Hans Selye, a Canadian endocrinologist and Father of stress, described stress as a complex, but uniform response elicited in mammals during threatened homeostasis (Selye, 1936; 1998). In more modern times, stress has been defined as the state of threatened homeostasis, which mobilizes a composite spectrum of adaptive physiological and behavioral response with an aim to restore the challenged body homeostasis (Kyrou and Tsigos, 2009). Stress response is usually referred as an orchestrated process, which involves various mechanisms for physiological and metabolic adjustments in the body to cope with the demands of a homeostatic challenge (Selye, 1936). These changes occur at the psychological (emotional and cognitive), behavioral (fight and flight), and biological levels (altered autonomic and neuro-endocrine function). Stress response is mainly influenced by the duration as well as the intensity of stressors (Armario et al., 1986; 1990). There are two key aspects of the body’s response to stress. On the one hand, the body responds to stress by releasing mediators to promote adaptation and cope with a stressor, which is a crucial determinant of health and disease. However, chronic persistent stressors produce pathophysiological changes due to maladaptive signaling (McEwen, 1998; Masood et al., 2003). Accordingly, the term allostatics (achieving stability through change) is used, and it refers to an active process by which the body responds to daily events and maintains homeostasis. However, allostatic load/overload either due to too much of stress or from inefficient management of allostatics (not turning off the response when it is no longer needed) leads to pathophysiological changes such as anxiety and depression (McEwen, 1998; McEwen and Wingfield, 2003).

It has been well described that a variety of stressors of different duration (short and prolonged) initiate the stress response in the form of activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic adrenomedullary system to increase the release of glucocorticoids and catecholamines in the blood stream, respectively. However, the repeated exposure of stress for few days/weeks results in the reduction of stress response in terms of the blunted HPA axis response and normalization of behavioral deficits (Groves and Thompson, 1970; Armario, 2006; Grissom and Bhatnagar, 2009). This blunted response to the stress stimulus during repeated exposure is referred to as ‘stress adaptation’ and it has been suggested to be a key protective mechanism against repeated stress exposure (Stone and Platt, 1982; Stone et al., 1985; Rabasa et al., 2011). Individuals have an inherent capacity to cope
with the psychological stress in the form of stress adaptation (Agrawal et al., 2011) and there is a reduction in the sensitivity of stress responsive elements, including the HPA axis during repeated stress episodes (Pfister and King, 1976). It may also be characterized in terms of the restoration of behavioral alterations and normalization of neuro-endocrinological changes, in comparison to the initial stress response (Cohen et al., 1983; Kant et al., 1985; Agrawal et al., 2011). The stress adaptive response in an organism, in terms of blunted HPA axis response, is generally observed against stressors which are not inherently harmful (McEwen, 2003). Studies have shown that the repeated exposure of mild stress produce stress adaptation (Ricart-Jané et al., 2002). Furthermore, stress adaptation has also been observed against repeated episodes of severe stress of varying durations (Cohen et al., 1983; Kant et al., 1985; García et al., 2000; Agrawal et al., 2011; Rabasa et al., 2011). The decline of the initial stress response after repeated exposure to same stressor (homotypic) might result due to the release of endogenous neuropeptides. Indeed, the mechanisms responsible for the unresponsiveness of stress system during repeated exposures of homotypic stress are not known.

Diverse animal models, acute as well as chronic, have been created to simulate the stress condition in animals akin to humans. Electric foot shock (Rabasa et al., 2011) and immobilization stressor (Bhatia et al., 2011; Rabasa et al., 2011) are more frequently employed stressors by different scientists to evaluate the anti-stress activity of pharmacological interventions. Electric foot shock is a complex stressor, which includes both physical and emotional components. This stress paradigm comprises acute or chronic exposures of foot shocks of varying intensity and duration on the electrified grid floor in an electric foot shock apparatus (Armario et al., 1986; 1990; De Vry et al., 1993; Rabasa et al., 2011). Electric foot shock remains the most widely used stimulus for producing the measured amount of discomfort in animals due to its experimental advantage of control over the intensity and duration. A number of studies have reported the development of adaptation in response to electric foot shock stress in terms of neural, endocrine, and behavioral responses in experimental animals (Ohi et al., 1989; Rabasa et al., 2011). Immobilization is also a frequently applied mixed stressor with both physical and psychological dimensions. The struggling and muscular exertion during the immobilization process constitute the physical components of stress. On the other hand, the limited movement during the
immobilized position and exposure in an open area comprise the psychological stress (Kvetnansky et al., 1970). Immobilization is well tolerated by laboratory rats and mice, and is used in both acute and chronic stress studies extending over weeks or even months. Both foot shock and immobilization stressors are sufficiently intense to activate the stress-responsive system in the body, including HPA axis and the sympathetic nervous system.

