonald, 2002). The summary of the literature by Machado, Herrera et al. (2014) suggests that chronic stress may stimulate inflammatory activities in the brain and disrupt sleep which might contribute to amyloid deposition. Furthermore, the authors found that stress speeds up the aging process, facilitates Aβ formation that accelerates hyperphosphorylation of tau protein, and increases the level of oxidative stress contributing to the early onset of AD. Taken together, chronic stress and high GCs levels should be considered as a risk factor in the onset and development of AD.

2.1 Noise as a stressor:

Noise can be defined as any sound that causes annoyance and interferes with task performance for instance, household items and transport vehicles are among the common daily noise. Being annoyed is a sign of stress, which means noise can evoke stress response like any other stressor (Stansfeld & Matheson, 2003).

Prolonged exposure to noise causes longer-lasting activation of the HPA axis, which may cause many problems because of the increase in the levels of stress hormones. The HPA axis receives auditory input via amygdala which is the brain structure responsible for encoding, storing, and the expression of fear memory. The lateral nucleus of the amygdala (LA) receives auditory input from two pathways. The first, via the auditory thalamic nucleus, delivers fast but poor quality information about the stimulus. The

second input is from primary auditory cortex through auditory association areas; this path is slower than the thalamic pathway but provides a precise representation about the stimulus. In response to auditory stimulus, LA integrates the information from both pathways to acquire the whole picture about this stimulus, and based on nature of this stimuli, the LA decides whether or not to process this information further and evoke the HPA axis (Eggermont, 2014). The HPA axis includes several anatomical structures found in both the central nervous system and the endocrine system. These structures include the paraventricular nucleus (PVN) of the hypothalamus which, in the face of aversive stimuli, releases the CRH that initiates a cascade of events ending in glucocorticoid release from the adrenal gland. Other regions such as brain stem noradrenergic neurons, sympathetic andrenomedullary circuits, and parasympathetic systems also contribute in regulation of stress response, by transmitting a wide range of sensory modalities into neurons of the PVN and influence CRH expression. Inadequate or excessive activation of the HPA axis disturbs hormones balance and elevates the cortisol levels, in which the general health and wellness become threatened (Smith & Vale, 2006).

In both human and animal population, a growing body of evidence links high cortisol levels to chronic exposure to external noise. Healthy industrial laborers working in noisy environments (>85 dB) exhibited heightened in the basal cortisol levels concurrent with irritability and fatigue at the end of the work day. The same workers showed significant decline in cortisol into its normal diurnal rhythm after using earmuffs for one week, in which the noise decreased by 33 dB (Melamed & Bruhis, 1996). In agreement with previous findings, animal studies revealed elevation in both ACTH and corticosterone in rats that were exposed to noise at different intensities (80, 85, 90, 95, 100, 105, and 110

dB) for 30 min in the presence of 60 dB of noisy background (Burow, Day, & Campeau, 2005).

2.3 General Adaptation Syndrome (GAS) theory of stress

Dr. Hans Selye is known as the father of the field of stress research, pioneering research on the biological effects of exposure to nocuous agents or stress. In 1936, Hans Selye created the general adaptation syndrome (GAS) model based on his rat experiments; he found that rats experience a typical syndrome in response to nocuous agents such as surgical injury, intoxications, or cold exposure. Dr. Selyle named this syndrome "general adaptation syndrome" and described its three stages: alarm, adaptation, and exhaustion. Briefly, in the first two stages, the body tries to adapt to the damaging agent through different symptoms depending on the nature of the agent or the drug employed type. If the injury was relatively slight, the animals will build up resistance and the organs functions return to normal. However, with severely damaging agents, the animals cannot maintain the resistance for a long time, and health problems and even death might occur. This stage in general adaptation syndrome is called exhaustion stage (Selye, 1998).

Hans Selye's general adaptation syndrome explains how our body responds to stress (Szabo, Tache, & Somogyi, 2012). The body senses the stress and tries to keep the homeostasis by showing stereotyped physiological effects like shrinkage in the spleen, lymph nodes, thymus, and adrenal gland enlargement. If the stress is severe or chronic, the body ends up in the exhaustion stage. Based on Selyes view, during the exhaustion stage, the neuroendocrine substrates that are released to protect the body are depleted with persistent stress, and this what increases the body's vulnerability to diseases. In 1995,

this hypothesis was modified by McEwen and Sapolsky, in which they proposed that the weakness that happens to the body during exhaustion stage is not because of depletion of adrenal hormones, but because of the persevered secretion of these hormones (McEwen & Sapolsky, 1995). (Fig2).

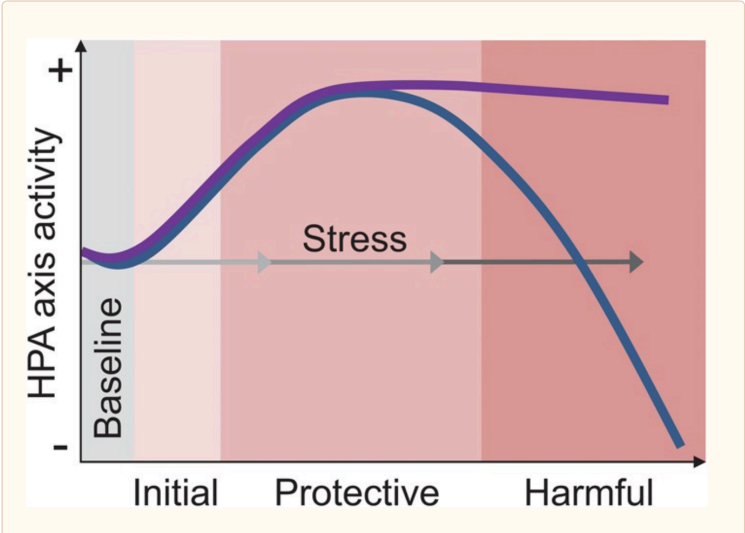

Figure 2. Two HPA axis-based models of stress. Selye's (1936) general adaptation syndrome proposed that the depletion of neuroendocrine factors with persistent stress makes the body susceptible to diseases (blue line). In contrast, current views hypothesize that abnormally elevated and/or long lasting neuroendocrine activity causes harm to the body (purple line). The three gray-gradient arrows represent mild, moderate, and sever stress. Reprinted with permission from (E. J. Kim, Pellman, & Kim, 2015), copyright (2015).

2.4 Glucocorticoids hypothesis of stress

Selye's model of the general adaptation syndrome is the current glucocorticoid-based hypotheses. The glucocorticoid cascade hypothesis of stress was formulated to explain a relationship between the activity of HPA axis in

response to stress and the adverse consequences of glucocorticoids actions upon the neurons and cognition (McEwen & Sapolsky, 1995). Glucocorticoids have been reported as mediators of stress effects on the hippocampus and many psychopathologies (E. J. Kim et al., 2015).

Countless stress studies have demonstrated that prolonged exposure to stress could shift the glucocorticoid actions from protective to harmful (E. J. Kim et al., 2015). CORT hormone acts through two types of receptors, mineralocorticoid (MR) and glucocorticoid receptors (GR). MR and GR differ in their CORT affinity and their expression in the brain areas. While MR demonstrates a higher affinity than GR by 10-folds, GR expresses more widely in the brain especially through the limbic system (Finsterwald & Alberini, 2014). Both receptors work in coordination with other stress mediators like CRH and autonomic nervous system to help the body cope with stress and keep homeostatic of the body. Under basal circulating CORT levels, the high-affinity receptors are extensively saturated. However, during stress when CORT levels increase, the low-affinity receptors GR start to be occupied along with MR. The continuous activation of GR receptors not only found to impair LTP (Pavlides, Watanabe, Magarinos, & McEwen, 1995), but also mediates the negative effects of severe or chronic stress on hippocampal morphology and function (Alfarez, Wiegert, Joels, & Krugers, 2002). Stress also causes MR: GR imbalance in the limbic system, which in turn alter the function of circuits underlying emotion, fear, memory, reward, and regulation of the hypothalamic–pituitary–adrenal (HPA) axis (Finsterwald & Alberini, 2014). Accordingly, it is believed that the

glucocorticoids secretion in response to severe or chronic stress may increase the brain's vulnerability to the insults, that in turn may contribute to development of some neurodegenerative diseases like Alzheimer's disease (Sterner & Kalynchuk, 2010).

3. The Present Study: Chronic Traffic Noise Exposure Contributes to Emergence of Cognitive and Motor Functions Impairment and Increases Amyloid-β Deposition in Mouse Model of Alzheimer's Disease.

3.1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that considers the most common form among dementia types. The annual incidence rate increases from about 1% in the aged population of 56 years to more than 6% among people over age 85 (Mayeux, 2003). AD characterized by extracellular accumulation of amyloid-β (Aβ) peptide in the brain due to disruption of the homeostatic mechanisms that regulate the proteolytic cleavage of the amyloid precursor protein (APP). Together with hyper phosphorylation of the microtubule-associated Tau protein in neurons, they activate a cascade of neurotoxicity that ultimately leads to cytoskeletal changes, neuronal dysfunction and cellular death that manifest with severe cognitive impairment and dementia in end-stage cases (Mohandas, Rajmohan, & Raghunath, 2009).

