Evidence on hepatic-hippocampal crosstalk in Chronodisruption and Nonalcoholic Steatohepatitis

Introduction

High fat diet induced hepatic steatosis is associated with alterations in neuro-behavior, which may affect brain synaptic plasticity through impairment in the oxidative and inflammatory status of the brain. Studies in animals have experimentally confirmed that feeding of high fat-high sugar diet causes nonalcoholic steatohepatitis (NASH) and neuro-inflammation affecting various components of neuro-behavior such as depression and anxiety disorders, cognition impairment, deficits in learning and memory etc. (Elwing et al., 2006; Surdea-Blaga & Dumitraşcu, 2011; Youssef et al., 2013). The development and progression of NASH may be mediated in part, due to the deficits in the mechanism of bile acids (BAs) metabolism. BAs acts as signalling molecules and play an important role in lipid solubilisation, digestion and hepatic glucose metabolism (Trauner et al., 2010). Also, their own synthesis and metabolism through the activations of various nuclear receptors such as farnesoid X receptor (FXR), pregnane X receptor (PXR) and liver X receptor alpha (LXRα) etc. is known (Lefebvre et al., 2009; Trauner et al., 2010; H. Wang et al., 1999). Obeticholic acid (OCA), a semi-synthetic bile acids has been used to treat primary biliary cholangitis and is under review for the treatment of NASH and other metabolic disorders (Chapman & Lynch, 2020; Younossi et al., 2019). Studies in humans and animal experimental models of cholestasis had shown adaptive responses in bile acid synthesis and transporters. Treatment with BA such as chenodeoxycholic acid (CDCA) in HepG2 human hepatoma cell line had increased the expression of BA transporter and metabolism (OSTα, OSTβ, SHP and BSEP) genes (Boyer et al., 2006). In patients with extrahepatic cholestasis, several BA transporters and metabolism genes (OSTα, OSTβ, MRP3 and OATP1B1) were significantly altered as compared to control subjects (Schaap et al., 2009). OSTα and OSTβ protein were found to be significantly increased in liver samples of NASH patients as compared to control (Malinen et al., 2018).

Neurotrophic factors such as Brain-derived neurotrophic factor (BDNF), Glial Cell-Line Derived Neurotrophic Factor (GDNF), Nerve growth factor (NGF), Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5) play an essential role in development and functioning

of nervous system (Dechant & Neumann, 2003; E. J. Huang & Reichardt, 2001; Lee & Kim, 2010; Miranda et al., 2019). These factors have also been reported vital in the pathogenesis of psychiatric disorders and neurodegenerative diseases. Neurotrophic factors like p75 neurotrophin receptor (p75NTR), BDNF, GDNF, NGF and ciliary neurotrophic factor (CNTF) are expressed in hepatic cell types and are known to regulate glucose and lipid homeostasis, insulin sensitivity, hepato-cellular injury and liver fibrosis (Kendall et al., 2009; J.-F. Li et al., 2013; Nakagawa et al., 2000; Rezende et al., 2012; Tao et al., 2019). In the condition of NAFLD/NASH or metabolic syndrome, several critical factors play an important role in the development of cognitive impairment and memory deficits such as high inflammatory cytokines (IL-1β, IL-6, and TNF-α), increase oxidative stress, inactivation of multiple neurotransmitter systems (noradrenergic, dopaminergic, cholinergic, histaminergic, etc.), decrease in the levels of BDNF, GDNF, NT-3, NT-4/5 and NGF (Castanon et al., 2015; Gladding et al., 2018; Mondal et al., 2020; Moreno-Navarrete et al., 2017; Uutela et al., 2012). BDNF and its receptor, tropomyosin receptor kinase B (TrkB) promote neuronal survival, neural plasticity, synaptogenesis and also have been associated as a mediator of hippocampal-dependent learning and memory (Miranda et al., 2019; Rattiner et al., 2004; Yoshii & Constantine-Paton, 2010). TrkB is known to interact with three major neurotrophins viz. BDNF, NT-3, NT-4 and have been implicated for survival and proliferation of neuronal cells (Ahmed et al., 2020; Yoshii & Constantine-Paton, 2010). The thyroid hormones tri-iodothyronine (T3) and thyroxine (T4) plays an important role in brain development and its deficiency during pregnancy lead to neuropsychiatric syndrome as evidenced in children with congenitally hypothyroid with mental retardation (Kempers et al., 2006). Depression has been positively correlated with thyroid functions and it has been observed that patients with thyroid disorders tend to develop depressive symptoms and vice-versa (Hage & Azar, 2012). Further, 60% of hyperthyroid patients have anxiety disorders (Kathol & Delahunt, 1986) and 31 to 69 % have symptoms of depressive-like disorders (Trzepacz et al., 1988). Also, patients with psychiatric depression showed low T3 levels (0.94 nmol/l) as compared to the control subjects (1.77 nmol/l) (Premachandra et al., 2006).

The gut-liver-brain axis is affected by high fat diet that causes ‘leaky gut’ and elevation in levels of liver derived products such as bile acids, ammonia, lactate, endotoxins, cytokines in circulation (Rutkowsky et al., 2018; Serino et al., 2012; B. Wang et al.,

2020; Yildirim et al., 2019). Under such circumstances, these products are known to traverse through the blood brain barrier and affect the hippocampal neurons (Butterworth, 2013; Ding et al., 2020; Lira et al., 2010). Various evidences suggests that systemic pro-inflammatory markers contribute to the pathogenesis of neurological disorders such as anxiety/depression, learning and memory deficits that have been observed in NASH patients and in rodents fed with high calorie diet (Del Rosario et al., 2012; Gladding et al., 2018; Klein et al., 2016; Vagena et al., 2019). In western diet fed C57BL/6 and LDLr$^{-/-}$mice, the systemic pro-inflammatory signalling involves humoral and neural routes where increased pro-inflammatory cytokines, levels of ammonia, lactate, and alterations in the permeability of blood-brain barrier plays a key role in neurobehavioral perturbations (Rutkowsky et al., 2018). Short-term high-fat diet feeding to male Swiss albino mice has been reported to impact hippocampal-dependent learning and memory with reduced synaptic density, increased BBB permeability and depression-like behavior (de Paula et al., 2021).

