Chapter 1

INTRODUCTION

1 Introduction

Post-traumatic stress disorder (PTSD) is described as the complex somatic, cognitive, affective, and behavioural effects of psychological trauma (Atwoli et al., 2015). It is characterized by intrusive thoughts, nightmares and flashbacks of past traumatic events, avoidance of reminders of trauma, hypervigilance, and sleep disturbance, all of which lead to considerable social, occupational, and interpersonal dysfunction (Atwoli et al., 2015).

1.2 Epidemiology

PTSD is a chronic incapacitating disorder with a 3.9% lifetime prevalence rate worldwide and a 6.4–7.8% rate in the USA (Pietrzak et al., 2011, Koenen et al., 2017). Trauma exposure is a required criterion for PTSD diagnosis, only 5.6% worldwide (8.3% in the USA) of those who experienced trauma developed the disorder (Koenen et al., 2017). This is due to numerous factors, including trauma type, variation in trauma response, social support, and individuals' endogenous factors.

1.3 PTSD in DSM-V

A Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criterion for PTSD is significantly different from DSM-IV. As per DSM-V, the stressor criterion (Criterion A) is more explicit concerning how an individual experienced “traumatic” events. Also, Criterion A2 (subjective reaction) has been eliminated. Whereas there were three major symptom clusters in DSM-IV: re-experiencing, avoidance/numbing, and arousal (Friedman et al., 2011, Kilpatrick et al., 2013), which are now four symptom clusters in DSM-V because the avoidance/ numbing cluster is divided into two distinct clusters: avoidance and persistent negative alterations in cognitions and mood (Friedman et al., 2011, Kilpatrick et al., 2013). It also includes irritable or aggressive behaviour and reckless or self-destructive behaviour. PTSD is now developmentally sensitive in that

diagnostic thresholds have been lowered for children and adolescents. Furthermore, separate criteria have been added for children age six years or younger with this disorder (Friedman et al., 2011, Kilpatrick et al., 2013).

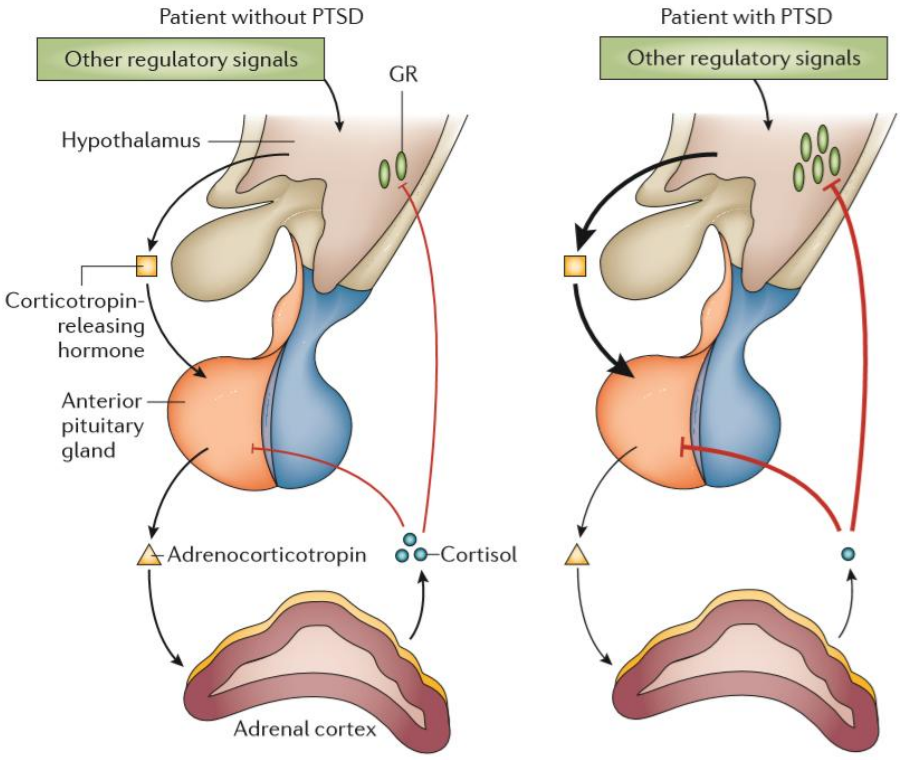
1.4 Pathophysiology of PTSD

1.4.1 Hypothalamic-pituitary-adrenal-axis (HPA-axis) theory of PTSD

The signs and symptoms of PTSD might reflect a persevering adaptation of the underlying neurobiological mechanisms to cope with extreme life-threatening situations. Recent research studies offer a very complex picture of the neurobiological dynamic of PTSD (Akiki et al., 2017). It mainly focus on exploring different aspects of neurobiological changes in PTSD patients, and its correlation with the clinical PTSD symptoms such as hyperarousal, impulsivity and cognitive deficits (Vermetten and Bremner, 2002).

