Analysis and Quantification of Physical Fatigue in Automobile Drivers: A Biomedical Approach

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Analysis and Quantification of Physical Fatigue in Automobile Drivers: A Biomedical Approach

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under the supervision of

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and

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Supervisors' Certificate

This is to certify that the work presented in the dissertation entitled *Analysis and Quantification of Physical Fatigue in Automobile Drivers: A Biomedical Approach* submitted by *Bibhukalyan Prasad Nayak*, Roll Number 509BM403, is a record of original research carried out by him under our supervision and guidance in partial fulfillment of the requirements of the degree of *Doctor of Philosophy* in *Biotechnology and Medical Engineering*. Neither this dissertation nor any part of it has been submitted earlier for any degree or diploma to any institute or university in India or abroad.

Aurobinda Routray

Mukesh Kumar Gupta

Dedication

This Thesis is dedicated to my wife and son for their immense support, persistent encouragement and an incredible patience.

Declaration of Originality

I, *Bibhukalyan Prasad Nayak*, Roll Number *509BM403* hereby declare that this dissertation entitled *Analysis and Quantification of Physical Fatigue in Automobile Drivers: A Biomedical Approach* presents my original work carried out as a doctoral student of NIT Rourkela and, to the best of my knowledge, contains no material previously published or written by another person, nor any material presented by me for the award of any degree or diploma of NIT Rourkela or any other institution. Any contribution made to this research by others, with whom I have worked at NIT Rourkela or elsewhere, is explicitly acknowledged in the dissertation. Works of other authors cited in this dissertation have been duly acknowledged under the sections "Reference" or "Bibliography". I have also submitted my original research records to the scrutiny committee for evaluation of my dissertation.

I am fully aware that in case of any non-compliance detected in future, the Senate of NIT Rourkela may withdraw the degree awarded to me on the basis of the present dissertation.

November 25, 2016
NIT Rourkela

Bibhukalyan Prasad Nayak

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Abstract

Vehicular accidents from fatigue due to sleep-deprived driving are rapidly increasing among heavy vehicle drivers. Consequently, a critical analysis of drivers' fatigue in real time, using established clinical parameters, and subsequent scoring is of dire need in automobile sector. Such a scoring system would be helpful in validating the fatigue detecting devices based on non-contact features that can be installed onboard. Therefore, this study aimed to analyze and quantify physical fatigue during sleep-deprived simulated driving and their utility in developing objective score for fatigue assessment. The genesis and progression of physical fatigue was also analyzed in apparently healthy and pathological condition such as tauopathy.

In the first set of experiments, the behavioral changes, cognition and motor performance in response to induced fatigue was analyzed in αCAMK-II-4R tau (transgenic/Tg) mice model of tautopathy ($n = 24$) and were compared with those of wild-type mice ($n = 24$). The mice were subjected to Accelerated Rotarod Test (ART), Open Field Test (OFT), Elevated Plus Maze Test (EPMT), Light and Dark Transition Test (LDT) and Forced Swimming Test (FST) for a comprehensive motor and cognitive performance analysis. Results showed that, genesis and progress of fatigue followed similar trend under physiological condition and pathological condition like tauopathy although the signs and symptoms of physical fatigue in mice models of tauopathy were more pronounced compared to healthy ones. Thus, a fatigue score system developed on healthy individuals may also be applied on pathological conditions that make the subjects vulnerable to fatigue.

Subsequently, to quantify the manifestations of physical fatigue, twelve seasoned drivers were subjected to simulated driving session for 30 h and electroencephalogram (EEG), electrocardiogram (ECG) and spirometer recordings were taken for each individual at 3h intervals. In addition, peripheral blood samples were collected and analyzed at 8 h intervals for random blood sugar (RBS), blood urea (BUN) and serum creatinine. Results revealed that, energy and entropy features of EEG showed significant discrimination across time points in α and θ-bands at Cz electrode. The power spectrum density of HF (high frequency) components of ECG decreased with advancing stress and fatigue indicating sympathetic predominance with severity in fatigue. Spirometer recording confirmed gradual decrease in FEV_1/FVC ratio (Forced Expiratory Volume at 1^{st} sec / Forced Vital Capacity) as fatigue progressed. On the other hand, all blood biomarkers increased with the progress of fatigue but RBS and creatinine showed better discrimination across time-points.

To quantify inducers of physical fatigue in drivers, 14 skilled heavy vehicle drivers were subjected to simulated driving session for 16 h and the load of central stress was measured by assessing the serotonin in platelet rich plasma and the extent of physical stress was measured by estimating the serum cortisol and insulin profiles at 4 h intervals. Serotonin increased by two fold (1421.9 ± 120 ng/mL at 0h to 2793.5 ± 153 ng/mL at 16h) at the end 16 h of simulated driving while serum cortisol and insulin increased significantly generating a state of insulin resistance. The data generated on above parameters were statistically validated and were found to be consistent against the clinically used subjective assessment tools namely customized sleepiness score, short form 36 ver.2 (SF36 v2) health survey score and Beck's depression inventory (BDI-II) for evaluating drowsiness, mental and physical feeling and mood disturbance, respectively at different stages of simulated-driving.

In conclusion, the results of the present study suggest that a precise functional staging of drivers fatigue can be established by integrating the variations in specific EEG parameters, power spectrum of HF components of ECG and, blood biomarkers particularly RBS and serum creatinine. Further, serum serotonin had a positive co-relation with the progression of central fatigue while serum cortisol and insulin had a positive co-relation with the progression of physical fatigue and therefore, in combination, they may be used in devising the scoring system for estimating fatigue progression in drivers. A staging system based on physiological parameters may be used for both healthy drivers as well as those who are prone to fatigue due to pathological condition.

Keywords**:** *Drivers' fatigue; αCAMK-II-4R tau; ART; OFT; EPMT; LDT; FST; Simulated driving; EEG; ECG; RBS; BUN; Creatinine; Spirometer; Subjective assessment; Serotonin; Cortisol; Insulin.*

Contents

List of Abbreviations

ABBREVIATION	EXPANSION
5-HT	5-Hydroxytryptamine (Serotonin)
5-HTRP	5-Hydroxytryptophan
AC	Approximate Component
ACTH	Adrenocorticotropic Hormone
ANN	Artificial Neural Network
ANS	Autonomic Nervous System
ART	Accelerated Rotarod Test
AVN	Atrio-Ventricular Node
BCAA	Branched-Chain Amino Acids
BDI	Beck Depression Inventory
BMI	Body Mass Index
BP	Bodily Pain
BUN	Blood Urea Nitrogen
BVP	Blood Volume Pulse
CCD	Charge-coupled Device
CFS	Chronic Fatigue Syndrome
CMR	Cerebral Metabolic Ratio
CMRCHO	Cerebral Metabolic Ratio of Carbohydrate
CMRO2	Cerebral Metabolic Ratio of Oxygen
CNS	Central Nervous System
Cortisol-21-HS-HRP	cortisol-21-hemisuccinate-horse radish peroxidase
Cortisol-3-O-CMO-BSA	Cortisol-3-O-carboxymethyl-oxime-bovine serum albumin
Cr	Creatinine
CSF	Cerebrospinal Fluid
CSMR	Complex Sensorimotor Reactions
CT	Computed Tomography
CTF	Combat and Technical Facilities
DC	Dark Chamber
DC	Detailed Coefficient

ABBREVIATION	EXPANSION
DHEA	Dehydroepiandrosterone
DWT	Discrete Wavelet Transformation
ECG	Electrocardiogram
EEG	Electroencephalogram
ELISA	Enzyme Linked Immunosorbent Assay
EM	Eye Movements
EMG	Electromyogram
EOG	Electrooculogram
EPMT	Elevated Plus Maze Test
FEV	Forced Expiratory Volume
FFA	Free Fatty Acid
FFF	Flicker Fusion Frequency
FFT	Fast Fourier Transform
FMCSA	Federal Motor Carrier Safety Administration
FS	Functional Status
FS	Functional Status
FST	Forced Swimming Test
FVC	Forced Vital Capacity
GABA	Gamma Aminobutyric Acid
GH	General Health Perceptions
HF	High Frequency
HOMA-2	Homeostatic Model Assessment-2
HPA	Hypothalamic-Pituitary-Adrenal axis
HR	Heart Rate
HRV	Heart Rate Variability
HRV	Heart Rate Variation
IL 1	Interleukin - 1
IR	Insulin Resistance
LA	Lactic Acid
LC	Light Chamber
LDT	Light and Dark Test
LF	Low Frequency
LFT	Lungs Function Test
LNAA	Large Neutral Amino Acids
MCS	Mental Component Score
MCS	Mental Component Summary
MH	Mental Health

ABBREVIATION	EXPANSION
NT	Neurotransmitter
OD	Optical Density
OFT	Open Field Test
OSA	Obstructive Sleep Apnea
PA CO2	Partial Pressure of CO2
PA O2	Partial Pressure of O2
PCS	Physical Component Score
PCS	Physical Component Summary
PERCLOS	Percentage Closure of Eyes
PET	Positron Emission Tomography
PF	Physical Functioning
PFT	Pulmonary Function Test
PRP	Platelet Rich Plasma
PSD	Power Spectral Densities
RBS	Random Blood Sugar
RE	Role Emotional
RIA	Radio Immuno Assay
RMO	Reaction to a Moving Object
RP	Role Physical
RT	Room Temperature
SAN	Sinu Atrial Node
SE	Shannon's Entropy
SEM	Standard Error of Mean
SF	Social Functioning
SF-36	Short-Form 36
SNS	Sympathetic Nervous System
SSMR	Simple Sensorimotor Reactions
Tg	Transgenic
TMB	3,3′,5,5′-Tetramethylbenzidine
TRP	Tryptophan
USP	United States Pharmacopia
VHF	Very High Frequency
VT	Vitality

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Chapter 1

Introduction

Vehicular and industrial accidents from human error induced by central fatigue with or without sleep deprivation are rapidly increasing. Fatigue in human operators is a major cause of many fatal accidents. It is more deadly in transportation systems. Literature suggests that fatigue is responsible for about 20-30% of total road fatalities [1–3]. A survey on road accidents in India has revealed that over 80 thousand people die in the traffic crashes annually, over 1.2 million are injured seriously and about 0.3 million disabled permanently. According to the Department of Road Transport and Highways, Government of India, the road accidents have been increased from 320,400 in 1994 to 829,800 in 2008 [4]. According to World Health Organization, more people die in road accidents in India than anywhere else in the world, including the more populous China. Considering the road fatalities an "epidemic", it will become world's 5th biggest killer by 2030 [5].

Fatigue causes lack of alertness and reduced mental and physical performance. There are several factors that promote the generation and growth of fatigue in vehicle operators. Among these, sleep deprivation, overwork, physical and mental conditions are important [6]. Manifestation of fatigue is not definite. It can be reflected in several ways. Fatigue can generate some changes in facial expression or eye movement, or can produce changes in physiological signals such as Electroencephalogram (EEG), Electrocardiogram (ECG) and Electromyogram (EMG) and some bio-chemical measurements such as glucose and urea. Further, the subjects themselves can realize fatigue, as it causes tiredness and drowsiness which affect their performance. Based on different nature of manifestations, researchers have suggested various methods to assess fatigue. These methods can be classified into following groups: questionnaire based subjective or self-assessments; Psychomotor performance measurement related to physical and mental ability; clinical investigations like blood, urine or cerebrospinal fluid (CSF) biochemical variations; ocular measurements like saccadic eye movement [7], Percentage Closure of Eyes (PERCLOS) [8]; measurement of physiological variables like EEG, EOG, EMG, ECG and spirometry findings. In addition, few methods such as measuring steering grip pressure, skin conductance, Blood Volume Pulse (BVP) etc. have been suggested to assess fatigue in drivers [9–11]. However, not a single method or parameter alone is sufficient to evaluate the genesis and development of

central fatigue or to design an applicable scoring system for physical fatigue as a whole in long distance drivers.

Identification of biochemical/physiological markers of physical fatigue and appropriate scoring of its genesis and progression in the proposed occupational groups may also help in validating the performance and diagnosis by wearable and/or on-board installed devices that are currently available (Appendix A).

1.1 Definition of Fatigue

According to the consensus of the scientific community "fatigue comprises of physiological, emotional and behavioral factors that can result in chronic physical or mental states" [12]. Fatigue is defined as a state to which the person reaches as a result of sustained activity wherein he or she declares being unable to continue the activity further or has started a subjective feeling of inhibition to continue the task in hand because of perceived reductions in efficiency [13]. Thus, fatigue is a nonspecific psychophysiological phenomenon that is marked by reduced efficiency and a declination to work [14]. Rasmussen et al. (2006) has defined fatigue more precisely as a complex state characterized by lack of alertness and reduced mental and physical performance, often accompanied by drowsiness [15].

1.2 Classification of Fatigue

According to the source of origin, physical fatigue can be classified into central and peripheral fatigue. Central fatigue is mainly due to increased cerebral metabolism leading to increased depletion of glucose and oxygen (leading to hypoxia). Cerebral hypoxia in turn causes a variety of mental aberrations: impaired judgment, drowsiness, dulled pain sensibility, excitement, disorientation, loss of time sense, and headache [16]. These factors play a role, but it is clear that other factors also contribute and that the ability to perform a task stops when the sensation of fatigue progresses to the sensation of exhaustion. Thus, persistent central fatigue invariably leads to motor inefficiency and inability to coordinate different skeletal muscles that is essential for smooth operation of an action. In addition, various affective symptoms are associated with central fatigue of which most consistent is the feeling of depression.

On the other hand, peripheral fatigue refers to fatigue of skeletal muscles that may arise from overuse of skeletal muscles or distorted motor commands from brain due to persistent central fatigue. Such peripheral fatigue culminates in mild to severe deficiency in muscle action. It appears as exhaustion.

1.3 Mechanism of Genesis of Fatigue

Long before its external manifestation, the fatigue is generated at molecular level in the human body. The fatigue that accompanies prolonged physical exercise or a monotonous activity is evolved from, (i) modification in the central nervous system (CNS) that reduces motor neuron impulse traffic to muscle and/or (ii) metabolic changes in muscle that ultimately lead to muscle exhaustion. Physical fatigue can originate within the muscle, which is known as peripheral fatigue, or within the central nervous system, which is known as central fatigue.

1.3.1 Peripheral Fatigue

The mechanism of genesis of peripheral fatigue can be manifold and is well established by numerous published research works. Several biochemical mechanisms i.e. depletion of phosphocreatine, accumulation of protons in the form of lactic acid (LA), depletion of glycogen and failure of neuromuscular transmission have been put forward to explain the peripheral fatigue [17]. The most investigated explanation for peripheral fatigue points towards the breakdown of muscle glycogen, a major store of energy inside muscle fibers. It results in the accumulation of lactic acid which impairs muscle function. Intracellular lactic acid deposition accounts at least for some types of peripheral fatigue. However, there are other types of fatigue where lactic acid does not accumulate. It is evidenced by a recent finding wherein the effects of acidosis were examined at body temperature rather than at room temperature and the inhibitory effects were minimal [18]. Other reasons that contribute to muscle fatigue are failure or inefficiency of CNS to produce action potentials (ionic dysregulation), depletion of energy stores, and protein degradation by reactive oxygen species and/or structural damage to muscles. Experiments have shown that transmission of the nerve signal through the neuromuscular junction can diminish at least a small amount after intense prolonged muscle activity, thus, further diminishing muscle contraction. Interruption of blood flow, through a contracting muscle, leads to almost complete muscle fatigue within 1 or 2 minutes because of the loss of nutrient supply, especially loss of oxygen [19].

1.3.2 Central Fatigue

Though the different mechanisms of peripheral fatigue are well studied and established, little is known about the mechanisms of central fatigue. The changes that appear at supraspinal level are termed as central fatigue [20]. The physiology of fatigue is associated with cortical excitation. Central fatigue is developed with gradual effort put in cerebral metabolism. The rate of this cerebral metabolism depends upon the nature of task. During various tasks, including driving, it increases [21]. A sequence of processes is

involved in this brain metabolism. These processes incorporate a number of chemical and electrical activities in the brain. Neuro-transmitters are the messengers involved in all these chemical and electrical activities. These are present in synthesized form for synaptic transmission. Synaptic transmissions are conducted through pumping of the neural membrane. This transmission requires consumption of oxygen and glucose [22]. It has been observed that the ratio of Cerebral Metabolic Ratio (CMR) of oxygen (CMR_{O2}) to that of carbohydrate (CMR_{CHO}) varies with any physical or mental activity. This variation affects the level of central fatigue [15]. Thus, a major question to be answered is how the above synaptic changes are initiated upon prolonged monotonous task that ultimately leads to signs and symptoms of central fatigue. To explain this, the biochemical changes of many neurotransmitters and peptides associated with chemical metabolism have been studied [23]. Norepinephrine, serotonin (or 5-HT), dopamine, gamma aminobutyric acid (GABA), acetylcholine, histamine, glutamate, adenosine, substance P, and IL-1 are few biochemical that are found to be responsible for the change in level of alertness and sleep [24][25]. However, the well-studied and established hypothesis centers on the role of excess 5-HT or serotonin as the main culprit for central fatigue generation and its manifestations. In addition, nutritional status also contributes to fatigue to a great extent. Since mechanism of central fatigue, unlike that of peripheral fatigue, is not well established, a comprehensive discussion on various factors that are hypothesized to generate central fatigue is given below.

Role of Neurotransmitters

5 hydroxytryptamine (Serotonin) In brain, the tryptophan is converted into 5 hydroxy tryptophan (5-HTRP) which is further transformed into niacin, serotonin or 5-hydroxytrtptamine (5-HT) and melatonin (Figure 1.1). These are directly responsible for cognitive changes. In 1988, Blomstrand and co-workers [26] suggested that changes in plasma amino acid concentrations could play a role in central fatigue by influencing the synthesis, concentration and release of neurotransmitters, particularly serotonin, in the brain. It has been observed that brain serotonin is involved in the control of arousal, sleepiness and mood. The serotonin could therefore, be linked to fatigue during and after vigorous sustained exercise. Furthermore, the synthesis and metabolism of serotonin in the brain has been shown to increase in response to exercise [27]. Barchas and Freedman [28], for the first time, showed an increased level of serotonin in the brain after rats had swum to exhaustion. Several studies have confirmed these early results and have also shown that sustained exercise causes an increase in the turnover of serotonin in some parts of the brain in experimental animals [27][29]. Furthermore, some recent studies have reported that the stress symptoms in rats in response to exercise can be significantly decreased by blocking the serotonin synthesis from tryptophan, thus, confirming the role of free tryptophan in the genesis central fatigue and its manifestation [30][31].

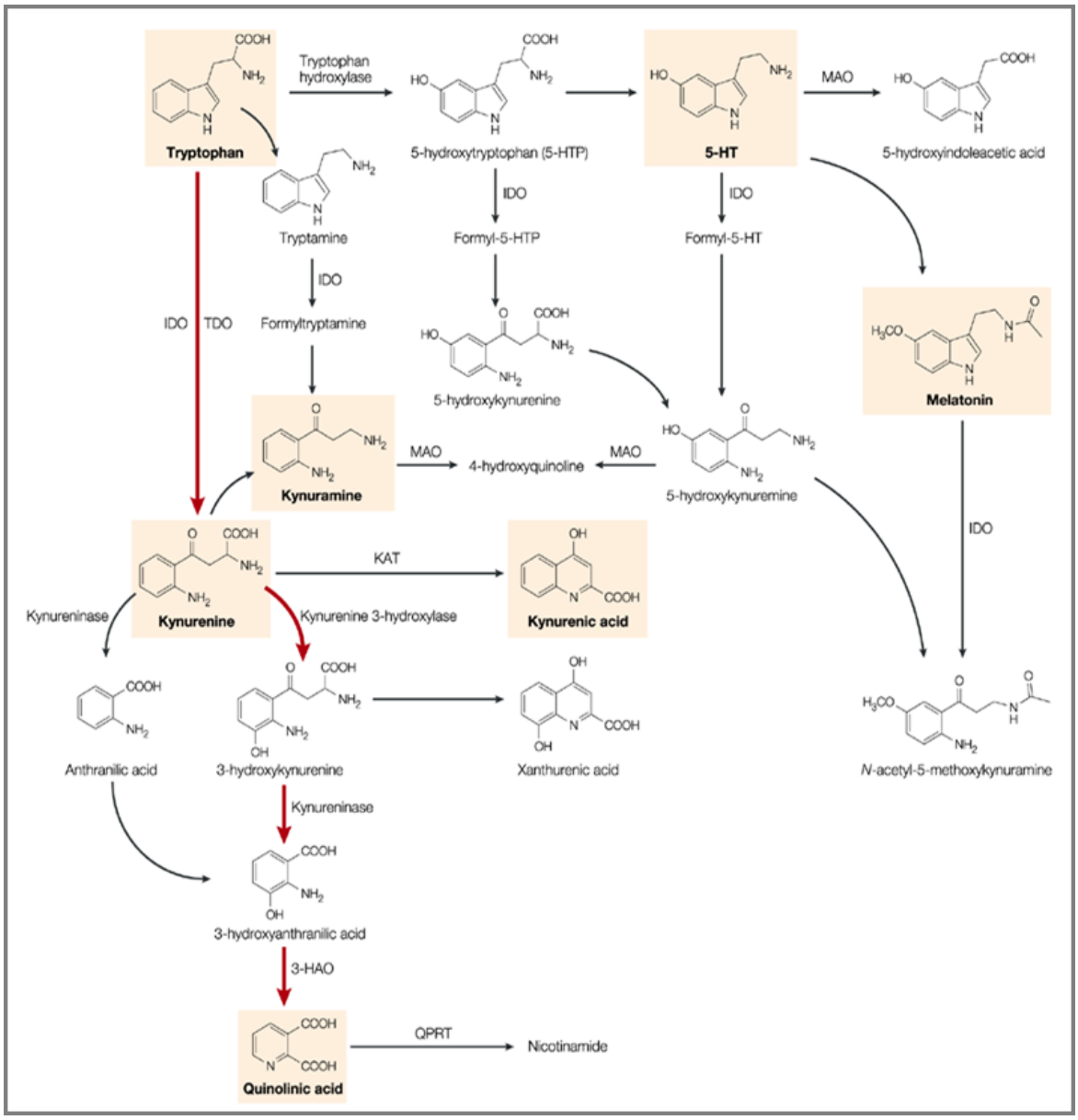

Figure 1.1: Production of Serotonin (5 hydroxytryptamine / 5-HT) and L-kynurenine from tryptophan in neuronal cells [32]. Free tryptophan after crossing blood brain barrier is utilized by neurons to produce serotonin and melatonin. In a minor pathway, it produces L-Kynurenine as shown in the figure.