Melatonin, an indoleamine neuroendocrine hormone synthesized in the pineal gland, shows a wide range of physiological functions including the hepatoprotective and neuroprotective roles (Alghamdi, 2022; Joshi et al., 2021; K. Li et al., 2018; Pan et al., 2006). Serum TG and bile acid concentrations were markedly reduced in day-time melatonin group as compared to night-time group (Yin et al., 2020). Oral melatonin (10 mg/kg) has been reported to improve bile acid synthesis in the liver of alcohol-fed C57BL/6J mice (Kim et al., 2017). Melatonin showed hepatoprotective potential by decreasing GCDCA induced mitochondrial ROS production in L02 cells by acetylation of superoxide dismutase 2 (SOD2), in turn promoting SIRT3/SOD2 signalling and alleviating mitochondrial functions (Y. Chen et al., 2015). Intraperitoneal administration of melatonin (12.5mg/kg) increased catabolism of cholesterol, fecal bile acid excretion, HDL/total LDL cholesterol ratio thus resulting in hypocholesterolemic effect in male Sprague-Dawley rats (Chan & Tang, 1995). In bile duct ligated male Wistar rats, melatonin induced fibro-suppressant and antioxidant effects via improving hepatic fibrotic changes, decreased collagen, MDA, lucigenin and GSH levels (Tahan et al., 2010). Oral administration of melatonin (100 mg/kg) had lowered total cholesterol, LDL-cholesterol, VLDL-cholesterol and total bile acids in Male Wistar rats treated with (75 mg/kg, i.p.) a-naphthylisothiocyanate (ANIT) (Ohta et al., 2007). The neuro-protective

effect of melatonin has been previously reported in various rodent models of seasonal affective disorder, LPS induced neuro-inflammation, HFD induced behavioral perturbations and Chronic unpredictable mild stress (CUMS) induced depression & anxiety (Arioz et al., 2019; Detanico et al., 2009; Nagy et al., 2015; Onaolapo et al., 2020; Spasojevic et al., 2016). Several studies have shown that administration of exogenous melatonin enhances cognitive performance, improves depression and anxiety-like behavior and locomotive behavior in rodent models of neurodegenerative diseases as well (B. H. Chen et al., 2018; Dwivedi et al., 2018; Jameie et al., 2019; Mehraein et al., 2011). Intraperitoneal administration of 10 mg/kg melatonin improves cognitive dysfunction, increased protein expression of BDNF, TrkB and also increased the immunoreactivity of myelin-basic protein (MBP) in dentate gyrus of mice subjected to scopolamine-induced amnesia (B. H. Chen et al., 2018). Intraperitoneal melatonin (10 mg/kg/day) for four weeks significantly prevented HFD and/or alcohol-induced cognitive and memory deficits, oxidative stress and neuro-inflammation in male Wistar rats (Dwivedi et al., 2018).

Neuro-behavioral perturbations are prominent features of many psychiatric, neurodegenerative and lifestyle disorders including Alzheimer's disease and other dementias. Hence, changes in mood, attention, memory, socializing, judgement and problem-solving abilities are imperative to the wellbeing of individuals. High calorie diet or chronodisruption is known to cause depression and anxiety-like disorders along with locomotor deficits. Also, alteration in neurotrophic growth factors in hippocampus of experimental rodents implies towards an analogy with reported diet/photoperiod induced perturbations in human behaviour. This chapter showcases alterations in bile acid metabolism and correlates its changes with the findings of BDNF-TrkB pathway so as to establish evidences on hepatic-hippocampal crosstalk in NASH.

Materials and methods

Experimental model: C57BL/6J mice male mice aged (6-7 weeks each weighing 20-22 g. Particulars of animal maintenance and ethical statement are provided in Materials and methods section.

Experimental groups:

1. Control
2. Chronodisruption (CD)
3. High fat-high fructose diet (H)
4. High fat-high fructose diet + Chronodisruption (HCD)
5. Chronodisruption + Melatonin (CDM)
6. High fat-high fructose diet + Melatonin (HM)
7. High fat-high fructose diet + Chronodisruption + Melatonin (HCDM)

Parameter tested:

1. Serum titres (Direct, indirect and total bilirubin) and Thyroid hormones (T3, T4 and TSH)
2. Quantitative RT-PCR: Bile acid metabolism markers genes (BA receptor genes: FXR and TGR5; BA synthesis genes: CYP7A1, CYP8B1 and CYP27A1; and BA transporter genes: ABCC2, ABCC4 and OST-β) in Liver and BDNF-TrkB pathway markers genes (BDNF, TrkB, SYN-1 and PSD-95; neurotrophins: NT3 and NT4) in hippocampus
3. Immunoblotting: BDNF, TrkB, SYN-1 and ERK1/2

The experimental protocol for the present study is depicted in Fig. 5.1. Detailed methodology is described in materials and methods section.

