CHAPTER 7

SUMMARY AND CONCLUSION

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Diabetes both T1DM and T2DM is known to manifest structural, physiological as well as psychological defects in brain. However, pathology for these undesirable neurological conditions can differ depending on the age of onset, cause, duration, as well as the molecular and metabolic alterations during diabetes. At mechanistic level, the cause of diabetic neuropathy in brain can be insulin deficiency or insulin resistance, hyperglycemia, inflammation, advanced glycation end product generation, amyloid beta formation and so on. Amongst these aspects, in this thesis we have focused on the insulin resistance which might contribute to spectrum of disorders observed in the brain of Type 2 diabetics. Several reports have laid the foundation for the association of stress as one of the causal factors for the generation of T2DM via glucocorticoid mediated peripheral insulin resistance. Also, neurological consequences observed in experimental models of T2DM are strikingly similar to those observed following chronic stress. However, there lacks clarity whether elevation in glucocorticoid levels (= stress) along with peripheral insulin resistance (= diabetes) can impair brain insulin signaling. This phenomenon of impaired brain insulin signaling has been coined as 'Type 3 diabetes or brain insulin resistance'.

This led to the genesis of hypothesis for the current study i.e. brain insulin resistance could be the culprit for central nervous system dysfunction observed in stress (elevated GC) induced diabetes. In view of this, dexamethasone induced T2DM rat model was developed. The molecular dissection of insulin signaling in different regions of brain demonstrated regional difference in onset of insulin resistance. Parallelly, neurobehavioral parameters were assessed where dexamethasone induced diabetic rat demonstrated depressive like behavior and altered motor cortex function. Interestingly, among different regions studied, hypothalamus was the prime region to depict impaired insulin signaling while no change was observed in cortex, cerebellum and hippocampus. Hypothalamic insulin resistance in this model corroborated with the alteration in appetite where there was reduction in food intake and body weight. This prompted us to further assess the molecular mechanism leading to hypothalamic insulin resistance mediated appetite loss, which otherwise is reported to result into hyperphagia. Insulin inhibits the expression of food intake stimulating orexigenic neuropeptides while activating the anorexigenic neuropeptides. Although there was a

decrease in anorexic signals i.e. *Pomc* and *Mc4r* with an increase in orexic signals i.e. *Npy* and *Agrp*, their effect on appetite stimulation was not propagated because of the reduction in neurotransmitters i.e. GABA and glutamate in insulin resistant hypothalamus. These results were in accordance with Tong *et al.*, where it was proved that alone appetite regulating neuropeptides are not sufficient to generate apt response towards feeding behavior (Tong *et al.*, 2008). Dexamethasone treatment and impairment in hypothalamic insulin signaling also decreased the levels of nutrient sensors- GLUT1, PPARγ and SirT1, responsible for appetite modulation. Thus, analysis of hypothalamic neuropeptides, neurotransmitters and nutrient sensors led to the conclusion that the complex cross talk of insulin signaling with these neural networks are decisive in appetite regulation. Hence, this study managed to unveil the unanswered observation of weight loss in murine models of dexamethasone treatment along with broadening the fact that hypothalamic insulin resistance apart from resulting in obesity can also be involved in weight loss. This study can be well related to stress and diabetes in patients with lean phenotype. The network of hypothalamic appetite regulation is laid early during the embryonic stage, thus susceptible to the intrauterine changes induced by maternal diabetes. The assessment of this hypothesis led to an observation that there exists sexual dimorphism in programming of the hypothalamic appetite regulation in offspring towards dexamethasone induced maternal insulin resistance, where as compared to male pups, female pups demonstrated hypothalamic insulin resistance and thus, might be more prone to metabolic disorders in adulthood.

Although hippocampal insulin signaling and cognitive behavior were not altered in dexamethasone induced T2DM rats, there were alterations in astrocytic and stemness components. Astrocyte specific expression of Gfap, Glast, Mct1, Mct4 and glycogen were remarkably reduced in hippocampus of dexamethasone treated rats. They also exhibited reduction in Nestin and Notch1-Hes5 expression, thus representing reduced pool of neural stem cells (NSCs). Astrocytes and NSCs are important cell type which can modulate brain microenvironment and can play noteworthy role in neuro-restorations in neurodegenerative diseases. Thus, any ill effects such as insulin resistance on these cells apart from neurons, might be one of the reason for neuronal deterioration as reported in diabetes. Hence, we further assessed the outcome of insulin resistance in astrocytes and NSCs by *invitro* silencing of the *Insr* gene. Further, we also elucidated plausible glucocorticoid mediated insulin insensitivity in these brain cells.