Several factors are reported to increase the risk of AD including chronic stress and noise exposure. Individuals who passed through stressful life events are more prone to develop AD than others (Wilson et al., 2006). Higher levels of cortisol associated with noise and stress are accompanied by atrophy of the hippocampus and prefrontal cortex, structures that regulate learning and memory (Rocher, Spedding, Munoz, & Jay, 2004) and impair emotion, cognition and spatial memory in both rodents and humans (Cui & Li, 2013; Cui, Wu, She, & Liu, 2012). Chronic stress is also shown to modulate motor performance (Metz, 2007), another independent symptom of cognitive impairment that uses as a functional impairment predictor of AD (Buchman & Bennett, 2011). At the molecular

level, the accumulation of amyloid plaques between the neurons causes widespread neuronal death, synaptic loss, perturbed long-term potentiation (LTP) and neural plasticity (J. J. Kim & Diamond, 2002; J. J. Kim, Foy, & Thompson, 1996) A repeated stress exposure accelerates the process of the amyloid plaques formation in different brain regions that modulate stress response like area in limbic system and prefrontal lobe (Jafari, Mehla, Kolb, & Mohajerani, 2018). In this context, a study using 3xTg-AD mice found that stress-level glucocorticoid administration promotes Aβ formation in these mice at earlier ages (Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006). Similarly, noise stimulates the nervous system and triggers stress responses and induces many non-auditory health problems, such as sleep disturbance, hypertension, cognitive decline, and cardiovascular diseases (Basner et al., 2014; Jafari, Kolb, & Mohajerani, 2018). All these stressors predispose to AD.

Although, several studies have measured the effect of auditory stresses (acute, moderate, and chronic), on animals (Cheng, Wang, Chen, & Liao, 2011; Cui et al., 2015; Tamura et al., 2012), few of the current studies addressed the role of traffic noise as a model of stress in the development of AD. A recent study by Hong Chen and his colleagues, shows that people living next to major roadways have a higher incidence of dementia (Chen et al., 2017). To further understand the relation between AD and traffic noise, we studied the impact of chronic noise exposure on APPNL-G-F mouse model of AD, the newly developed model that expresses APP at wild type levels, overcoming by this the problem of APP overexpression, the phenomena that presents in previous APP transgenic mouse models. These mice develop part of AD-associated pathologies, including amyloid plaques, synaptic loss, and microgliosis and astrocytosis which recapitulate the

neuropathology observed in patients with AD. In addition, they exhibit an age dependent impairment in cognition and memory starting at age 6 months. (Saito et al. 2014; Mehla et al. 2018). This line will allow us to study the effect of traffic noise exposure as a model of stress on CORT hormone, behavioral deficits, and Aβ pathology away from the impact of other features and artifacts that present in most previous models, such as APP over expression.

3. 2 Material and methods

3.2.1 Animals

Twenty-one APPNL-G-F mice (11female,10 male) were used in compliance with the Canadian Council of Animal Care and the regulations of the Province of Alberta and the University of Lethbridge Animal Welfare Committee. Food and water were available for the duration of the experiments. The animals were housed 3–4 per cage in standard shoe-box cages, in temperature and the humidity-controlled room with a 12 h light: dark cycle.

3.2.2 Experimental design

Traffic noise exposure Procedure. When the mice reached age 2 months, they were randomly assigned into two groups, control group (n=11, 5male and 6 female) and traffic noise group (n=10, 5 male and 5 female). Traffic noise (TN) exposer was done by transferring the TN group cages from the housing room into another room and placing inside a sound chamber. Traffic noise generated by a speaker at 75 dB, 8h/day from 8:00 A.M. - 4:00 P.M. for 30 days (Tamura et al., 2012) (Cui et al., 2015). At the same time,

their unstressed counterpart's cages were transferred into another room inside sound chamber and kept undisturbed.

3.2.3 Plasma CORT assay

Blood collection from the submandibular vein was performed a day before and after noise exposure at the beginning of the light period (8 a.m.) (Barriga, Martin, Tabla, Ortega, & Rodriguez, 2001). A swift lancing motion was used to puncture the vein without the use of the anesthesia. An approximately 0.1 ml of submandibular blood was collected in heparin-coated tubes. The samples were centrifuged at 6000 rpm at 4 °C for 15 min to obtain the plasma which was stored at −80 °C until further analysis (Browne et al., 2014; Golde, Gollobin, & Rodriguez, 2005). For the quantitative determination of Corticosterone in plasma (ng/ml), a commercially available ELISA kit was used according to the manufacturer's instructions (Jafari, Mehla, Afrashteh, Kolb, & Mohajerani, 2017). A microplate reader (Synergy HT BioTek) was used to read CORT absorbance at 450 nm wavelength. KC4 Bio-Tek® Microplate Data Collection and Analysis software were used in the determination of corticosterone concentration in samples. The coefficient of variation for all samples was determined using the same standards and controls across all plates to reduce intra-plate variability (Barriga et al., 2001; Jafari, Mehla, Afrashteh, et al., 2017; Malisch et al., 2007).

3.2.4 Behavioral assessments

A battery of standard tests was used to assess different behaviors related to AD throughout the study. These behavioral tests are pre-pulse inhibition (PPI), novel object recognition (NOR), elevated plus-maze (EPM), rotarod test(RRT), balance beam test

(BBT), and Morris water task (MWT). All Behavioral tests were conducted once when the mice have 4 months of age, except MWT and PPI, they were also performed in the beginning of the experiment when the animals are 2 months old (Fig. 3). This step was done to confirm that both groups are the same in terms of spatial memory and cognition before starting traffic noise paradigm, also to use them as a reference to compare the animal performance before and after TN exposure. The behavioral tests were carried out respectively as mentioned above, and no more one test a day.

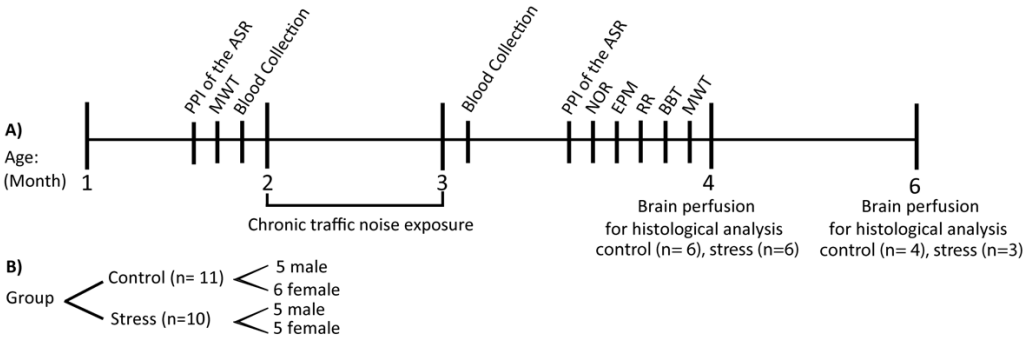

Figure 3. Experiment design and flowchart of animal group: A) Timeline of procedure, PPI and MWT were performed before the blood collection, which was conducted a day before stress paradigm and repeated a day after. Stress group was exposed to Traffic noise exposure(TNE) for one month at the same time control group was kept undisturbed. At 4 months, PPI and MWT were reran plus another four behavioral tests which are NOR, EPM, RR, and BBT. Lastly, mice were sacrificed for further histological analysis at two time points, at age 4months (5 control, 6 stress), and at age 6 months(4control,3stress). B) The flow chart of animal distribution, 21 mice were randomly divided into two groups, 11 mice (5 male and 6 female) were assigned as a control group, and the rest of the mice were considered as a stress group, they were 10 mice (5 male and 5 female).

3.2.4.1 Pre-pulse inhibition (PPI)

Pre-pulse inhibition is a test used to assess learning, and sensory gating; the ability of an animal to suppress irrelative sensitive, motor, and cognitive information (Valsamis &

Schmid, 2011). We measured the animal's ability to attenuate their response to a startle stimulus (startle pulse) preceded by a non-startle stimulus(pre-pulse). PANLAB Harvard Apparatus was used (Fig. 4), the test started with acclimation period, in which the animal was placed in a holder inside the acoustic box and left for 3-5 minutes, this stage helps the animal to adapt to the environment. Habituation followed accumulation period, when the animal received 10 startle stimuli (110 dB, and each stimulus 20 ms duration with a 1 ms rise/fall time) in presence of a constant background throughout the session(65dB). Immediately afterward is pre-pulse inhibition phase; different acoustic stimuli were repetitively introduced to the animal in pseudo-random order (30s ITI), ten trials from each stimulus (40 in total) (Shoji, Takao, Hattori, & Miyakawa, 2016). These trials are 10 startle pulse (the same one used in habituation phase), 10 pre-pulse (80dB,8kHz,20ms), 10 pre-pulse pulse (pre-pulse preceding startle pulse), and 10 trials where there is no stimulus. The animal movement as a result to the stimulus was detected and converts into a voltage signal and stored in the computer hard drive. The data was used to calculate pre-pulse inhibition(%) using the following equation (Longenecker & Galazyuk, 2011)

$$\text{PPI \%} = \frac{\text{Pulse} - \text{Prepulse Pulse}}{\text{Pulse}} \times 100$$

Figure 4. pre-pulse inhibition experiment. (Left) the PANLAB Harvard Apparatus consists of four acoustic startle boxes connected to the computer hard drive. (Right) shown the set up inside the acoustic startle box.