Kirby et al. and Meeusen et al. [33][34] conducted experiments separately in rats during exercise and measured the concentration of 5-HT in the extracellular fluid in specific areas of the brain by microdialysis technique. They showed that exercise stimulates serotonin release from the nerve cells in some areas of the brain. The role of serotonin in inducing fatigue was further confirmed by a novel experiment conducted by Wilson and coworkers [35] where administration of a serotonin re-uptake inhibitor to human subjects decreased the physical performance in terms of exercise time to exhaustion during standardized cycle exercise at 70% of the maximal oxygen uptake as compared with a placebo condition. Thus, any task that involves decision making, planning and coordination of limbs increases the neuronal

metabolism. This in turn leads to increased blood supply to the brain to compensate for the increased requirement of nutrition and oxygen by the active nerve cells. This increased blood supply in specific condition increases the delivery of plasma-free tryptophan across the blood brain barrier. Free tryptophan, after its entry into CNS, is utilized for synthesis of serotonin (Figure 1.1). However, the rate-limiting step in the synthesis of serotonin is the transport of tryptophan across the blood-brain barrier into the brain [20]. Tryptophan is transported via the L-system (the amino acid transporter system), which also transports the other large neutral amino acids (LNAA) including the three branched-chain amino acids (BCAA). At this transporter level, diet has a role in accelerating or decelerating the fatigue genesis. The free tryptophan that enters central nervous system, majority is utilized in serotonin pathway; however a minor portion is utilized in pathway leading generation of another important molecule, L-kynurenine. L-kynurenine acts non-specifically on neuronal cells of various brain regions inducing depression. Thus, central fatigue persisting for a prolonged period not only transmits to periphery but also is associated with depression. The prolonged central fatigue can disturb the equilibrium of autonomic nervous system leading to distortion in peripheral metabolism (Figure 1.2). This disturbance can lead to excess metabolism of fat and protein increasing the free fatty acid (FFA) level and other metabolites. tryptophan is the only essential amino acid that binds to albumin [36]. At rest, 90% of the total plasma tryptophan is in the bound form to albumin. But excess FFA and glucose competes with tryptophan and accelerates their release from albumin, thus, increasing the level of free tryptophan. It is hypothesized that the concentration of the free tryptophan governs the rate of uptake in the brain [20]. At the same time, other metabolites, particularly, CO_2 and LA, leads to vasodilation and thereby, increases the blood supply to brain. Reentry of free tryptophan into CNS establishes a vicious cycle of central fatigue → peripheral fatigue → central fatigue again.

Many studies, conducted on exercising animals, have confirmed a relationship between the increase in the plasma concentration of free tryptophan and the increase of the tryptophan concentration in the brain but no such relationship was found for the total concentration of tryptophan [27][37][29]. Therefore, it is suggested that, it is the free tryptophan concentration that competes with the BCAA or FFA or glucose for entry into the brain.

Norepinephrine It has dual role; as a neurotransmitter and as a neuro-hormone. The part of brain which controls attention and responding actions are affected by norepinephrine. It has been observed that Dopamine and Norepinephrine are responsible for enhanced concentration ability and less reaction time. tryptophan is one of the eight essential amino acids which is found in food but not synthesized in the human body.

Dopamine According to the original central fatigue hypothesis, extra cellular serotonin deposition in different region of brain was believed to be the prime cause of central

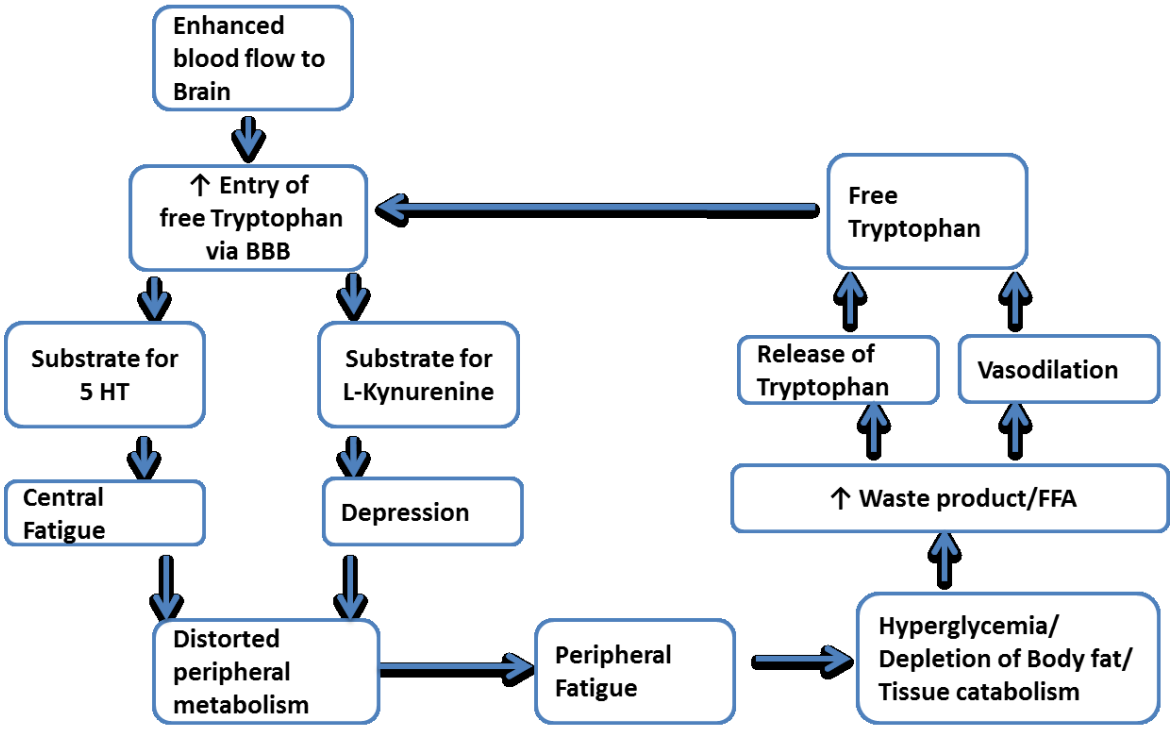

Figure 1.2: Vicious cycle of central and peripheral fatigue. (BBB: Blood brain barrier, 5HT: 5 hydroxytryptamine, FFA: Free fatty acid). With central task, blood flow to brain increases and the delivery of free tryptophan across BBB also increases. tryptophan is utilized by neurons to produce 5 HT/serotonin that gives rise to symptoms of central fatigue. In a minor pathway, tryptophan is also utilized to form L-Kynurenine, the mediator of mood disturbances. If the central fatigue is prolonged, it distorts the firing of neurons at commanding centers of brain. In addition, the autonomic nervous system that originates from the brain is affected and the effect spreads to periphery via hypothalamic pituitary axis. The vital signs and metabolic activity is disturbed so that peripheral fatigue ensues. Either the waste products of metabolism (lactic acid) or excess fat and branched chain amino acids produced from tissue catabolism displaces tryptophan from albumin. Thus more free tryptophan is available and can cross BBB leading to aggravated central fatigue.

fatigue [20]. The prolonged repetitive activities and exercises produce this extra cellular serotonin. However, several other factors along with serotonin are believed to orchestrate the genesis of central fatigue. The revised central fatigue hypothesis advocates the correlation of increased serotonin to dopamine ratio with feeling of fatigue. It points that arousal, motivation, and performance are elevated with low ratio of serotonin to dopamine [38–40]. Recent findings have, however, provided support for a significant role of dopamine and norepinephrine in performance during exercise or prolonged and repeated action under hot ambient [41].

Melatonin Melatonin, an essential hormone secreted by pineal gland, plays an entraining role in circadian physiology [42][43]. The daily rhythm in melatonin is a conserved in vertebrates with high values always occurring at night and thus acts as a sleep inducer [44].

On the contrary, serotonin is synthesized more during day time. Melatonin is synthesized from the metabolism of serotonin and with the exposure to light; the conversion of serotonin to melatonin is sharply decreased [45]. Thus, any disturbance in the rhythmic release of both serotonin and melatonin could be a strong generator of fatigue. It has been observed that excess serotonin can induce production of melatonin through positive feedback [46]. Thus, during development of central fatigue from any type of task or exercise, the excess serotonin thus produced can also increase melatonin turn over leading to drowsiness even during day time.

Role of Nutrition

Various hypotheses have been put forward to link nutritional status to the genesis of central fatigue. One most investigated hypothesis is free tryptophan (TRP) model. Large intake of diet rich in tryptophan increases free TRP in peripheral circulation. The gradual entry of tryptophan to the brain results in cumulative increase in TRP pool of brain which is responsible for releasing neurotransmitter serotonin [26][37][47][48]. Serotonin is directly responsible for central fatigue and is associated with sleep and drowsiness. tryptophan which forms serotonin is easily available in protein rich foods [23]. Studies show that when protein food is taken, tryptophan is readily absorbed by brain [23]. So protein can be considered as food influencing the thinking process, whereas carbohydrate intake is more responsible for drowsiness. However, the amount of tryptophan transported into the brain depends not only on the concentration of tryptophan in the bloodstream, but also on the concentrations of the other LNAA [49], mainly the BCAA (branched chain amino acids) since these make up approximately 75% of the LNAA. As it has been mentioned earlier, tryptophan is the only amino acid that binds to albumin in the plasma and equilibrium between bound and free tryptophan exists [36]. Thus, any component in diet that competes with tryptophan for binding with albumin will also affect the free TRP level.

Other NTs like norepinephrine and dopamine are more readily synthesized in body than serotonin. It has been found that tyrosine and phenylalanine that act as raw material for synthesis of dopamine and norepinephrine are plentiful in protein containing food. Thus, food rich in proteins makes a person vulnerable to early fatigue that sustains for a longer period [50].

1.4 Methods of Evaluating Fatigue

The methods of quantifying fatigue in specific occupational groups can be classified as follows: questionnaire based subjective or self-assessments; psychomotor performance measurement related to physical and mental ability; clinical investigations like blood, urine or CSF biochemical variations; measurement of physiological variables like EEG, ECG,

EMG, EOG, O_2 saturation of cerebral or peripheral vessels, or pulmonary parameters measured by spirometry. However, not a single method or parameter, alone, is sufficient to discriminate successive stages of central fatigue and the resultant peripheral fatigue. The basic principles of above methods and the significance of using specific biomedical equipment for assessing fatigue were outlined below.

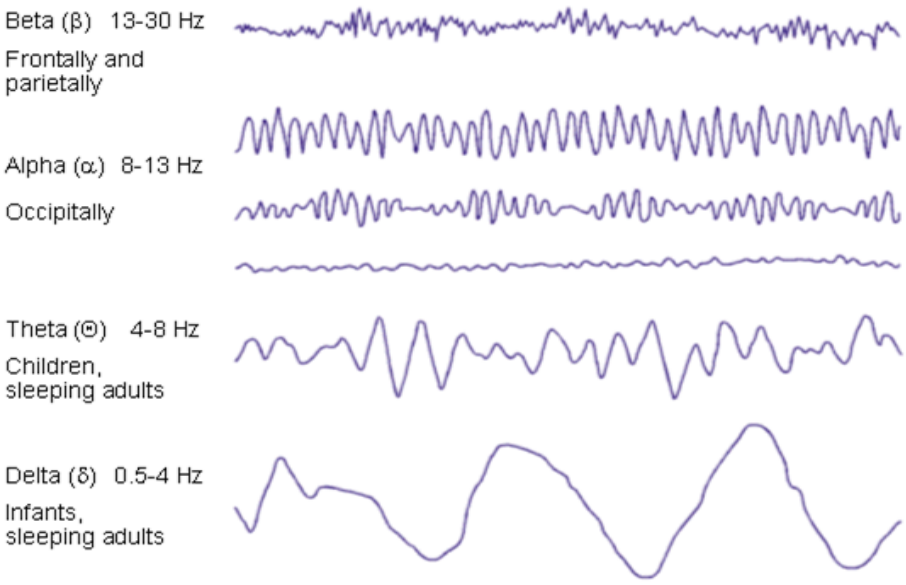

Figure 1.3: Different frequency bands of an EEG signal [51]. Alpha (α) waves are rhythmical and are found in almost all normal adult people when they are awake and in a quiet, resting state. Beta (β) waves are recorded mainly from the parietal and frontal regions during specific activation of these regions. Theta (θ) waves occur normally in the parietal and temporal regions in children. Delta (δ) waves occur in very deep sleep, in infancy, and in serious organic brain disease.

1.4.1 Neurophysiological Methods

EEG (Electroencephalogram) EEG gives abundant information on brain function during physiological conditions that involve increased, decreased or distorted brain metabolism. An EEG signal contains distinct frequency bands that are shown in Figure 1.3.

Alpha (α) waves are rhythmical waves that occur at frequencies between 8 and 13 Hz and are found in the EEGs of almost all normal adult people when they are awake and in a quiet, resting state of cerebration. Beta (β) waves occur at frequencies between 13-30 Hz, and may go as high as 80 Hz in specific circumstances. They are recorded mainly from the parietal and frontal regions during specific activation of these parts of the brain. Theta (θ) waves have frequencies between 4 and 8 Hz. They occur normally in the parietal and temporal regions in children, but they also occur during emotional stress in some adults, particularly during disappointment and frustration. Delta (δ) waves include all the waves of the EEG with frequencies less than 3.5 Hz, and they often have voltages two to four times

greater than most other types of brain waves. They occur in very deep sleep, in infancy, and in serious organic brain disease [51]. They also occur in the cortex of animals that have had subcortical transections separating the cerebral cortex from the thalamus. Therefore, δ waves can occur strictly in the cortex independent of activities in lower regions of the brain.

When the awake person's attention is directed to some specific type of mental activity/task, the alpha waves are replaced by synchronous, higher-frequency but lower-voltage β waves [51]. Thus, tasks like driving can increase the beta component of EEG waves. If the task is combined with sleep deprivation, the θ component is invariably increased due to the presence of drowsy state [52]. Similarly, any mental task that generates central fatigue, if persists for a prolonged period distorts the emotion. This is characterized by irritation, frustration and ultimately depression with the duration of central fatigue. In such state the δ component is expected to increase in EEG signal.

Electromyogram (EMG) EMG signals represent neuromuscular activity and are effective biological signals for expressing movement intent and performance ability for external device control. Thus, fatigue of any origin can disturb the EMG output. In prolonged monotonous exercise like driving, the central fatigue that persists can ultimately affect EMG since central nervous system (CNS) can significantly affect muscle force generated after muscle contraction. CNS regulates muscle force production by varying two main parameters: the recruitment of new motor units, and the modulation of firing rates of active motor units. The firing behavior of motor units can be assessed by parameters such as the firing rate, firing variability, synchronization of motor unit firings, and the common modulation of motor unit firings. Such physiological parameters of muscle can be assessed by EMG data analysis. The behavior, influence, and causality of EMG parameters on the increasing force fluctuation during fatigue have been extensively studied. De Luca et al. [53] and Garland and co-workers [54] reported a decrease in the firing rate of most motor units during a short-lasting fatiguing task. Adam and De Luca [55] later found that this initial decrease was followed by an increase as the muscle continued to contract and progress toward exhaustion. Researchers have also proved the gradual increase in firing rate following eccentric exercise [56].

1.4.2 Cardiopulmonary Parameters

Electrocardiogram (ECG) The ECG (or EKG) is the graphical recording of the electrical activity of the heart. It records the heart electrical current (voltages/current) by means of metal electrodes placed on the surface of the body, these metal electrodes are placed on arms, legs or chest (precordium).

The heart is a pumping organ that pumps oxygenated blood to whole body. The electrical activities that give rise to cardiac wall contraction are generated in the heart muscle cells

itself, a property called auto-rhythmicity. The impulse is generated at the sino-atrial node or SAN (pace maker of heart) spreads through the atria to reach atrio-ventricular node (AVN). Next, it passes through inter-ventricular septal A-V bundle, Purkinje fibers, endocardium of the ventricles to reach the epicardial surfaces. The entire electrical activity is reflected in ECG recording. The normal electrocardiogram (Figure 1.4) is composed of a P wave, a QRS complex, and a T wave. The P wave is caused by electrical potentials generated during atrial depolarization (excitation leading to contraction). The QRS complex is caused by the ventricular depolarization and the spread of corresponding impulse through the ventricular walls. The T wave is caused by potentials generated as the ventricles recovered from the state of depolarization.

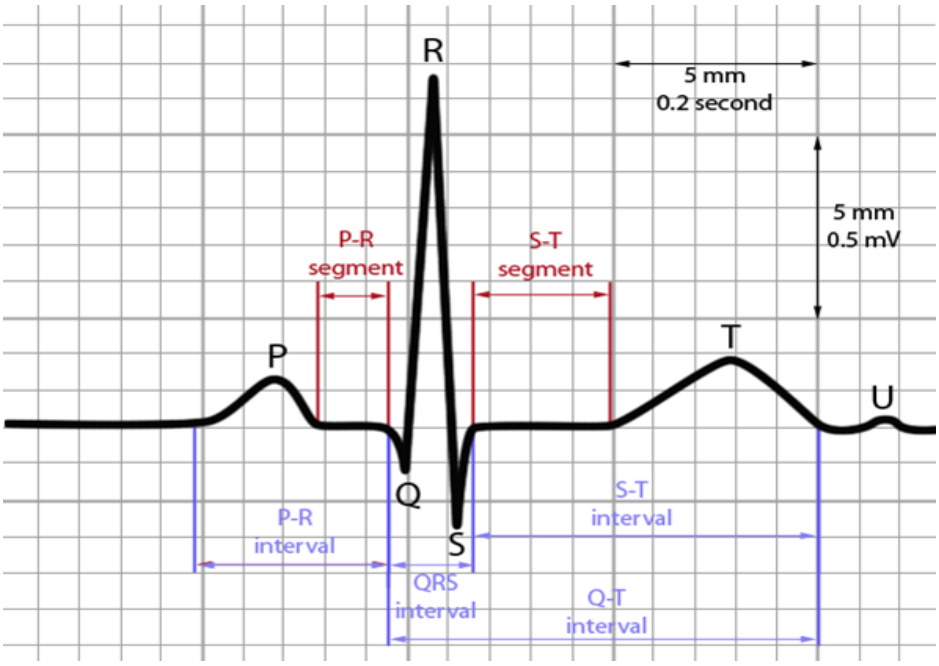

Figure 1.4: Different components of a typical ECG signal [57]. P wave represents atrial depolarization (activation); QRS complex represents ventricular depolarization (activation) while T wave represents ventricular repolarization (relaxation). PR interval indicates duration of excitation pulse to move from sino-atrial node (SAN) to atrio-ventricular node (AVN) while QRS interval indicates passage of pulse through the ventricles.

The function of the heart in part is controlled by autonomic nervous system (ANS), which provides both afferent (sensory) and efferent(motor) nerves to the heart, in the form of sympathetic terminations throughout the myocardium and parasympathetic to the sinus node, atrial muscle and atrio-ventricular node [58]. The control by ANS is closely linked to the heart rate (HR). Stimulation of the parasympathetic nerves to the heart causes a decrease in the rate of rhythm of the sinus node, and the excitability of the A-V junctional fibers between the atrial musculature and the A-V node while stimulation of the sympathetic nerves accelerates self-excitation and, therefore, increasing the heart rate.

Any task including driving leads to disturbance in the autonomic nervous system

(ANS) that is reflected in the electrocardiogram pattern in the form of change in HR. This ANS mediated heart rate variation (HRV) is regarded as a critical index of neurovisceral integration and the self-regulatory activity of the individual. Since beat to beat variation of heart is not uniform over time, HRVs indicate the heart's ability to respond to multiple physiological and environment stimuli, among them, breathing, physical exercise, mental stress, hemodynamic and metabolic changes and sleep [59]. This autonomic disturbance can be initiated centrally (if central fatigue persists for a longer period) from hypothalamus that acts as the central control room for ANS or peripherally [60][61] (from peripheral fatigue) due to metabolic disturbances.

For analyzing HRV, the indices can be obtained either by linear or non-linear methods. In linear methods it can be computed both in time domain or frequency domain (FFT). The HRV time domain analysis is mainly based on statistical methods or geometric methods. The statistical approach is particularly applied for long period heartbeat time series (24 h) and calculated rather from direct measurements of RR intervals or differences between them. All indices that are measured in time domain analysis and their significance is given in the Table 1.1.

Table 1.1: The indices and significance of Heart Rate Variability (HRV) in time and frequency domains

Statistical Indices	Definition	Significance
SDNN	Standard deviation of all normal RR intervals recorded in a time interval, expressed in ms [58].	
SDANN	Standard deviation of the normal RR intervals means, every 5 minutes in a time interval, expressed in ms [58].	
RMSSD	Root-mean square of differences between adjacent normal RR intervals in a time interval, expressed in ms [58].	
pNN50	Percentage of adjacent RR intervals with a difference of duration greater than 50ms [58].	
High-frequency component (High Frequency - HF),	Range: 0.15 to 0.4 Hz	HF component corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart;
Low frequency component (Low Frequency - LF),	Range: 0.04 and 0.15 Hz,	LF components corresponds to the joint action of the vagal and sympathetic activity on the heart, with a predominance of the sympathetic ones;
Very Low Frequency (VLF) and ultra-low frequency (ULF) indices	Range: 0.0015-0.04 Hz	The physiological explanation is not well established and seems to be related to the renin-angiotensin-aldosterone system [62].
LF/HF		It reflects the absolute and relative changes between the sympathetic and parasympathetic components of the ANS and their resultant effect on heart [62].

On the other hand, the geometric approach is based on the conversion of the series of RR intervals into a geometric pattern such as Poincaré plot [63] and Lorenz plot [64]. The Poincaré plot is a geometric method for dynamic analysis of HRV, which represents a temporal series within a Cartesian plane in which each RR interval is correlated with the preceding interval and define a point in the plot [65]. The analysis of Poincaré plot can be performed in a qualitative manner (visual), by assessing the figure formed by its attractor, which is useful for showing the degree of complexity of RR intervals [66], or quantitative, by adjusting the ellipse of the figure formed by the attractor, from which three indexes can be obtained: SD1, SD2 and SD1/SD2 ratio [65]. But for short term recording of ECG, frequency domain analysis of HRV is more relevant. Usually the FFT (Fast Fourier Transform) of tachogram and the corresponding energy in Very Low Frequency (VLF, <0.04Hz), Low Frequency(LF, 0.0Hz, 0.15Hz) and High Frequency(HF, 0.15Hz,0.4Hz) bands are considered to be sensitive indicator of HRV trend in response to a task. The spectral power density is the most widely used, when it deals with studies with individuals at rest or resting monotonous task like driving [67].. The various indices of FFT analysis of HRV and their significance is given in the Table 1.1.

Thus, recording the ECG and studying the beat to beat variation (HRV) or changes in different intervals (PR interval, QT interval) or peaks (R peak, T peak) can give important clue about fatigue genesis and progress.

Spirometer Recording Spirometry is the most commonly used measure in the clinical assessment of true lungs function [68]. It measures the air moving in and out of the lungs in the form of a series of lungs volumes and capacities (Figure 1.5) and the outcomes are collectively termed Pulmonary Function Tests (PFTs) or Lungs Function Tests (LFTs) [69]. Spirometer recording are used as an assessment and diagnostic measure rather than an action required in normal daily activities [70]. At the same time, since peripheral fatigue initiates from skeletal muscles, the efficiency of respiratory muscles is also decreased both in acute fatigue (from moderate to severe exercise) and slow fatigue (from prolonged monotonous task like driving). Thus, the effect of fatigued respiratory muscles on lungs function can be assessed from spirometer findings. Muscles that are typically considered during respiration are the diaphragm, the abdominal muscles, the intercostals, anterior scalenes, and serratus anterior. It has been shown that, the diaphragm fatigues in a short period. Although extra-diaphragmatic muscles are able to compensate, they too fatigue with time and thus, compromise the lungs performance. By monitoring the decrease in lungs performance from spirometer recording, an approximate estimate of peripheral fatigue can be obtained.

The most important indicators of pulmonary functional status that can be measured from spirometer recordings are forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) from a full inhalation [72] while the ratio, FEV_1/FVC is the most sensitive indicator that differentiates restrictive to obstructive profile of pulmonary function [73].