Experimental design

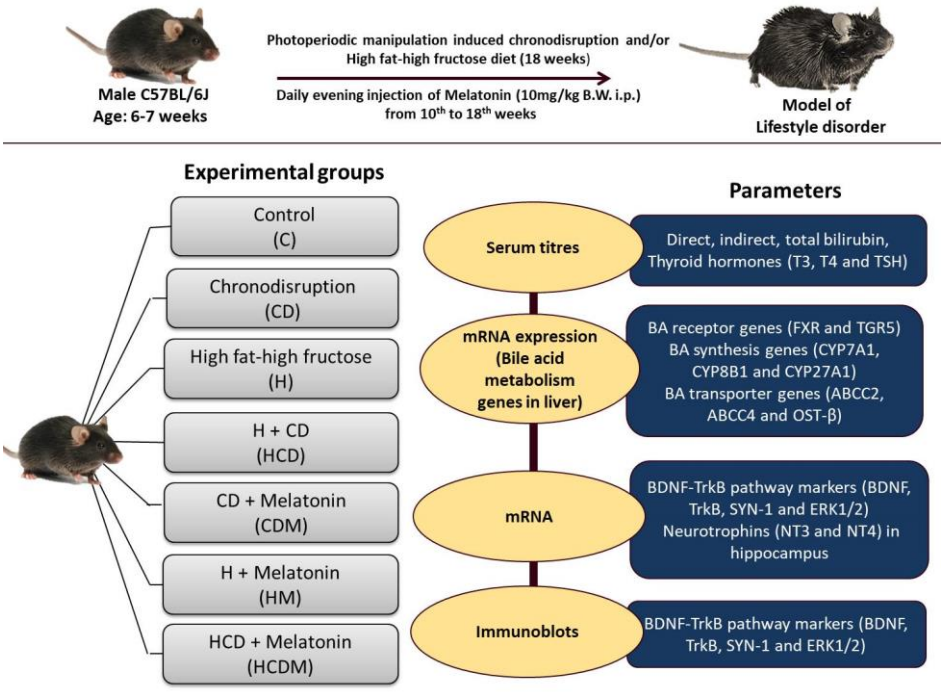

Figure 5.1: Flow chart of experimental protocol followed for hepatic-hippocampal crosstalk in high fat-high fructose diet and/or chronodisruption induced Nonalcoholic steatohepatitis in C57BL/6J mice.

Results

High fat-high fructose diet (H) and photoperiodic manipulation induced chronodisruption (CD) causes alterations in bilirubin levels in C57BL/6J mice

Direct, indirect and total bilirubin levels were moderately higher in H and HCD groups whereas; direct bilirubin levels were significantly (p<0.05) elevated in H group (Fig. 5.2). However, no change was observed in direct bilirubin in CD group. The indirect and total bilirubin levels were low in CD group as compared to control. Melatonin treated (CDM, HM and HCDM groups) did not induce any changes in the bilirubin levels and the same were comparable to control.

High fat-high fructose diet (H) and/or chronodisruption (CD) induced alterations in hepatic bile acid metabolism genes of C57BL/6J mice

Effect of High fat-high fructose diet (H) and/or chronodisruption (CD) was assessed in liver through mRNA levels of genes associated with bile acid (BA) metabolism viz. BA receptor (FXR, TGR5) (Fig. 5.3a & b), BA synthesis (CYP7A1, CYP8B1 and CYP27A1) (Fig. 5.3c, d & e) and BA transporter (ABCC2/MRP2, ABCC4/MRP4 & OST-β) genes (Fig. 5.4a, b & c). mRNA levels of FXR showed no significant change in any of the experimental groups. TGR5 showed a decrement in CD and H groups with a significant decrement (p<0.05) in HCD group. Melatonin treated groups (CDM, HM and HCDM) had recorded no major change in FXR with a moderate improvement in TGR5 mRNA. Bile acid synthesizing genes CYP7A1 and CYP8B1 showed a gross non-significant decrement in CD, H and HCD groups but specifically, CYP8B1 was unchanged in CD group. CYP27A1 showed a reciprocal non-significant upregulation in CD, H and HCD groups. Melatonin treatment had accounted for an improvement in mRNA levels of CYP7A1 and CYP8B1 genes and a decrement in CYP27A1 gene. BA transporter genes (ABCC2, ABCC4 & OST- β) had recorded a moderate to significant increment in mRNA levels of the said genes. Melatonin treatment caused an improvement as evidenced by the observed decrement in all the melatonin treated groups.

High fat-high fructose diet (H) and/or chronodisruption (CD) induced alterations in neurotrophic factors in hippocampus of C57BL/6J mice

In order to study the independent/cumulative effects of high fat-high fructose diet (H) and/or chronodisruption (CD) induced NASH, we assessed the hippocampal neurotrophic BDNF-TrkB pathway in whole hippocampus of C57BL/6J mice (Fig. 5.5). The mRNA levels of BDNF-TrkB pathway (BDNF, TrkB, SYN-1 and PSD-95) genes and Neurotrophin genes (NT3 and NT4) were assessed. Further validation was obtained by assessing the protein immunoblots of BDNF, TrkB, ERK1/2 and SYN-1 in hippocampus of control and treated mice. A non-significant decrement was recorded in mRNA levels of BDNF, TrkB and SYN-1 in CD, H and HCD groups. The protein levels of BDNF recorded no change in CD and H groups but HCD showed a decrement as compared to the control. The TrkB protein levels showed no change and were comparable to the control. Melatonin treatment had accounted for varying degrees of increment in mRNA and protein levels of BDNF and TrkB as compared to their respective disease controls (CD vs CDM; H vs HM and HCD vs HCDM) with BDNF protein in CDM group being an exception since it did not show an improvement. Protein levels of SYN-1 showed a moderate increment in all the disease control groups (CD, H and HCD) whereas; melatonin treatment had accounted for a moderate decrement (Fig. 5.7). This trend is contrary to the published literature. Protein levels of ERK1/2 did not show any major change in CD, H, HCD and CDM groups. The melatonin treated (HM and HCDM) groups had recorded for moderate increment in their protein levels. mRNA levels of NT3 and NT4 were non-significantly lowered in CD, H and HCD groups but, only NT3 showed a significant decrement ($p<0.05$) in CD group. Melatonin was able to non-significantly up-regulate NT3 and NT4 in CDM, HM and HCDM groups (Fig. 5.6).

High fat-high fructose diet (H) and/or chronodisruption (CD) mediated alterations in T3, T4 and TSH hormone levels in serum of C57BL/6J mice

Circulating titres of T3, T4 and TSH hormone in serum of high fat-high fructose diet (H) and/or chronodisruption (CD) induced NASH in C57BL/6J mice was done using anti-T3, anti-T4 and anti-TSH ELISA kits (Fig. 5.8). It was observed that high fat-high fructose diet (H) and/or chronodisruption (CD) significantly ($p<0.001$) lowered T3 levels and significantly ($p<0.001$) higher T4 levels in CD, H and HCD groups. TSH levels were

significantly higher in H (p<0.05) and HCD (p<0.01) groups; but, a non-significant increment was recorded in CD group. Melatonin treatment was able to significantly improve (p<0.001) T3 and T4 levels in CDM, HM and HCDM groups. However, TSH levels did not improve following melatonin treatment as their levels were recorded to be higher than control.