3.2.4.2 Rotarod test

Rotarod test is used to assess motor performance in rodent (Deacon, 2013). The apparatus consists of a horizontal rod, which automatically accelerates at different speeds. The rod is divided into 4 sections using flanges (22 cm), in which four animals can be ran at the same time (Fig. 5). Falling plate sensor is placed below the running rod to monitor each animal's latency-to-fall separately. On the testing day, mice were gently placed on the rod, and the mice latency (sec) to maintain themselves on the accelerating rod before falling off was recorded. Three speeds (8, 16, and 4-40 rpm) were conducted in three different days, and in each speed, the animal underwent 3 trials with 3-5min inter-trial interval, the average of the trials was calculated and used to compare the groups (Brooks & Dunnett, 2009).

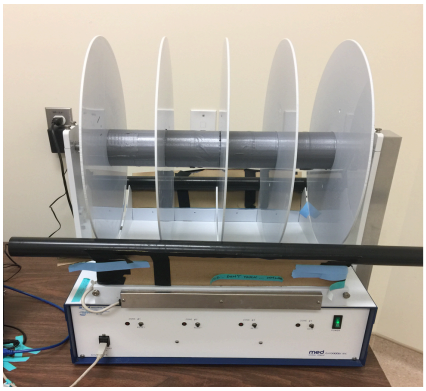

Figure 5. Rotarod test apparatus. The rotarod consists of a circular rod divided into four sections, and turning at a constant or increasing speed.

3.2.4.3 Novel object recognition (NOR) test

Novel object recognition test is used to assess recognition memory, the ability of the

animal to recognize the familiar object from the new one. In brief, the test is ran in a white square box (47 cm width × 50 cm length × 30 cm height) (Fig.6). During the familiarization phase (Fig.6, Phase A), the mouse was placed in the box in presence of two similar objects in opposite and symmetrical corners and given 5min to explore them. Then, the mouse was returned into his home cage. 5 min later, the test phase (Fig.6, Phase A) was conduct by replacing one of the familiar objects with a new one, and return the mice to the box for 3min of exploration. The second phase was filmed (30 frames/second) to calculate the time that mouse spent with the old object and the time with the new one (Jafari, Kolb, et al., 2018).

Phase A **Phase B**

Figure 6. The Novel Object Recognition Test. (Left) the training phase, the animals explore two identical objects. (Right) the testing phase, the animal is exposed to the familiar object from the training phase and a novel object.

3.2.4.4 Elevated plus maze (EPM) test

Elevated plus maze test is used to measure anxiety response in a rodent. The test exploits the struggling between animal fear from high open spaces and their innate behavior to

explore novel environments. The apparatus is made of a matte black Plexiglas consisting of two crossed arms 48 cm above the ground, resulting in four arms meeting in the center. Two symmetrical arms is enclosed by 21cm high walls, and two symmetrical open arms without walls, each arm is 5 cm in width and 27 cm in length (Fig.7). The test was recording using a digital camera fixed at the end of the open arm. The session started by placing the mouse with his front paws in middle of one of the open arms facing the maze center and recorded for 5 min. Time that mouse spent in each arm, and the number of his entries to each arm was calculated (Jafari, Faraji, et al., 2017).

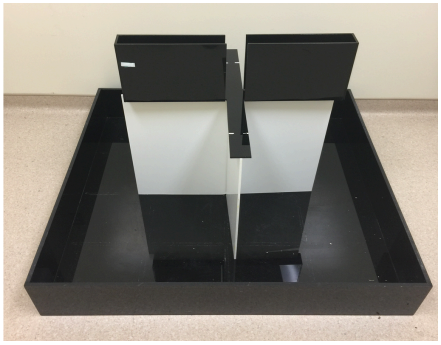

Figure 7. The elevated plus maze apparatus. It consists of elevated platform with two open arms and two closed arms.

3.2.4.5 Balance Beam Test (BBT)

Balance beam test is used to assess balance and motor coordination. The test consists of a round beam (1 cm diameter, 100 cm long) that is elevated 50cm above the ground (Fig.8). Mice were encouraged to cross the beam and reach a black box at the end of the beam. Mice were pre-trained a day before the actual test to reduce the natural aversion of the mice to cross over unprotected places, and this will help to assess motor coordination accurately. In the actual test, 3 trials for each mouse were conducted and used to calculate

latency to cross the beam and the number of foot slips (Stover, Campbell, Van Winssen, & Brown, 2015; Tamura et al., 2012).

3.2.4.6 Morris water maze(MWM)

Morris water maze test is used to evaluate learning and spatial memory (Vorhees & Williams, 2006). The task is carried out in a circular white tank (153cm in diameter) in which was divided imaginary into four quadrants by two perpendicular axes creating four starting positions for the trials, North(N), South(S), East(E), and West(W). 10-12cm2 circular platform was placed inside the pool in the north-east quadrant. The pool was filled with water~1.0 cm above the platform at 23± 1 °C. Un-toxic white paint was added to the water to hide the platform. Animals in this task rely on three distal cues around the pool to navigate into the hidden platform (Fig.9). On each day of the eight acquisition days, the animal underwent four trials to locate the hidden platform. The trial begins by placing the mouse facing the wall in the water from the starting position N, W, E, and S in turn. A 60s was given to the mouse to find the platform. However, the trial was stopped if the mouse found the platform before passing the given time. On the first two acquisition days, if the mouse failed to find the platform within the given 60s, he was guided into it and left for 30s. About 5 minutes was a given interval between the trials. Several measurements such as escape latency (sec), swimming path (m), and swimming speed(m/s) were monitored using an automated tracking system (HVS Image Hampton, U.K.) (Baldi, Efoudebe, Lorenzini, & Bucherelli, 2005; Jafari, Mehla, Kolb, & Mohajerani, 2017).

Retention or memory recall was evaluated using a probe test that was done 24hours after

the last training day (on the day 9). During a probe test (60s in duration), the platform was removed out of the pool, and the mouse performed the task only from the east starting position. The percentage of the time that mouse spent in the platform quadrant (NE) was calculated, and compared with the spent time in the other three quadrants (Maei, Zaslavsky, Teixeira, & Frankland, 2009).

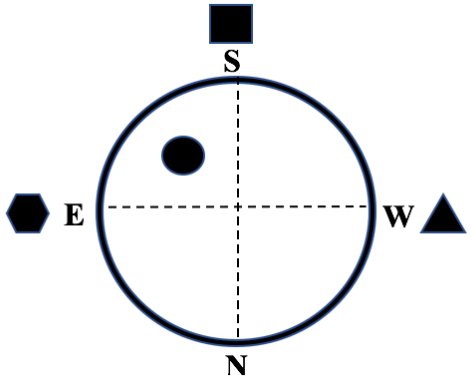

Figure 8. Schematic representation of Morris water maze test. The maze consists of circular pool divided imaginary into four quadrants. The hidden platform sits in the east south quadrant. The test rans in present of three different visual ques round the pool.

3.2.5 Histology

24h before perfusion, the amyloid-β plaques detector (Methoxy XO4 fluorescent dye) was intraperitoneally injected into the animal based on its weight (10 mg/kg). Then the animal was sacrificed using an overdose of i.p Euthansol. After the death was assured, animal's body was pinned out on cork board. Precisely, the heart was exposed by cutting through the abdomen and the rib cage. A small incision cut in the right atrium was done using sharp scissors. At the same time (~50 ml) 0.9% phosphate buffered saline (PBS) was

pumping into the left ventricle, followed by (~50ml) of 4% paraformaldehyde (PFA), until the blood turned to nearly clear. Once completed, the head was detached from the body and brain was carefully extracted. The brain was fixed by immersion 24h in 4%PFA followed by 48h in 30% Sucrose. Brain was sliced using cryostat and placed on the top of Superfrost Microscope slides (Faraji et al., 2017). NanoZoomer (Hamamatsu Photonics K.K.,Japan) was used to image the brain sections for the detection and quantification of amyloid-β plaques.