PTSD is characterized by abnormal regulation of hormones in the HPA-axis (the center of the mammalian neuroendocrine stress response) (Daskalakis et al., 2013). During stress exposure, neurons in the paraventricular nucleus (PVN) secrete corticotrophin releasing hormone (CRH) which stimulates production and release of adrenocorticotrophin (ACTH) into the anterior pituitary. It is transported to the adrenal gland, where the hormone glucocorticoids (cortisol/corticosterone) are produced (Daskalakis et al., 2013). These glucocorticoids are stimulated by ACTH and released by the adrenal cortex (Yehuda and Antelman, 1993, Heim and Nemeroff, 2001). Glucocorticoids plays a key role in physiological management of stress, as it participate in regulating immunity, metabolism and brain functions (Drouin et al., 1992). Glucocorticoids activates norepinephrine system (NE), (sympathetic nervous system, SNS) and send negative feed-back to HPA-axis to reinstate homeostasis in stressful situation (Daskalakis et al., 2013). However, during PTSD, the HPA-axis functions are disrupted due to increased

glucocorticoid receptor (GR)-mediated response which down-regulate cortisol production (Drouin et al., 1992), which fails to reset the HPA-axis homeostasis (Yehuda and Antelman, 1993). Such failure leads to lowering of cortisol level with elevated levels monoamines (serotonin and NE) (Daskalakis et al., 2013). Previous study in human with PTSD suggested that the activation of the HPA-axis leads to sustained increase in level of CRH with hypocortisolism (Yehuda and Antelman, 1993). These above changes may cause oversensitive response to traumatic event and associated with fear reponse.



Yehuda R. Post-traumatic stress disorder. New England journal of medicine. 2002 Jan 10;346(2):108-14.

Figure 1.1 HPA-axis disruption in the pathophysiology of PTSD

1.4.2 Neurochemical and neuroanatomical theory of PTSD

The clinical hallmark of PTSD is the involvement of cortico-limbic dysfunction, which includes the prefrontal cortex (PFC), hippocampus (HIP), and amygdala (AMY) (Quirk and Mueller, 2008). Such neuroanatomical and functional changes in PFC, HIP, and AMY are also observed in PTSD animals (Krishnamurthy et al., 2013). PFC and HIP

inhibits while AMY activates HPA axis mediated corticosterone release (Wang et al., 2014). Any abnormalities in the PFC-AMY pathway lead to impairment of extinction fear response and associated memories (Koenigs and Grafman, 2009, Milad et al., 2009), whereas dysfunction in the HIP-PFC pathway leads to poor learning process, which is involved in controlling fear response (Acheson et al., 2012).

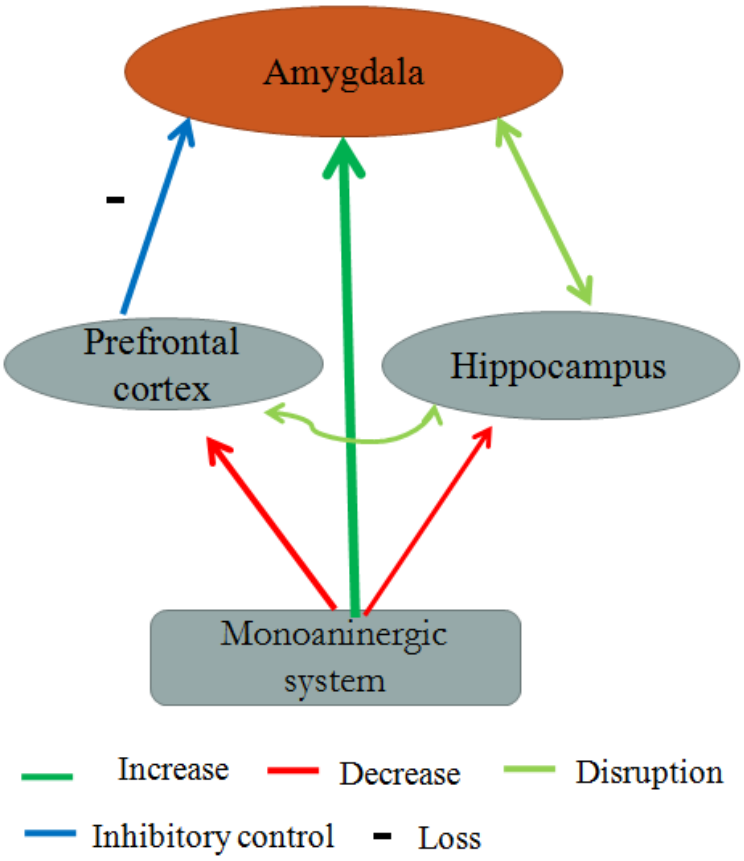


Figure 1.2 the dynamic relationship between AMY, PFC, and HIP. Green and red arrow represents the increased and decreased action of the monoaminergic system, respectively. The Blue arrow with a minus sign represents the loss of inhibitory control over AMY. The light green arrow represents the disruption of the projection of HIP with AMY and PFC.

Further, alteration of the monoaminergic system was also identified in PTSD patients that may disturb the dynamic relationship between AMY and PFC, contributing to the development of vigilance, impulsivity and intrusion of memories (Sherin and Nemeroff, 2011). Serotonin (5-HT) originates in the brainstem and projects to different areas of brain such as the AMY, the HIP and the PFC that are crucial in the processing of traumatic experiences (Kelmendi et al., 2016). Further, increased levels of catecholamines

such as dopamine (DA) and norepinephrine (NE) are also responsible for symptoms such as increased startle response and arousal (Sherin and Nemeroff, 2011).