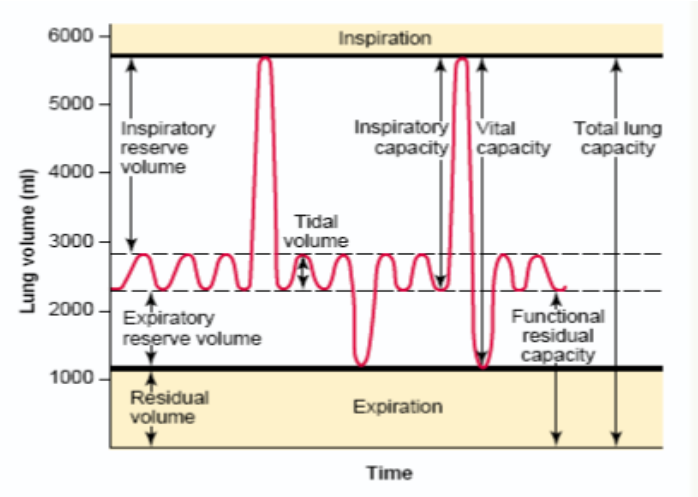

Figure 1.5: Respiratory excursions during normal breathing and during maximal inspiration and expiration [71]. Such a pneumatogram gives an estimation of different lungs volumes (tidal volume, inspiratory reserve volume, expiratory reserve volume, residual volumes) and lungs capacities (inspiratory capacity, functional residual capacity and forced vital capacity). All volumes can be measured directly from spirometer recording except residual volume while capacities are measured indirectly.

While studying the peripheral fatigue, the accurate measurement requires the subject's forced maximum effort during expiration [74] which helps the researchers to study the limitation of fatigue on performance. Spirometer recordings, at regular intervals, during any task give a pneumatogram that can give abundant information on the severity of peripheral fatigue.

1.4.3 Endocrine Parameters

Central fatigue generally correlates poorly with traditional markers [75] and is frequently associated with other psychosocial factors, such as depression, sleep disorder, anxiety, and coping styles [76][77], which suggests that dysregulation of the body's stress systems may serve as an underlying mechanism of fatigue. At the other end, physical fatigue can be well assessed by classical markers like cortisol, insulin etc. In fact, the neural, endocrine, and immune systems orchestrate together in regulating the body's response to stress and maintain the homeostasis. Two main pathways have been postulated to explain by which psychogenic stress is relayed from the brain to the body or how the central fatigue spreads to periphery: (1) via the hypothalamic-pituitary-adrenal (HPA) axis with the resultant release of glucocorticoids (cortisol in humans; corticosterone in rodents) and (2) via the sympathetic nervous system (SNS), with the resultant release of catecholamines (noradrenaline and adrenaline). In addition, the level of another important NT, 5-HT gets increased in brain after prolonged stress/exercise. 5-HT is believed to generate the general manifestations of stress and the resultant fatigue. These neuroendocrine stress systems coordinate the response of many other physiologic systems to a stressor, including the

immune and cardiovascular systems, as well as energy production and/or utilization and behavior, therefore, bringing the physiologic systems back to equilibrium [78]. But if the stress remains persistent for a prolonged period can ultimately lead to genesis and progress of fatigue. Steroid hormones are produced by adrenal cortex, which include the most widely studied of the stress-related hormones like cortisone, hydrocortisone, testosterone, estrogen, 17-hydroxyketosteroids, dehydroepiandrosterone (DHEA), DHEA sulfate, pregnenolone, aldosterone, androstenedione, progesterone, and other intermediates to hormone production [79]. However, cortisol produced by the outer cortex, is a major stress hormone that has more prolonged effects on human body. The cortisol production responds to all those stressors that increase energy requirements of the body. It includes fasting, infection, intense exercise, pain or emotional or mental stress. In initial stages because of excessive mental stress or physical work the serum cortisol levels rise, glucose utilization declines and insulin resistance increases, gluconeogenesis in the liver increases, and blood glucose levels increase rapidly [80]. Adrenal hyperfunction can be marked by a tendency toward insulin resistance, hypertension, mild obesity, and elevated serum lipid and triglyceride levels [80].The final stage of the stress response is the adrenal deficiency at extreme fatigue, the body's ability to synthesize cortisol and other corticosteroid hormones is greatly diminished [81]. Thus, during tasks that involve prolonged monotonous session (in drivers, shift-workers, pilots, surgeons), the biological system is expected to behave as described above. The persons with low cortisol levels drag themselves through the day and fell exhausted [79]. By studying the variation of stressors like serotonin and cortisol (indicator of spread of central fatigue to periphery) can give information about the stages/severity of fatigue. The level can also help in establishing the impact of any monotonous task like driving in combined with sleep deprivation on the circadian rhythm (biological clock).

Many researchers have assessed the level of brain serotonin and correlated it with the progression of central fatigue [34][35]. However, some practical problems are observed while estimating serotonin level in CSF in healthy human subjects since it involves invasive procedure thus, creating ethical objections. But it has been observed that, in rodents [82] as well as in depressive patients [83][84], the level of serotonin in platelets and platelet rich plasma (PRP) are well correlated with that of specific neuronal cells and extracellular fluid of CNS. Thus, PRP serotonin can be reliably considered as the indicator of CNS serotonin level [82].

Another important physical stress indicator is insulin since cells develop resistance to insulin concerning the breakdown of glucose when the task is combined with sleep deprivation. Stress normally causes the adrenal glands to produce more cortisol, which raises blood sugar levels so that the cells can utilize more glucose to generate more energy as a response to the stressor. The elevated blood sugar, in turn, requires higher levels of insulin to bring the glucose from the blood into the cells [85]. This cycle is repeated frequently and

thus the cells may become insulin resistant to protect themselves from high level of glucose, especially when no energy-consuming physical exercise is taken in response to the stress. The increase in insulin resistance results in the utilization of more and more insulin to get glucose into the cells [80].

1.4.4 Biochemical Parameters

Blood biochemical profile can give abundant information on genesis of fatigue and can act as important indicators of level of fatigue. Various blood parameters that represent either the inducers or outcome of events leading to fatigue can reliably reflect the gradual genesis of central as well as peripheral fatigue. Blood glucose level indicates the rate of oxidative metabolism of live cells. Thus, with increase in fatigue, more glucose is required by the cells for energy production. Earlier researchers found profound alterations in glucose metabolism during peripheral fatigue, in some situations resembling patients with type-2 diabetes, during sleep deprivation [86]. Another important blood parameter that is of great interest in performance study is creatinine. Creatinine has been found to be a fairly reliable indicator of kidney function as well as muscle degradation out of overuse from prolonged fatigue [87]. Any condition that impairs the function of the kidneys or increase the physiological load to kidney will probably raise the creatinine level in the blood [87]. Similarly, the blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function as well as fatigue. Urea is a substance secreted by the liver, and removed from the blood by the kidneys. The liver produces urea in the urea cycle as a waste product of the digestion of protein that usually takes place at the peak of the development of fatigue. Very few studies in general population and almost no study in drivers have been performed to measure the above blood parameters during the progress of fatigue. It is proposed that measuring above parameters in real time may give significant information on genesis of central fatigue in our target group.

1.5 Origin of the Problem

The main challenges in preventing road accidents by fatigued drivers are:

- An accurate and objective estimation of the severity of fatigue during driving
- A critical analysis of physiological and biochemical parameters that can discriminate fatigue stages
- A physical fatigue scoring system may have application in designing and validating the on-board systems for alerting the drivers

The current study was carried out to address these problems. It is obvious that prolonged monotonous exercise such as driving combined with sleep deprivation initiates fatigue in

brain (central fatigue) and if it persists, ultimately spreads to periphery. The resultant peripheral fatigue further aggravates the central fatigue and initiates a vicious cycle. A series of external and internal factors play a role in further progression of physical fatigue. If the driving task is persistent without any fatigue countermeasure, a state of exhaustion is reached, which grossly compromises the level of alertness and accounts for major road accidents. However, before the end point of fatigue is reached, the progression of fatigue is manifested through a series of subjective and objective parameters. The subjective parameters constitute both specific and non-specific symptoms of fatigue that can be assessed precisely by questionnaire-based subjective assessment tools. The results of subjective assessment tool would reflect the exact state of fatigue at a particular time point and can be used as reference for fatigue intensity in target occupational groups. However, the subjective assessment score cannot be used alone to accurately estimate the fatigue during any type of task due to its non-specificity and a wide subject to subject variation. At the same time the objective assessment of fatigue can be conducted by measuring the variation in a multitude of physiological and biochemical parameters reflecting both central and peripheral fatigue during driving task.

By investigating the variations of carefully selected physiological and biochemical parameters during simulated driving under sleep deprivation till the point of complete exhaustion, an insight into the generation and progression of fatigue in drivers can be obtained. At the same time, by correlating the variations of each parameter with the subjective assessment score (that has been taken as reference of fatigue intensity) would assist us screening those parameters that may have significant potential for discriminating the fatigue stages. Ultimately, the screened parameters with subjective assessment score can be used to devise a fatigue scoring system in drivers for validation of alert systems under development.

Thus, quantifying drivers fatigue in terms of a series of physiological parameters during simulated condition of driving can assist in finding the rationale of using those parameters that can be utilized for discrimination of different stages of fatigue genesis and progress. Such factors may in turn be utilized to establish a functional scoring system that might be useful in validating various non-invasive devices (Appendix A) under development to predict the level of fatigue in drivers onboard and warn them before any mishap occurs. Again, this scoring system may also be projected to other occupational workers who are exposed to prolonged monotonous task like driving i.e. pilots, shift workers, surgeons or physicians etc.

1.6 Objectives of Research Work

The objectives of the current research work were:

i. To compare the trends in psychomotor manifestations of physical fatigue between

healthy and diseased condition (that make individual prone to fatigue) in mice models.

ii. To quantitatively analyze the central fatigue in human drivers, during sleep-deprived simulated driving, by Electroencephalogram (EEG) recording.

iii. To quantitatively analyze peripheral fatigue during sleep deprived simulated driving by

   (a) various indices of heart rate variability (HRV) obtained from ECG recordings,

   (b) lungs functional status using spirometer recordings, and

   (c) key blood biomarker (blood glucose, urea and creatinine) profile using biochemical analyzer.

iv. To establish the interdependency between the central manifestations (from EEG) and peripheral manifestations (from ECG and blood biomarkers) of driver fatigue by correlation analysis.

v. To analyze and assess the major stress inducers (serotonin, cortisol and insulin) that lead to fatigue in human drivers during sleep-deprived simulated driving.

vi. To perform a comprehensive subjective assessment of central and peripheral fatigue during simulated-driving by established subjective assessment tools and compare them with the objective parameters assessed above.

Chapter 2

Literature Review

2.1 Fatigues in Drivers

Driving involves various activities such as perception, psychomotor movements, reasoning, visual and auditory processing, decision making and reaction etc. Such activities, if persistent, can give rise to both physical and central fatigue in addition to visual fatigue, a part of central process [88]. While central fatigue represents a psychological phenomenon with a downturn in psychodynamic process, physical fatigue indicates excess metabolism by peripheral muscles due to motor load [14]. At the same time visual fatigue in drivers can be explained separately that is due to the stress developed in eyes.

2.1.1 Peripheral Fatigue in Drivers

Physical fatigue in drivers is characterized by a decline in motor activities, deficiency in muscle power generation and disturbance in co-ordination of multitasking. Since driving involves a series of tasks like changing the clutches and gear, movement of steering wheel, braking etc., the resultant physical fatigue is mainly caused by repeated muscle movement. However, this fatigue becomes a complex phenomenon when it is contributed by many other factors in driving task like excessive work load, irregularities in time, sleep deprivation, medical conditions etc. [88]. During repetitive movements, the muscles reserve excessive lactic acid and generate more carbon-dioxide resulting slower muscular contraction and expansion. In long term, excess nitrogenous products like ammonia reduce the concentration of glutamate, an excitatory neurotransmitter causing cerebral dysfunction. Again, release of creatinine put load on kidney functions further compromising overall activities. NTs like serotonin and dopamine are some of the chemicals responsible for drowsiness [89] while L-kynurenine acts as an inducer of depression [90, 91] in long distance drivers.

2.1.2 Central Fatigue in Drivers

The key feature of central fatigue is decreased cognitive performance. The contributing factors may be prolonged mental processes, monotonous physical tasks or both [88]. All

types of mental task involve generation of NTs for neuronal integration and communication and thus, involve energy expenditure. Thus, consumption of increased oxygen and glucose compensates the energy demand. The amount of glucose consumption by a particular region of the brain varies with the complexity of task or stimulus presented to the subject. It has been observed that a sensation of fatigue increases with the increased glucose consumption and that in turn prevents the depletion of metabolic energy in the brain [92]. Since driving involves a series of demanding acts like reasoning, decision making, perception and recognition, the task puts a strong effect on mental exhaustion/fatigue.

2.1.3 Visual Fatigue in Drivers

Visual fatigue plays an important role in driving specially at night when the eyes are highly stressed. The intermittent glare of light coming from the opposite direction enhances the stress in eyes. This is called the visual fatigue. It is directly reflected on eye blink rate and PERCLOS and other ocular behavior. It is further enhanced by the sleep deprivation and other type of fatigues [93]. At higher stages of visual fatigue the long duration blink increases [94, 95] which results an increase in the PERCLOS values.

2.2 Causes of Driver's Fatigue

Driver's fatigue can be due to both intrinsic as well as extrinsic factors. Intrinsic factors like sleep deprivation; associated sleep disorders; distorted circadian rhythm; irregular working hours; psychological factors like anxiety, mood, expectation; and long and monotonous driving sessions can affect fatigue tremendously. At the same time, it is also dependent on environmental stimuli and the ambience of driving [96].

2.2.1 Sleep Deprivation

Sleep is an essential component to reenergize brain functions. It enables our brain to perform important housekeeping tasks, such as organizing long-term memory, integrating new information, and repairing and renewing tissue, nerve cells and other biochemical factors [97]. Persistent sleep deprivation may lead to a behavior similar to illusion, hallucination and inconsistency [98]. Road traffic and safety studies consider sleep deprivation as a major factor for road accidents. A survey report by National Highway Traffic Safety Administration, USA, estimates that 0.1 million motor vehicle accidents a year are caused by driver fatigue from sleep deprivation [99]. Apart from sleep deprivation that is invariably associated with long distance driving, various clinical conditions that affect normal sleep cycle can make drivers vulnerable to major accidents by decreasing their normal efficiency. It is reported as a major factor responsible for fatigue [100]. The relevant sleep disorders that can increase sleep debt in drivers are sleep apnea, insomnia and narcolepsy. Obstructive

sleep apnea (OSA) is a common condition that can significantly affect daytime functioning and the person suffers from excessive daytime sleepiness and fatigue [101]. Since several studies have established a correlation between OSA and increased risk for crashes in commercial vehicle operators, the importance of identifying OSA has become the subject of focused attention by the Federal Motor Carrier Safety Administration (FMCSA). On the other hand insomnia is characterized by difficulty in initiating or maintaining sleep or both. It may result from emotional disturbances like anxiety, depression as a consequence of prolonged driving itself thus establishing a vicious cycle. It may cause tiredness, lack of energy and concentration during daytime [102]. Narcolepsy causes excessive daytime sleepiness even after adequate nighttime sleep. A person with narcolepsy may become drowsy or fall asleep, often at inappropriate times and places. Drivers with narcolepsy at are higher risk of causing day time accidents.

2.2.2 Psychological Disorders

Central fatigue out of prolonged monotonous task invariably results in depression or mood disorders. Thus, the drivers who are already suffering from a transient state of depression or chronic depression are vulnerable to develop fatigue at lower level of driving load [103].

2.2.3 Distorted Circadian Rhythm

Circadian rhythm is the daily periodicity of different types of processes of human body like physiological, biochemical etc. Many functions such as brain activity, hormone production and some other biological activities are dependent on this rhythm [104]. So the performance as well as generation of fatigue is affected by the circadian rhythm that is disturbed by any type of task spanning over day and night or involved sleep deprivation (i.e. shift workers, cruise operators, and long distance drivers etc.). An experiment conducted over 24 hours showed that the driving performance is affected by diurnal variations [105]. Eleven heavy vehicle operators were subjected to simulated driving for 30 minutes at 6 times a day. They drove at a constant speed of 80 km/h and the driving performance was measured in terms of the mean and standard deviation of lateral position, the variation in speed and the reaction time to a secondary task. The found that the driving performance is worst (significant variation in speed and lane of driving with high reaction time) between 02.00 AM at night to 06.00 AM in the morning. Studies have also correlated the performance level in drivers with the variation in stress hormones that follows circadian rhythm. Sluiter and co-workers investigated the circadian variation of stress hormones like cortisol and adrenaline in 10 coach drivers during a long distance trip of 48 h. They concluded that the circadian rhythmicity of adrenaline secretion is disturbed significantly while that of urinary cortisol remained unaffected along the deterioration of driving performance [106]. Adrenaline is a monoamine and a key neurotransmitter in sympathetic system. Cortisol is a

corticosteroid that initiates the stress leading to fatigue.

2.2.4 Nature of Driving Session

The type, duration and topography of driving scenario can also influence fatigue development. A boring view, a monotonous highway can lead to illusory state called highway hypnosis [107]. This declines the driver's sense and slows their reaction time. Moreover, the comfort of modern highway and the sophisticated car create the situation for falling asleep.

2.2.5 Personal Factors

The personal factors of driver such as age, gender, medication, stress, mood and type of personality are also some of the important contributors to fatigue generation. In addition attitude of drivers also contribute a lot to fatigue induction. International report has confirmed that many drivers, particularly young males, continue to drive tired despite being aware of their tiredness. It is likely that some drivers fail to fully appreciate the risks associated with driving in this state [108]. For many drivers the goals and rewards of completing the journey outweigh the calculated/known risk.

2.2.6 Extrinsic Factors

Various external factors either related to vehicle or environment or social interaction can affect the generation and progress of fatigue in drivers [109] such as

- The quality and ergonomics in the location of vehicle steering, brakes, tyres etc. as well as the comfort inside the vehicle
- Distraction such as music, cell phone, passengers etc.
- Environmental factors such as road condition, traffic, weather, surroundings etc.

Thus, while assessing the fatigue level in drivers during simulated driving condition, these factors must be taken into consideration.

2.3 Manifestations of Fatigue in Drivers

2.3.1 Direct Manifestation

Ocular behavior These manifestations can be broadly classified into two groups; movement of eyelids (blink) and changes in size and motion of the pupil. A number of parameters have been defined in literature from the above manifestation such as blink rate,

blink duration, PERCLOS, saccade etc. [110]. However ocular behavior alone cannot represent the intensity of fatigue particularly during associated drowsiness in long distance driving.

Micro sleep Microsleep is a very common experience by the drivers, people working in front of computer screen, even passengers inside a vehicle. It results from severe sleep loss [111]. In microsleep driver experiences total attention lapse. The driver may not be aware of the microsleep and loss of attention. So the risk of the accidents is highest in this state. The duration of microsleep is found to be varying from few seconds to several minutes.

Physiological signals Driving involves various physical and mental activities. It is also influenced by emotion, anxiety and some other psychological factors. All these physical and mental activities are reflected in one or more physiological signals such as EEG, ECG, and EMG. Among these, the EEG is considered to be the most significant reflector of fatigue [96].

2.3.2 Indirect Manifestation

The major indirect manifestation is attention impairment and performance degradation. After sustained work like driving, the subject (here driver) gets cumulative attention impairment due to cognitive load and external distractions. The result of attention impairment can be observed in the behavior of a sleep deprived vehicle operator. Two common effects of attention impairment are increase in performance error and the increase in response or reaction for a given task [112]. It has been observed that the 'reaction time' becomes relatively longer once the driver becomes fatigued. Phillip et al [113] conducted an experiment on 13 drivers where after the controlled sleep, subjects drove five identical 200 km sessions on a separated lanes motorway (100 km one way and 100 km the other) and Each driving session lasted 105 min. The subjects were tested for reaction time at 10 min interval by personal PALM organizer and found a significant delay in reaction times with increased driving performance. Other than the above types of manifestation, fatigue may be reflected on skin temperature and conductance, head nodding, yawning, distorted speech etc.

2.4 Quantification of Drivers' Fatigue

Similar to fatigue in general, the drivers may exhibit some easily observable physiological features from which their fatigue can be inferred. Physiological features may broadly be classified into: (i) contact features, including the brain activity, heart rate variability, and skin conductance which can easily be detected by EEG, ECG, EMG, GSR (Galvanic skin

response), PPG (Photoplethymsography) [114] and, ii) non-contact features, including the eye movements (EM), head movement, and facial expressions which can easily be observed from the dynamic images provided by a CCD camera. Consequently, two approaches are feasible for research: the contact feature-based methods and the non-contact feature-based methods (Figure 2.1).

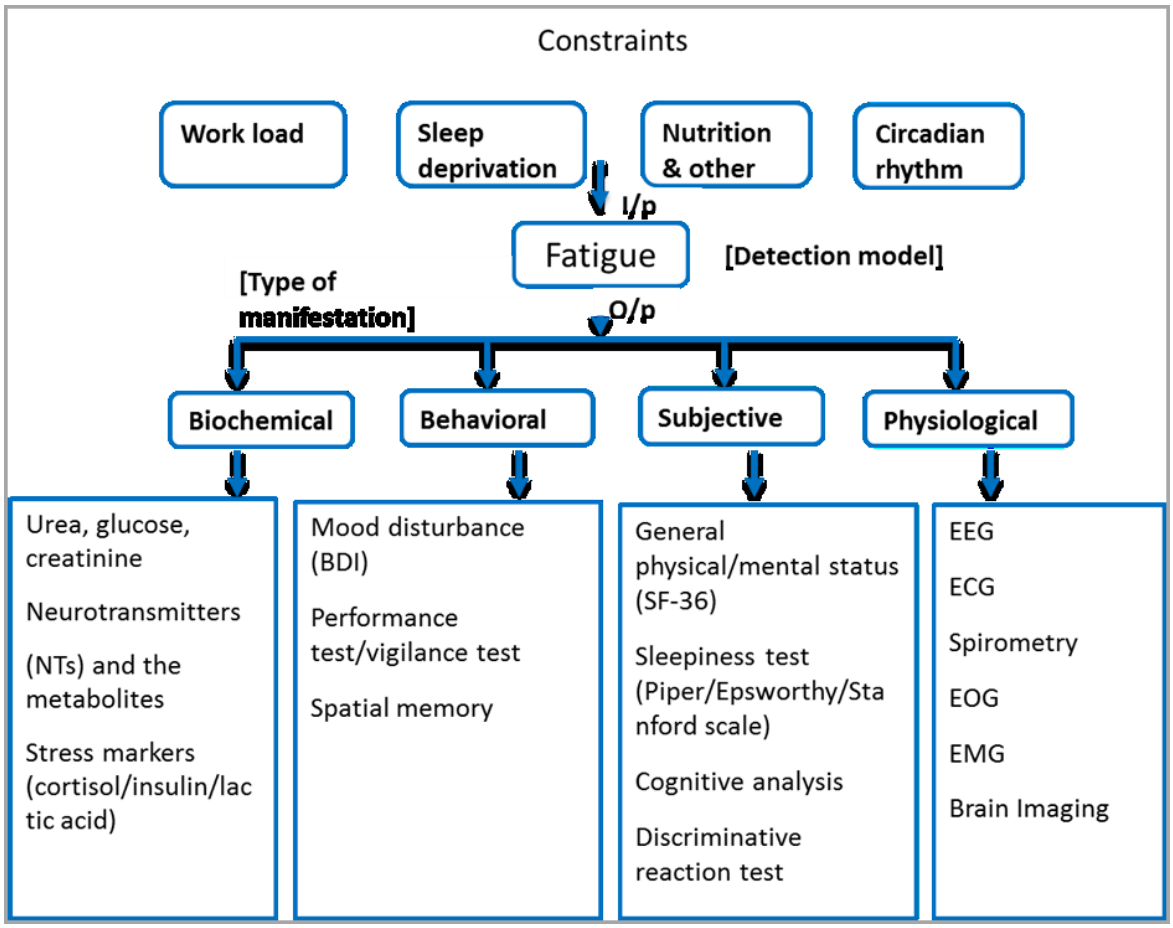

Figure 2.1: Approaches for Fatigue Measurement. Various constraints shown here can affect the severity and the rapidity of onset of physical fatigue. At the same time, the various outcomes of fatigue (biochemical, physiological along with subjective parameters) can be utilized to quantify the level of physical fatigue.