3.2.5.1 Quantification of amyloid-β (Aβ) plaques

Six brain sections were chosen ,these sections correspond with Bregma ~3.20, ~2.96, ~0.98, ~ -2.06, ~ - 3.08, ~ -5.34mm based on the mouse brain atlas (Paxinos & Franklin, 2001; Spijker, 2011) (Fig.16B). The percentage of the area covered by plaques and the number of plaques were calculated in the whole brain sections (Saito et al., 2014) (Fig. 16D-16E).The plaque area(%) also was calculated in different brain regions of interest (i.e. frontal pole, olfactory area, isocortex, Anterior cingulate area, nucleus accumbens, Hippocampus, posterior parietal area, cortical amygdala area, entorhinal area, retrosplenial area, hindbrain, and midbrain) (Fig. 16F). The quantification of plaque area (%) was done in both hemispheres using ImageJ 1.4.3.67 software (Moon et al., 2009). The image of the brain section of interest was uploaded in ImageJ and using required options under “Edit”, “Image”, “Process”, and “Analyze” tabs. The number of plaques in the brain section was quantified using ilastik-1.3.2rc2 software (Jafari, Kolb, et al., 2018).

3.2.6 Statistical analysis

All data were analyzed using SPSS Statistics 24.0 The level of statistical significance was sat to $P < 0.05$ for all tests. The results were presented as the mean ± SEM. Analysis of variance (ANOVA) was used to determine the statistically significant difference between stress and control groups regarding different depended variable of behavioral tests, CORT assay, plaques area (%), and plaques number. Repeated measures (ANOVA) was conducted to compare MWT, PPI, and CORT in each group at two-time points. The F-values, P-values, estimations of the effect size (partial $\eta 2$), and observed power were informed for the statistical analyses. The Bonferroni test was used as post hoc test to compare group mean in different measurements. For all behavioral tests, the number of mice was: control =11; stress=10, while the number of animals in histology part was: at age 4 months (control= 5; stress= 6), at age 6 months (control= 4; stress= 3).

3.3 Results

There were not sex-associated differences in either behavioral and histological assessments ($P \geq 0.218$). Thus, male and female in each of control and stress groups were pooled together.

3. 3.1 The effect of TNE on plasma corticosterone levels

The basal corticosterone level (ng/ ml) before traffic noise exposure was the same among the control and traffic noise groups ($P= 0.991$). The traffic noise group exhibited elevated corticosterone levels after 30 days of traffic noise exposure compare to both the control group and their first assessment ($P < 0.001$) (Fig. 9A).

3.3.2 Behavioral tests results

3.3.2.1 The effect of TNE on cognition and motor coordination

No difference was noticed in the startle stimulus response (ASR) between the control and traffic noise group before and after the TNE paradigm (P= 0.788, P= 0.643 respectively). However, the ASR significantly reduced in both groups at age 4months (post-stress) compare to when they were at age 2months (pre-stress) ($P \leq 0.01$) (Fig. 9B2). In respect of PPI (%), the difference was significant between the two groups after TNE exposure, which was higher in the control group (P= 0.01) (Fig. 9B1).

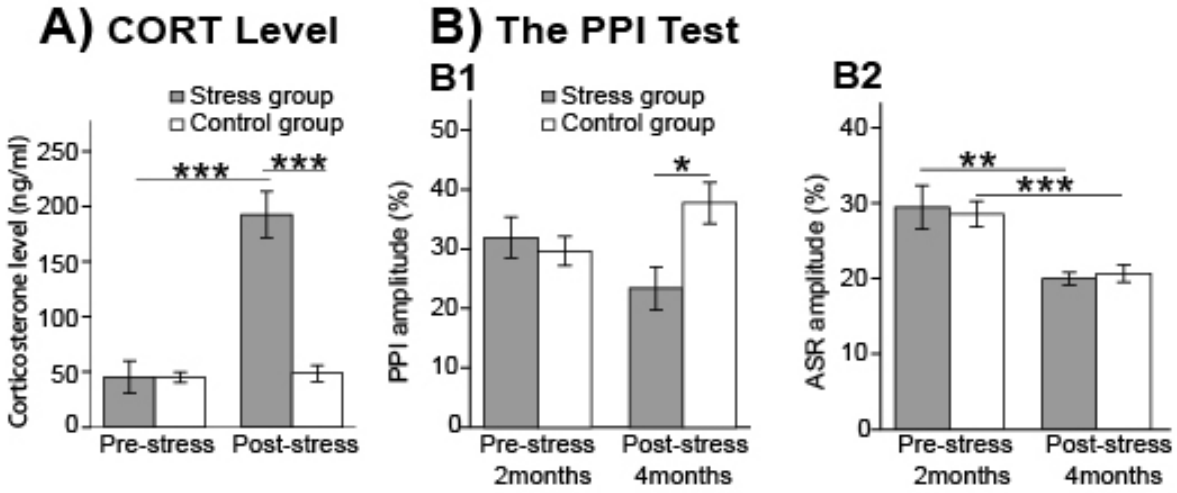

Figure 9. The CORT levels and PPI results. A) The corticosterone (CORT) levels(ng/ml) before and after traffic noise exposure (TNE). It was similar in both groups before TNE and rose significantly in stress group after TNE compare to the control group and the first measurement as well. B) Pre-pulse inhibition and startle amplitudes (%): B1) Percent PPI was identical among both groups pre-stress and significantly increased in stress group compare to control group after stress exposure. B2) No difference observed among the groups before and after TNE in startle response, the significant difference was exhibited between the first and second measures, in which ASR percentage was lower in the second assessment. Data was shown as the mean ± S. E. M. (*P<0.05, **P<0.01***P<0.001)

3.3.2.2 Novel object recognition

During the test phase, control group spent significantly greater time exploring the novel object than the previously explored object ($P \leq 0.001$), while the traffic noise group spent significantly longer time with the familiar object ($P= 0.003$). The old-time ratio was significantly higher in the traffic noise group compared to the control group ($P \leq 0.001$) (Fig. 10A-C).

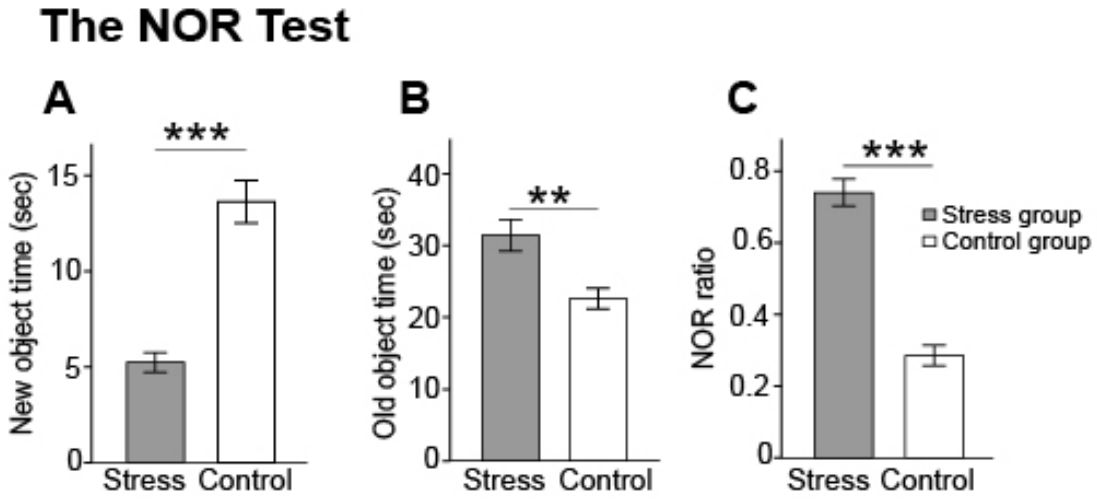

Figure 10**.** The novel object recognition results: A) The control group showed significantly longer time exploring the novel object B) Time spent exploring the familiar object was significantly greater in the stress group C) Old object time ratio was significantly higher in stress group. Data was shown as the mean ± S. E. M. (*P<0.05, **P<0.01***P<0.001)

3.3.2.3 Elevated plus maze test

The traffic noise group significantly spent a higher time in the closed arm ($P= 0.002$), and

a lower time in the open arm ($P \leq 0.001$) in comparison with their controls. The noise group was also showed significantly less number of entries into the open arm ($P= 0.036$) (Fig. 11A-C).