1.4.3 Pathology of PTSD Experimental models

The pathophysiology of PTSD includes abnormalities in HPA-axis function and the monoaminergic system. Monoaminergic theory of PTSD is well-validated and the current treatment strategies are based on it. However, targeting monoaminergic systems is useful only for 30 percent of PTSD patients due to its complex pathology (Alexander, 2012). Therefore, there is a need to ascertain a new pathology that can be a therapeutic target for PTSD. Recent evidence suggests that mitochondrial dysfunction is one of the pathological causes of PTSD (Garabadu et al., 2015). Krishanamurthy et al. have demonstrated mitochondrial dysfunction in the animal model of PTSD. Further, study has shown improvement of mitochondrial function mitigates the morphological changes in different brain regions and improves the neurobehavioural outcome of PTSD (Krishnamurthy et al., 2013). It has been reported that there is an aberrant expression of mitochondrial respiratory chain enzymes in different brain regions in the single prolonged stress (SPS) model of PTSD (Xing et al., 2013). Atypical respiratory enzyme activities could lead to increased reactive oxygen species (ROS) production and may cause a loss in the mitochondrial integrity through a decrease in the mitochondrial membrane potential (MMP) (Zangar et al., 2004, Xing et al., 2013). A decrease in MMP can cause leakage of the cytochrome-C (Zangar et al., 2004), which activates factors related to intrinsic pathway of apoptosis (Shi, 2001). Several authors have reported the apoptosis in the HIP, PFC, and amygdalar tissues of PTSD-subjected rodents (Zangar et al., 2004, Li et al., 2013). Therefore, based on evidence, mitochondrial dysfunction and associated changes may be pharmacologically amenable to drugs in PTSD.

1.5 Pharmacotherapy of PTSD

The Food and Drug Administration (FDA) approved drugs for PTSD are selective serotonin reuptake inhibitors (SSRIs), sertraline, and paroxetine. Clinical studies have shown the superiority of paroxetine in managing the main cluster symptoms of PTSD (Marshall et al., 2001, Jeffereys, 2012). Further, the use of paroxetine is safer compared to other SSRIs as it produces a positive effect on cognition in depressed patients (Prado et al., 2018). Furthermore, the use of paroxetine did not produce any significant effect on non-depressed or normal individuals (Prado et al., 2018). Even in preclinical studies, paroxetine alleviated the PTSD-like symptoms only in stressed rodents (Takahashi et al., 2006, Krishnamurthy et al., 2013). Non-FDA approved drugs like tricyclic antidepressants (imipramine and amitriptyline) (Frank et al., 1988) and monoamine oxidase (MAO) inhibitors (phenelzine and moclobemide) can also be prescribed (Önder et al., 2006). These drugs are also found to restore the normal function of the HPA-axis in response to stress (Szymańska et al., 2009, McEwen et al., 2010).