2.4.1 Electroencephalogram (EEG)

Maslov and co-workers suggested that in any task that demands constant attention for prolonged period, fatigue slowly deepens and is followed by chronic fatigue and overfatigue, with weakening of the inhibitory processes and nervous processes [115]. The authors estimated the fatigue and exhaustion level in the operators who controlled the combat and technical facilities (CTF) of submarines and miners separately. All hierarchical levels of the body, from socio-psychological to subcellular were examined by incorporating complex of methods for estimation of the brain functional status (FS) that involved time of simple and

complex sensorimotor reactions (SSMRs and CSMRs, respectively) to photic and acoustic stimuli, reaction to a moving object (RMO), flicker fusion frequency (FFF). The results were compared to energy level of whole EEG signal recorded from the operators both before and after the cruise. The study concluded that as fatigue became chronic (of prolonged duration) and shifted towards a state of over-fatigue, there occurred a considerable increase in the FFF, a reduction in the CSMR time. Though the performance of the correction tests improved among operators; a trend towards premature reaction appears in the RMO tests. The results also well correlated with an increase in relative energy content of whole EEG signal. Thus, EEG that represents brain neuronal functions can provide abundant information on the human cognitive states. Following this fact, Lal and co-workers [1] attempted to develop an algorithm based on the changes in all the major EEG bands (δ, θ, α aed β bands) during the fatigue to detect different levels of fatigue genesis and progress.

While studying the EEG recordings in truck drivers on board, Papadelis and co-workers concluded that the occurrence of α wave bursts indicates a high level of sleepiness and a high possibility of an upcoming severe driving error [116]. Torsvall and co-workers recorded ambulatory EEG and electrooculogram (EOG) from train drivers during a night and day trip and they observed that the spectral power at α band increased during night driving relative to the daytime levels for the sleepy group [117]. In most studies, the EEG data were subjected to Fourier spectral analysis and alterations in α and θ bands were generally reported [1, 117]. Combining the EEG power spectrum estimation, principal component analysis, and fuzzy neural network model, Jung et al [118] designed a system to estimate and predict the drowsiness level of a driver. Budi et al [119] assessed the four EEG activities (at δ, θ, α and β binds) during a monotonous driving session, and got the results for conditions stable δ and θ activities over time, a slight decrease of alpha activity, and a significant decrease of beta activity. While analyzing the literature, it was obvious that EEG is most extensively studied parameter in drivers' fatigue (central component) evaluation. However, there was hardly any work that has attempted to correlate EEG findings with blood biochemical indicators to score fatigue. The correlation of EEG with multiple parameters is essential arrive at a scoring system of central fatigue in drivers.

2.4.2 Electrocardiogram (ECG)

The ECG is another contact feature that can give useful information on stages of fatigue development combined with sleep deprivation. The raw ECG may not be relevant. But the FFT of raw ECG signals after proper correction for noise gives the LF (low frequency), VFH (very low frequency), HF (high frequency) components as well as the LF/HF ratio all of which are important to derive information about fatigue. However, any comprehensive study on ECG signal variation in drivers during driving task is grossly lacking. At the same time many authors have studied ECG variation, particularly all frequency components as well as

HRV during other demanding tasks leading to fatigue. In one such study among marathon racers, the fast Fourier transforms (FFTs) was used to estimate the power spectral densities of the RR interval variability [120]. They derived the HRV of female Marathon racers and found a significant decrease in power level of HF components. They also concluded that the higher the rate of fall in HF power content, the faster to recovery from fatigue after the task. Apart from HF power, a detectable trend couldn't be established in other indices of HRV during the progress of fatigue from racing. The spectral parameters obtained from the spectral analysis of the HRV signals are used as the input parameters to the artificial neural network (ANN) for the classification of the different cardiac classes. In one such study, Papadelis and co-workers recorded ECG signal from sleep deprived drivers exposed to real time driving condition inside the lab with an objective to measure alertness level and find out a countermeasure [116]. However, a statistical significant variation was not observed in HRV with time. Recently, Zhao and coworkers [121] measured HRV from ECG signal recorded before and after a continuous stretch of 90 min of simulated driving in 13 drivers. They observed significant difference in HRV. However the study was for a shorter period and didn't reflect the cardiac response when the fatigue is in progress. On the contrary, the literature investigating variation of HRV indices during prolonged on road driving is lacking.

2.4.3 Spirometer Recording

Though an extensive literature can be found describing spirometer recording in various clinical assessments, there is dearth of literature that analyzes spirometer findings during exercise in normal population or in drivers. Thus, in the current study, it was decided to monitor lungs function during fatigue progress in drivers. It is hypothesized that on prolonged driving, central fatigue spreads to periphery that can compromise respiratory function.

2.4.4 Biomarkers and Stress Hormones

During acute or chronic stress, whether of physiological or pathological origin, various hormones are secreted to cope with stress and maintain the homeostasis so that fatigue development can either be halted or slowed down. By measuring these stress hormones during occupational stress like driving can give important clues on fatigue stages. The various stress hormones are cortisone and its derivatives, melatonin, prolactin and testosterone etc. However the key hormone is cortisol since its variation is significant when the work is over prolonged period and involves sleep deprivation like driving, shift-working etc. Shinkail and co-workers conducted an experiment in shift workers to study the effect of stress/fatigue out of disturbed circadian rhythm on salivary and serum cortisol [122]. They collected saliva and blood samples at 4-h intervals in experimental short-term shifts and measured both salivary and serum cortisol by radioimmunoassay. They found that

both salivary cortisol and the blood cortisol both showed lower levels than normal during disturbed circadian rhythm. Individuals with chronic fatigue syndrome (CFS) are good candidates for studying the variation of various biophysical parameters during fatigue genesis and development. The fatigue profile in these patients resembles the fatigue from prolonged monotonous task like driving though the mechanisms of onset may differ. On analyzing the trend in urine corticosteroids over one night patients with CFS, Jeries and co-workers [123] concluded that urinary free cortisol and cortisone concentrations showed a significant normal diurnal rhythm, but levels were lower across the cycle in CFS patient. A study investigating the variation in cortisol level and the impact of disturbed circadian rhythm on its trend during prolonged sleep deprived driving can give an insight into driver's fatigue.

2.4.5 Subjective Assessment

Questionnaire based investigations represent a possible approach to the study of drivers' fatigue. Subjective symptoms of fatigue are in a sense regulator of activity, which prevent individuals from excessive decrements in performance or reaching a state of exhaustion. Prolonged driving is usually accompanied with sleep deprivation that makes the drivers vulnerable to accelerated central fatigue. At the same time sleep deprived driving also induces mood disturbance or depression in drivers. To evaluate the effect of sleep deprivation, sleepiness score are used as important assessment tools. At the same time, subjective assessment tools that measure the general physical and mental and/or the affective components of behavior are also essential to get an insight into driver's fatigue level. The researchers have used either established or modified subjective assessment tools for monitoring fatigue in drivers or general population that are outlined in the Table 2.1.

Table 2.1: Subjective assessment tools used for performance assessment

Tools	**Significance**	**Supporting Literature**
Sleepiness Scale (i.e. Piper' Sleepiness Scale, Stanford Sleepiness Scale, Epworth Sleepiness Scale)	Measures the drowsiness level from sleep deprived condition alone or associated with general or specific activity.	[124–126]
Depression inventory (i.e. Beck' Depression inventory/BDI-II)	Measures affective component of thought (mild mood disturbance to major depression)	[127]
Subjective feeling of well-being (i.e. SF-36 v2 health survey)	Subjective feeling of physical and mental well-being during a disease process/intervention/task	[128]
Brief Pain Inventory (BPI)	Psychosomatic pain associated with any condition	[129]

2.5 Fatigue Study in Animals

Animal models are important tools in investigating the cause and mechanism of action of a physiological and/or pathological phenomenon. They are particularly useful in biomedical engineering because pathological conditions alter the normal physiological parameters and cannot be performed in human subjects [130, 131]. However, studies investing the course of fatigue genesis and development in healthy and/or pathological animals are scarce. While exploring the causative factors of fatigue, several studies in experimental animals have shown that physical exercise increases the synthesis and metabolism of brain 5-HT or serotonin [82, 132]. The role of serotonin as a key NT in inducing fatigue is supported by studies where the brain concentration of serotonin has been altered by means of pharmacological agents. When the serotonin level was elevated, the performance was impaired in both rats and human subjects. Again a decrease in serotonin level caused an improvement in running performance in rats [31]. The precursor of serotonin is the amino acid tryptophan and its synthesis in the brain is thought to be regulated by the blood supply of free tryptophan in relation to other large neutral amino acids (including the branched-chain amino acids, BCAA) as well as free fatty acids since these compete with tryptophan for transport into the brain [47].

Few studies have effectively used different animal models to investigate the effect of exercise or task induced fatigue on performance level that can be projected to general population as a whole. Beck and co-workers used rat models to analyze the physiological impact of handling and exercise testing during the light and dark periods and to assess the time to exhaustion at aerobic capacity intensity (TE), based on hematological parameters (total white cell count) [133]. They found that, with increased duration of task (forced swimming), the total white cell count increased with decrease in exercise tolerance. Similar study has also been conducted on trained race horses to observe the impact of circadian rhythm disturbance on their performance. Tortonese and co-workers induced experimental jetlag in race horses and found dysregulation in circadian clock genes but the racehorses managed to adapt and improved the performance after light-dependent rapid resetting of neuroendocrine systems [134]. The study predicted similar finding in trained target groups (drivers, shift workers, pilots).

2.6 Overall Framework of Experiments

On the basis of literature reviewed in the preceding sections, the experiments are designed in the current study to address the challenges in evaluating fatigue in our proposed occupational group i.e. automobile drivers. When the driving task, in case of long distance driving, is persistent without any fatigue countermeasure, a state of exhaustion is reached that grossly compromises the level of alertness and accounts for major road accidents. However, before

the end point of fatigue is reached, the progression of fatigue is manifested through a series of subjective and objective parameters. The subjective parameters constitute both specific and non-specific symptoms of fatigue. In the current study, an attempt has been made to assess the subjective symptoms of fatigue in drivers very precisely by questionnaire based subjective assessment tools. The results of subjective assessment tool would reflect the exact state of both central and peripheral fatigue at a particular instant of time and can be regarded as standard. Simultaneously, the objective assessment of fatigue has been conducted by measuring the variation in a multitude of physiological and biochemical parameters reflecting both central and peripheral fatigue during driving task. Few of these objective parameters can be the outcomes while others are inducers of fatigue in drivers. In the current study, the physiological/biochemical parameters that indicate either outcome or inducers have been addressed separately with utmost precision. The outcomes of central fatigue have been assessed from EEG parameters while that of peripheral fatigue from HRV study, spirometer recording and blood biomarker analysis. Similarly a separate experiment was designed to assess the variation in inducers of fatigue (serum cortisol, serotonin and insulin) to investigate the stress progressing to fatigue, thus exploring the genesis and stages of advancing fatigue in drivers. All these parameters were correlated with the findings of subjective assessment to derive the rationality of each parameter in developing a scoring system for drivers' fatigue. The immediate application of such a scoring system is to validate the devices under development to detect and alert drivers onboard based on non-contact features of fatigue. However, it is also essential to decipher whether a device used for onboard fatigue detection can be used equivalently both for physiological fatigue (experienced by healthy drivers) and pathological fatigue (drivers suffering from medical conditions that make them prone to fatigue). To solve this problem, an attempt has been made in the current study to establish and compare the trends in fatigue genesis and progression of physical fatigue using rodent models.

Chapter 3

Comparison of Trends in Psychomotor Manifestations of Fatigue between Healthy and Pathological Conditions in Rodent Models

Physical fatigue is expressed as a subjective phenomenon and has been scored through subjective assessment tools by many authors during an array of task or exercise in different occupational groups. However in the current project an attempt has been made to estimate the physical fatigue during simulated driving in automobile drivers by assessing a series of objective parameters. The objective is to determine those objective parameters that can be used for scoring fatigue in drivers due to their potential to give inter stage variation.

However, it's a challenge to predict whether a scoring system thus developed can be used both for physiological fatigue and fatigue accelerated from a pathological condition. Thus, it's necessary to study the trend in genesis and journey of fatigue in a biological model since a similar trend in both normal and pathological conditions with different intensity can make the same scoring system to be effective for both groups by adjusting the cut-off values of each parameters. Before evaluating physical fatigue in our target occupational group, a study was conducted to 1) evaluate the fatigue progress in apparently normal mouse subjected to fatigue-inducing task and, 2) to evaluate change in the fatigue genesis and progress in under pathological condition such as tauopathy. αCAMKII-4R tau transgenic (Tg) mice were chosen for the study. These mice have mutation in AA386 of tau. Such mice not only show symptoms of tauopathies but also have reduced density of $5HT_6$ receptors in cortical areas and therefore, exhibit the feature of cognitive and memory deficit that is typical of physiological central fatigue [135]. Due to neuro-degeneration, the fatigue process is accelerated in these mice model with induced exercise. The Figure 3.1 explains the suitability of this animal model for our experiment.

The signs and symptoms of central fatigue is initiated by accelerated production of serotonin (5 hydroxytryptamine/5HT) from free tryptophan that crosses blood brain barrier

in excess due to increased blood supply to brain at the peak of a task or exercise. In addition, free tryptophan is also utilized for production of L-kynurenine in a minor pathway when tryptophan turnover is very high (chronic fatigue or over-fatigue) and is hypothesized to be a key contributor to depression [136], [137]. Thus central fatigue is invariably associated with depression if it persists for a prolonged period. However, both serotonin and L-kynurenine acts on specific receptors to give rise to symptoms of central fatigue. As shown in the Figure 3.1, serotonin acting on $5HT_6$ receptors show positive effects like cognitive enhancement and memory development. But unfortunately, in most of the rodent models constructed to study tauopathies, the density of normal $5HT_6$ receptors in cortical areas is reduced [138] with expression of dysregulated $5HT_6$ receptors out of polymorphism leading to alteration in the transmission of several NTs responsible for motor, memory and cognitive enhancement [139]. Thus such mice exhibit feature of cognitive and memory deficit that is typical of physiological central fatigue. At the same time serotonin acts on neurons containing $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_2$ and $5HT_3$ receptors and induce stress that ultimately ends in the symptoms of central fatigue like tiredness, inability to concentrate, lack in decision making etc. [140]. Such neurons in the target mice model are intact thus developing typical features of central fatigue. At the same time, L-kynurenine acts non-specifically on neuronal cells to produce symptoms of depression both in wild and transgenic mice [141] during prolonged fatigue. Thus, it is hypothesized that in αCAMKII 4R tau mice, in contrast to wild mice, the motor, cognitive and memory deficit from task induced fatigue will be accelerated due to dysregulated expression of $5HT_6$ receptors while the symptoms of central fatigue will be prominent due to overexpression of $5HT_{1A,1B,1C}$, $5HT_2$ and $5HT_3$ receptors in neurons.

3.1 Materials and Methods

3.1.1 Animals and Environmental Condition

Two groups of mice were chosen for the experiments: Group-I (test): αCAMKII-4R tau Tg mice; Group-II (control): non-transgenic (non-Tg) wild mice. The animals were obtained from Experimental Animal Division, RIKEN BioResource Center (Koyadai, Tsukuba) and the experiments were conducted at the Alzheimer Laboratory of Dr. Y. Tatebayashi at the Brain Science Institute (BSI), RIKEN, Tokyo, Japan during May - Aug 2003. The collected data were compiled and analysed using the appropriate statistical package in SPSS during Dec 2010 - March 2011 at NIT Rourkela. Twenty four female mice from each group within the age range of 12-16 months were subjected to various behavioral assessment tools (Appendix B) to observe and compare motor as well as cognitive performance after induced fatigue on a rotarod apparatus. During the experiments, the animals were put on a standard rodent laboratory diet (10% fat, 70% carbohydrate, and 20% protein). The animal experiments were performed as per the guidelines for laboratory animal care laid down by

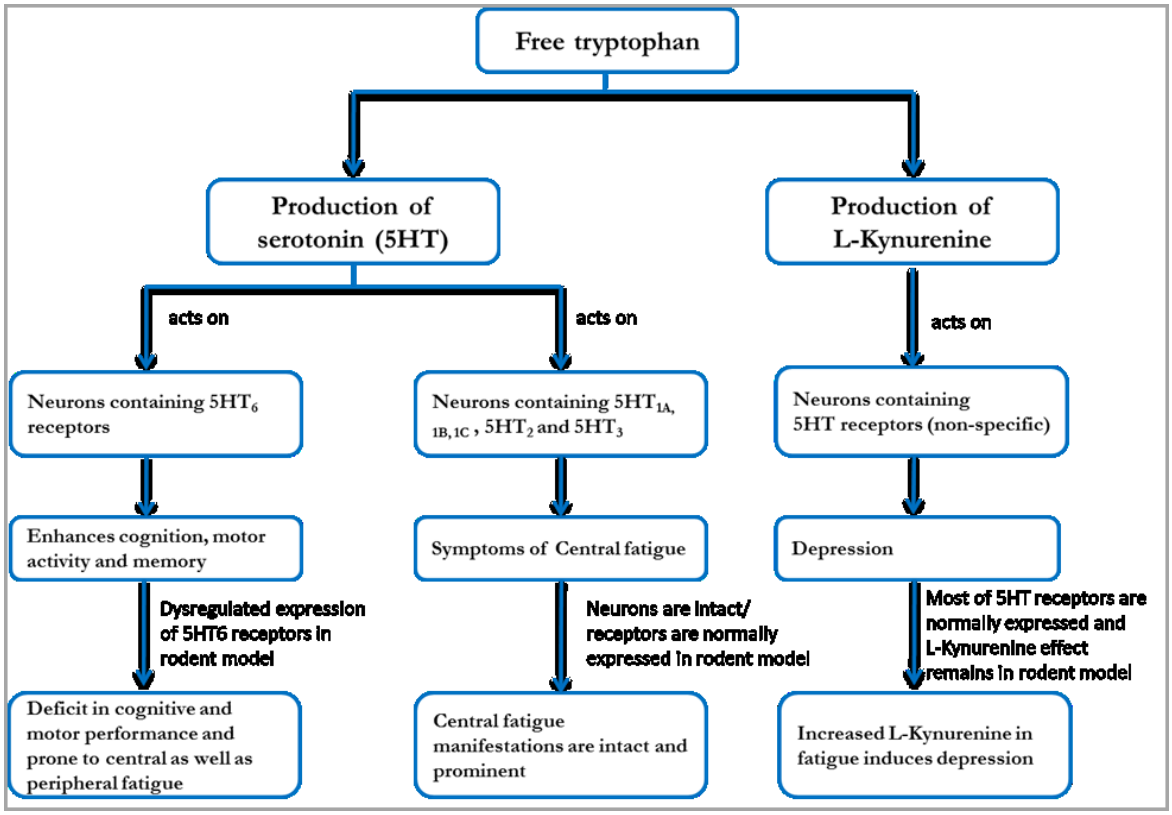

Figure 3.1: Rationality of choosing the mouse model (αCAMKII-4R tau) for fatigue analysis. Serotonin acts on $5HT_6$ receptors and shows positive effects like cognitive enhancement and memory development. But unfortunately, in most of the rodent models constructed to study tauopathies, the density of normal $5HT_6$ receptors in cortical areas is reduced. While $5HT_1$ receptor specific action and (that leads to central fatigue symptoms) and non-specific action of serotonin (that leads to depression) are intact in these models. Thus the combined features make the models vulnerable to accelerated fatigue, hence novel models for fatigue study.

NIH, USA [142] and the experiment protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the BSI, RIKEN, Japan. All animals were individually coded, and investigators were blind to group designations throughout testing.

3.1.2 Design of Experiments

Mice in each group were subjected to a series of micro-processor controlled behavioral tests after a session of exercise on rotarod in a sequence as outlined in Table 3. On Day 1, all tests were conducted till the animals are completely exhausted. However two tests i.e. Accelerated Rotarod Test (ART) and Forced Swimming Test (FST) were repeated for 3 days to analyze fatigue progression after the animals are experienced from repeated exposure to same task. This repeat measure also allowed the assessment of learning ability and adaptability of animals. Each animal was subjected to a 10 minutes of ART at a fixed speed of 2 rpm before start of the experiments with visual cues placed in front for inducing

peripheral, central and visual fatigue.

Table 3.1: Design of experiment for fatigue study on mice model

Sequence in a day	Test	Day 1	Day 2	Day 3	Significance
1	Accelerated Rotarod Test (at 2 rpm)	10 min	10 min	10 min	For induction of fatigue (peripheral, central and visual)
2	Accelerated Rotarod Test	5 min	5 min	5 min	Effect of fatigue on motor performance and learning/deciding skill
3	Open Field Test	2 h			Effect of fatigue on locomotor ability and cognition (thought process)
4	Elevated Plus Maze Test	10 min			Effect of fatigue on locomotor ability and development of anxiety
5	Light and dark Transition Test	1 h			Confirming the role of fatigue on development of anxiety and its impact in destabilizing the thought process
6	Forced Swimming test	5 min	5 min	5 min	Effect of extreme fatigue on development of severe anxiety that initiates affective symptoms i.e. depression/helplessness

3.1.3 Behavior and Motor Assessment

Accelerated Rotarod Test (ART)

Motor coordination and balance in response to induced fatigue were tested with a rotarod test in accordance with the established protocol [143] with minor modification. Briefly, each animal (non-Tg/Tg mice) was placed on a horizontally oriented rotarod with 3 cm diameter (UGO Basile Accelerating Rotarod, Italy) suspended above a cage floor. Rodents naturally try to stay on the rotating cylinder and avoid falling to the ground. At first, the speed of rotarod was kept constant at 4 rpm for 10 minutes with visual cues placed in front of the animal for inducing peripheral and visual fatigue. This first phase on rotarod induced fatigue in the rodents. After 2 minutes of rest, the animal was again kept on the rotarod and the drum

was accelerated from 4 to 40 rpm in 5 minutes. The experiment was conducted in 3 trials over 3 days (0, 24 and 48 h) to observe the learning and reacting capability of animals. The time each animal was able to maintain its balance on the rod before fall was measured. The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning.

Open Field Test (OFT)

Locomotor activity was measured using an open field test. Briefly, each mouse was placed in the center of the open field apparatus (UGO Basile, Italy) and the behavior was recorded for 120 minutes. Data acquisition and analysis were performed automatically using Image OF software. The activities like total distance traveled (in meter) and time spent in the center were measured. In addition, the tracings generated by the movement of each animal over 2 h were recorded by a camera incorporated to OFT apparatus. The total distance travelled indicates the motor performance of the animal and the tracings of each animal over 120 minutes give information about the cognition.