Both clinical and preclinical studies have described the important role of neuropeptides in body’s response against the stressors (Zukowska-Grojec and Vaz, 1988; Morgan et al., 2001). The renin–angiotensin system (RAS) is a complex, and is one of the best-studied enzyme-neuropeptide systems in the body. The RAS components are also present in the CNS and play an important role in modulating the sensory, emotional and behavioral responses (Von Bohlen and Albrecht, 2006). The brain Ang II, acting primarily through AT$_1$ receptor stimulation, is a multifunctional peptide with an important role to control the central sympathetic system activity and stress response (Saavedra, 2011). The AT$_1$ receptor expression closely follows the HPA axis and the receptors are detected in all stress responsive brain areas including the prefrontal cortex, hippocampus, hypothalamus, the median eminence and the subfornical organ (Tsutsumi and Saavedra, 1991). Research evidence suggests the critical role of central renin–angiotensin system in modulating the stress response as the different types of stressors (isolation, immobilization/restraint, cold restraint and immunological) influence the release of angiotensin II and expression of its receptors in the brain as well as in the peripheral tissues (Yang et al., 1996; Bregonzio et al., 2008). A number of preclinical studies have shown the effectiveness of renin-angiotensin inhibitors in attenuating the stress response and anxiety. Peripheral administration of losartan, a selective AT$_1$ receptor antagonist, significantly attenuated and anxiogenic behavior in hypertensive rats (Srinivasan et al., 2003). Another AT$_1$ antagonist, valsartan, was shown to produce anxiolytic-like effects in the elevated plus maze test (Braszko et al., 2003a). Peripheral administration of candesartan also decreased the hypothalamic-pituitary-adrenal response to isolation stress (Armando et al., 2001). In addition, clinical studies have also shown the significance of angiotensin receptor antagonists in attenuating the stress-associated anxiety and improving the mood in depressed patients. It has been reported that chronic administration of candesartan resets the HPA axis and improves the anxiety.
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and depression of patients with type 2 diabetes mellitus (Pavlatou et al 2008). In addition, angiotensin converting enzyme inhibitors, captopril and enalapril, decrease anxiety and depression in hypertensive patients (Braszko et al., 2003b). Nasar et al also demonstrated that the use of renin angiotensin modulators is associated with a lower frequency of anti-depressant usage in patients (Nasr et al., 2011). On the contrary, some studies have also addressed the anti-stress role of angiotensin neuropeptides (Hein et al., 1995; Ichiki et al., 1995). Studies have also described the role of angiotensin in cellular adaptive mechanisms in response to repeated neurogenic stress (McDougall et al., 2000). However, the role of angiotensin II in stress adaptive response in repeated immobilization and foot shock stress subjected mice is not explored.