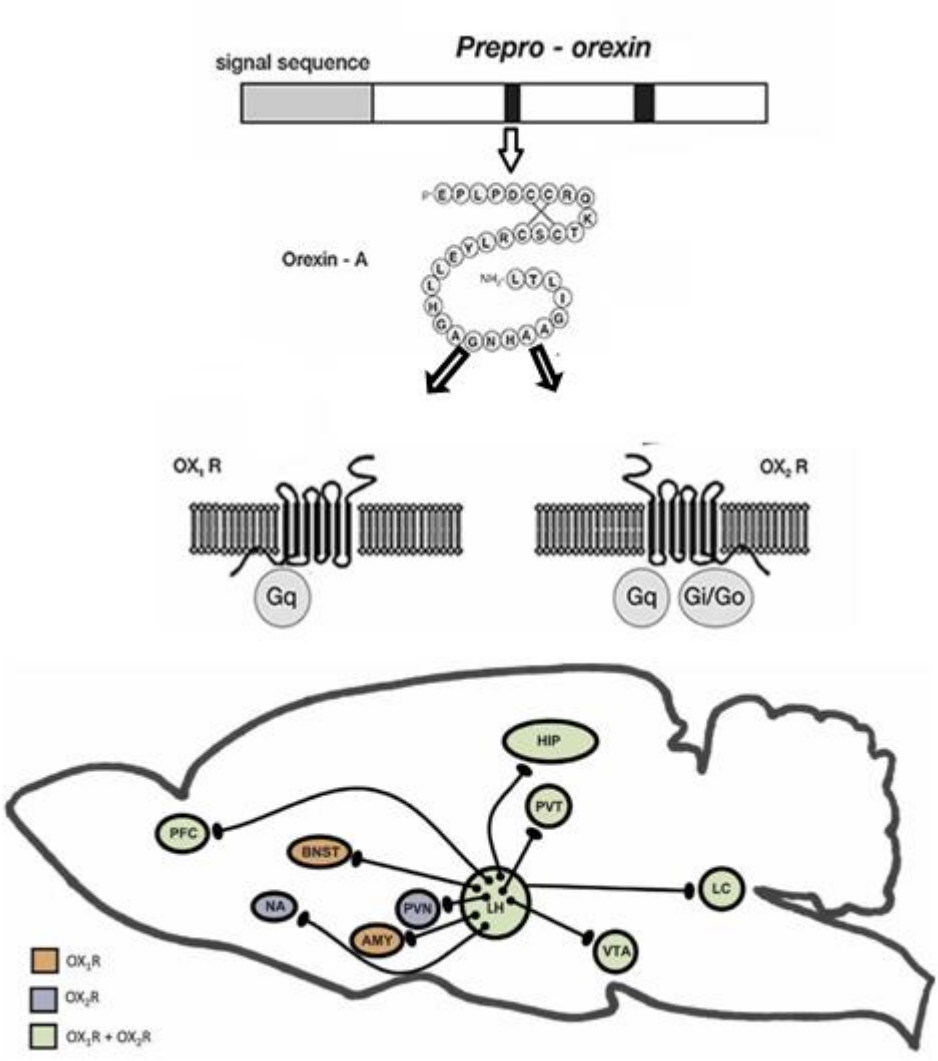
1.6 Limitations of current therapies

Although SSRIs are associated with an overall response rate of approximately 60% in patients with PTSD, only 20% to 30% of patients achieve complete remission (Berger et al., 2009). Further, prolonged use of these drugs may aggravate anxiety due to adaptive changes in 5-HT auto-receptors (Mnie-Filali et al., 2013). Benzodiazepines (BZDs) are prescribed as adjunctive therapy with SSRIs until SSRI reaches their therapeutic level (Saeed and Bruce, 1998). However, BZDs have withdrawn because they risked addiction to alcohol abuse patients (Nutt, 1986). Therefore, there is a need to develop a novel treatment strategy for the remission of PTSD symptoms.

1.7 Orexin and PTSD

1.7.1 Orexin receptors and projections in different brain regions

Orexin (also known as hypercretin) is a neuropeptide hormone produced in the hypothalamus. It influences sleep, arousal, appetite, and energy expenditure, and defective orexin signalling is associated with narcolepsy. Orexin neuropeptide is categorised into two types, orexin-A and orexin-B that promote wakefulness. Orexin-A mediates its effect through both orexin receptor-1 (OX1-R) and orexin receptor-2 (OX2-R), while orexin-B selectively acts on OX2-R (Sakurai et al., 1998). Both the receptors are glycoprotein coupled receptors but differ in their function (Bonaventure et al., 2015). OX1-R is selectively expressed in the AMY, hypothalamus, and PVN. OX1-R regulates the fear acquisition and regulation of HPA-axis and CRH systems (Salehabadi et al., 2020). However, OX2-R predominantly present in locus coeruleus (LC) and HIP and plays a critical role in wakefulness (Wang et al., 2018). Input from the limbic system (AMY and bed nucleus of the stria terminalis) might be important to regulate orexin neurons' activity upon emotional stimuli to evoke emotional arousal or fear-related responses. Thus, both the receptors are crucial for panic disorders and its antagonism may mitigate core symptom (fear response and anxiety) of PTSD.



Grafe LA, Bhatnagar S. Orexins and stress. Front Neuroendocrinol. 2018;51:132-145.

Figure 1.3 Orexin projections to stress-relevant brain regions and the expression of orexin receptors in different brain regions

Orexin are directly innervate the PFC and HIP, orexin activation during stress underlie cognitive impairments. Orexin receptors in the nucleus accumbens (NA) and ventral tegmental area (VTA) mediate stress-induced reward seeking (Grafe and Bhatnagar, 2018). Orexin regulates activity in the HPA-axis through projections to the PVN, where neurons expressing CRH are located (Winsky-Sommerer et al., 2004). Further, orexin neurons densely project to the paraventricular nucleus of the thalamus (PVT), which plays a role in regulating neuroendocrine and behavioural adaptations to repeated or chronic stress (Kirouac et al., 2005). Orexin mediates behaviours relevant to affect and

mood in humans (depression, anxiety, and fear) through actions in the Bed Nucleus of the Stria Terminalis (BNST) and the AMY (Sutcliffe and de Lecea, 2000). Orexin also have inputs to brain regions important for arousal, such as the LC, which in turn can regulate activity in the limbic, thalamic and hypothalamic structure that are directly regulated by orexin-A (Grafe and Bhatnagar, 2018).

1.7.2 Orexin regulates HPA-axis function

Orexin is an endogenous neuropeptide that is normally responsible for wakefulness (Winrow et al., 2012). However, the level of orexin neuropeptide is modulated during stressful events (Winrow et al., 2012). The activation of the orexinergic system leads to hyperarousal, sleep disturbance, and associated anxiety, which are the primary symptom of PTSD (Klenowski et al., 2016). Orexin-A has been shown to promote the HPA-axis response to acute stress at all levels (Johnson et al., 2012). For example, the central infusion of orexins activates CRH neurons. Additionally, optogenetic stimulation of orexin neurons increases cFos expression in the PVN (Bonnavion et al., 2015). Moreover, CRH neurons in the PVN abundantly express the OX2-R (Johnson et al., 2012). Central administration of orexins also increases downstream HPA hormones, ACTH, and corticosterone, which can be reversed with a CRH antagonist (Bonnavion et al., 2015). Orexin promotes ACTH release through its actions on both OX1-R and OX2-R in the pituitary (Date et al., 2000). Orexin stimulates the glucocorticoid secretion via OX1-R in the adrenal gland, though studies in both rat and human adrenal glands have indicated that both orexin receptors are present (Kok et al., 2002).