Elevated Plus Maze Test (EPMT)

EPMT was conducted as per established protocol described elsewhere [144]. The EPM apparatus (UGO Basile, Italy) consists of two open arms (25×5 cm) and two enclosed arms of the same size, with 15 cm high transparent walls. The arms and central square were made of white plastic plates and were elevated to a height of 55 cm above the floor. Plastic ledges of 3 mm height were provided for the open arms to prevent animals falling from the apparatus. Arms of the same type were arranged at opposite sides to each other. Each mouse was placed in the central square of the maze (5×5 cm), facing one of the closed arms. Mouse behavior was recorded during a 10 minute test period. The illumination level was 120 lux at the center of the maze. For data analysis, we used the following parameters: the percentage of entries into the open arms, the time spent in the open arms (s), the number of total entries, and total distance travelled (cm). Data acquisition and analysis were performed automatically using Image EP software. EPMT predicts the state of anxiety in animals. Usually, the animals suffering from anxiety and fear avoid visiting open arms, thus, decreasing the frequency of entry into the open arms.

Light and Dark Test (LDT)

LDT was conducted as per established protocol described elsewhere [145]. The apparatus used for the test consists of a cage (21 × 42 × 25 cm) divided into two sections of equal size by a partition containing a door (UGO Basile, Italy). One chamber was brightly illuminated (400 lux), whereas the other chamber was dark (4 lux). Briefly, mice were placed into the dark side and allowed to move freely between the two chambers with the door open for 10

min. The total number of transitions between LC and DC and time spent in each side were recorded automatically using Image LD software. In addition, the tracings produced by each animal over the time span were recorded. Usually, mice prefer to stay in dark during relaxed and fearless condition. With the development of anxiety, the animal's thought process is destabilized and frequently migrates between the light and dark chambers (LC and DC). Thus, the time spent in LC and total number of transitions is expected to increase in anxiety condition compared to relaxed and healthy animals.

Forced Swimming Test (FST)

FST was conducted as per the established protocol [146] with some modifications. Briefly, each mouse was subjected to two trials during which they were forced to swim in a Plexiglas cylinder (20 cm height$\times$10 cm diameter) filled with water. The first trial was conducted on 1st day that lasted 5 minutes. This trial is meant for training the animals. The first trial was conducted after all tests on day-1 to simulate extreme fatigue while the animals were subjected to additional fatigue by allowing them to swim during the experiments. Another 2 trials were conducted on days 2 and 3 (at 24 hours of interval) to observe the trend of fatigue outcome. Each trial lasted for 5 minutes during which the immobility and the distance traveled were recorded. Images were captured at one frame per second. For each pair of successive frames, the amount of area (pixels) within which the mouse moved was measured. When the amount of area was below a certain threshold, mouse behavior was judged as "immobile". When the amount of area equaled or exceeded the threshold, the mouse was considered as "moving". The optimal threshold by which to judge was determined by adjusting it to the amount of immobility measured by human observation. Immobility lasting for less than a 2 sec was not included in the analysis. Data acquisition and analysis were performed automatically, using Image-PS software. The duration of immobility indicates the instance of severe depression or hopelessness in animals. At the peak of major depression, the animals usually loose the charm of survival which is reflected in the FST outcome.

Statistical Analysis

All experimental outcomes are expressed as means ± SEM. Differences between means were analyzed using Student's t-test. In all analyses, the null hypothesis was rejected at the 0.05 level, calculated using the SPSS software version 20.0 (SPSS Inc., IL, USA).

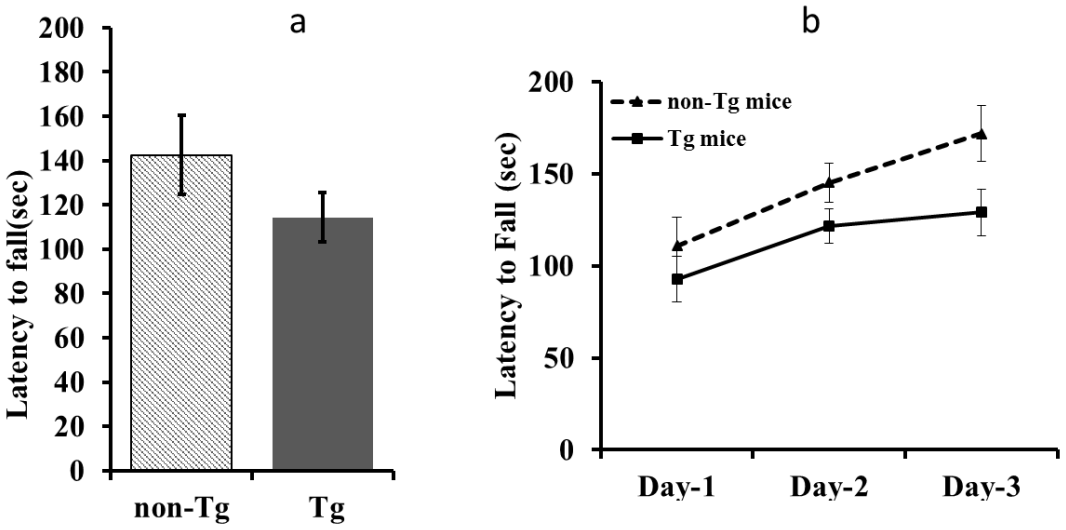

Figure 3.2: Results of accelerated rotarod test (ART) comparing the motor coordination between αCAMkII-4R Tau (Tg) and wild-type (non-Tg) mice. Averaged time of stay on rotarod before fall in 3 trials over 3 days (a) was significantly higher in wild-type mice compared to αCAMkII-4R Tau mice. The performance on rotarod at 3 time points (b) showed increase in latency to fall in both the groups indicating learning and adaptability. However, the learning is much slower in Tg mice. In (a) and (b); values represent means $\pm$ SEM, n= 24 per group

.

3.2 Results and Discussion

3.2.1 ART Results

The rotarod test for motor performance and learning ability was conducted in both groups of mice at three time points (0, 24 and 72 h) after a phase of fatigue induction by allowing the animals to stand on rotarod at a fixed speed on the beginning of each day experiments. The duration of stay on rotarod without fall averaged over 3 days was significantly higher in case of non-Tg mice compared to that of Tg mice (Figure 3.2(a), p=0.031, α=0.05, n=24).The durations of stay at individual time points (0, 24 and 48 h) were also significantly higher in non-Tg mice except at 24 h (Figure 3.2(b), p=0.0061, p=0.072 and p=0.0066 at 0, 24 and 48 h, respectively). As we can observe in ART, the latency to fall gradually increased over 3 days in both the groups confirming their ability to learn a skill. However, the learning ability in case of αCAMKII 4RTau mice are significantly slower than the wild mice as shown by the trend line of Tg animals nearer to X-axis (Figure 3.2(b)). Tg mice underperformed in both motor coordination as well as learning ability that indicates the worsening impact of fatigue on αCAMKII 4R tau mice. But, the most significant result can be observed in the trends (decrement) of motor performance and learning ability that is similar in both groups of mice though the healthy mice performed better.

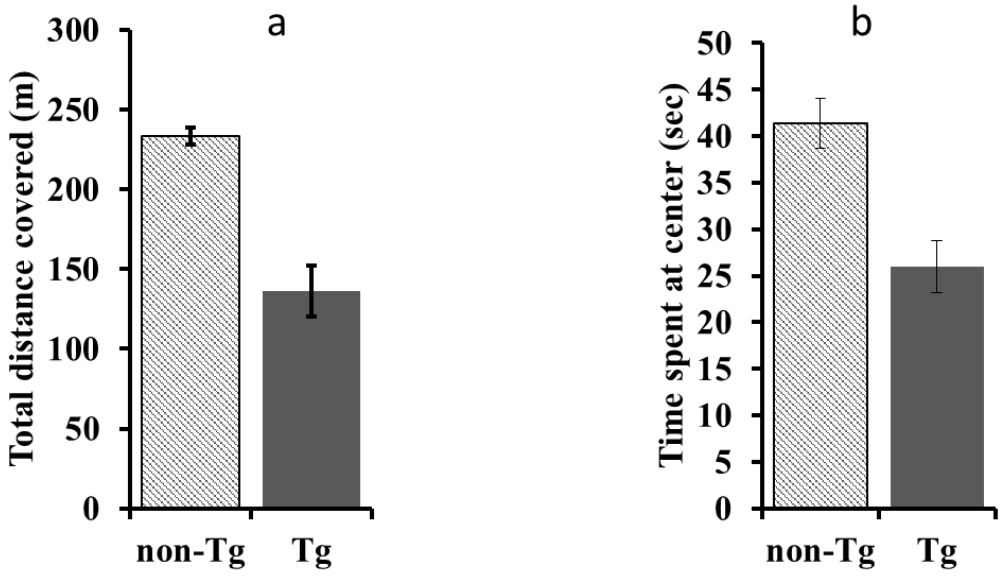

Figure 3.3: Results of open field test (OFT) comparing the spontaneous locomotor and cognitive performance between αCaMK-II-4R tau and wild-type mice: αCaMK-II-4R tau mice covered significantly shorter distance over 120 minutes (a) and spent less time at the center (b) compared to that of wild-type mice. In (a) and (b), values represent means $\pm$ SEM, n= 24 per group.

3.2.2 OFT Results

In OFT, significant difference was found in total distance covered over 120 minutes (Figure 3.3(a)), p=0.00005, α=0.05, n=24) and duration of stay at center (Figure 3.3(b)), p=0.0009, α=0.05, n=24). It can be observed that non-Tg mice outperformed the αCAMKII-4R tau mice in locomotor ability as shown by significantly larger distance covered by the former. A shorter stay of Tg mice at the center confirms the state of anxiety and a strong sense of nervousness in them from prolonged central fatigue.

While observing and comparing the tracings of both group of mice (Figure 3.4), some important clues to the cognitive performance of αCAMKII-4R tau mice could be noted. The tracings of Tg mice in general appear to be more dense indicating confused, disorderly movement of the animals that indicate the effect of aggravated central fatigue. While the tracings of non-Tg mice show central clear zone that indicate that the movements gradually confined to the periphery or the border of squared field. Thus, as time progressed, non-Tg animals learnt that the escape route might be present at periphery that indicates their cognitive or decision making ability.

3.2.3 EPMT Results

In the elevated plus maze, the percentage of entries into open arms was significantly higher in wild mice compared with αCAMKII-4R tau mice (Figure 3.5(a)); p = 0.023, α=0.05, n=24).

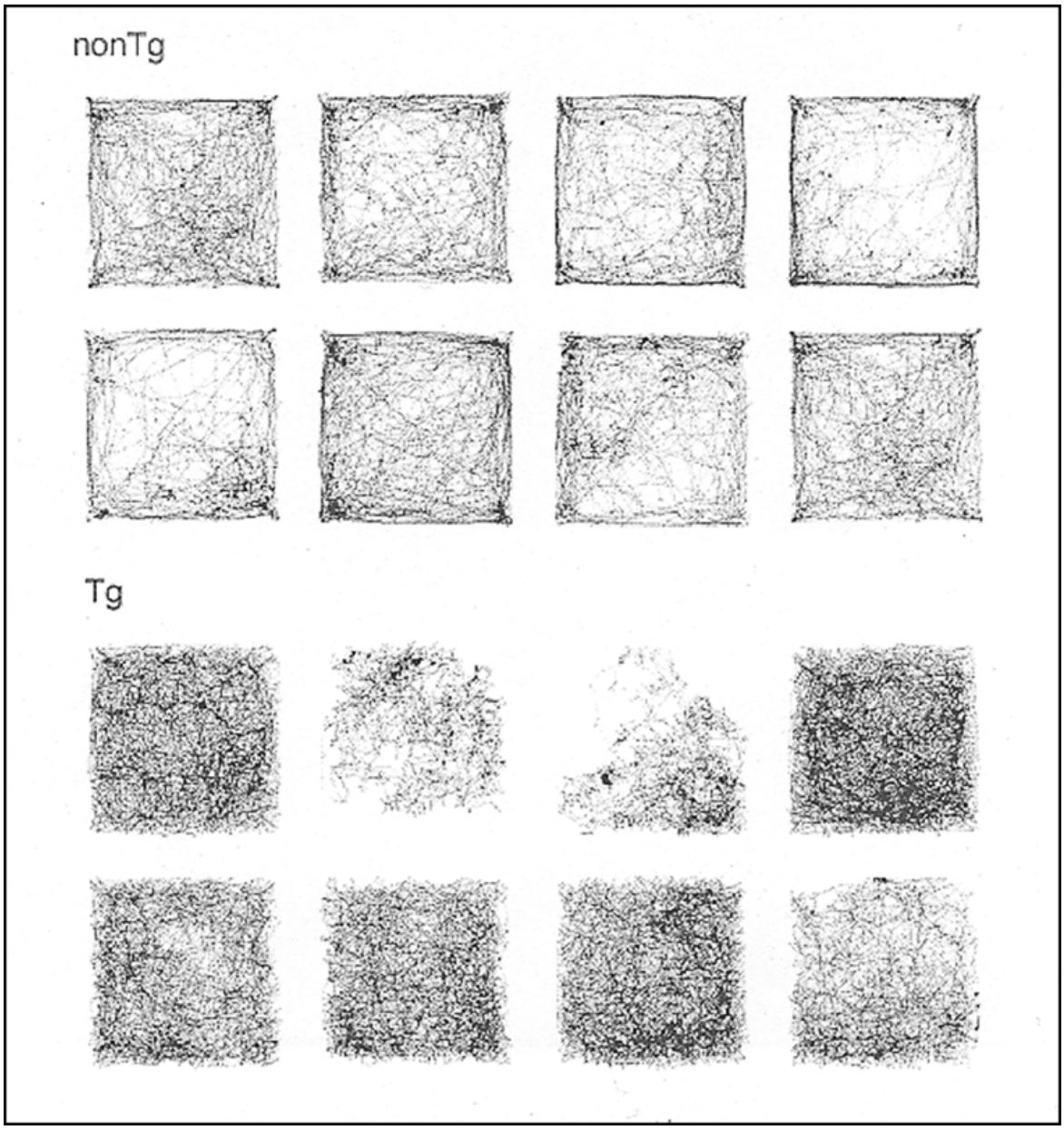

Figure 3.4: Movement tracings of αCAMkII-4R Tau and wild-type mice over 120 minutes inside open field apparatus recorded by Image-OF software. n= 8 per group

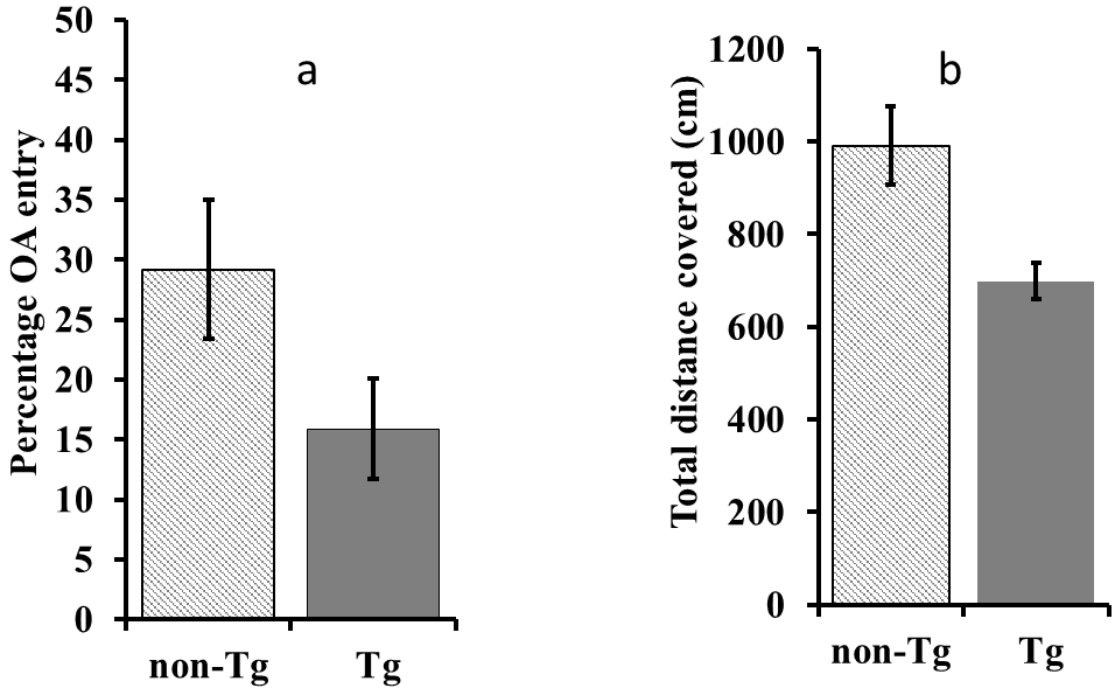

Figure 3.5: Results of elevated plus maze test (EPMT) comparing anxiety state between αCaMK-II-4R tau and wild-type mice. αCaMK-II-4R tau mice exhibited lower percentage of open arm entry (a) and covered significantly less distance (b) compared to wild-type mice. In (a) and (b), values represent means $\pm$ SEM, n= 24 per group.

The lower percentage of open arm entry in the case of αCAMKII-4R tau mice revealed their timid and anxiety behavior. Though the total distance travelled by wild animals was more than non-Tg animals confirming the motor deficit in Tg animals, the difference fell at the transition of significance level (Figure 3.5(b)); p = 0.0511, α=0.05, n=24). There were no significant differences in times spent in open arms and on the center of the maze (not shown). Thus the results of EPMT that shows less frequent visit of open arms by Tg mice is consistent with the shorter time spent at the center of Open Field revealing a significant state of anxiety and fear associated with central fatigue due to aberrant Tau.

3.2.4 LDT Results

Analysis of the light/dark transition test revealed significant differences between wild and Tg mice in the time spent in light chamber over 10 minutes (Figure 3.6(a), p=0.007; n=24), number of transitions between light and dark chambers (Figure 3.6(b), p=0.0002; n=24) and latency to first entry into LC (Figure 3.6(c), p= 0.000, n=24). The αCAMKII-4R tau mice exhibited significantly number of transitions between light and dark chambers compared to wild mice that proved their restless behavior. Similarly, a longer latency period to enter into LC in case of αCAMKII- 4R tau mice confirm that these Tg animals are in a worse state of fatigue induced anxiety compared to wild ones. In addition, the tracings

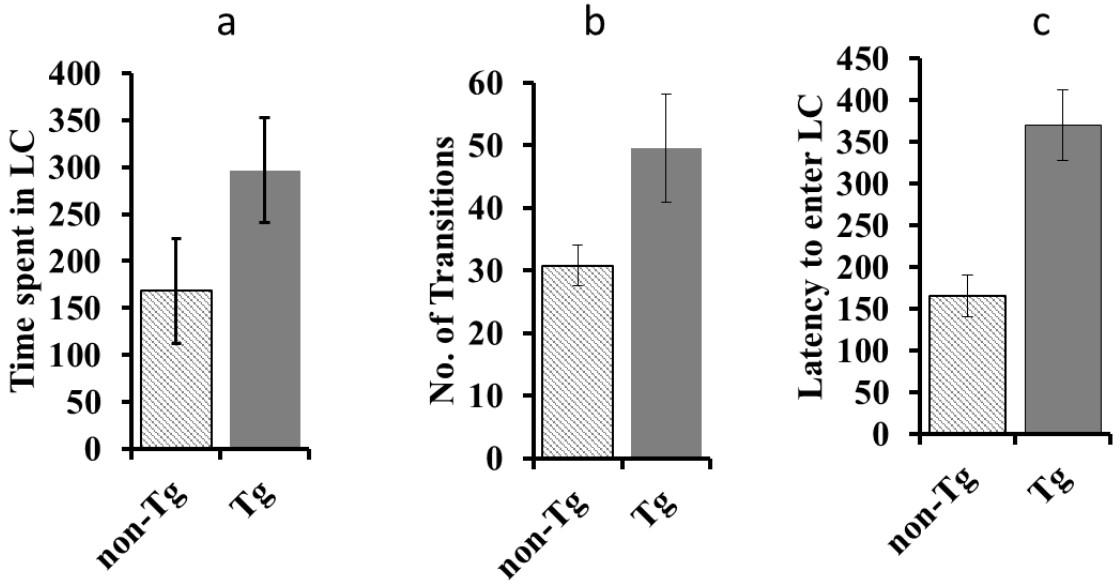

Figure 3.6: Results of light and dark transition test (LDT) for comparing the anxiety state between αCaMK-II-4R tau and wild-type mice: αCaMK-II-4R tau mice spent longer time in LC (a), made more transitions between light and dark chambers (b), exhibited longer latency to first entry into LC (c) compared to wild mice. In (a), (b) and (c), values represent means $\pm$ SEM. n= 24 per group.

recorded during LDT (Figure 3.7) revealed some important information about the mouse model. Most of the Tg mice exhibited dense tracing (5 out of 8) that indicates random and frequent movements of those animals reflecting restlessness and loss of concentration. One can observe large dark spots that represent wondering around a central point for a period of time representing indecisiveness and freezing of thought process. Such spots are not found in the tracings of wil-type non-Tg mice. Again, the transition point between LC and DC appears to be more concentrated in case of Tg animals reresenting significantly more number of transitionsbetween LC and DC. On the otherhand,the wild mice exhibited an orderly movement observed from their sharply defined tracings. Thus, the thought process in αCAMKII 4RTau mice is moderately disturbed due to their vulnerability to fatigue compared to wild mice in addition to anxiety which affects their ability to plan and decide.