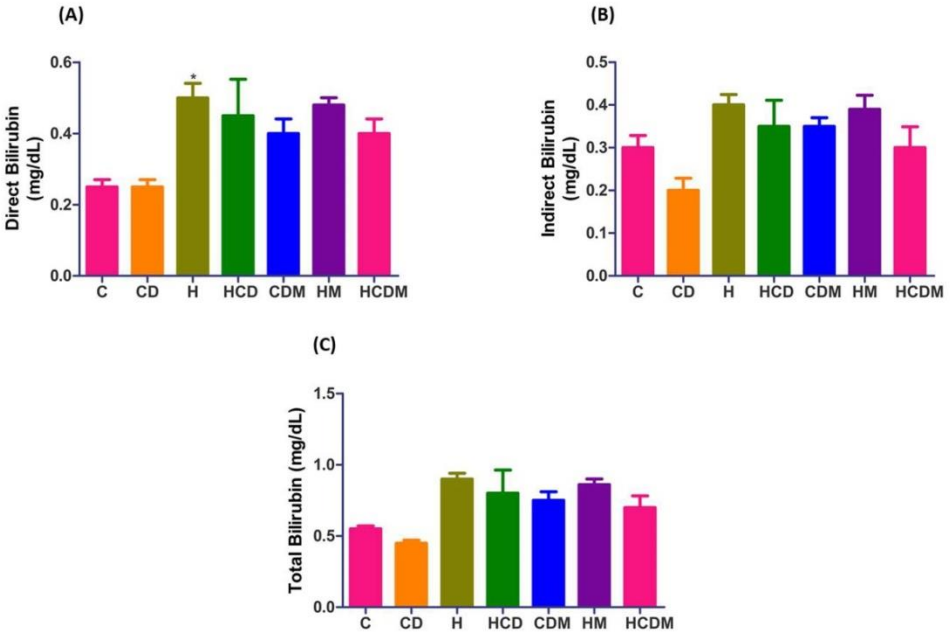

Figure 5.2: Levels of (A) Direct bilirubin (B) Indirect bilirubin and (C) Total bilirubin in C57BL/6J mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin (i.p. 10mg/kg) was administered intraperitoneally in CDM, HM and HCDM groups from 10th week to 18th weeks of the experimental regime. Results are expressed as mean ± S.E.M. *$p < 0.05$, H compared to Control (C). ###$p < 0.001$ is when CDM compared with HM with H group respectively.

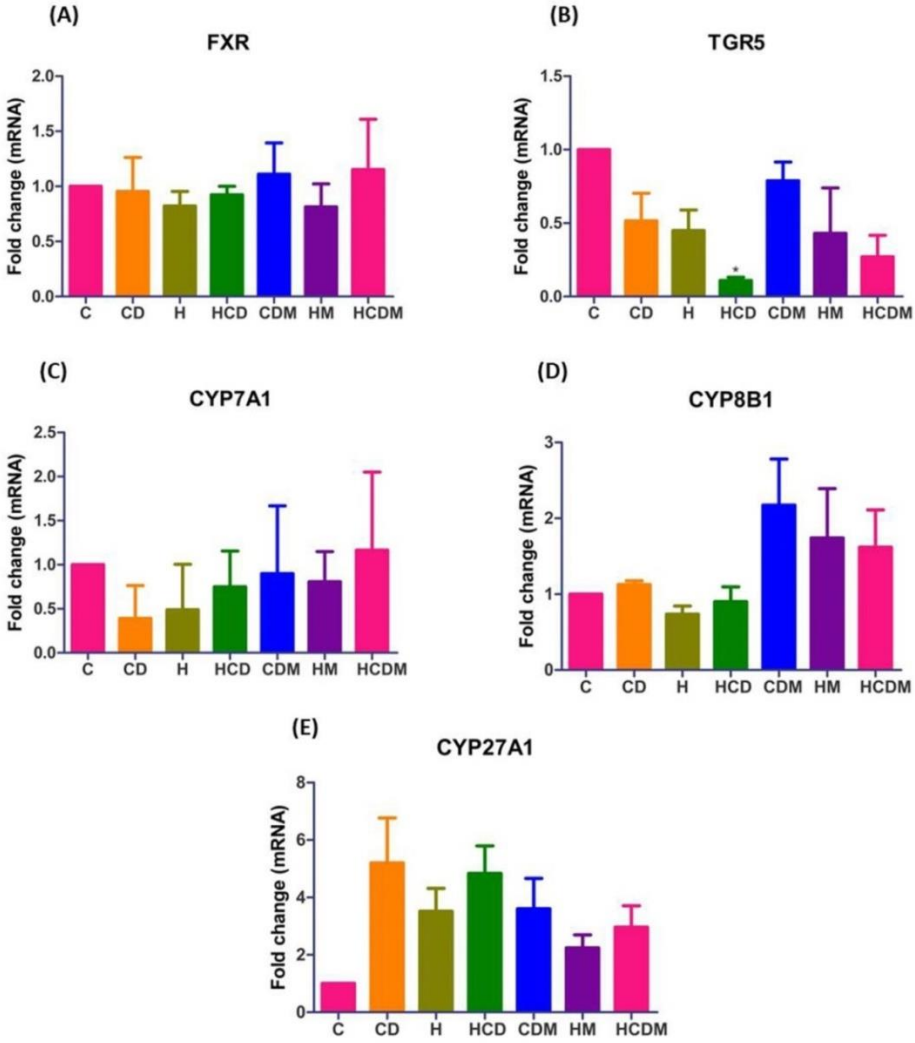

Figure 5.3: Changes in the mRNA gene expression of bile acid receptor (A) FXR, (B) TGR5, and bile acid producing genes (C) CYP7A1, (D) CYP8B1 and (E) CYP27A1 in the mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups from 10^{th} week to 18^{th} weeks of the experimental regime. Results are expressed as mean ± S.E.M. *$p < 0.05$, is when HCD compared to Control (C).

