Concise Summary

Of the thesis entitled

Hepatic hippocampal crosstalk in circadian desynchrony and nonalcoholic steatohepatitis

Lifestyle disorder is a modern term to highlight the perturbations taking place in terms of food intake, sedentary lifestyle, chrono-endocrinological deviations, sleep-wake cycle and chronic inflammation. Non-alcoholic fatty liver disease (NAFLD)/Nonalcoholic steatohepatitis (NASH) is a chronic liver disease afflicting about one third of the world's population and is estimated to be around 9-32% in the general Indian population and has been recognized as a major health burden. NAFLD is multifactorial disease with broad spectrum and can progress to nonalcoholic steatohepatitis (NASH), characterized by inflammation, apoptosis and ballooning degeneration. Patients with NASH have been reported with deficits in learning, memory and executive functioning. NAFLD/NASH and the metabolic syndrome have been linked to neurobehavioral perturbations through systemic inflammation, altered gut microflora, neuroinflammation and sleep apnea.

Circadian misalignment, dysregulation, jet-lag or chronodisruption are the terms often used to depict alterations in the circadian clock due to changes in the photoperiodic regime/sleep wake cycle. Circadian desynchrony and development / progression of lifestyle disorders is well established. Several studies have revealed that chronodisruption affects the circadian release of hormones and their titres, neuro-psychological behavior, spatial-temporal changes in brain, altered gut microflora and systemic low-grade inflammation triggering other health risks. Melatonin is a neurohormone with powerful antioxidant, anti-inflammatory, anti-neoplastic, anxiolytic and anti-apoptotic properties. Previous study in our lab had shown that evening injections of melatonin improves synchronization of core circadian clock genes in C57BL/6J mice subjected to high fat diet/chronodisruption. In our study, neuro-behavioral test for locomotion, depression, anxiety, spatial learning and memory along with alterations in BDNF-TrkB pathway in the hippocampus was studied in rodent models to decipher the hepatic-hippocampal crosstalk.

The bile acids are known to cross the blood-brain barrier (BBB) and impact hippocampal neurons resulting into the shifts in behavior. In this study, it is hypothesized that high fat diet in the combination with photoperiodic regime causes fatty changes in liver that has subtle impacts on bile acids culminating in altered neuronal functions.

The entire thesis is divided into five chapters for the sake of convenience wherein; a variety of protocols were employed for interpretation of the proposed hypothesis.

In **Chapter 1**, the C57BL/6J mice were treated with high fat-high fructose diet and/or photoperiodic manipulation induced chronodisruption. Validation of the disease condition was done through a series of experimental protocols. Briefly, C57BL/6J mice were subjected to 60% fat and 20% fructose supplemented in drinking water (H diet) and/or phase advance of (8-h) photoperiodic manipulation induced chronodisruption (CD) for 18 weeks to induce NASH. In our study, at the end of 18 weeks we had recorded elevated levels of AST, ALT, blood glucose, triglycerides, total lipids, LDL-cholesterol and VLDL-cholesterol in chronodisruption (CD), high-fat high-fructose diet (H) and a combination of the two (HCD) groups. Histopathological evaluation showed distorted hepatic chords, ballooning hepatocytes and macrovesicular steatosis in disease control (CD, H and HCD) groups. On the other hand exogenous melatonin treatment accounted for the improvement in the elevated titres of liver functional test (AST, ALT), lipid markers (triglycerides, total lipids, LDL-cholesterol and VLDL-cholesterol) and significant decrement in the hepatic fat accumulation in the liver of chronodisruption (CDM; CD + melatonin), high-fat high-fructose diet (HM; H+melatonin) and a combination (HCDM; H, CD and M. At ZT0, ZT6, ZT12 and ZT24 CD groups, significantly lowered levels of melatonin were recorded as compared to control. Also, the genes regulating the lipid metabolism in the liver were altered in CD, H and HCD groups. Evening melatonin treatment improved the circadian melatonin, mRNA expression of core clock genes (Bmal1 and Clock), and the lipid metabolism genes in (CDM, HM and HCDM) groups respectively.

In **Chapter 2 and 3**, we have studied the alterations in inflammatory cytokines and gut microbiota compositions in C57BL/6J mice subjected to high fat-high fructose diet and/or photoperiodic manipulation induced chronodisruption respectively. It was

observed that titres of TNF-α, IL-6, IL-10, IL-12 and IL-17 were significantly altered in serum of disease control (CD, H and HCD) groups. Similarly, the mRNA levels of pro- and anti-inflammatory genes (TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-17, MCP-1and Nf-κB) in liver and hippocampus showed a similar trend due to high fat-high fructose diet and/or chronodisruption. Further, to establish the connection between liver-gut-brain axis we studied the gut microbiota composition of all the experimental groups and it was recorded that the Firmicutes: Bacteroidetes ratio which is an important indicator of gut dysbiosis was increased in disease control (CD, H and HCD) groups. Along with the changes at phylum level we also found that genus Ruminococcaceae and Desulfovibrionales abundance was significantly increased in (CD, H and HCD) groups compared to alistipes. Exogenous melatonin administration was able to improve the inflammatory cytokines titres in circulation, mRNA expression levels in liver and hippocampus, Firmicutes: Bacteroidetes ratio as well as gut dysbiosis. Corticosterone rhythm and titres were higher in disease control (CD, H and HCD) mice whereas; the same was reversed in melatonin treated groups. These changes are attributed to anti-inflammatory, anti-oxidant and gastro-protective properties of melatonin.

In the light of above mentioned findings in liver and gut it was thought pertinent to assess the neuro-behavior aspects of lifestyle disorder. Hence, in **Chapter 4**, we have studied the diurnal and nocturnal behaviors of the mice separately to evaluate, locomotor deficits, anxiety, depression, spatial learning and memory. C57BL/6J mice were subjected to series of neuro-behavioral tests to evaluate the mental fitness upon exposure to high fat-high fructose diet and/or chronodisruption. It was recorded that, the diurnal and nocturnal locomotor activity, anxiety-like behavior, depression, spatial learning and memory was significantly affected in CD, H and HCD groups. Exogenous melatonin administration was able to make moderate to significant corrective changes neuro-behavioral perturbations albeit with some exceptions. Melatonin has anti-depressive, anxiolytic, anti-stress and neuroprotective properties and the observed changes are attributable to the said properties.

In **Chapter 5**, changes in expression of genes governing bile acid metabolism, key genes of the BDNF-TrkB pathway and neurotrophic factors were studied along with the serum

titres of bilirubin and thyroid hormones. Melatonin treatment to the disease groups was able to improve the mRNA levels of the bile acid (BA) metabolism genes as well as BDNF-TrkB pathway in high fat-high fructose diet and/or chronodisruption. Exogenous melatonin mediated improvement was observed pertaining to the expression of BA metabolism genes and BDNF-TrkB pathway which confirms the neuro-hepatoprotective role of melatonin at molecular, histopathological, physiological as well as psychological levels.

Taken together, the study reveals that exogenous melatonin acts as subtle modulator of hepatic-hippocampal crosstalk via. Improving hepatic circadian clock, hepatic-neuroinflammation, gut-microbiota composition, diurnal/nocturnal neuro-behavioral and BDNF-TrkB pathway in the experimental model of lifestyle disorder.