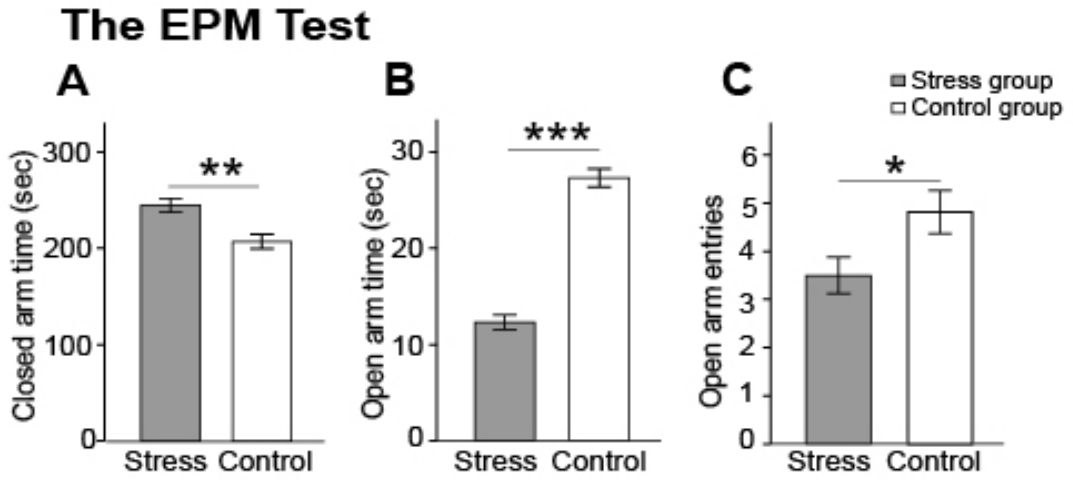

Figure 11. The elevated plus maze results: A) A significant higher stay time(sec) in the closed arm, and B) lower spent time in the open arm, and C) less number of entries into the open arm for stress group compare with respective controls. Data was shown as the mean ± S. E. M. (*P<0.05, **P<0.01***P<0.001)

3.3.2.4 Rotarod test

The results expressed the time (sec) that mice remain on the accelerating rod, and this time was significantly lower in the traffic noise group compared to their controls in all the 8,16, and 4-40 (rpm) speeds ($P \leq 0.001$ in all of them) (Fig. 12A)

3.3.2.5 Balance beam test

The traffic noise group showed significantly longer time(sec) to cross the beam ($P \leq 0.001$), and recorded a higher number of foot slips while crossing the beam ($P \leq 0.001$) compare to the control group (Fig. 12B1 and B2).

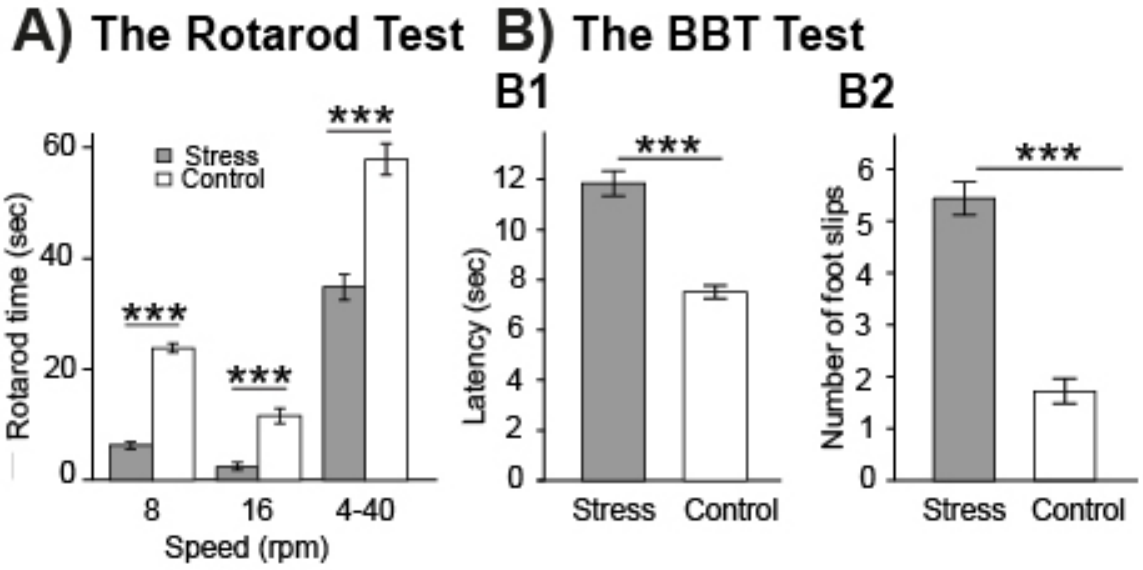

Figure 12**.** The RR and BBT results. A) The rotarod: Stress group significantly exhibited shorter rotarod latency (sec) overall the speeds compare to control group. B) The balance beam: B1) Stress group recorded significantly greater latency, and B2) more foot slips than control group. Data was shown as the mean ± S. E. M. (*P<0.05, **P<0.01***P<0.001)

3.3.2.6 Morris water test

At 2 months of age (pre-stress), Both chosen groups expressed an identical latency(sec), traveled distance and speed, and we did this step to use the data as a reference and to make sure that there was no difference between the chosen groups in their spatial memory ability from the beginning. After TNE, even though both groups significantly exhibited

daily improvement in their latency and the selected path to find the platform ($P \leq 0.01$), traffic noise group significantly demonstrated longer latency and path throughout all the acquisition days ($P \leq 0.001$). No significant differences were observed between the groups in swim speed (data not presented) ($P= 0.06$), (Fig13. A and B). Regarding probe test post stress, both traffic noise and control group significantly stayed longer time (%) in the target quadrant than the other three ($P \leq 0.001$). There was a significant difference between the control and traffic noise group in the target quadrant time (%), in which it was less in the traffic noise group ($P=0.001$), (Fig. 13C). In first trial difference, traffic noise group showed no significant difference between the latency on day one and day eight, while the difference was significantly in control group($P=0.027$), (Fig.13D)

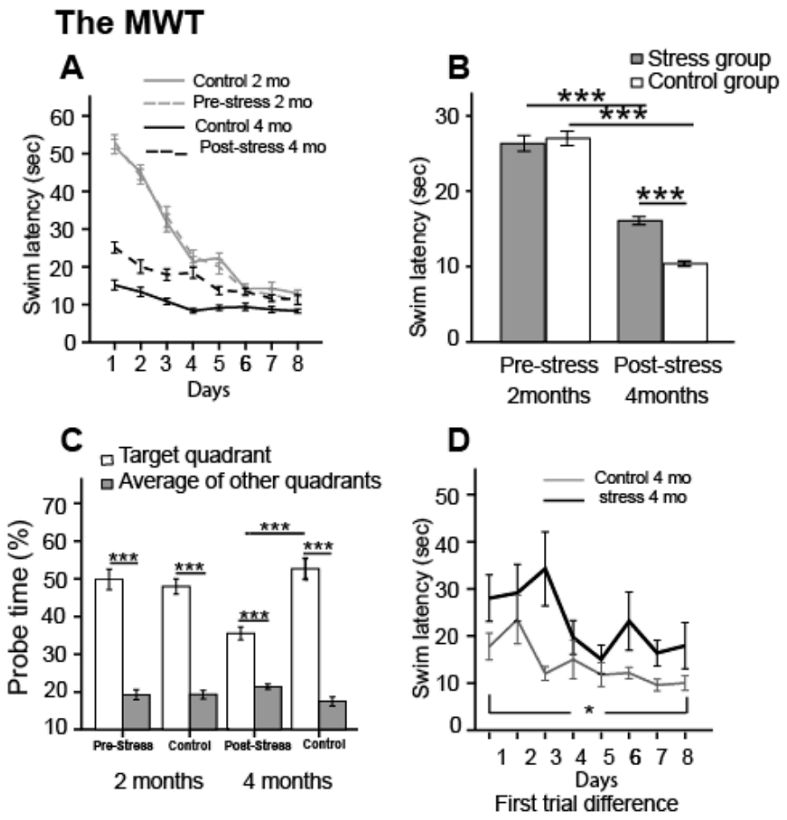

Figure 13**.** The Morris water results: A) No difference was observed between stress and control group in the escape latency at 2 months (pre- stress), while stress group shows significantly longer latency than control group after exposure to TNE. B) The averaged time of the eight training days for both groups pre-and post-stress exposure, in which stress group spent significantly greater time to find the platform post-stress. Latency was significantly reduced for both groups at 4months in comparison with 2 month. C) Probe time (%), stress group spent significantly less time in the target quadrant compare to their controls in the second assessment. Both groups at both assessment stages significantly spent higher time in the target quadrant compare to the averaged time of the other three quadrants. D) First trial difference over the eight training days post stress. Data was shown as the mean ± S. E. M. (*P<0.05, **P<0.01***P<0.001)

3.3.3 Histology

A deposition of Amyloid-β plaques was exhibited in all brain sections. At age 4 months, the plaque area (%) and the number of plaques were significantly higher in the traffic noise group than the control group in all brain sections except the sixth one (P≤ 0.024, P≤

0.041, Fig. 15D-15E). Similarly, the plaque area (%) was also higher in all chosen brain regions except the hindbrain and midbrain regions. (P≤ 0.054, Fig. 15D). At age 6 months, the plaques area (%) was higher in the traffic noise group than the control group in all six brain sections. (P≤ 0.032, Fig.16 C1-C6).