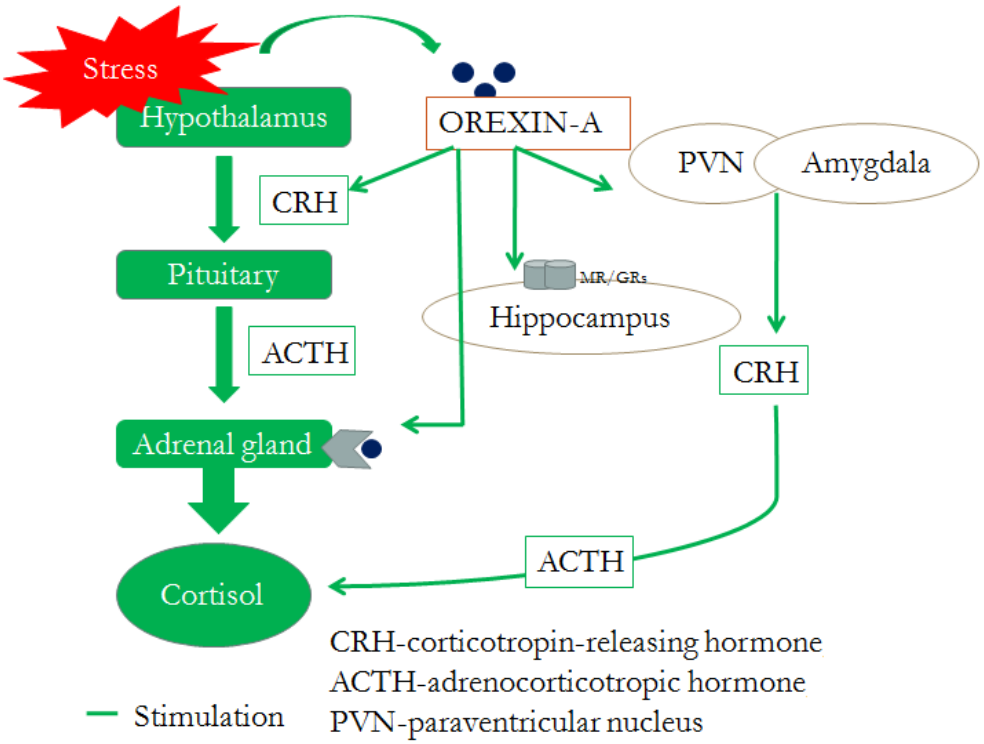


Figure 1.4 role of orexin-A in the regulation of hypothalamic and extra-hypothalamic stress system.

Thus, the activation of the orexinergic system stimulates the CRH-secreting neurons of the hypothamic and extrahypothalamic system that stimulate pituitary ACTH and adrenal "cortisol" secretion. Since, the activation of orexin-A stimulates glucocorticoid and mineralocorticoid receptor (MR) in the hippocampus and glucocorticoid production directly from the adrenal gland. Therefore, orexin could also participate in the regulation of HPA-axis in PTSD.

1.7.3 Orexin modulates catecholamine's and monoamines

Orexin-containing neurons not only innervate target neurons via efferent nerves, but also accept projections from their target neurons, particularly monoaminergic (i.e., noradrenergic (Walling et al., 2004), serotonergic (Brown et al., 2002), dopaminergic (Korotkova et al., 2003), and cholinergic neurons (Burlet et al., 2002). Orexin and monoaminergic neurons form a negative feedback pathway in the dorsal raphe nucleus (DRN) and LC (Brown et al., 2002). Thus, the activity of orexin neurons may increase the activity of monoaminergic neurons. Orexin excites both noradrenergic and serotonergic

neurons in the amygdala (Bisetti et al., 2006). Subsequent postsynaptic release of NE and 5-HT onto orexin neurons may reflect negative feedback since orexin neurons are directly inhibited by 5-HT (5-HT1A receptor-mediated) (Bisetti et al., 2006). NE also has direct effects on orexin neurons via α1 adrenergic neurons (Yamanaka et al., 2006). Preliminary studies of orexinergic signalling within the central nucleus of amygdala (CeA) suggested that the neuropeptides induce postsynaptic excitation (Bisetti et al., 2006). Orexin-A stimulates the monoaminergic neurons such as serotonergic neurons and noradrenergic neurons in AMY and potentiates the conditioned fear responses and arousal activity respectively (Walker and Davis, 2002). In more detail, orexin-A infusion into the LC increases norepinephrine release at efferent targets of the LC noradrenergic system that are associated with arousal (i.e., dentate gyrus) (Walling et al., 2004). Furthermore, *in-vitro* analysis suggested that orexin also excites serotonergic neurons in the DRN (Bisetti et al., 2006). Therefore, orexin-A involves in the regulation of the monoaminergic system, which is one of the main etiological factors involved in the pathophysiology of PTSD. Based on evidences it can presumed that orexin would participate in the regulation of HPA-axis and monoaminergic system in PTSD, and its pharmacological modulation may ameliorate the PTSD-like symptoms in animal model of PTSD.