3.2.5 FST (Porsolt) Results

In the FST (Porsolt), t-test showed significant group effects on immobility (Figure 3.8(b); trial 1: p=0.0011; trial 2: p=0.009; α =0.05, n=24) and distance traveled (Figure 3.8(a); trial 1: p = 0.001; trial 2: p = 0.001; α=0.05, n=24). The αCAMKII-4R tau mice showed significantly higher percentage of immobility that represents the state of severe mood disturbance and a sense of doom while the wild mice fought hard for survival. The total displacement covered by swimming was significantly higher in wild mice at each 1

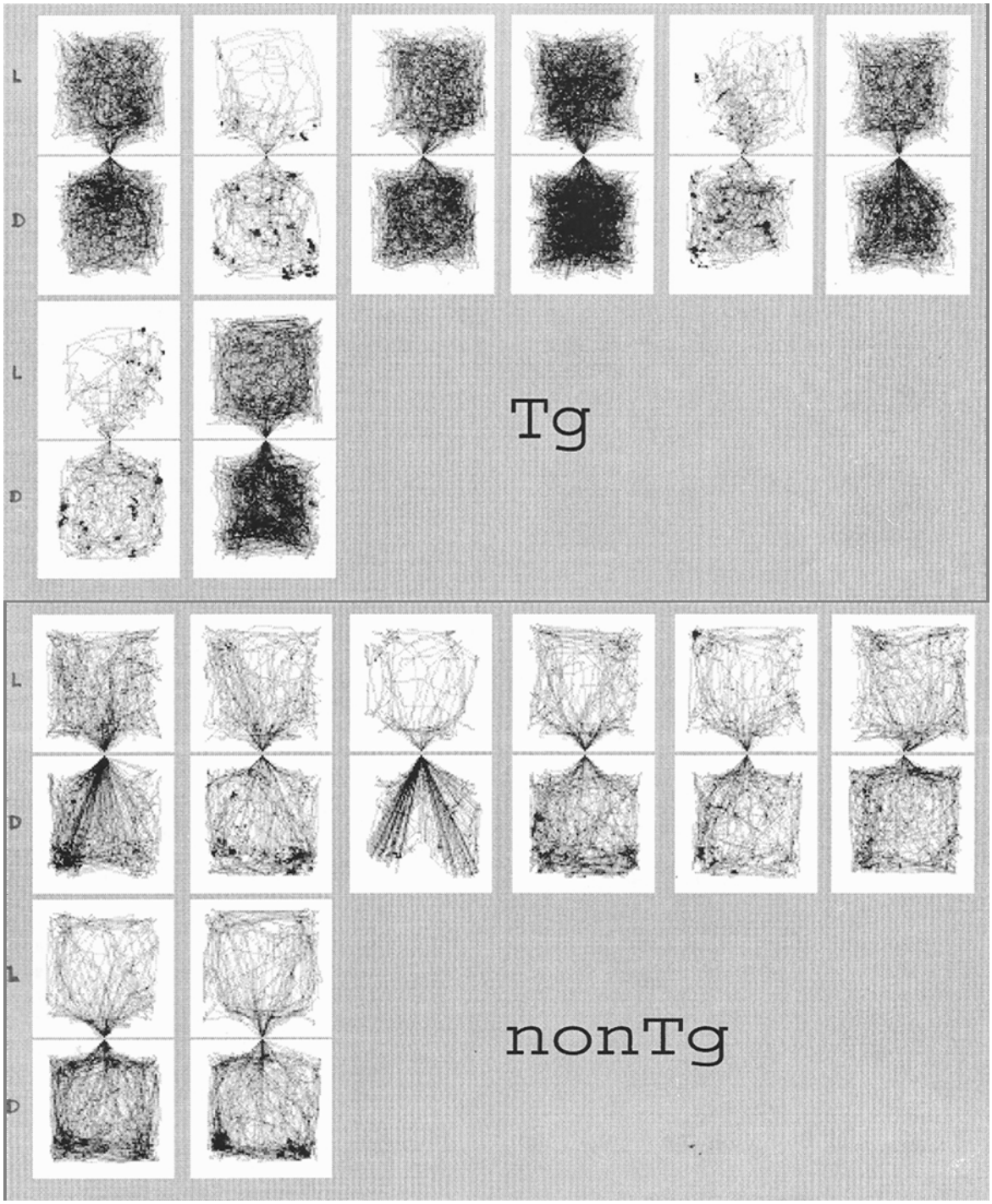

Figure 3.7: Movement tracings of αCAMkII-4R Tau and wild-type mice in light and dark chambers in LDT that compares state of anxiety and cognition. n=8 per group

minute blocks (total 5 minutes) in both trials that showed deficit in locomotor ability of neurodegenerative mice. The distance covered in 2nd trial was lower that of 1st trial in both the groups which indicates development of fatigue and depression in both, however, the extent was more for αCAMKII-4R tau mice. Locomotor deficit as observed in FST is consistent with the finding of OFT.

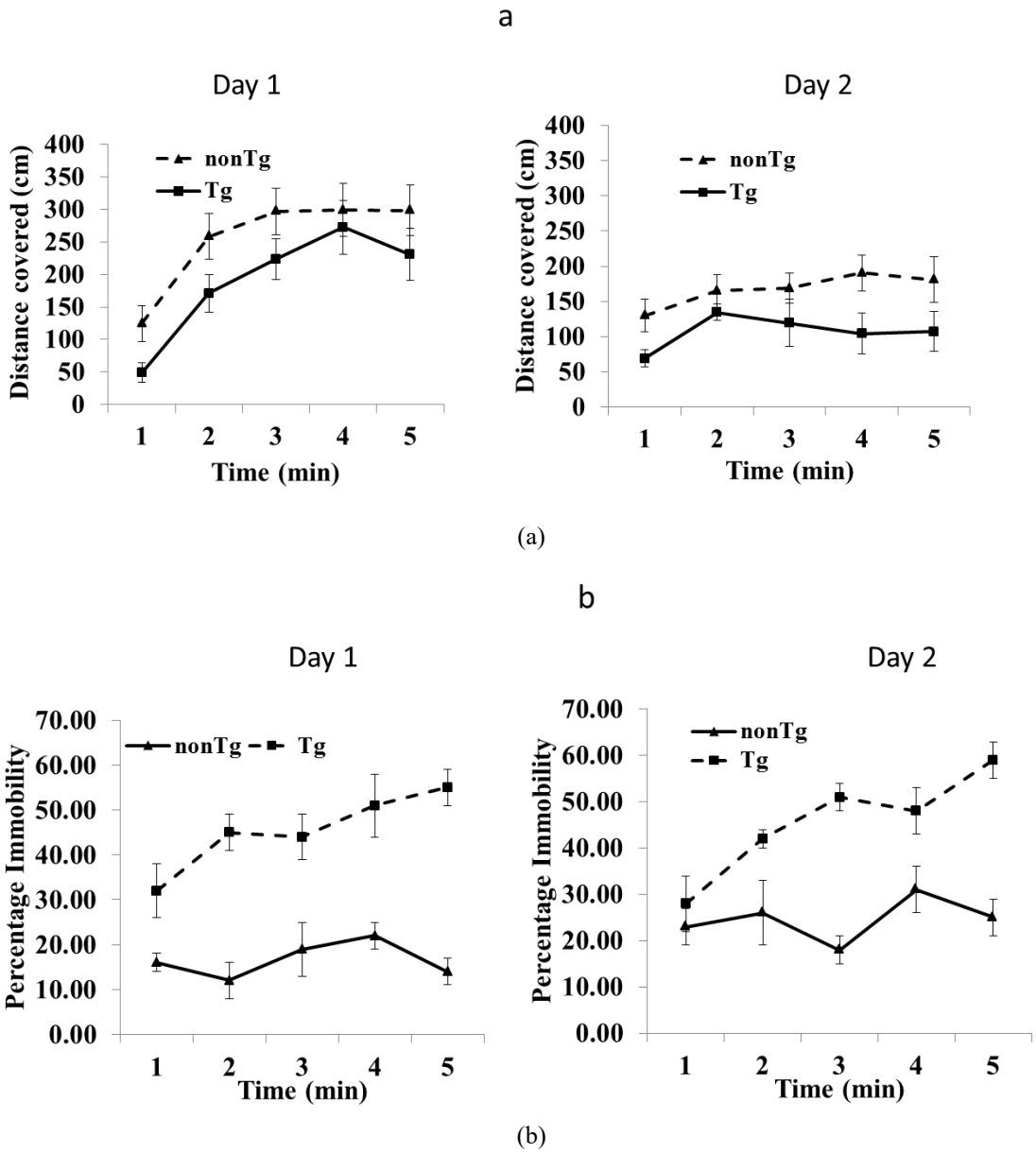

Figure 3.8: Results of forced swimming test (FST) for comparing the state of depression between αCaMK-II-4R tau and wild-type mice. αCaMK-II-4R tau mice covered shorter distance by swimming (a) and exhibited significantly higher percentage of immobility (b) in two trials over two days compared to that of wild mice. n= 24 per group.

3.3 Summary and Conclusion

The results of fatigue study on mice can be summarized as:

i. In ART, the wild mice stayed for a longer period on rotarod before fall. Over 3 days, the latency to fall increased. The Tg mice exhibited significant lower latency to fall though it increased over 3 days with the same trend as that of wild mice. The persistent lower performance in Tg mice showed that they adapted poorly to endurance training compared to normal.

ii. The wild mice covered more distance in OFT and EPMT, swum a longer period in FST that reflects better tolerance to induced fatigue. The Tg mice covered significant less distance in OFT, EPMT and FST that shows pronounced impact of fatigue on locomotor performance.

iii. The wild mice spent more time in open arm of EPMT and showed lower latency to first visit to LC in LDT that show the fearless attitude. Tg mice were timid as shown from significant less time spent in open arm of EPMT. Fear and a lower level of confidence in doing a task are gradually developed with advancement of central fatigue.

iv. Wild mice spent more time at the border of OFT in search of outlet and preferred to stay more time in DC of LDT reflecting their intactness of decision making ability after prolonged fatigue. Tg mice spent more time in random movement in OFT and spent equivalent time in both DC and LC that reflects the deterioration in decision making or cognitive performance due to fatigue.

v. Wild mice made fewer transitions between LC and DC in LDT and showed less immobility in FST that reflects lower level of anxiety associated with prolonged fatigue or over fatigue. Tg mice made frequent transitions between LC and DC and gave in early in FST developed due to prolonged fatigue. The disturbance in affective component (depression) is a key symptom of prolonged central fatigue. However, the trend in performance level in FST over 48 h was similar to that of wild mice.

The study showed that the signs and symptoms of physical fatigue in mice models of tauopathy were more pronounced compared to normal ones and, however the genesis and progress of fatigue followed similar trend under physiological condition and tauopathy that is known to be associated with accelerated fatigue. Thus, a fatigue score system developed on healthy individuals may also be applied on pathological condition such as tauopathy.

Chapter 4

Quantifying the Manifestations of Central and Physical Fatigue in Drivers during Simulated Driving Session

In this chapter, the variation in manifestations of fatigue amongst heavy vehicle drivers have been investigated at progressing stages of fatigue in the process of simulated driving with sleep deprivation. For discrimination of fatigue stages, it is quite essential to quantify a set of parameters that reflect manifestations of both central and peripheral fatigue in drivers.

Thus, the current study attempts a multidimensional analysis of driver's fatigue through investigating the variations in key blood biomarkers (RBS, blood urea and serum creatinine), heart rate variability (HRV) from ECG, lung's functional status from spirometer recording and brain's functional status (FS) from EEG parameters during simulated driving conditions. The EEG signal was passed through a band pass filter for discrete wavelet transformation (DWT). Next, the relative energy and entropy features at different EEG bands were obtained at different stages of simulated driving. The ECG signal was also analyzed for obtaining R-R interval followed by heart rate (HR) measurement. This data was utilized for obtaining various indices of HRV. Changes in EEG frequency bands (raw data) have direct correlation with the fatigue stages in general population [147, 148]. It is hypothesized that, the relative energy and entropy at different EEG bands can also act as more refined and reliable indicator of progressing stages of physical fatigue in drivers. To test this hypothesis, the EEG parameters were co-related with the cardiovascular and blood biochemical parameters and were validated against clinically proven subjective assessment tools. The biochemical parameters (RBS, creatinine and urea), proposed to be investigated in this study, are established biomarkers that are clinically correlated to physical work and exercise whereas EEG is known to give abundant information on central fatigue [149, 150]. In this process, it is to be seen that the correlation among these physiological and biochemical parameters may further help in developing and validating an application for alerting fatigued drivers.

4.1 Materials and Methods

In order to attain the above discussed goals, a systematic plan of work was developed and implemented as depicted below:

Table 4.1: Design of experiment and time lines of tests conducted on human drivers

STAGES	ACTUAL TIME	ELAPSED TIME	BLOOD COLLECTION	ECG	SPIROMETER	EEG
0	08:30 h	0 hr	YES	YES	YES	YES
1	08:30-11:30 h	3 hrs		YES	YES	YES
			LUNCH			
2	12:00-15:00 h	6.5 hrs		YES	YES	YES
3	15:00-18:00 h	9.5 hrs	YES	YES	YES	YES
			SNACK			
4	18:30-21:30 h	13 hrs		YES	YES	YES
			DINNER			
5	22:00-01:00 h	16.5 hrs	YES	YES	YES	YES
6	01:00-04:00 h	19.5 hrs		YES	YES	YES
7	04:00-07:00 h	22.5 hrs		YES	YES	YES
			BREAKFAST			
8	07:30-10:30 h	26 hrs	YES	YES	YES	YES
9	10:30-13:30 h	29 hrs		YES	YES	YES
			LUNCH			
10	14:00-17:00 h	32.5 hrs	YES	YES	YES	YES

Table 4.2: Scheduled task at each stage of experiments that lasted for 3 hours

Duration	Task	Purpose
60 min	Simulated driving on PC	To generate mental, physical and visual and fatigue
30 min	Auditory and visual tasks	To enhance mental and visual fatigue
60 min	Simulated driving on PC	To generate mental, physical and visual and fatigue. EEG was recorded at this phase
30 min	Obtaining ECG for HRV measurement Spirometer recording	To assess the cardiopulmonary status with severity of fatigue

4.1.1 Experimental Design

The entire experiment was conducted inside a temperature controlled laboratory and was divided into a number of identical stages shown in Table 4.1. The subjects were kept awake during the whole course of experiment. To begin with, the blood samples were collected from each subject at 8:30 h (stage 0) to measure blood biomarkers that are considered as control. An EEG, ECG and a spirometer recording was also obtained at 0 hour of elapsed time of simulated driving for obtaining reference/control values. Further blood samples

were collected at 8 hours interval to repeat the measure of blood biomarkers while EEG, ECG and spirometer recordings were repeated at 3 hours interval as shown in Table 4.1. The whole experiment lasted for 32.5 hours including all activities (Table 4.1) while each stage of scheduled tasks (Table 4.2) lasted for 3 hours. The stages were repeated till most participants complained of extreme fatigue. Thus, the simulated driving protocol comprising of prolonged monotonous driving tasks with sleep deprivation reflected an identical environment of on road driving.

4.1.2 Driving Simulation Setup

The subjects were put to driving task using a pre-installed simulator setup (Appendix C). The simulator setup had a driving module and an audiovisual component. The driving module consisted of driving simulator software (Euro Truck Simulator, SCS software, Denmark) and a software compatible steering wheel (capable of 90º rotation) with a vibration leg pad and hydraulic brake system (DriveForce™ GT, Logitech, India). The audiovisual component comprised of a LCD projector with screen and a high definition sound system (Inspire-M 4500™, Creative India Ltd.) to recreate an environment of highway driving. The driving module and the audiovisual unit were installed in a PC (HP desktop, Intel Dual Core, Microsoft 7 OS, 4GB RAM and Nvidia Graphic card). The projector and the screen were positioned optimally so that the drivers can get a clear visibility. The whole setup was arranged keeping drivers ergonomics equivalent to on-board driving.

4.1.3 Selection of Human subjects

Twenty male human volunteers, with mean age of 30.5± 9.5 years, from a pool of experienced heavy vehicle drivers, were randomly selected for the study. All participants were subjected to clinical examination by the registered medical practitioner and were apparently healthy at the start of the experiments. The volunteers were prohibited from driving, tobacco chewing, alcohol consumption, stimulant drinks (tea/coffee) and any medication 24 h before the start of the experiment and during the entire period of experimentation. An informed written consent was obtained from all the participants.

4.1.4 Blood Sample Collection

Peripheral blood samples were collected from each subject at designated time period (Table 4.1) by puncturing the brachial vein. Blood was collected in heparin (72 USP units/ 2ml, Crest Diagnostics) for plasma glucose and urea estimation and in clot activator (silicon coated, Crest Diagnostics) for serum creatinine estimation. The samples were stored in refrigerator at 4-8°C until analysis. The samples were analyzed for plasma glucose, urea and serum creatinine within 24 h of collection.

4.1.5 Spirometer Recording

Pulmonary function was assessed by spirometry to measure the lungs volumes and the lung capacities. Since FEV_1/FVC ratio is the most sensitive indicator of pulmonary functional status, FEV_1 and FVC data was isolated from the complete spirometer data for further analysis. Briefly, each volunteer was subjected to the spirometer recording according to the schedule given in Table 4.1. The recordings were taken by a portable spirometer (SP250, Schiller, Switzerland). Periodic calibration checks showed variations of less than 1% (<10 ml per liter) for volume and no detectable variation for time. The subjects were put to one-to-one explanation, demonstration and practice to prepare them for the specific maneuver required for FVC measurement. The required information regarding the subject (height, weight and BMI) were fed to the in-built software (SDS104, Schiller, Switzerland) and the necessary test module was selected. To complete a FVC maneuver, each subject was instructed to exhale as soon as possible into the mouthpiece after a forced inspiration with nostrils closed (it avoids any loss of air through nostrils). Each subject made at least two, and if necessary up to four, attempts to perform FVC maneuver to meet the ATS acceptability and reproducibility criteria [151].

4.1.6 HRV Analysis from ECG Recording

Various indices of HRV were obtained from ECG in following steps,

Acquisition of ECG data ECG data were acquired from all the subjects at defined stages (Table 4.1) using a six-lead ECG machine (Recorders and Medicare Systems, India). Six pre-cordial electrodes were placed on designated area of the subject in supine position and ECG profile from each lead was recorded. The total testing time lasted for about five minutes per individual. Data were stored in the cardio software (Recorders and Medicare Systems, India).

Analog to Digital Conversion of ECG data Since HRV indices are derived from HR data, the procurement of an enhanced R-wave is essential to obtain accurate R-R intervals. A standard lead II is usually considered for HRV analysis because the ECG waveforms obtained from this lead contains high-peaked R-waves [152]. Therefore, the raw ECG data (5 minutes duration in each stage) from lead II of each subject were sampled at 500 Hz that showed prominent R-waves (Figure 4.1(d)).

Noise Elimination The digitalized EEG signal from lead II was filtered with a band pass filter having higher and lower cut-off frequency of 0.5 and 20 Hz, respectively, to remove the artifacts. A bandpass filter with a center frequency of 17 Hz maximizes the signal (QRS complex)-to-noise (from T-waves, power source, EMG etc.) ratio [118]. The band

pass filtering helped in removing low and high frequency artifacts but preserved R peak (Figure 4.1(a)). The resulting signal was squared to make the result positive and to emphasize the large difference resulting from QRS complex (Figure 4.1(c)).

Detection of R wave The rectified signal was sorted in descending order and from the first two data points (first two highest peaks) were averaged to get an estimation of R-peak amplitude.70% of the average R-peak was taken as threshold to detect R-peak from the rest of the signal as shown in Figure 4.1(d). A blanking interval of 300ms was taken in sample search procedure (during blanking period no search was done). A smaller interval constant was purposely appropriate to minimize artifacts from fluctuation of the baseline [152].

HRV calculation The averaged HRs of all subjects, obtained by processing the ECG waveforms from type-II lead described above, were exported in a text format to the Kubios HRV (heart rate variability) software version 2.0 (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland) for analysis of the following HRV parameters (i) time domain: geometrical index i.e. Poincare plots; and (ii) frequency domain: power spectral density of VLF, LF, HF components. However, the HF components were considered for analysis because the physiological significance of VLF and LF components has not yet been established. They are recommended for the short-term HRV analysis by The European Society of Cardiology and the North American Society of Pacing and Electrophysiology.

4.1.7 EEG Recording

Data Acquisition A 20 channel EEG machine (RMS, Maharashtra, India) was used for obtaining the EEG of each subject. The first sets of EEG data were recorded before starting any task assuming that each subject is at zero fatigue. Next, the procedure was repeated at the final phase of each stage. Briefly, three sets of EEG data were recorded in each stage (3 minutes data during driving simulation on PC, 2 minutes data after the game with open eye and no activity condition, and then 2 minutes data with closed eye and no activity condition). Only the first 3 minutes data was used for signal processing. Nineteen scalp electrodes were used in addition to reference and ground to collect the signals from locations Fp1, Fp2, F3, F4, F7, F8, Fz, T3, T4, T5, T6, C3, C4, Cz, P3, P4, Pz, O1, and O2. International 10 – 20 system was used for placing electrodes on scalp. The sampling frequency was kept at 256 Hz.

Signal Processing The EEG signal was characterized in the wavelet domain using energy and entropy measures at different frequency bands on MATLAB (R2010b, MathWorks, USA). The analysis involves preprocessing of the collected data and computation of features based on Discrete Wavelet Transform (Appendix D) for estimation of fatigue. The signals at 5 different stages (at an approximate interval of 8 hours) were analyzed and presented to

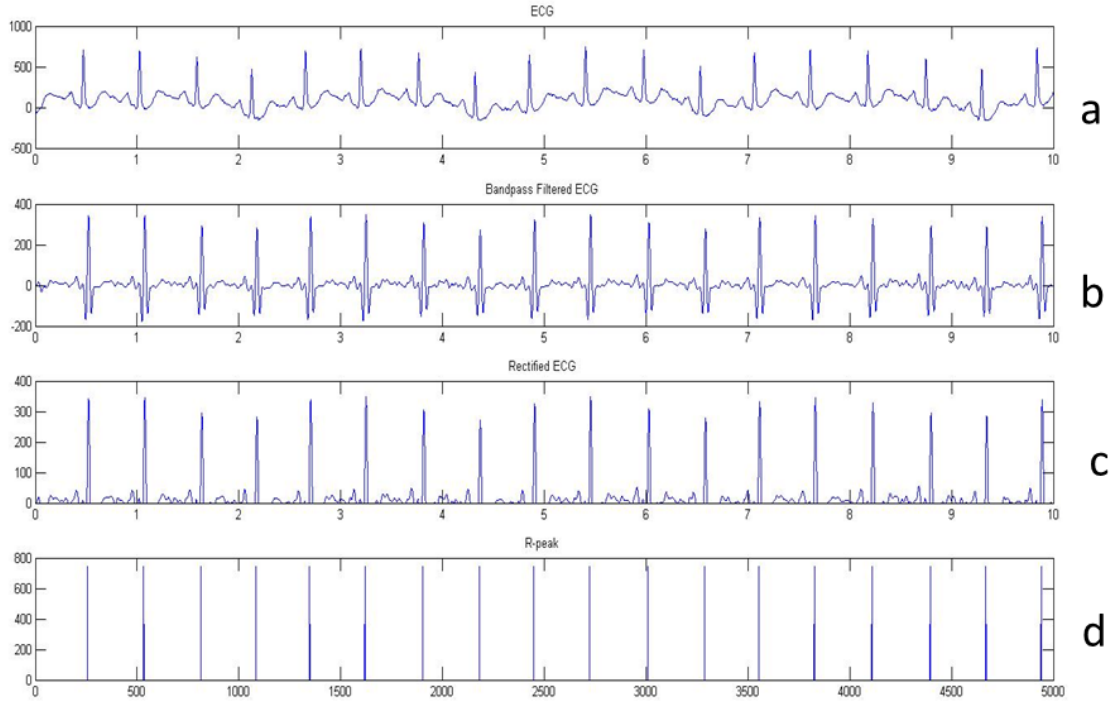

Figure 4.1: Stages of ECG signal preprocessing in a subject to isolate R-peaks showing sampled EEG signal from lead II (a) EEG signal after passing through band pass filter (b), squared EEG signal for positive QRS (c) and the prominent R-peaks after thresholding above 70% of averaged R-peak amplitude (d).

match the information obtained from blood collection (Stages-0, 3, 5, 8 and 10). These 5 stages indicate 5 different levels of fatigue.

Preprocessing The raw EEG data is contaminated with numerous high frequency noises due to surrounding thermal and power frequency noises and low frequency noises of 0-16 Hz due to eye movements, respiration and heart beats [153]. Thus, raw EEG was processed by using a band pass filter with cutoff frequencies of 0.5 Hz and 30 Hz. The filtering process was followed by normalization to zero mean and unity standard deviation, ensuring the removal of any unwanted biases that may have crept into the experimental recordings. Artifacts due to eye movements and eye blinks can also generate potentials with a magnitude much higher than that of original EEG [153]. The frequency bandwidth of these artifacts overlaps with the useful EEG band (in band artifacts), disturbing the parent shape of the spectrum. As the wavelet coefficients represent the correlation of signal with the mother wavelet, the signal will generate high amplitude coefficients at places where artifacts are present. These coefficients were eliminated using a simple thresholding technique expressed as:

$$T_j = \text{mean}(C_j) + 2 \times \text{std}(C_j) \tag{4.1}$$

where, C_j is the wavelet coefficient at the jth level of decomposition [153]. If the value of any coefficient is greater than the threshold, it is reduced to 0.1 time the actual coefficient value [154]. This generated a new set of wavelet coefficients which was used for further analysis.