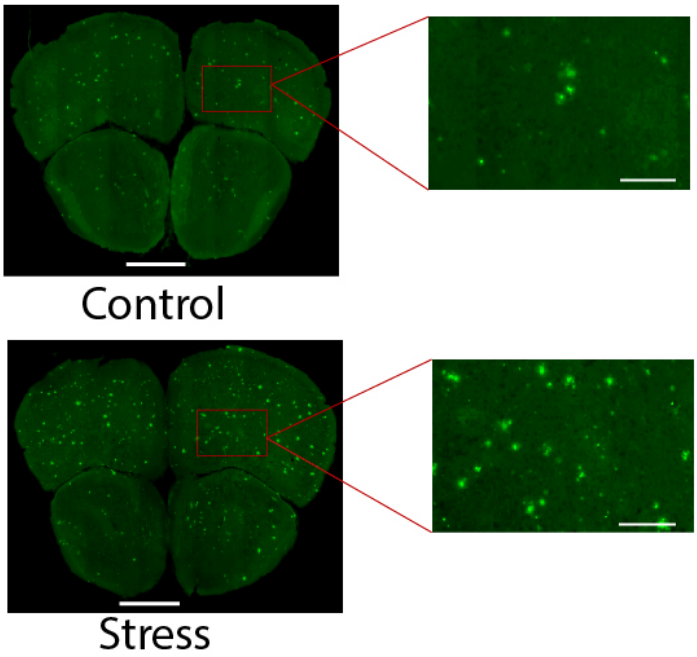

Figure 14. Brain sections samples illustrate Aβ plaques at age 4 months in the control and stress group. Scale bar is 2.5 mm

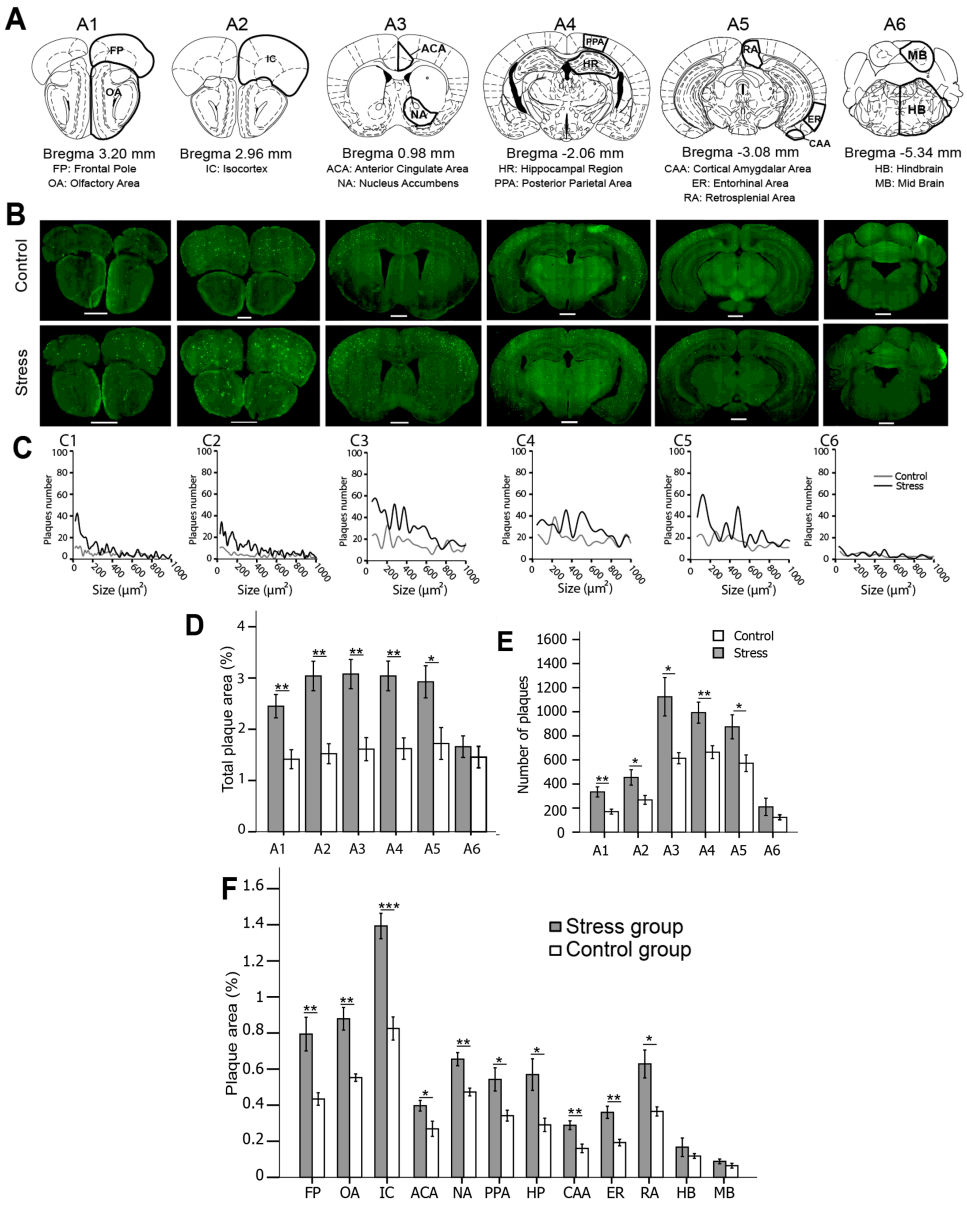

Figure 15. The Aβ plaques area (%) quantification at 4 months (5 control, 6 stress): A) (A1-A6: Coronal mice brain sections correspond with bregma ~3.20, ~2.96, ~0.98, ~ -2.06, ~ - 3.08, ~ -5.34mm were chosen to quantify Aβ plaques. B) Brain sections samples from control and stress group corresponding to the chosen bregmas. C) Distribution of plaque size in the chosen brain sections. D-E) The Aβ plaque area (%) and the number of plaques in the chosen brain sections. F) The percentage of Aβ plaques area in some brain regions. Aβ, FP, frontal ploe; OA, olfactory area; IC, isocortex; ACA, anterior cingulate area; NA, nucleus accumbens; PPA, posterior parietal area; HR, hippocampal region; CAA, cortical amygdalar area; EA, entorhinal area; RA, retrosplenial area; HB, hindbrain; MB, midbrain. Results represented as mean ± S.E.M. (*P<0.05 or **P<0.01). Scale bar: 2.5 mm.

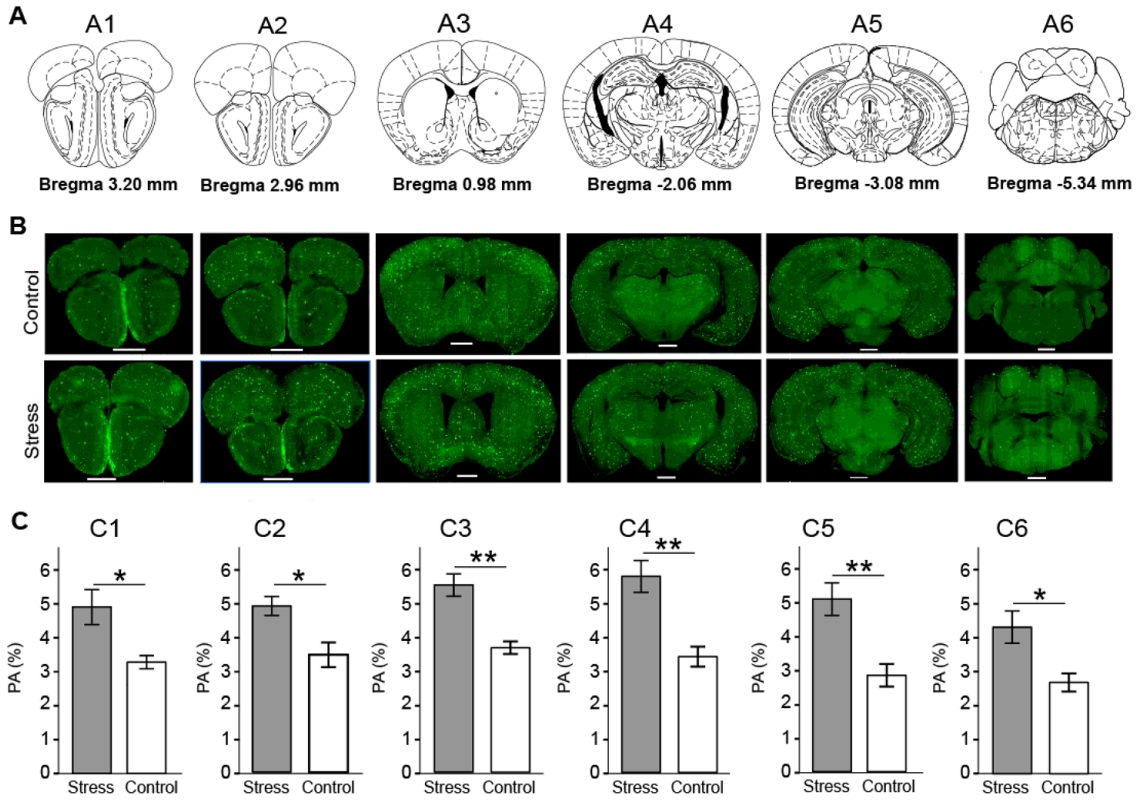

Figure 16 **.** The Aβ plaques area (%) quantification at 6 months (3stress, 4control): A) (A1-A6: Coronal mice brain sections correspond with bregma ~3.20, ~2.96, ~0.98, ~ -2.06, ~ -3.08, ~ -5.34mm were chosen to quantify Aβ plaques area (%). B) Brain sections samples from control and stress group. C) (C1-C2) The Aβ plaques area (%) in the chosen brain sections. Results represented as mean ± S.E.M. (*P<0.05 or **P<0.01). Scale bar: 2.5 mm.