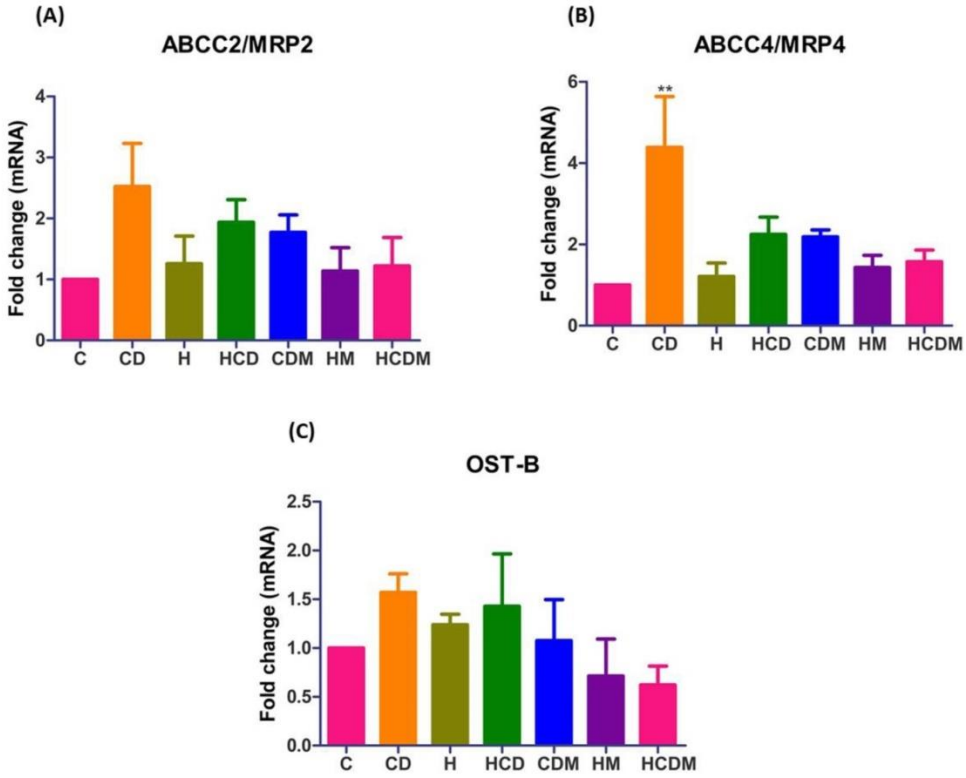

Figure 5.4: Changes in the mRNA gene expression of bile acid transporters genes (A) ABCC2, (B) ABCC4 and (C) OST-β in liver of mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups. Results are expressed as mean ± S.E.M. **$p < 0.01$ is when CD is compared with Control (C).

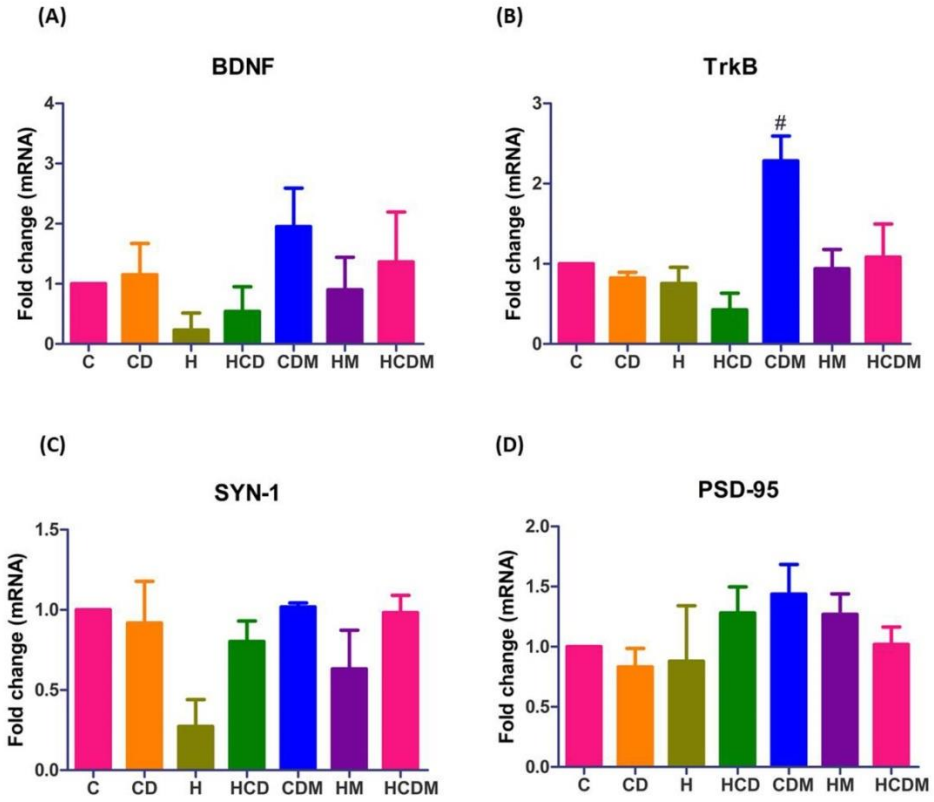

Figure 5.5: Changes in the mRNA gene expression of brain-derived growth factor and receptor (A) BDNF, (B) TrkB, and synaptic markers genes (C) SYN-1, (D) PSD-95 in hippocampus of mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups. Results are expressed as mean ± S.E.M. #$p < 0.05$, is when CDM compared with CD group respectively.