The function of insulin signaling as well as the impact of insulin resistance on astrocyte was explored using *in vitro Insr* gene silencing. *Insr* knockdown (KD) in astrocytes negatively affected survival and proliferation. It also compromised the astrocytic machinery involving glucose uptake and glycogen synthesis. Post glutamate challenge, INSR KD cells demonstrated altered gene expression of glutamate (decreased *Glt1*, increased *Glast*) and lactate transporters (decreased S*lc16a3*, increased *Slc16a1*) in astrocytes along with steep reduction in glycogen synthesis and lactate efflux. These results signified the vital role of insulin in machinery of astrocytic lactate shuttle. Thus, this metabolic alteration in astrocytes will make it incompetent to fulfil neuronal and other brain cell needs during excitotoxicity and neurotransmission, eventually leading to cell death. Thus, our results clearly concluded the critical role of insulin per se for astrocytic survival, proliferation and function. Having recognized the role of insulin signaling in astrocytes, we further subjected them to dexamethasone treatment *in vitro*. Post dexamethasone exposure, astrocytes demonstrated impaired insulin signaling, a similar phenomenon has been established in peripheral cells. Thus, to the best of our knowledge, ours might be the first report claiming that dexamethasone can blunt the insulin responsiveness in astrocytes. This decrease in insulin signaling by dexamethasone was followed by decline in insulin stimulation in glucose uptake, glycogen synthesis and lactate production. Altogether, we conclude that astrocytic insulin resistance at cellular level might prove detrimental for overall brain function.

Further, the regulatory role of insulin in NSCs fate determination was explored. Our results manifested that insulin concentration was vital for the deciding survival and proliferation in NSCs. Also, differentiation fate steered towards neurogenesis with higher concentrations of insulin (0.22 μM and 4.3 μM) while lower dose (0.04 μM or below) promoted astrogliogenesis. Thus, signifying the decisive role of insulin in these cells. However, there has always been a confusion regarding the *per se* role of insulin because of its overlap with IGF signaling. Thus, as in astrocytes, neural stem cells isolated from postnatal 0-day rat brain were rendered insulin resistant by *Insr* gene silencing. As seen with astrocytes, *Insr* KD decreased the survival and the entry into synthesis phase in the cell cycle by NSCs. Further, it hampered the neurogenesis significantly without altering gliogenesis. Thus, demonstrating that insulin signaling was vital in channelizing NSCs towards neurogenesis. Subsequently, when assessed for interference in insulin signaling by dexamethasone in NSCs, interestingly it also did

reduce insulin mediated Akt signaling. However, GCs did not directly hinder the insulin action of survival and fate determination in NSCs. To the contrary insulin rescued dexamethasone mediated cell death and cell cycle arrest. Also, when evaluated the effect of these hormones on differentiation, dexamethasone and insulin in combination promoted neurogenesis (as evident from *Map2*) and astrogliogenesis (as evident from *Gfap* and *S100b*) better than their alone treatment. Thus, confirming the existence of cross talk between these two hormones in deciding the outcome of stem cell fate between neurogenesis or gliogenesis.

Concluding, the highlights of the study undertaken in this thesis are:

- There exists a regional difference in the onset of TYPE 3 diabetes/ brain insulin resistance and its corresponding behavioral functions in response to glucocorticoid induced diabetes.
- The prime target of glucocorticoid (dexamethasone) induced brain insulin resistance is the hypothalamus leading to appetite change. The interactions of hypothalamic insulin resistance with that of other neural circuits is responsible for the reduction in appetite in glucocorticoid induced diabetic model.
- There is a sexual dimorphic modification in hypothalamic appetite regulation in response to maternal diabetes induced by glucocorticoid, and thus can predispose the risk of diabetes, obesity and metabolic disorders in later life.
- *Insr* gene silencing in astrocytes and NSCs can serve as an ideal *in vitro* model to elucidate the role of insulin resistance in these brain cells. Also, *in vitro* exposure to glucocorticoid lead to development of insulin resistance in both astrocytes as well as neural stem cells.
- Insulin resistance can alter the astrocytic survival, proliferation, metabolism and lactate shuttling to brain cells, thus can prove detrimental in overall brain functioning.
- Insulin resistance modulates the fate of neural stem cell survival, proliferation and differentiation, thus clarifying the role of insulin in NSC plasticity.