3.4 Discussion

We studied the effect of traffic noise as a model of stress on development of AD, our subject was knock-in APPNL-G-F mouse, the newly adapted model by Alzheimer's researchers. The results showed that chronic traffic noise exposure caused an elevation in plasma corticosterone levels, impaired cognitive, motor coordination, and spatial learning in male and female mice. Chronic traffic noise also promoted the production of Aβ plaques in different brain regions. Sex differences were not observed; thus, the results were pooled together.

3.4.1 TNS elevated CORT hormone levels:

There is a large body of evidence indicates that the effect of stress on HPA axis may be implicated in the progression of AD (Csernansky et al., 2006; Weiner, Vobach, Olsson, Svetlik, & Risser, 1997). Our results showed a significant increase in plasma corticosterone in the stress group compared to the control group after stress exposure. Patients with sporadic AD were found to have elevated cortisol in the early stages of the disease. This association between high cortisol levels and the severity of cognitive decline has been documented in AD patients (Pedersen, Wan, & Mattson, 2001). In addition, both corticosterone and dexamethasone administration increased the levels of APP, β-C-terminal fragment, and both forms of Aβ ($A\beta_{40}$ and $A\beta_{42}$) in the tissues which may indicate an increase in Aβ plaques formation (Green et al., 2006). Our findings were consistent with a study done by Zhihui and his collogues using rats which showed a significant increase in corticosterone after 30 days of noise exposure. They also reported a decrease in corticosterone levels 2 months after mild stressors were applied for several weeks in transgenic AD mice (TG2576) (Cuadrado-Tejedor et al., 2012), which indicates that results can vary by the time of assessment and stressors used.

3.4.2 TNS caused behavioral deficits:

Beside the hormonal changes, animals exposed to stress exhibited a decline in PPI amplitude in which demonstrates a deficit in sensorimotor gating and reflects an inadequate organization of cognitive resources. Deficits in PPI were observed in both patients and animal models of some neuropsychiatric diseases (Valsamis & Schmid, 2011). This decline was attributed in other researches to entorhinal cortex damage, the

first anatomical region to be affected in patients with AD (Goto, Ueki, Iso, & Morita, 2002). In contrast, a human study found that there were no differences in the PPI amplitude between AD patients and healthy controls (Hejl, Glenthoj, Mackeprang, Hemmingsen, & Waldemar, 2004). This could be attributed to other factors such as the anxiety level that modulate PPI (De la Casa, Mena, & Ruiz-Salas, 2016), the threshold for detecting noise (Ford et al., 1995), and the interval time between the pre-pulse and startle pulse, which might not be enough to evoke attentional mechanisms (Braff & Geyer, 1990). In regard to the ASR, no significant differences were observed between the stress and the control group before and after exposure to traffic noise. However, the ASR in both groups decreased significantly post stress exposure at 4 months. The reduction in ASR amplitude is normal with aging, and it is consistent with studies that support reported slower neuronal process speed, global attentional capacity and decreased startle response with age (Ellwanger, Geyer, & Braff, 2003; Kok, 2000; Krauter, Wallace, & Campbell, 1981; Parham & Willott, 1988).

We also measured the novel objects recognition capacity of mice to evaluate cognition. Normally , animals exhibit tendency to explore new objects than previously presented ones (Antunes & Biala, 2012). In our study, stress group spent less time with the novel objects and longer time with the familiar ones. This poor exploration performance of the stressed group indicates a traffic noise effect on the NOR test. The deterioration in NOR was found previously to be related to entorhinal and perirhinal cortex lesions (Norman & Eacott, 2005). Other brain structures such as hippocampus, nucleus accumbance (Sargolini, Roullet, Oliverio, & Mele, 2003), and nucleus baselis (Bartolini, Casamenti, & Pepeu, 1996) also have been found to play a role in the response to NOR test.

Interestingly, these regions have been shown to be damaged at the early stages in animal models of AD (Mustafiz et al., 2011; Tamagnini et al., 2012). The NOR test is a reliable test that does not require reinforcement stimuli and is widely used to evaluate new drugs for AD (Zhang et al., 2012).

Furthermore, higher anxiety level is considered a remarkable symptom of AD and some other neuropsychiatric diseases. In this study, stressed animals exhibited signs of anxiety which was demonstrated by an increase in the time spent by mice in closed arms in comparison to open arms, as well as the increased number of entries to the closed arms. On the other hand, there were no differences observed between the two groups in locomotive behaviors. Thus, our EPM data is in favor of higher anxiety-like behavior in the stress group (Walf & Frye, 2007).

Motor function impairment is another non-cognitive symptom recognized in AD patients. Here, we show that animal in the stress group revealed a deficiency in both RR and BBT motor tasks. In RR, they stayed less time on the rod before they fell of the rode in all used speeds (8, 16, 4-40 rpm). Similarly, stressed animals took longer time to cross the beam and made more foot slips compared to non-stressed ones using the BBT. Results by clinical studies, indicated that dysfunction in fine motor dexterity may serve as early phenotypic marker of AD (Buchman & Bennett, 2011). The control of different aspects of movement such as posture, balance, and speed requires integration of sensory, visuospatial, and cognitive information which is processed by multiple interconnected cortical and subcortical motor regions (Bakker et al., 2008; Rosano, Brach, Longstreth, & Newman, 2006). Consequently, damage in these motor-related brain regions and/or loss of the links between them lead to motor dysfunction (Agosta et al., 2010). The

development of AD pathology in motor regions causes motor function deficits (Buchman & Bennett, 2011). In addition, stress hormones has also been suggested as a factor that modulate the motor performance (Metz, Schwab, & Welzl, 2001) (Harle et al., 2017). However, the significance of motor dysfunction as an early sign of AD is still controversial (Albers et al., 2015).

Beside the motor and cognitive functions, we also evaluated the learning and spatial memory using MWT. The test demonstrated that animals exposed to stress has a defective learning and spatial memory, as they spent longer time to find the hidden platform. stressed animal also showed long term learning deficits as they show no improvement in first trial latency over the training days (Vorhees & Williams, 2006) (Tomas Pereira & Burwell, 2015). To eliminate the effect of motor function impairment on the results of the test, we looked at the traveled distance difference, and we found that stress mice took longer path to locate the platform, which indicates that they had difficulties to remember the location of the hidden platform. These results are consistent with previous results done in our lab (Jafari, Kolb, et al., 2018; Jafari, Mehla, et al., 2018) and other research findings (Cuadrado-Tejedor et al., 2012; Han et al., 2017). In contrast, others reported that stress has no effect on the learning and spatial memory (Luine, Martinez, Villegas, Magarinos, & McEwen, 1996) (Conrad, 2010). Of note, the differences in the used stressor (mild, acute, and chronic) and training time are all critical factors that could cause the inconsistency between the experiments. In addition, retention memory was also affected in stressed animals, which spent less time in the target quadrant in comparison to non-stressed groups. Previous animal research shown that higher levels of CORT hormone and increased Aβ burden in the hippocampus and surrounding brain areas could mediate the decline in MWT performance. (J. J. Kim & Diamond, 2002; Landfield,

Waymire, & Lynch, 1978; McEwen & Magarinos, 2001). Of note, we did not measure CORT hormone at the time of behavioral tests, but even if the hormone levels decreased over the time, we expect that the damage has been done during the times when the CORT levels were high.