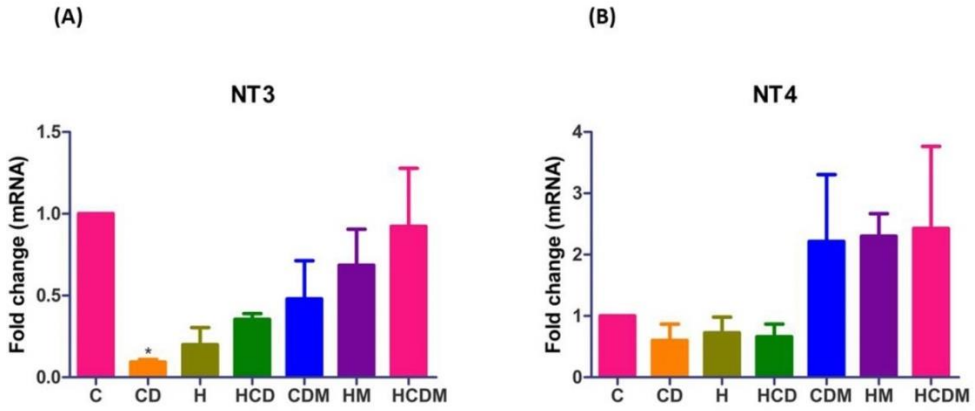

Figure 5.6: Changes in the mRNA gene expression of neurotrophins (A) NT3 and (B) NT4 in hippocampus of mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups from 10th week to 18th weeks of the experimental regime. Results are expressed as mean ± S.E.M. *$p < 0.05$, is when CD compared to Control (C).

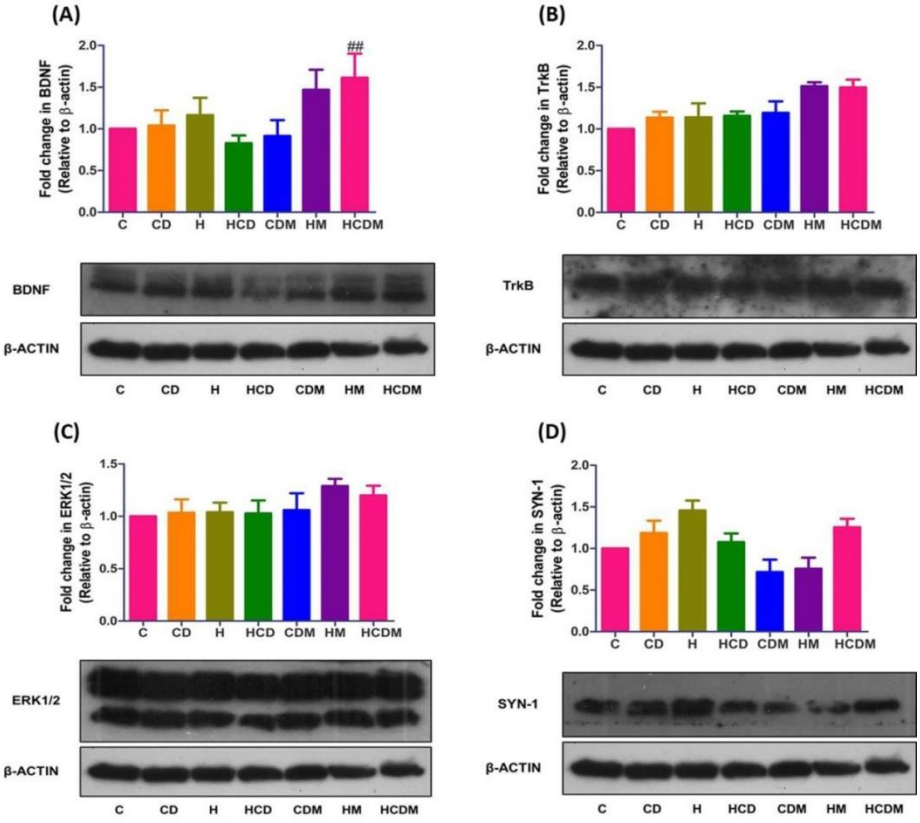

Figure 5.7: Changes in the protein expression of (A) BDNF, (B) TrkB, (C) ERK1/2 and (D) SYN-1 in hippocampus of mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups. Results are expressed as mean ± S.E.M. ##$p < 0.01$, is when HCDM compared to HCD.

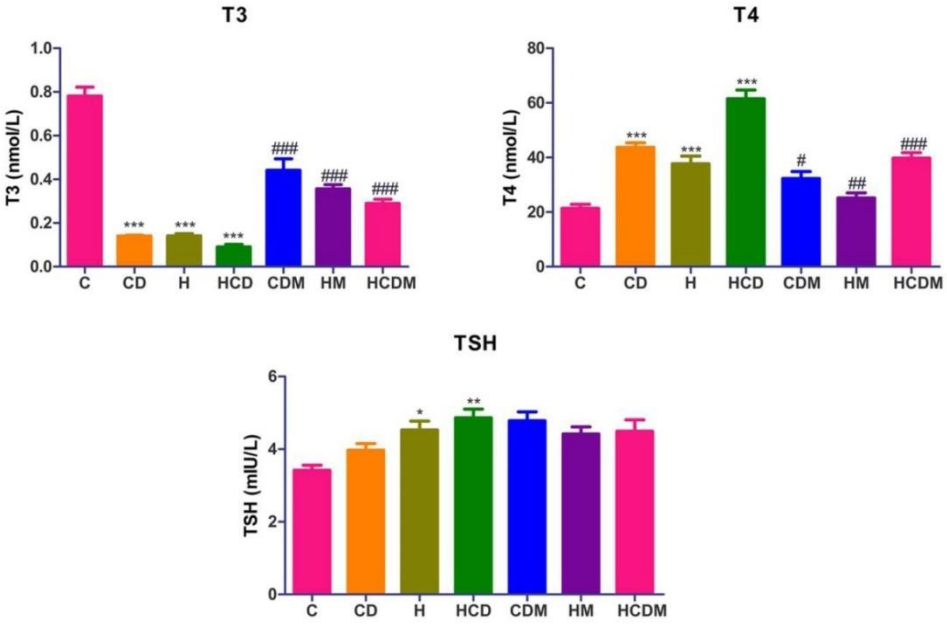

Figure 5.8: Alterations in thyroid hormones T3, T4 and TSH in CD, H and HCD groups in mice subjected to high fat-high fructose diet and/or chronodisruption. Exogenous melatonin was administered intraperitoneally in CDM, HM and HCDM groups. Results are expressed as mean ± S.E.M. *p < 0.05, **p < 0.01, and ***p < 0.001 is when CD, H and HCD compared to Control (C). #p < 0.05, ##p < 0.01, and ###p < 0.001 is when CDM compared with CD, HM with H and HCDM with HCD group respectively.