Opioid receptors are widely distributed on the HPA axis and stress responsive brain regions (Sauriyal et al., 2011). Endogenous as well as exogenous opioids have been reported to play an important role in modulating the stress, anxiety, depression and post-traumatic stress disorder (PTSD) (Land et al., 2008). Stress-induced release of corticotropin-releasing hormone (CRH) triggers the release of endorphin that in turn inhibits the over-activation of HPA axis (Nakagawasai et al., 1999). A significant reduction in inhibitory opioid tone has also been reported with the development of depression (Burnett et al., 1999). The activation of μ-opioid receptors in the amygdala is associated with the stress coping behavior (Barfield et al., 2010). Furthermore, transgenic mice with low β-endorphin exhibit the anxious behavior, with deficits in coping ability (Barfield et al., 2010). Moreover, an increase in enkephalin and delta opioid receptors in the basolateral amygdala and nucleus accumbens produces the anti-stress effects (Marotti et al., 1996; Chen et al., 2004; Kung et al., 2010). In response to acute and chronic stress, the levels of nociceptin (N/FQ) are increased in the hippocampus; hypothalamus and medio-dorsal forebrain, which tend to produce stress adaptation (Cruz et al., 2012; Delaney et al., 2012). An earlier study by our own laboratory described that opioid receptor antagonist, naltrexone, reverses the behavioral adaptation in response to repeated water immersion stress exposure (Agrawal et al., 2011). However, the role of endogenous opioids in stress adaptation following repeated stress exposure of variable duration is not explored yet.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase, which exists in 2 isoforms, α and β and regulates many neuronal functions including
neurotransmission, neuroplasticity, metabolic and neuronal polarity (Gould and Manji, 2005). The GSK-3β pathway has been implicated in the development of a wide variety of neuropsychiatric diseases, such as Alzheimer’s disease, schizophrenia, attention deficit hyperactivity disorder (ADHD), and bipolar disorders (Gould et al., 2004). GSK-3β is widely expressed in the different brain regions, including the amygdala, prefrontal cortex and hippocampus, which are considered as stress sensitive brain regions (Seo et al., 2015). Research evidence has shown that the hyperactivity of GSK-3β may contribute to depression (Gould et al., 2004; Zhang et al., 2013). Pharmacologic inhibition of GSK-3β in the hippocampus produces antidepressant-like behaviors in the forced swim test in mice (Gould et al., 2004). Moreover, insufficient GSK-3β signaling reduces immobility time in the forced swim test and increases exploratory activity (O’Brien et al., 2004). Lentivirus-induced continuous over-expression of GSK-3β in the hippocampal dentate gyrus in chronic mild stress-subjected mice significantly decreased sucrose preferences in the sucrose intake test and increased immobility time in both forced swim and tail suspension tests (Zhang et al., 2013). Moreover, mice with GSK-3β inactivated gene possess anxiolytic and 'pro-social effects' at the behavioral level in the dark light emergence and open field tests (Latapy et al., 2012). Administration of flupirtine has been shown to attenuate stress-induced deleterious effects by decreasing GSK-3β activity, suggesting the role of activation of Akt/GSK-3β signaling pathways during stress induction (Huang et al., 2015). Other studies have shown that exposure to stress of different types including immobilization, swim, and restraint stress increases GSK-3β activity in the hippocampus and frontal cortex of animals (Chen et al., 2012; Seo et al., 2015).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is a stress-regulated transcription factor and has a pivotal role in controlling inflammatory and innate immune responses (Piva et al., 2006). Exposure to stress has been documented to trigger the signaling cascade involving the activation and potentiation of NF-kB in different stress sensitive brain regions, including the frontal cortex, hippocampus, amygdala and hypothalamus (Madrigal et al., 2001; 2002). Stress-induced activation of NF-kB has also been recognized in humans and transgenic mice subjected to psychological or immobilization stress, respectively (Bierhaus et al., 2003). A study by Koo et al demonstrated that stress induces inhibition of
neurogenesis in the adult hippocampus leading to 'pro-depressive effects' and administration of an inhibitor of NF-kB was shown to attenuate the deleterious effects of stress (Koo et al., 2010). A previous study by our laboratory has also demonstrated the anti-stress effects of NF-kB inhibitor in restraint stress-induced behavioral changes (Manchanda et al., 2011).

Although, the role of GSK-3β and NF-kB signaling has been described in stress-associated alterations, yet their potential role in the stress adaptive processes has not been explored. Therefore, the present study was designed to explore the role of GSK-3β and NF-kB signaling in immobilization and electric foot shock-induced stress in mice. To elucidate the role of GSK-3β and NF-kB in stress and stress adaptation, the protein levels of p-GSK-3β-S9 (phosphorylation of glycogen synthase kinase β at N-terminal serine, Ser 9), total GSK-3β and NF-kB was examined in the prefrontal cortex (stress sensitive) region of brain. The measurement of p-GSK-3β-S9 and NF-kB protein levels in the mouse brain following repeated exposure to stress paradigm may help to elucidate the relationship between stress adaptation and signaling cascade involving GSK-3β and NF-kB.