Computation of Energy-based Features The energy in different frequency bands of EEG signal is associated with different functionalities of brain. These frequency bands are obtained at various levels of resolution in a wavelet decomposition scheme. The energy at jth level of decomposition can be expressed as,

$$E_j = \sum_{k=1}^{L} |C_j(k)|^2 \tag{4.2}$$

where, $C_j(k)$ = the wavelet coefficient (approximate or detailed) and L = total number wavelet coefficients at the jth level. Thus, the relative energy of a particular band represented by the resolution level j is given by,

$$p_j = \frac{E_j}{\sum_j E_j} \tag{4.3}$$

These relative energy parameters can be used as features for fatigue classification.

Computation of Entropy based features Standard Shannon's entropy (SE) was applied to improve the EEG classification in the presence of uncertainties associated with the energy bands. The SE in the wavelet domain was defined as,

$$SE = -\sum_j p_j \ \log_2(p_j) \tag{4.4}$$

4.1.8 Subjective Assessment

The subjective assessment of fatigue due to monotonous driving with sleep-deprivation was based on questionnaire-based tests. Two questionnaires-based tests were conducted at five time points corresponding to the blood sample collections (Table 4.1).

Questionnaire-based test 1: SF-36 v2 Health Survey SF-36 version 2 health survey tool is a multipurpose health survey that measures overall physical and mental status, functional status, and health-related quality of life at a particular instant out of task/disease/intervention [155]. From the scoring of SF-36 survey, one can obtain the overall physical status and mental status of the subject in the form of Physical Component Score (PCS) and Mental Component Score (MCS), respectively. The PCS evaluates 4 peripheral components i.e., Physical Functioning (PF), Role Physical (RP), Bodily Pain (BP), and

General Health Perceptions (GH), while MCS evaluates four central components namely Vitality (VT), Social Functioning (SF), Role Emotional (RE) and Mental Health (MH). The SF-36v2 health tool (Appendix E) was used with partial modification. Briefly, the SF-36 was completed by each of the subject during the simulated driving condition. The responses to the questionnaire were fed to the software (QM Certified Scoring Software, QualityMetric, USA) and the scores were determined. The score of each subject at stage-0 (0 hour of elapsed time of driving), when each participant is assumed to be free from any type of fatigue, was considered as reference.

Questionnaire-based test 2: Sleepiness Score Questionnaire for estimating the sleep depth was prepared to assign a sleepiness score and evaluate the impact of sleep-deprivation during long-distance driving. A set of questions were selected from standard sleepiness scales (Stanford Sleepiness Scale, Piper Fatigue Scale and Epworthy Sleepiness Scale) for the purpose (Appendix F). The questions were asked through an interactive session at 5 stages (stages 0, 3, 5, 8, 10) matched with 5 time points of blood collection (Table 4.1). Subject's self-assessment was used for final fatigue level assessment on a scale 1–10 with 10 being the most fatigued.

4.1.9 Statistical Analyses

All experimental outcomes are expressed as means ± SEM. Data were analyzed by repeated measure of ANOVA using the SPSS software version 20.0 (SPSS Inc., IL, USA). Differences were considered statistically significant at $p<0.05$. For obtaining the interdependency of various parameters, Pearson's correlation coefficients and the corresponding levels of significance were calculated.

4.2 Results and Discussion

4.2.1 Variation in Blood Biomarkers

To investigate the effect of physical fatigue three blood biomarkers (RBS, Urea and Creatinine) were analyzed (Figures 4.2-4.5).

Variation in Blood Glucose A gradual increase in RBS was observed as the subjects (drivers) continued the simulated driving under sleep-deprivation. A significant increase in the RBS level (104.85±2.08 mg/dL) was observed at the elapse of 16.5 h (Stage 5) which further increased to 125.15±2.87 mg/dL at the elapse of 26.0 h (Stage 8) and then dropped to 107.77±1.64 mg/dL at the elapse of 32.5 h (Stage 10) (Figure 4.2(A)). A positive co-relation between the duration of simulated driving and the level of RBS may thus, suggest that the RBS level, in combination with other tests, may serve as a valuable indicator of

fatigue. Interestingly, at 26.0 h of simulated driving, the RBS level rose by 34.3% and was higher than the normal RBS level (80-120 mg/dL) (Figure 4.2(B)). Previous studies have documented that sleep deprivation lead to increase in RBS level that resembles type 2 Diabetes Mellitus [156, 157]. Similar increase in the RBS level was also reported to occur upon mild exercise [158]. Thus, it is likely that sleep deprivation coupled with mild physical exercise of prolonged driving may have predisposed the drivers to a hyperglycemic-like state. The drop in the RBS level at 32.5 h of driving was probably due to uncontrollable sleepy state of the experimental subjects which lead to partial compensation of the sleep debt [156, 157].

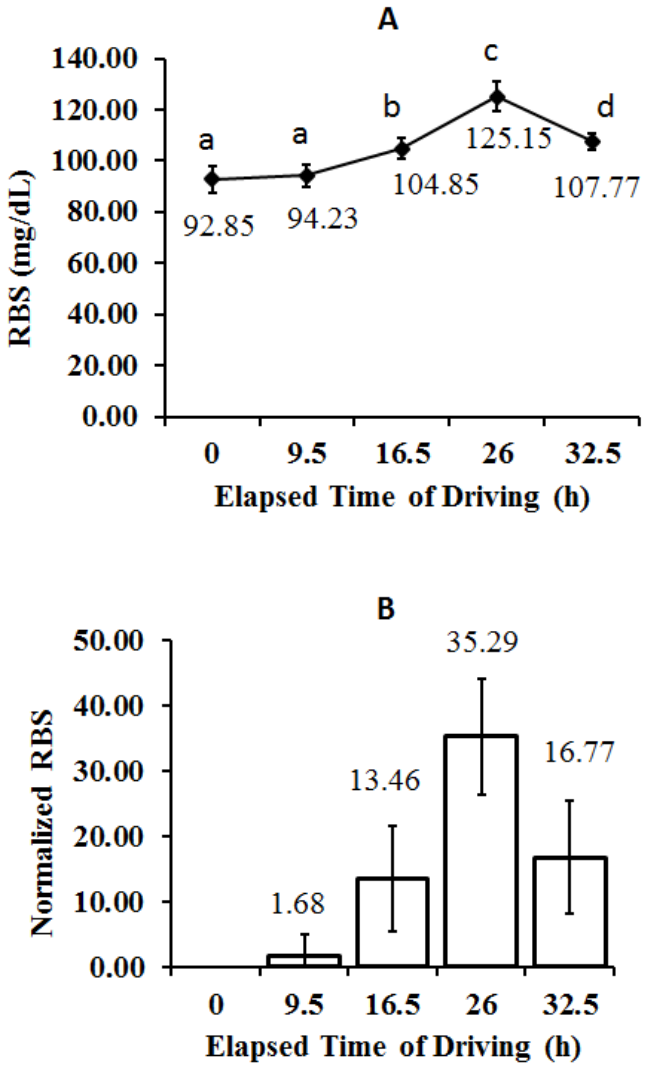

Figure 4.2: Trends in random blood sugar (A) and normalized plasma random blood sugar (B) in experimental subjects at five time points (Stages 0, 3, 5, 8 and 10: Refer Table 4.1). Different alphabets (a, b, c) over the curve (A) indicate statistical difference among stages ($p<0.05$).

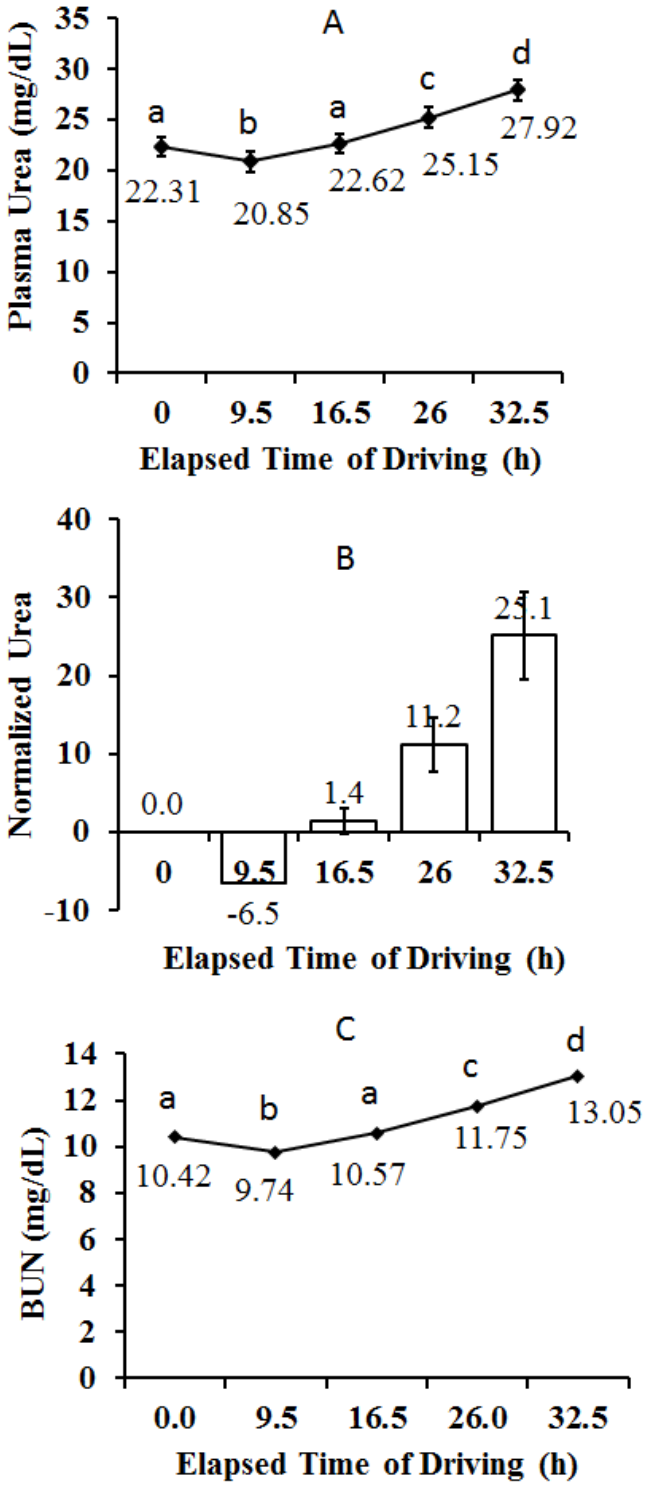

Figure 4.3: Trends in plasma urea (A) normalized plasma urea (B) and BUN (C) in experimental subjects at five time points (Stages 0, 3, 5, 8 and 10: Refer Table 4.1). Different alphabets (a, b, c) over the curve (A and C) indicates statistical difference among stages ($p<0.05$).

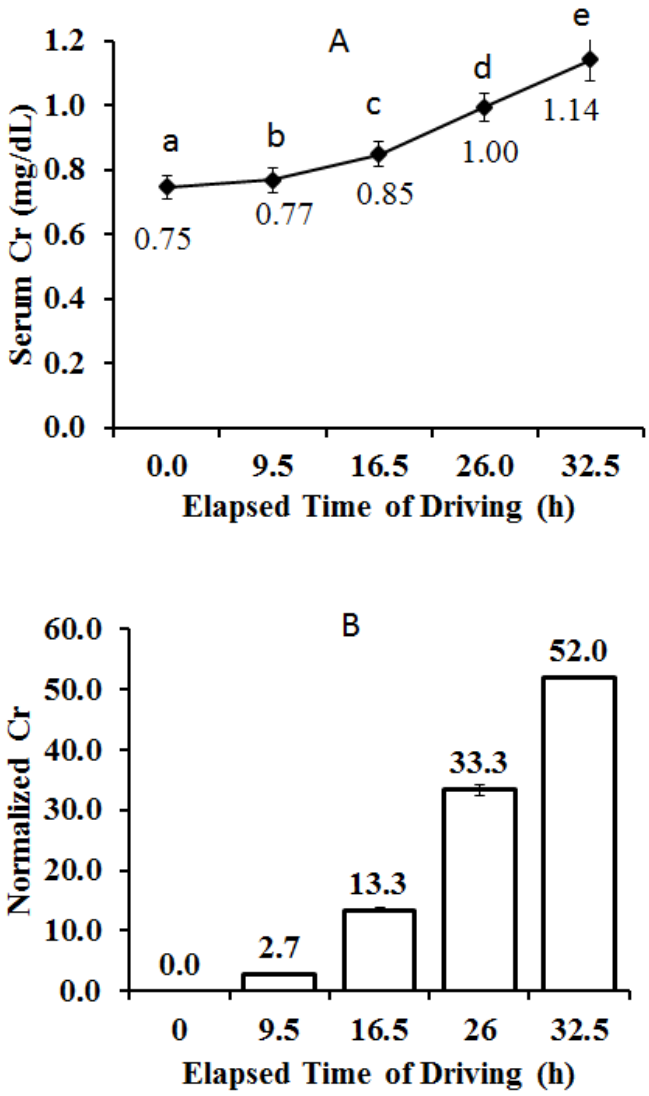

Figure 4.4: Trends in serum creatinine (Cr) (A) and normalized serum Cr (B) in experimental subjects at 5 time points (Stages 0, 3, 5, 8 and 10: Refer Table 4.1). Different alphabets (a, b, c) over the curve (A) indicate statistical difference among stages ($p<0.05$).

Blood Urea Level Blood Urea Nitrogen (BUN) was calculated as Urea (mg/dL) / 2.14 [159] and the trend in blood urea level and the normalized blood urea level over Stage 0 (reference level) have been shown in Figures 4.3(A) and 4.3(B). BUN was estimated to evaluate the influence of fatigue on the protein metabolism. It was observed that, BUN level gradually increased from 10.42±2.4 mg/dL to 13.05±1.2 mg/dL at the elapse of 32.5 h (Stage 8) of simulated driving (Figure 4.3(C)). Interestingly however, the level of BUN was remained within the normal range (8-24 mg/dL) throughout the period of study. Similar observation has also be made by Lemon and Mullin [160] who reported that BUN was increased during prolonged exercise but remained within the normal range. The increase in BUN was due to metabolic events within the skeletal muscle. During prolonged submaximal exercise, a combination of metabolic events such as intramuscular depletion of glycogen,

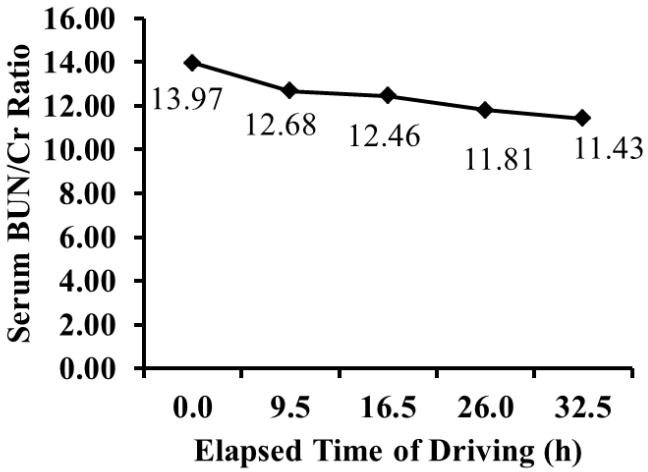

Figure 4.5: Trend in BUN/Cr ratio in experimental subjects at 5 time points (Stages 0, 3, 5, 8 and 10: Refer Table 4.1).

marked elevation of muscle temperature and altered concentration of electrolytes occur which are all known to promote the gluconeogenesis and deamination of amino acids [161]. Increased deamination may thus lead to increase BUN. Previous studies have also confirmed that during exercise that lasts longer, there is possibility of increased synthesis of urea in liver and skeletal muscles due to exercise induced arginase production, a key enzyme in urea synthesis pathway [162]. Again the stress that culminates in fatigue, can also stimulate the secretion of glucocorticoids which are involved in breakdown of amino acids and thereby, increase in the level of BUN [163]. Thus, it is likely that prolonged mild exercise and stress of the simulated driving under sleep-deprived condition, in combination with the altered neuro-hormonal profile, may have led to increased BUN level. Conversely, BUN level, in combination with other tests, may serve as a valuable indicator of fatigue under simulated driving condition.

Creatinine Levels in Serum The level of serum creatinine (Cr) gradually increased from 0.75±0.02 mg/dL at the start of driving session to 1.14±0.03 mg/dL (25% increment) at the elapse of 32.5 h of driving (Figure 4.4(A), (B)). Previous studies have reported that, during sub-maximal exercise, creatine is produced from the breakdown of muscle phosphocreatine and are released into the bloodstream wherein they can undergo hydrolysis to result in an increase in the serum creatinine level [164]. A gradual increase in the creatinine level has also been observed by Refsum and co-workers [165] during prolonged skiing.

In three subjects, the creatinine level crossed the upper cut-off limit of normal creatinine range (0.6-1.2 mg/dL) to reach a level of 1.28, 1.31 and 1.32 mg/dL at the elapse of 32.5 h (Stage 8). Increased level of creatinine is known to be associated with impaired kidney function. However, in the current study, impaired kidney function was probably not the cause of elevated creatinine as interpreted from the level of BUN/Cr ratio (Figure 4.5). Though

the BUN/Cr ratio decreased with increase in the duration of simulated driving, it remained within the normal range (11-16) [166]. Again a consistent fall in BUN/Cr ratio in presence of a consistent rise in both BUN and Cr indicates that the rate of increase in Cr is more than that of BUN during course of driving. It indicates catabolic state in tissues [166]. It can be observed from the trend Cr level (Figure 4.4(A)) that the peripheral tissue catabolism took a sharp rise after 9.5h of simulated driving.

4.2.2 ECG Results

ECG data, obtained from each subject in each stage were analyzed to derive different indices of HRV.

HRV analysis in Time Domain Since short-term, stage-wise ECG recordings were taken from each subject, the time domain indices of HRV were assessed by geometrical instead of a statistical approach [59]. The geometric methods present RR intervals in geometric patterns and several approaches are used among which Poincaré plots are extensively studied. These plots represent a temporal series within a Cartesian plane in which each RR interval is correlated with the preceding interval and define a point in the plot [65, 167]. The Poincaré plot can be analyzed qualitatively by assessing the figure formed by its attractor, which is useful for showing the degree of complexity of RR intervals [66], or quantitatively by adjusting the ellipse of the figure formed by the attractor, from which three indexes can be obtained: SD1, SD2 and SD1/SD2 ratio [59]. The SD1 represents the dispersion of points that are perpendicular to the line of identity and provide an index of instantaneous recording of beat-to-beat variability whereas SD2 represents the dispersion of points along the line of identity and represents the HRV in long-term records [166]. Thus, SD1 variation is more relevant in the present stu4y.

The Poincaré plots of an average subject in each time point during simulated driving session are shown in Figure 4.6. The Poincaré plots at all-time points were comet shaped (i.e. an increase in the dispersion of beat-to-beat RR intervals is observed with increase in RR intervals) which is considered as normal plot [168]. Thus, the subjects were physiologically healthy without any conduction anomaly of the cardiac muscles. However, with the advancement of time points during simulated driving, the SD1 component gradually increased. This increased SD1 represents increase in instantaneous HRV with increased intensity of fatigue. Thus, SD1 may give valuable information to assess the fatigue.

Fast Fourier Transform (FFT) analysis FFT analysis was preferred in the current study due to short term recording of ECG in a task (driving) that involved prolonged monotonous activity at resting condition. The power spectral densities (PSD) of HF (Figure 4.7) and LF (Figure 4.8) components of ECG were considered to ascertain the severity of fatigue at different duration of simulated driving. Results showed that, PSD of HF component

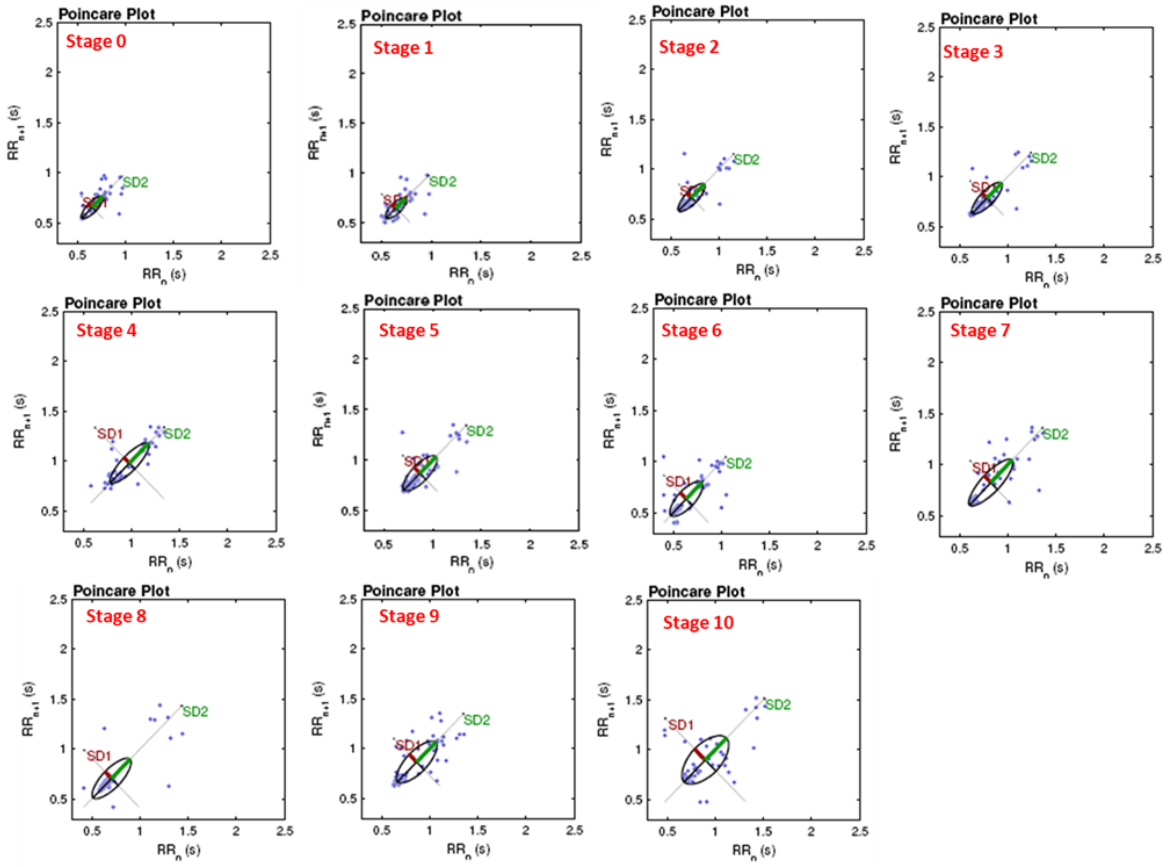

Figure 4.6: Poincare plots of an average subject at eleven stages (Table 4.1) of fatigue progression during simulated driving session. There was gradual increase in SD1 which indicates instantaneous beat to beat variation.

decreased with increase in the duration of simulated driving and thus, has a good potential for inter-stage discrimination of fatigue (Figure 4.7). However, the PSD of LF component and the LF/HF ratio varied with time and did not show a consistent pattern (Figure 4.8 and 4.9).