Discussion

Multiple metabolic and inflammatory pathways are involved in the transition from simple steatosis to NASH. Homeostasis of Bile acids (BA) is crucial in NASH and has been implicated for its involvement in hepatic lipid, glucose and energy metabolism via activation of nuclear bile acid receptor FXR (farnesoid X receptor) and a membrane G protein-coupled receptor TGR5 (Takeda-G-protein-receptor-5) (T. Li & Chiang, 2014; Trauner et al., 2010). FXR is a nuclear bile acid receptor involved in bile acid synthesis and metabolism and its dysfunction is implicated in the pathology of cirrhosis, cholestasis and atherosclerosis (Boyer et al., 2006; Fiorucci et al., 2005; Zhang et al., 2012). $FXR^{-/-}$ mice have been reported to exhibit less obstructive cholestasis with higher serum BAs as compared to control mice (Stedman et al., 2006). These mice also show alterations in multiple neurotransmitter system (glutamatergic, GABAergic, serotoninergic, and norepinephrinergic neurotransmission) in hippocampus and cerebellum resulting in altered neuro-behavior (F. Huang et al., 2015). G-protein-coupled bile acid receptor (Gpbar1 or TGR5) plays an important role in multiple cell signalling pathways and has become an attractive target for the treatment of metabolic diseases such as type 2 diabetes (T2D), NAFLD/NASH, atherosclerosis and obesity (Guo et al., 2016). Organic solute transporter α/β (OST-α and OST-β) are bile acid transporter important for the recycling of bile acids in the enterohepatic circulation and is directly regulated by the bile acid nuclear receptor, farnesoid X receptor (FXR). In patients with NASH, higher serum BA, overexpression of OST-α and OST-β along with increased taurine- and glycine-conjugated primary and secondary BAs were reported as compared to healthy subjects (Ferslew et al., 2015). Significantly higher OST-β protein expression was reported in the liver from patients having confirmed case of NASH (Malinen et al., 2018). An increased hepatic OST-α & OST-β expressions are found in both humans and experimental murine models of cholestasis (Soroka et al., 2010). In the present study, alterations in the key genes regulating bile acid metabolism in healthy and steatotic liver of C57BL/6J mice have been investigated wherein; high fat high fructose and/or circadian desynchrony mediated NASH was induced over a period of 18 weeks. Further, alterations in the expression levels of BDNF-TrkB pathway marker genes (BDNF, TrkB, SYN-1 and ERK1/2) along with the neurotrophic growth factors (NT3 and NT4) and synaptic plasticity marker (PSD95) have also been investigated in hippocampal tissue.

The results of hepatic mRNA expression of BA receptor (FXR, TGR5), BA producing (CYP7A1, CYP8B1 & CYP27A1) and BA transporters (ABCC2, ABCC4 & OST-β) were altered in CD, H and HCD groups. However, expression of TGR5 expression was downregulated in CD and H group with significant decrement recorded in HCD group. TGR5 is an important membrane receptor which is activated by bile acids and plays a fundamental role in BA metabolism (Guo et al., 2016). In mouse model of diet-induced obesity, chenodeoxycholic acid (CDCA) treatment significantly decreased blood glucose levels and improved glucose tolerance (X. Chen et al., 2017). In liver, bile acids are synthesized through CYP7A1 mediated classic (neutral) and CYP27A1 mediated alternative (acidic) pathways (Chiang, 2009). In Dimethyl-nitrosamine-induced liver fibrosis, significant decrement in mRNAs of hepatic bile acid synthesis genes (CYP7A1, CYP8B1 & CYP27A1) was reported with an altered liver function test (Song et al., 2016). Hepatic mRNA levels of CYP8B1 were reported to be decreased in NASH patients while, an increase in CYP7B1 was implied towards a shift of BAs from the classical pathway to alternative synthesis pathway (Lake et al., 2013). Reports on C57BL/KsJ$^{-db/db}$ with hepatic steatosis, had shown a significant decrement in the hepatic mRNA expression of CYP7A1 and CYP8B1 as compared to wild-type C57BL/6 mice (Lim et al., 2016). Similar observation were made in our study wherein; a decrement in CYP7A1 & CYP8B1 (classical) and an increment in CYP27A1 (alternative) were recorded in CD, H and HCD groups, suggesting a shift of synthesis of BAs from classical to alternative pathway. Various studies on NAFLD had concluded to have a more profound ABCC2/MRP2 expression as compared to other transporters in steatotic liver of mice (Canet et al., 2015; Hardwick et al., 2012). Polymorphisms of ABCC2 have also been implicated in NAFLD and clinical studies had suggested that allelic variants of ABCC2 correlate with clinical and histological spectra of NAFLD (Sookoian et al., 2009). Male Sprague–Dawley rats fed with MCD diet for 8 weeks were reported to have higher expression of ABCC2 in a steatotic liver (Hardwick et al., 2012). In out study, the BA transporter genes (ABCC2, ABCC4 & OST-β) were found to be upregulated in CD, H and HCD groups that corroborate with the published reports on experimental models and clinical studies. Thyroxin (T4) is converted to triiodothyronine (T3) by TGR5 (a BA receptor) mediated activation of deiodinases (Watanabe et al., 2006). In the present

study, downregulated TGR5 in all three disease control (CD, H and HCD) groups was in agreement with lowered circulating titres of T3 and TSH and increment in T4 levels. Exogenous melatonin treatment improved the T3, T4 and TSH titres in CDM, HM and HCDM groups. Our findings are in agreement with other studies that had reported melatonin mediated improvement in thyroid gland functioning and secretion of T3 and T4 in an experimental model of NASH or circadian desynchrony model (Jiménez-Aranda et al., 2013; Krotewicz & Lewinski, 1994; Vriend, 1983; Vriend et al., 1982).