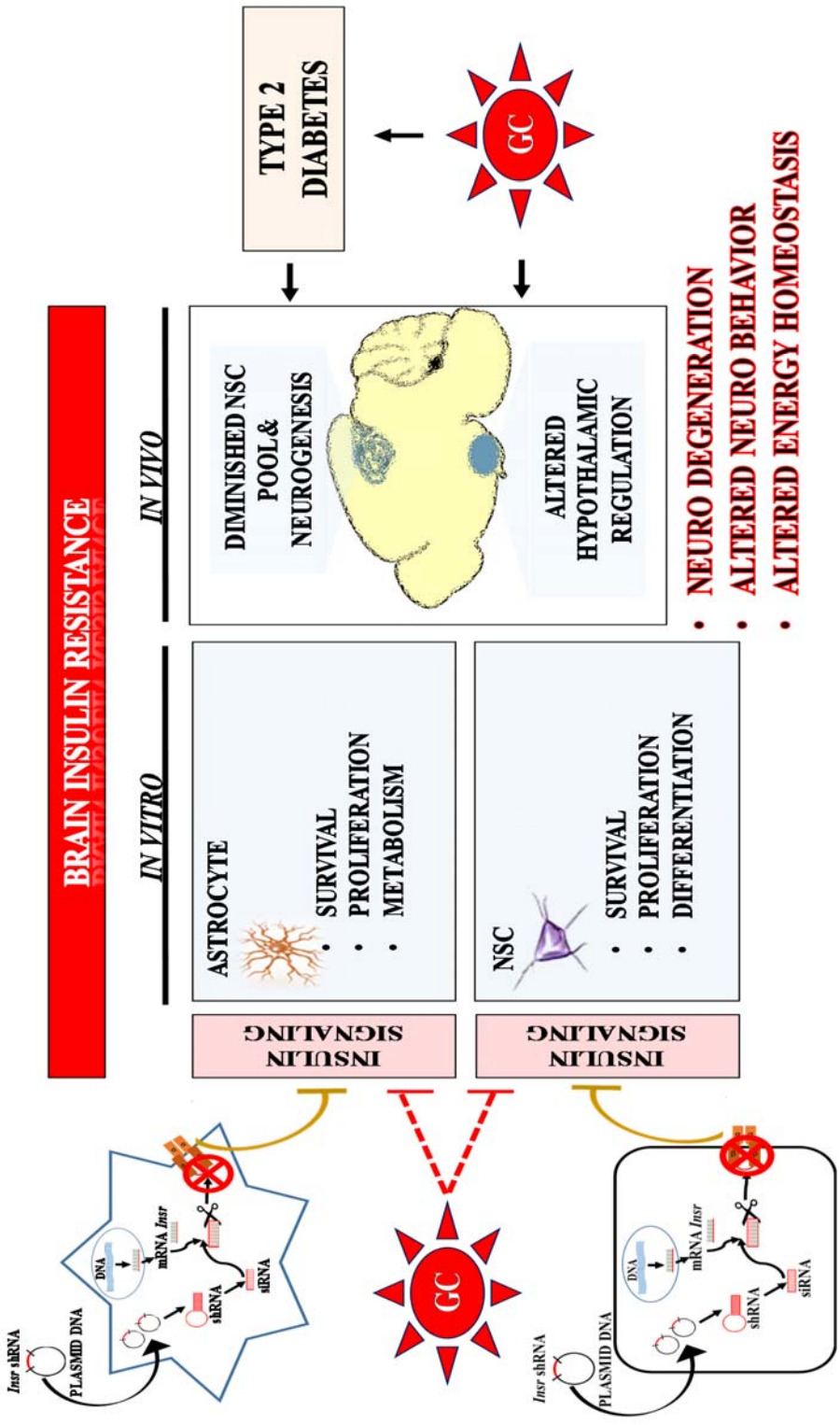

Figure 7. 1 Summary of "Cellular and molecular aspects of insulin resistance in brain". Hypothalamic insulin resistance leading to reduction in appetite change as well as hippocampal reduction in stem cell pool was prominent in GC-glucocorticoid (dexamethasone) induced diabetic rat. Further elucidation at cellular level by *in vitro Insr* gene silencing demonstrated the role of insulin signaling in survival, proliferation and function of astrocytes and neural stem cells. Also, i*n vitro* exposure of dexamethasone impaired insulin signaling in astrocytes and neural stem cells

Thus, the molecular and cellular aspects of insulin resistance in brain was analysed systematically. In the broader context, this study can be extrapolated to human subjects where early or later life stressful events leading to type 2 diabetes can extend its effect on brain causing type 3 diabetes. Also, vice versa brain insulin resistance can become a root cause for the generation of metabolic disorders. It also emphasizes on the fact that responses to insulin signaling vary in different brain regions and its cells. Hence, cellular and regional status of insulin resistance in particular can decide the onset or severity of neurodegeneration. Thus, implying that designing of neuro-regenerative or neuroprotective therapeutic strategies should involve regional as well as cellular facets of brain.