1.8 Animal model of PTSD

To develop new treatment strategy it is important to use animal models which would manifest at least the cardinal symptoms observed in PTSD patients. Models such as restraint stress, predator stress and SPS are commonly used for PTSD. The restraint model involves 2 to 6 h restraint, while in predator stress rodents are exposed to its natural predator (cat) to develop PTSD-like symptoms (Lindauer et al., 2006, Zoladz et al., 2008). In the SPS model the animals are exposed to variables stressors (includes 2 h restraint then 20 min forced swim followed by exposure to ether vapour) and left

undisturbed for seven days (Liberzon et al., 1997, Liberzon et al., 1999). Further, in the SPS paradigm hypercorticosolism and enhanced negative feedback of the HPA axis has been reported (Kohda et al., 2007, Pooley et al., 2018). However, clinical studies on PTSD indicate neuroendocrine abnormalities and HPA axis dysfunction which is characterised by hypocortisolism (Yehuda et al., 1993, Manson et al., 2005). These neuroendocrine findings are specific to PTSD and serve as pathophysiological markers for animal models of PTSD (Yehuda and Antelman, 1993). The stress-re-stress (SRS) model of PTSD shows hypocortisolism akin to clinical condition (Yehuda and Antelman, 1993). In this model, the animals are exposed to three consecutive variable stresses; 2 hr restraint, forced swim and halothane-induced anaesthesia. A brief reminder of stress showed stable anxiety state in rodents and hormonal abnormalities similar to PTSD patients (Yehuda and Antelman, 1993). SRS model also has some limitations as there are no measures of fear responses which are considered to be responsible for the development of most of the symptoms (Prajapati et al., 2020). The mentioned limitation is not observed in our novel SRS model of PTSD. In this model, foot shock (FS) was used in place of forced swim test (FST) as a SRS to induce PTSD-like symptoms, we observed, FS induced PTSD phenotypes (cognitive dysfunction, fear response and anxiety-like behaviour) and hypocorticosteronemia was more robust compared to the existing model of PTSD (Prajapati et al., 2020). Further, the repeated exposure of FS as a re-stress cue is considered as one of the most relevant experimental animal models that exhibit psychological, physiological, and endocrinological stress in animals (Prajapati et al., 2020). Therefore, in the present study, we used novel SRS model for the pharmacological evaluation.

1.9 Cognitive flexibility and PTSD

Cognitive flexibility is an important executive function and refers to the ability to adapt behaviours in response to changes in the environment (Durairaja and Fendt, 2020). Of note, many brain disorders are associated with impairments in cognitive flexibility. Cognitive flexibility is the ability to perform reversals or to transfer attention within the same perceptual dimension (IDS: intradimensional set shift) or between different perceptual dimensions (EDS: extra-dimensional set shift) (Waltz, 2017). Impairments in cognitive flexibility are characterized by a delay in inhibiting previously learned intrinsic rules, usually followed by difficulties in learning new rules (Waltz, 2017). During PTSD, the executive function is compromised due to exaggerated fear and patients are not flexible to take a decision or unable to cope with the traumatic memory. Cognitive inflexibility is one of the clinically observed major symptoms of PTSD together with fear and anxiety (Keith et al., 2015). Cognitive flexibility regulates the core symptoms of PTSD, such as fear response or negative emotional state and anxiety (Ramaswamy et al., 2016, Thomas and Stein, 2017). The abnormality in cognitive flexibility causes the inability to shift from negative to positive emotional state (Ramaswamy et al., 2016, Thomas and Stein, 2017). However, cognitive inflexibility has not attracted much interest in animal models of PTSD. Therefore the inclusion of cognitive inflexibility in animal models would provide new insight of the pathophysiology and pharmacotherapy of PTSD.

1.9.1 PTSD, cognitive inflexibility, and cholinergic system

Acetylcholine (ACh) signals through both muscarinic as well as nicotinic receptors. Further, modulation of alpha-7nicotinic acetylcholine receptor (α-7nAChR) has been observed in the schizophrenic and Alzheimer's patients (Haydar and Dunlop, 2010). In addition to this, studies have shown that, the α-7nAChR is predominantly present in the

brain mainly in PFC and HIP (Takada-Takatori et al., 2008). Moreover, pathophysiological and functional changes are observed in PFC, HIP and amygdalar regions in PTSD patients (Krishnamurthy et al., 2013, Jin et al., 2014). PFC and HIP inhibit while AMY activates fear-induced memory and anxiety behaviour (Wang et al., 2014). A wealth amount of data indicate that cholinergic transmission in the prefrontal cortex facilitates the process of cognitive flexibility through the cortico-basal-ganglia–thalamic circuit (Ragozzino et al., 2002). For instance, systemic administration of cholinesterase inhibitors to boost cholinergic transmission improved reversal learning and attentional set-shifting deficits in old rats (Nikiforuk et al., 2015). Conversely, mice with forebrain cholinergic signaling impairment, which includes the whole cortical mantle, HIP and AMY showed consistent deficits in reversal learning assessed using the Morris Water Maze (Martyn et al., 2012), as well as a severe reversal learning impairment in a visual discrimination task (Kolisnyk et al., 2013). Since cholinergic system plays an essential role in regulating executive function and decision-making ability (cognitive flexibility) during traumatic event conditions, there is still limited research on how cholinergic function is affected during PTSD. Therefore, assessment of cholinergic function in PFC, HIP and AMY would give a better understanding of the pathophysiology of cognitive inflexibility in PTSD.