Recent studies have proposed that melatonin ameliorates alcohol-induced altered bile acid synthesis by enhancing miR-497 expression in C57BL/6 mice (Kim et al., 2017). In our study, BA metabolism genes viz. BA receptor (TGR5), BA producing (CYP7A1, CYP8B1 & CYP27A1) and BA transporters (ABCC2, ABCC4 & OST-β) were restored following melatonin treatment to the disease control groups. Taken together, these results of BA producing genes indicate that exogenous melatonin administration improves expression of CYP7A1 and CYP8B1 mRNA that promotes the conversion of cholesterol to bile acid through BA classical synthesis pathway.

Emerging data from various experimental rodents and human studies suggest that not only fibrotic or cirrhotic liver disease; but also the NAFLD/NASH is linked with a risk of cognitive impairment and dementia (Elwing et al., 2006; Yilmaz & Ozdogan, 2009; Youssef et al., 2013). In middle-aged adults, MRI studies revealed a strong association of NAFLD with lower brain volume wherein; total cerebral brain volume, hippocampal and white matter hyperintensity volumes and presence/absence of covert brain infarcts were measured (Weinstein et al., 2018). Study by Gorelick et al., and Debette et al., had shown a link between liver steatosis and brain structure & function wherein; common risk factors such as lack of physical activity, obesity and diabetes epitomized as the risk factors for cognitive impairments, dementia and brain aging (Debette et al., 2011; Gorelick et al., 2011). Various evidences from patient studies had reported on a direct association between metabolic syndrome and common mental health disorders viz. depression and anxiety (Banerjee et al., 2020; Elwing et al., 2006; Surdea-Blaga & Dumitraşcu, 2011). The neurocircuitry of anxiety, depression and mood disorders involve portions of limbic system such as amygdala, cortex, hippocampus, hypothalamus and

thalamus (Price & Drevets, 2010). Neurotrophins exert many biological effects by activating tropomyosin-related kinase (Trk) receptors, which in turn, triggers cascades such as, phosphatidylinositol 3-kinase PI3K/AKT, phospholipase Cγ (PLCγ), and Mitogen-activated protein kinase (MAPK) pathways. Four major neurotrophins (BDNF, NGF, NT3 and NT-4) have been reported in mammals that govern growth, maturation, differentiation and maintenance of neuronal cells (Dechant & Neumann, 2003; E. J. Huang & Reichardt, 2001; Lewin & Barde, 1996). Brain Derived Neurotrophic Factor (BDNF) is a crucial mediator of neuronal vitality and is involved in plastic changes related to learning and memory. Alterations in BDNF expression are reported to be in synchrony with aging pathology and prevalence of psychiatric disease/ mental disorders (Bramham & Messaoudi, 2005; E. J. Huang & Reichardt, 2001). In diet induced obese model of zebrafish, mRNA levels of BDNF were reported to be downregulated whereas; significant increment in TrkB expression was recorded in brain (Montalbano et al., 2016). Our study, had recorded a downregulation in mRNA expression of BDNF, TrkB, NT3 and NT4 in the disease control (H and HCD) groups with moderate changes in CD group. The TrkB protein levels were however found to be unchanged. A report on Wistar rats fed with HFD had recorded reduced BDNF, PSD-95 and NT3 levels in brain (Arcego et al., 2018). In our study, although the BDNF mRNA levels in H and HCD groups had shown a decrement, the protein levels were unchanged in all the three disease control groups. These results are in agreement with reports on high fat diet fed / 8h phase advance photoperiod protocols in wistar rats wherein; no significant changes in BDNF levels were reported (Alzoubi et al., 2013; Sugiyama et al., 2020). Herein, it is interesting to note that, other research groups had reported loss of both long and short-term memory in HFD fed wistar rats (Alzoubi et al., 2013) and our study too had recorded significant shifts in behavioural patterns (depression, anxiety, spatial learning and memory) in CD, H and HCD groups (Chapter 4). These findings throw light on the fact that, dietary/photoperiodic manipulations can alter behavioural traits irrespective of measureable changes in hippocampal BDNF levels.

Melatonin exerts neuroprotective effects mediated by anxiolytic, anti-apoptotic, anti-amyloid, anti-inflammatory and anti-oxidant mechanism as well as by improving the levels of brain derived neurotrophins (Esposito & Cuzzocrea, 2010; Gupta et al., 2003;

Sugiyama et al., 2020; Vincent, 2018; X. Wang, 2009; Xu et al., 2019; Yousaf et al., 2010). In the present study, exogenous melatonin treated (CDM, HM and HCDM) groups showed moderate improvement in the expression of BDNF, TrkB, SYN-1, NT3 and NT4 as compared to their respective disease control (CD, H and HCD) groups. These results corroborate with findings of other research groups wherein, melatonin is shown to increase BDNF-TrkB immunoreactivity in dentate gyrus with recovered cognitive impairment of scopolamine-induced amnesia in male ICR-mice (B. H. Chen et al., 2018). Also in another study, 100 mg/L melatonin supplemented in drinking water reversed cognitive decline and restored BDNF levels in hippocampus of HFD-fed aged rats (Xu et al., 2019). Intraperitoneal melatonin along with exercise had significantly elevated the protein expression of BDNF and its receptor TrkB in brain of HFD-fed Wistar rats (Sugiyama et al., 2020). These findings corroborate the results obtained in our study that showcases an increase in protein levels of BDNF and TrkB in hippocampus of HM and HCDM treated mice.

Taken together, this study unravels the role of exogenous melatonin in modulating hepatic-hippocampal crosstalk via improving the inflammatory status in liver and brain (chapter 2), the gut microflora (chapter 3) and also improving the nocturnal/diurnal neuro-behavioral perturbation (chapter 4) caused due to high fat-high fructose diet and/or chronodisruption subjected C57BL/6J mice. The cumulative effect of high fat-high fructose diet and chronodisruption has shown a combinatorial effect on the development of lifestyle disorder. Exogenous melatonin mediated improvement in the gut microflora and restoration of neuro-behavior (depression, anxiety, locomotion, spatial learning and memory) without major changes in BDNF-TrkB pathway is an important finding of our study. The controlled experimental conditions enables us to decipher the said changes however, in human subjects, single nucleotide polymorphisms, age groups, ethnic/geographical variations, sex-specific changes and comorbidities may significantly alter the inferences and hence needs further scrutiny.