3.4.3 TNS exacerbated deposition of Aβ:

Long term exposure to stress were not only associated with behavioural deficits, it also accelerates the production of AD neuropathology. In this study, Aβ plaques area (%) and plaques number were measured at 4 months in six coronal brain sections including the cortical and subcortical regions essential in the response to stress and the development of AD. Aβ deposition was significantly higher in most sections taken from stressed mice except in the sixth section taken from the posterior part of the brain which is believed to be the least part affected by plaques in AD. Similar findings have been demonstrated in a study using (Tg2576) mouse model of AD, they found that exposure to stress for six months exacerbates Aβ plaques production, with more plaques developed in the cortex and hippocampus (Dong et al., 2004). Previous studies found that higher levels of cortisol could augment Aβ formation. A recent study found that corticotropin-releasing factor (CRF) and CRF1 receptors, that regulate the stress-induced glucocorticoids, could mediate Aβ production in AD Tg2576 mice. CRF1antagonists treatment blocked the effect of stress and led to a significant decrease in Aβ levels and Aβ deposition as well (Park et al., 2015). Another study using 3×Tg-AD mice demonstrated that corticosterone administration mediates Aβ formation by increasing either APP production or cleaving process of this protein (Green et al., 2006) . Another human study revealed a strong association between cortisol levels, cortical burden of Aβ, and global cognition, in which

they found that cognitively normal older people with Aβ and high level of cortisol suffer faster cognitive decline than those with lower cortisol levels. Suggesting that higher levels of cortisol plus Aβ burden have a combined affect in cognitive decline that has been seen in preclinical stage of AD (Pietrzak et al., 2017).

3.5 Conclusion and future direction

In conclusion, the findings of this study imply that long term exposure to traffic noise could have a cumulative effect on AD-like pathology and cognitive deficits in a mouse model of AD. Since the strain that we used containing mutated genes associated with familial AD, our results and conclusion are relevant to the familiar AD and can't be extended to the sporadic AD. In agreement with previous stress studies (Cuadrado-Tejedor et al., 2012; Cui et al., 2015; Jafari, Kolb, et al., 2018), we found that chronic traffic noise remarkably increased the levels of corticosterone hormone, negatively affected learning and memory, caused anxiety-like behavior, and disturbed balance and motor function. Besides, it enhanced the production of Aβ pathology, which was determined by measuring the percentage of plaque area and counting the number of plaques as well. Our combined results suggest that chronic traffic noise exposure could be one of the environmental risk factors that lead to the early onset and accelerate the development of AD. Further studies are needed to consider the relative effectiveness of some elements like the intensity and duration of exposure, alterations in corticosterone hormone days after exposure, the role of microglia and astrocyte cells, and using other AD models to study the effect of traffic noise on other AD pathology like Neurofibrillary tangles.

Summary of results

Table 1. Results of the Univariate ANOVA in comparing the two groups in corticosterone levels and different measures of the behavioral tests

	Significant Main Effects			
CORT Level (ng/ml)	**F**	**P**	**η^2**	**Power**
Day before TNE	000	0.991	000	0.050
Day after TNE	42.498	***≤0.001	0.810	1000
PPI of the ASR Test				
ASR amplitude (%) before TNE	0.074	0.788	0.004	0.058
ASR amplitude (%) after TNE	0.222	0.643	0.012	0.073
PPI amplitude (%) before TNE	0.292	0.565	0.015	0.081
PPI amplitude (%) after TNE	8.224	*0.010	0.302	0.777
NOR Test				
New object time (sec)	34.551	***≤0.001	0.696	.1.000
Old object time (sec)	11.373	**0.003	0.374	0.892
NOR ratio	95.300	***≤0.001	0.834	1.000
EPM Test				
Closed arm time(sec)	13.477	**0.002	0.415	0.936
Open arm time(sec)	147.231	***≤0.001	0.886	1.000
Open arm entries	5.066	*0.036	0.211	0.570
RR Test				
8 rpm speed	252.833	***≤0.001	0.930	1.000
16 rpm speed	36.694	***≤0.001	0.659	1.000
4-40 rpm speed	39.400	***≤0.001	0.675	1.000
BBT				
Latency(sec)	63.416	***≤0.001	0.769	1.000
Foot slips	90.191	***≤0.001	0.826	1.000
MWT				
Latency (sec) before TNE	0.103	0.748	0.000	0.062
Distance (m) before TNE	0.148	0.700	0.000	0.067
Speed(m/s) before TNE	0.440	0.508	0.001	0.102
Probe time (%) before TNE	0.315	0.581	0.017	0.083
Latency (sec) after TNE	85.154	***≤0.001	0.118	1.000
Distance (m) after TNE	38.238	***≤0.001	0.057	1.000
Speed(m/s) after TNE	3.824	0.060	0.006	0.497

CORT, Corticosterone; ASR, acoustic startle reflex; PPI, prepulse inhibition; BBT, balance beam test; TNE, traffic noise exposure; MWT, Morris water task; NOR, novel object recognition; RR, rotarod; η^2, effect size. Asterisks indicate *P<0.05, **P<0.01, or ***P<0.001.

Table 2. Results of the repeated measures ANOVA to compare the repeat of the CORT assay, PPI of the ASR, and MWT before and after TNE per group

	Significant Main Effects			
CORT Level (ng/ml)	**F**	**P**	**η^2**	**Power**
Control group	0.180	0.680	0.016	0.067
Stress group	33.232	***≤0.001	0.769	0.999
PPI of ASR in the control group				
PPI amplitude (%)	3.603	0.072	0.153	0.439
ASR amplitude (%)	14.390	***0.001	0.418	0.950
PPI of ASR in the stress group				
PPI amplitude (%)	2.923	0.104	0.140	0.367
ASR amplitude (%)	9.828	**0.006	0.353	0.842
MWT in the control group				
Latency (sec)	265.574	***≤0.001	0.275	1.000
Distance travelled (m)	131.561	***≤0.001	0.158	1.000
Speed (m/s)	34.765	***≤0.001	0.047	1.000
Probe time (%)	1.894	0.184	0.086	0.259
MWT in the stress group				
Latency (sec)	67.282	***≤0.001	0.100	1.000
Distance travelled (m)	29.543	***≤0.001	0.047	1.000
Speed (m/s)	8.268	**0.004	0.014	0.819
Probe time (%)	20.239	***≤0.001	0.558	0.988

CORT, Corticosterone; ASR, acoustic startle reflex; PPI, prepulse inhibition; BBT, balance beam test; TNE, traffic noise exposure; MWT, Morris water task; NOR, novel object recognition; RR, rotarod; η^2, effect size. Asterisks indicate *P<0.05, **P<0.01, or ***P<0.001.

Table 3. Results of the Univariate ANOVA for comparing the two groups in Aβ plaque quantifications at 4 and 6 months.

Age) Four months	**F**	**P**	**η^2**	**Power**
A1)				
Total plaque area (%)	11.735	**0.008	0.660	0.861
Number of plaques	10.868	**0.009	0.547	0.834
Plaque area in FP (%)	11.153	**0.009	0.553	0.843
Plaque area in OA (%)	11.596	**0.008	0.563	0.857
A2)				
Total plaque area (%)	17.477	**0.002	0.660	0.959
Number of plaques	5.659	*0.041	0.386	0.564
Plaque area in IC (%)	34.158	***≤0.001	0.791	0.999
A3)				
Total plaque area (%)	15.287	**0.004	0.629	0.934
Number of plaques	7.924	*0.020	0.468	0.708
Plaque area in ACA (%)	6.541	*0.031	0.421	0.626
Plaque area in NA (%)	16.435	**0.003	0.646	0.949
A4)				
Total plaque area (%)	14.563	**0.004	0.618	0.923
Number of plaques	9.238	**0.014	0.507	0.772
Plaque area in PPA (%)	6.956	*0.027	0.436	0.652
Plaque area in HF (%)	7.420	*0.023	0.452	0.680
A5)				
Total plaque area (%)	7.294	*0.024	0.448	0.672
Number of plaques	5.713	*0.041	0.388	0.568
Plaque area in CAA (%)	13.996	**0.005	0.609	0.913
Plaque area in ER (%)	16.848	**0.003	0.652	0.953
Plaque area in RA (%)	8.829	*0.016	0.495	0.753
A6)				
Total plaque area (%)	0.457	0.516	0.048	0.093
Number of plaques	1.120	0.317	0.111	0.158
Plaque area in HB (%)	0.708	0.422	0.073	0.117
Plaque area in MB (%)	1.577	0.241	0.149	0.203
Age) Six months				
A1) Total plaque area (%)	11.020	*0.021	0.688	0.756
A2) Total plaque area (%)	8.763	*0.032	0.637	0.661
A3) Total plaque area (%)	26.985	**0.003	0.844	0.982
A4) Total plaque area (%)	20.148	**0.006	0.801	0.943
A5) Total plaque area (%)	16.040	**0.010	0.762	0.888
A6) Total plaque area (%)	10.282	*0.024	0.673	0.728

FP, frontal pole; OA, olfactory area; IC, isocortex; ACA, anterior cingulate area; NA, nucleus accumbens; PPA, posterior parietal area; HR, hippocampal region; CAA, cortical amygdalar area; EA, entorhinal area; RA, retrosplenial area; HB, hindbrain; MB, midbrain; η^2, effect size; Asterisks indicate *P<0.05, **P<0.01 or ***P<0.01

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