1.10 Orexin and cognitive flexibility

Several classical neurotransmitter systems, including acetylcholine, dopamine, and noradrenaline, are important for cognitive flexibility (Prado et al., 2017). However, the role of neuropeptide in the regulation of cognitive flexibility has also been studied. The neuropeptide orexin, which released by neurons in the lateral hypothalamus, is a major player in maintaining feeding, arousal, and motivational behaviour. Orexin regulates behavioural and neuroendocrine responses during stressful conditions as these events lead

to the impairment of cognitive flexibility and function (Durairaja and Fendt, 2020). Also, patients with psychiatric disorders such as panic disorder are associated with significant dysregulation of hypothalamic orexin activations (Grafe et al., 2017). It has been shown that stress worsens cognitive flexibility due to orexin neuropeptide activations (Grafe et al., 2017). Piantadosi et al. illustrated that stimulation of prefrontal cholinergic neurons leads to the release of orexin from hypothalamic neurons, which play an important role in cognitive activation since high orexin activates the executive functions via activation of cortical cholinergic neurons (Piantadosi et al., 2015). Recent studies demonstrated that orexins mediate the impairments in adaptations to repeated stress and subsequently produced cognitive inflexibility in rats (Grafe et al., 2017). Overall, the above information provides evidence for a broader role for orexins in traumatic event induced cognitive inflexibility in PTSD.

1.11 Orexin and mitochondrial function

Orexin affects the HPA-axis and neurotransmitters involved in the pathophysiology of stress and anxiety. However, the downstream pathways of orexin signaling are not clearly understood. Evidence has suggested that orexin plays an important role in regulating energy homeostasis and other physiological processes by activating various downstream pathways such as the Mammalian target of rapamycin (m-TOR) (Li et al., 2014a). m-TOR is a serine/threonine protein kinase and a master regulator of energy homeostasis and metabolism (Liu et al., 2016). The mitochondrial dynamics is directly regulated by the m-TOR pathway. The m-TOR acts as a node for downstream signalling to regulate mitochondrial dynamics and protein translation in the body (Klann et al., 2004). Activation of m-TOR phosphorylates the mitochondrial fission process protein (MTFP-1), a downstream substrate responsible for activation of mitochondrial dynamin-related protein (Drp-1) (Morita et al., 2017). Reports have suggested that the mammalian target

of rapamycin complex-1 (mTORC-1) facilitates mitochondrial fission by enhancing Drp-1 activity and reduces mitochondrial fusion (Lerner et al., 2013, Morita et al., 2017). Further, several reports suggested that, mitochondrial dynamics play an essential role in regulating energy balance, mitochondrial biogenesis, and homeostasis during a stressful situation (Zemirli et al., 2018). However, no studies till now have investigated the role of mitochondrial dynamics in PTSD. Therefore, the assessment of mitochondrial dynamics in an animal model of PTSD would not only give a new insight for pathology of PTSD but can also validate it as a pharmacological target for PTSD. Dysregulation of the mTORC-1 pathway has been observed in a neurological disorder such as stress, anxiety and depression (Abelaira et al., 2014). Kim et al. demonstrated the loss of mTORC-1 signalling in the hippocampus of anxious and depressive patients and this effect was significantly mitigated by rapamycin (Kim et al., 2010).

Rapamycin, a specific inhibitor of m-TOR, has been shown to be useful in the treatment of certain diseases. Clinically, rapamycin is preferred as an immunosuppressant in humans and is useful in preventing kidney transplants' rejection. The various study suggested anticancer property of rapamycin in its higher doses (Li et al., 2014b). Preclinically, rapamycin is used for the pharmacological validation of the m-TOR pathway's role in the pathophysiology of neurological diseases (Li et al., 2014b). Studies have shown the anxiolytic and anti-stress effect of rapamycin in chronic unpredictable stress (Fifield et al., 2013). Study has shown that, the down regulation of hippocampal m-TOR signalling in rats exposed to chronic unpredictable mild stress (Wang et al., 2008). However, there are limited reports on the role of mTORC-1 and associated modulation in mitochondrial dynamics in PTSD.

1.12 Hypothesis

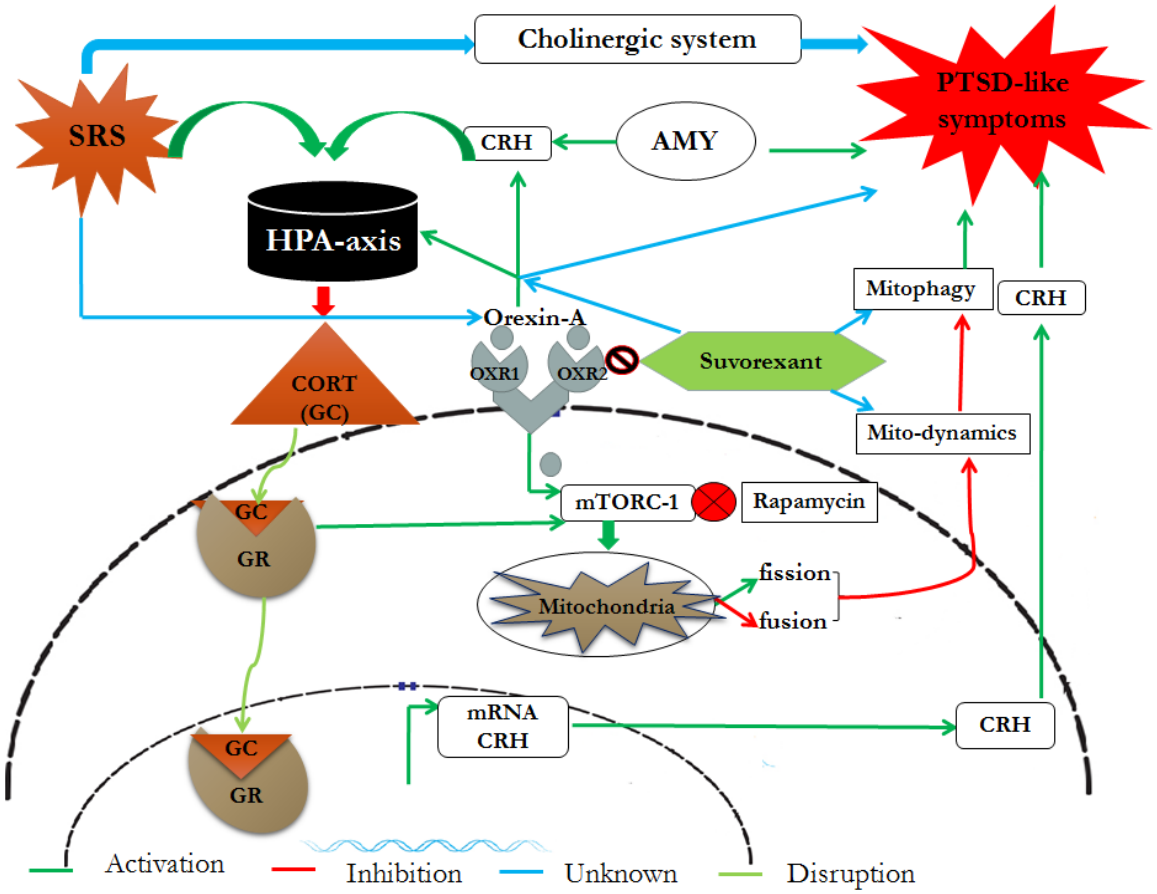


Figure 1.5 proposed hypothesis of pharmacological evaluation of orexin antagonist in the experimental model of PTSD. Green, red, blue, and light green arrow denotes activation, inhibition, unknown and disruption respectively.

Orexin-A is potentially regulates the HPA-axis and extra-hypothalamus stress system (AMY) and stimulates the monoaminergic neurons in different brain regions. Clinical investigations have found dysfunction of the HPA-axis and dysregulation of the monoaminergic system as core pathophysiological factors for PTSD. Further, evidences suggest that orexin-A is involved in the pathophysiology of stress and panic anxiety disorder. However, role of orexin-A in PTSD remains unclear. Therefore, we aimed to evaluate the role of orexin-A in the rodent model of PTSD and, wheather the pharmacological antagonism of orexin can mitigate PTSD-like symptoms. Despite this, PTSD is associated with the negative alteration in cognitive function due to enhanced fear response. Cognitive inflexibility is one of the clinically observed major symptoms of PTSD together with fear and anxiety. The mechanism of cognitive inflexibility involves hypoactivity of cholinergic system in the prefrontal cortex responsible for executive function. During PTSD, the executive function is compromised due to exaggerated fear and patients are not flexible to take a decision or unable to cope up with the traumatic memory. Therefore, the inclusion of cognitive inflexibility in animal models would provide new insight into pathophysiology and pharmacotherapy of PTSD. Moreover, use of cognitive enhancer could facilitates cholinergic transmission and may improve cognitive inflexibility and associated fear and anxiety in PTSD. Thus, another aim of the present study to validate cognitive inflexibility in SRS model of PTSD. The pathophysiology of PTSD involves the disruption of the HPA axis which is manifested as a hypocoriticoism. This reduction in corticosterone level leads to dysregulation of glucocorticoid and glucocorticoid receptor dimer. Such dysregulated dimer alters the mitochondrial function through activation of mTORC-1 pathway, which acts as a node for downstream signalling to regulate mitochondrial dynamics and protein translation in the neuronal cell. Activation of m-TOR phosphorylates MTFP-1 (a downstream substrate

responsible for activation of mitochondrial Drp-1). Mitochondria dynamics include process of fission and fusion which are regulated by different proteins such as Drp-1, mitochondrial fission protein-1 (Fis-1), optic atrophy-1 (Opa-1), and mitofusin-1 and -2 (Mfn-1, Mfn-2). This dysfunction of mitochondrial dynamics directly impair mitophagy, which plays a crucial role in maintaining healthy mitochondria and controls the neuro-behavioural outcome in pathological conditions. Hence, evaluation of mitophagy impairment through mitochondrial dynamics could be a valuable strategy in controlling mitochondrial homeostasis during PTSD condition Mitochondrial dysfunction is an experimental pathology of PTSD and reports suggest that orexin plays an important role in regulating mitochondrial function, and also participates in the activation of mTORC-1 pathway. Therefore, finally we aimed to determine the effect of orexin antagonist on mitochondrial dynamics in rats, exhibiting PTSD-like symptoms. Moreover, inclusion of m-TOR inhibitor could potentiate the effect of orexin antagonist in ameliorating SRS-induced alteration on mitochondrial dynamics and mitophagy.

1.13 Objectives

Objective-1: Evaluation of the anti-PTSD potential of orexin antagonist in rats

Objective-2: Validation of cognitive inflexibility in SRS model of PTSD

Objective-3: Determination of the effect of orexin antagonist on mitochondrial dynamics in rats exhibiting PTSD-like symptoms