Figure 4.10 shows the distribution of mean PSD at different frequency bands. The PSD of HF components (0.15-0.4 Hz; yellow band) decreased with the advancement in simulated driving time. The HF component corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve (parasympathetic influence) to the heart [59, 169]. Thus, with increase in driving time and exhaustion, the parasympathetic influence decreased and sympathetic predominance remained strong either directly or indirectly. On the contrary, the PSD of LF components that reliably indicate sympathetic influence on heart, increased only at midnight (Stages 5 and 6, Table 4.1). The sudden sympathetic surge at Stages 4 and 5 reflects peaking of stress in midnight out of prolonged driving task in sleep-deprived condition while the decrease in sympathetic influence subsequently resulted from respiratory modulation (impact of fatigued respiratory muscles on heart). At the same time, the continuous decrement in parasympathetic/vagus effect (as indicated by trend in PSD of HF components) till the end stage can be explained to be resulted from decrease in respiratory efficiency, an indirect action of vagus nerve. Thus, with the increase in intensity

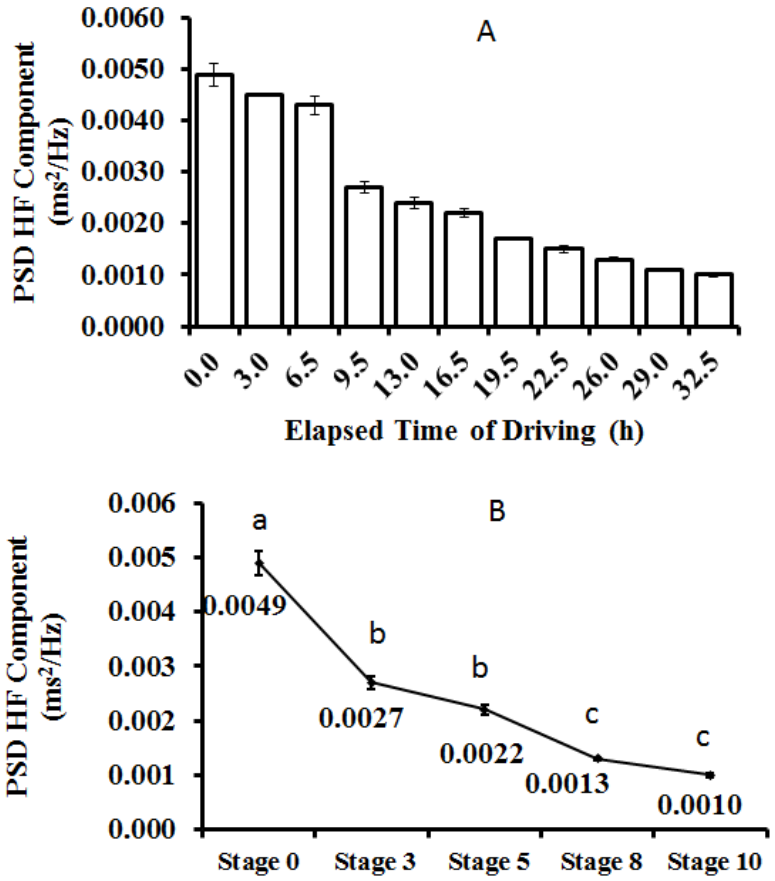

Figure 4.7: Power spectral density (ms^2/Hz) of HF components (0.15-0.4 Hz) of an average subject at eleven stages (A) and at 5 stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1). Different alphabets (a, b, c) over the curve (B) indicate statistical difference among stages ($p<0.05$).

of fatigue in drivers during driving task, the sympathetic predominance increased (increase in HR) and, at the same time, there was an overall increase in instantaneous HRV. The SD1 component in Poincare plots also confirmed an increasing trend in instantaneous HRV with fatigue severity. Although several previous studies have reported the impact of exercise in athletes or in normal population on HRV, data on HRV in the long distance drivers are scarce.

4.2.3 Results of Spirometer Recording

The FVC, FEV_1 and FEV_1 to FVC ratio of 12 subjects were measured at different Stages (Table 4.1). As shown in Figure 4.11 and 4.12, both FVC and FEV_1 gradually increased with the elapse of driving time to 16.5 h (Stage 5) and thereafter decreased (Stage 6-10).

However, the changes in the FVC and FEV_1 were statistically non-significant although a trend was found. Previous studies have shown that FEV_1/FVC ratio is a sensitive indicator of restrictive and/or obstructive functional status of the pulmonary system [168]. A ratio of

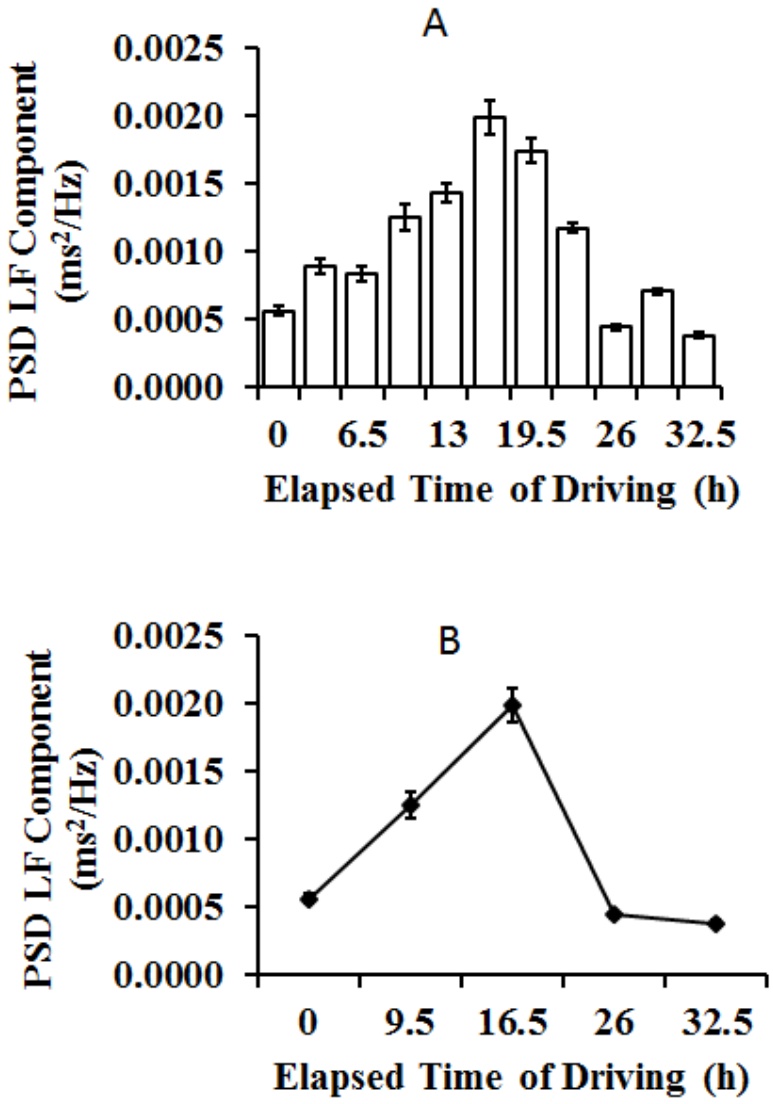

Figure 4.8: Power spectral density (ms^2/Hz) of LF components (0.15-0.4 Hz) of an average subject at eleven stages (A) and at five stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1).

less than 0.7 indicates obstructive profile found in pathological conditions such as asthma, chronic bronchitis etc. that cause the obstruction of the pulmonary tracts. On the other hand, a ratio greater than 0.9 indicates a restrictive profile that may arise from a pathological change in lungs parenchyma (lungs tissue) or a pathological/physiological restriction to the expansion of lungs [170, 171]. From Stage 5 onwards, the FEV_1/FVC ratio gradually increased and showed a restrictive profile in the drivers (Figure 4.13). Since the medical history of the drivers indicated no pulmonary abnormalities, the restrictive profile with gradual increase in fatigue is likely due to the onset of fatigue in respiratory muscles. Furthermore, a parallel increase in the level of BUN (Figure 4.3) may have inhibited the respiratory muscle function at these stages.

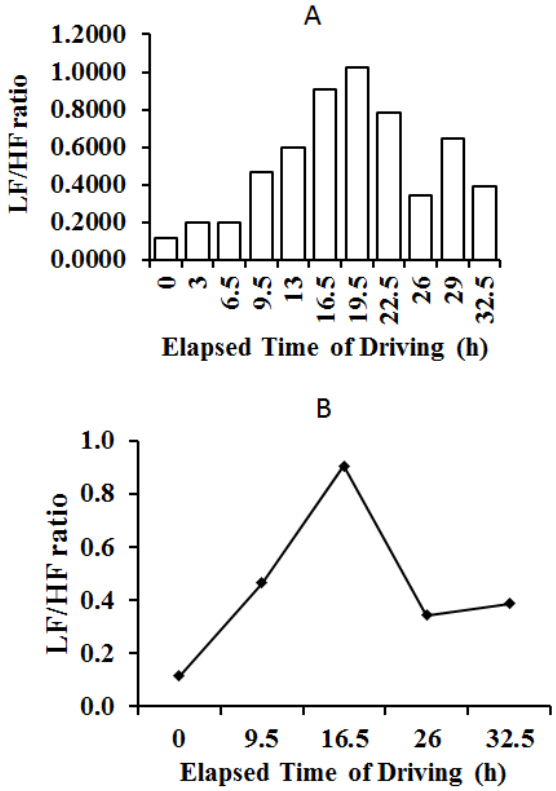

Figure 4.9: The LF/HF power spectral density ratio of an average subject at eleven stages (A) and at five stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1).

Though FVC and FEV_1 can give an insight into the spread of central fatigue to periphery and subsequent progression of peripheral fatigue, a continuous trend was not obtained in these parameters. Thus, these pulmonary parameters cannot reflect the graded genesis and progression of peripheral fatigue from a complex task such as driving wherein the peripheral fatigue is slowly initiated from prolonged central fatigue and subsequently, none of them can be used for developing a score for driver's fatigue.

4.2.4 EEG Results

Energy-based Analysis The relative energy at each level of wavelet decomposition was calculated at different Stages of the simulated driving. Each of these decomposition levels represents a particular band, e.g., detailed coefficient-1 (DC-1) represent β, DC-2 represent α, DC-3 represent θ and DC-4 along with approximate component (AC-5) represents δ (δ_1 and δ_2 bands respectively) band of the signal. Figure 4.14 shows the mapping of relative energy variation of all these bands in spatial domain, derived from interpolation of data between each electrode and its neighborhood electrodes. It can be seen that at earlier stages of fatigue most of the energies are concentrated in the low frequency δ band and as the fatigue increases it spreads into the other bands. The gradual increase in relative energy is distinctly

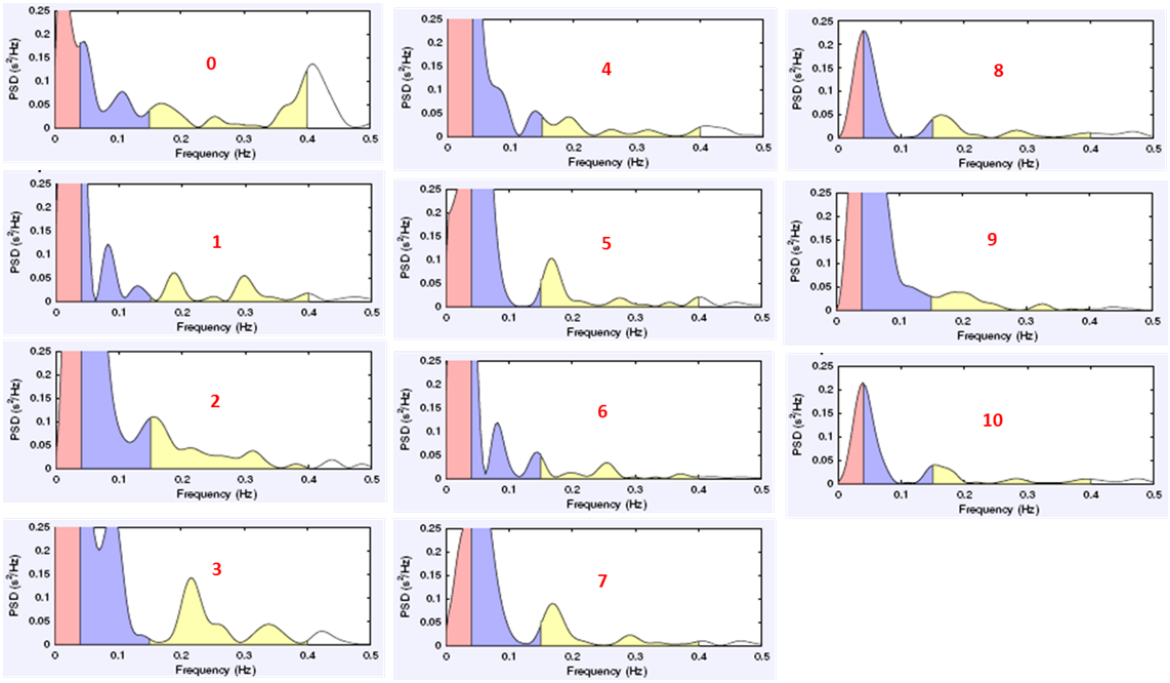

Figure 4.10: Power spectral density (ms^2/Hz) of HF components (0.15-0.4 Hz) of an average subject at eleven stages (Table 4.1). It decreased with progression of peripheral fatigue as indicated by area under yellow zone.

seen in β, α, and θ bands with elapsed time of simulated driving. The inter-stage variation of relative energy was highest in θ band followed by α and β bands. Again, the variations of relative energies of above frequency bands were most significant in Cz electrode followed by the Fz and O. The variations of relative energies of α and θ bands at electrode location C_z are presented in Figures 4.15(A) and 4.15(b). In both α and θ bands, the mean ($\pm$SEM) relative energy increased along with elapsed time of driving task.

Further analysis revealed that energy variation at different stages did not differ substantially. Thus, it is not rational to use the data for formulating an effective scoring system of fatigue in drivers. For example, the variation of relative energy of α band from θ h (Stage 0) to 32.5 h (Stage10) of simulated driving was approximately 3 times. To augment the sensitivity in discriminating fatigue levels across the stages, various derived parameters were predicted and examined. Interestingly, the relative energy of a derived parameter represented by $(\alpha + \theta)/(\delta_1 - \delta_2)$ ratio was found to be the most relevant. The variation of this parameter is shown in Figure 4.15(C).The parameter showed significant inter-stage variation $[F(4, 11) = 7.44, p = 0.0001]$ with the progression of fatigue. An increase in RE was approximately 6 times from 0 h (Stage 0) to 32.5 h (Stage 10) of the simulated driving. Such type of derived parameters may be helpful in devising the stages of fatigue with high degree of resolution. From the energy analysis, it can be further inferred that the RE of the derived band $(\alpha + \theta)/(\delta_1 - \delta_2)$, along with that of θ and α bands, can be of practical value to devise a scoring system for the drivers' fatigue.

The increased energy in α-band was in accordance with the established fact that the

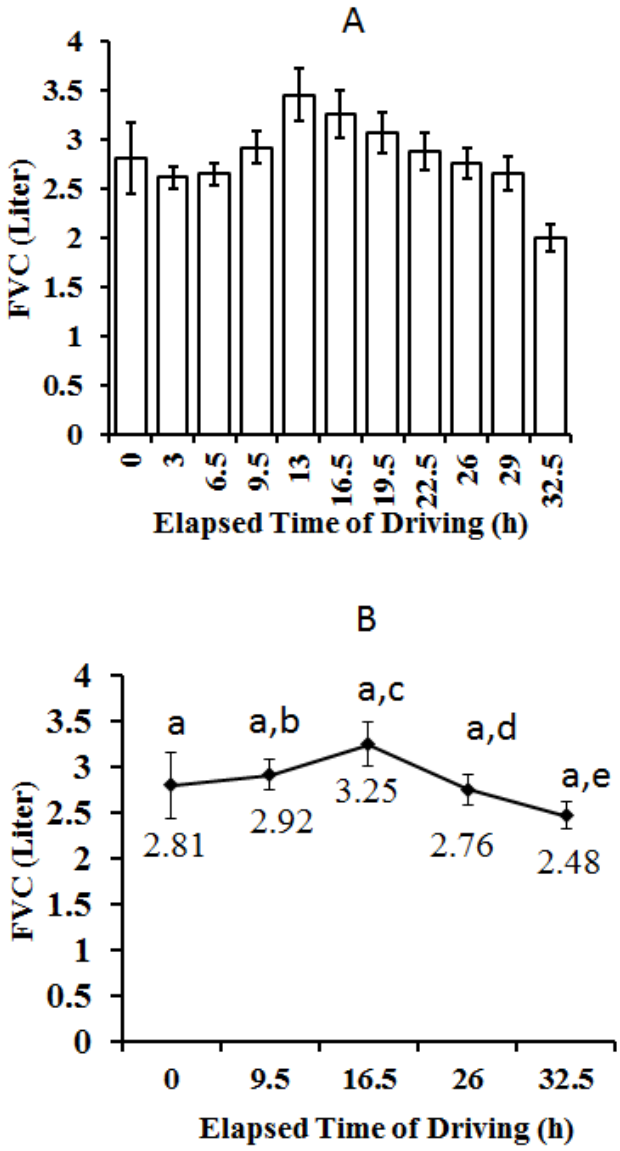

Figure 4.11: The forced vital capacity (FVC) of an average subject at eleven stages (A) and at five stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1). Different alphabets (a, b, c) over the curve (B) indicate statistical difference among stages ($p<0.05$).

frequency of the α rhythm increases with high blood glucose level, high body temperature, high level of adrenal glucocorticoid hormones, and low arterial partial pressure of CO_2 ($PaCO_2$) or high arterial pressure of O_2 (PaO_2) [52]. Thus, any persistent task, similar to prolonged driving, increases the demand of nutrition and oxygen (O_2) by the brain and thereby, leads to a compensatory increase in glucose (major energy source for the brain cells) and O_2. When the central fatigue spreads to periphery due to uninterrupted task without adequate rest, the peripheral stress hormone, glucocorticoid, is increased in the blood. In both situations, the biochemical conditions increase the frequency of α-band as well as the

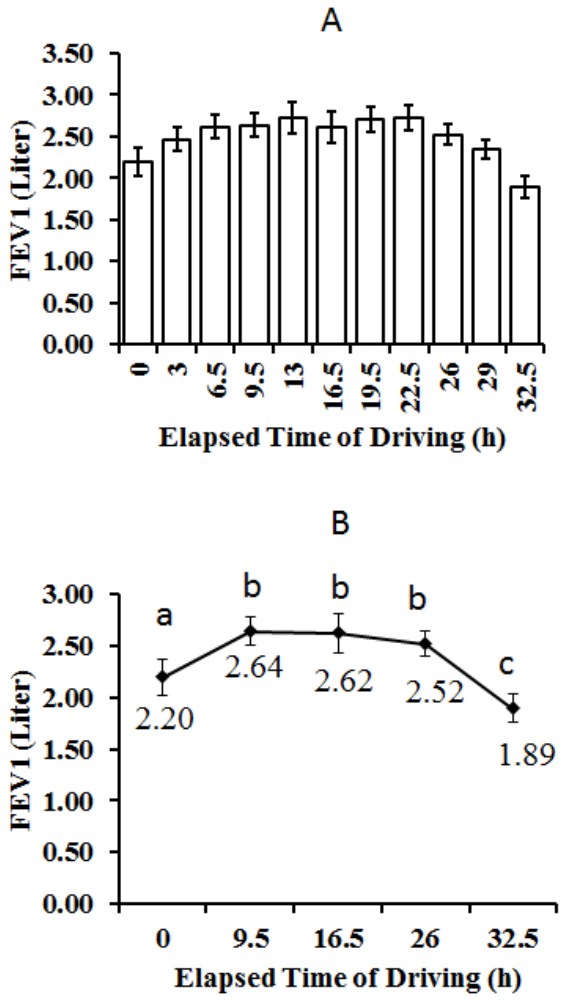

Figure 4.12: The forced expiratory volume at the first second (FEV_1) of an average subject at eleven stages (A) and at five stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1). Different alphabets (a, b, c) over the curve (B) indicate statistical difference among stages ($p<0.05$).

relative energy content. The current study reproduced the fact in the long distance drivers.

Entropy-based Analysis Variation in Shannon's entropy (SE) across 5 Stages was significant $[F(4, 11) = 20.86, p = 0.000]$ only at Cz electrode. The variation in SE (Mean $\pm$ SEM) of 12 experimental subjects at Cz electrode is shown in Figure 4.16 that clearly indicates an increase in SE with elapsed time of driving task or increasing fatigue level. SE is considered as a measure of randomness in system and provides a measure of flatness of energy spectrum [172]. The significance of this entropy can be best understood in terms of probabilistic concept. A signal having very high energy content in a particular wave group of EEG indicates lack of randomness in terms of frequency of that particular signal. Hence the entropy value will be lower for such signals. On the other hand uniform distributions of energy in all the wave groups indicate the presence of randomness associated with the

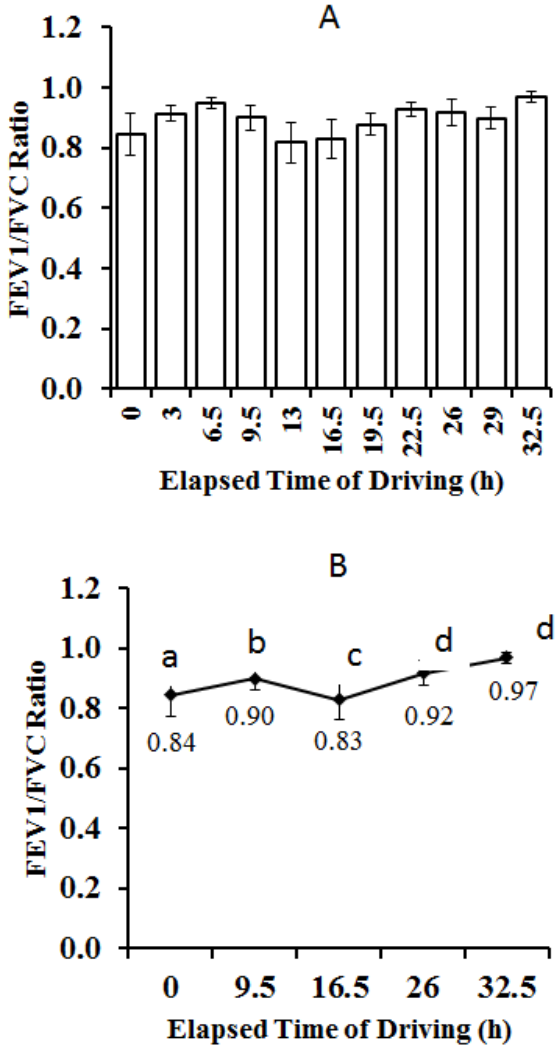

Figure 4.13: The FEV_1/FVC ratio of an average subject at eleven stages (A) and at five stages matched with blood biomarker analysis (B) of fatigue progression (Refer Table 4.1). Different alphabets (a,b,c) over the curve (B) indicate statistical difference among stages ($p<0.05$).

signal resulting in higher entropy value. Thus, a gradual increase in SE with advancing stages of central fatigue in drivers during simulated driving session indicated that the energy distribution becomes more uniform in all frequency bands of EEG as fatigue progresses.

The interpolated SEs between all possible electrode pairs were also derived and an entropy mapping of brain was obtained (Figure 4.17). Such mapping with specific color code depicts a global variation of SE at all locations on the scalp (corresponding to complete brain surface). It is evident that the SE distribution is more diffuse with increasing fatigue level in experimental drivers. However the stage wise variation in SE over brain surface is more prominent at δ followed by θ band. It can be concluded that, an estimation of SE of EEG signal during a monotonous task like driving can improve the classification of fatigue stages in the presence of uncertainties associated with the energy bands.

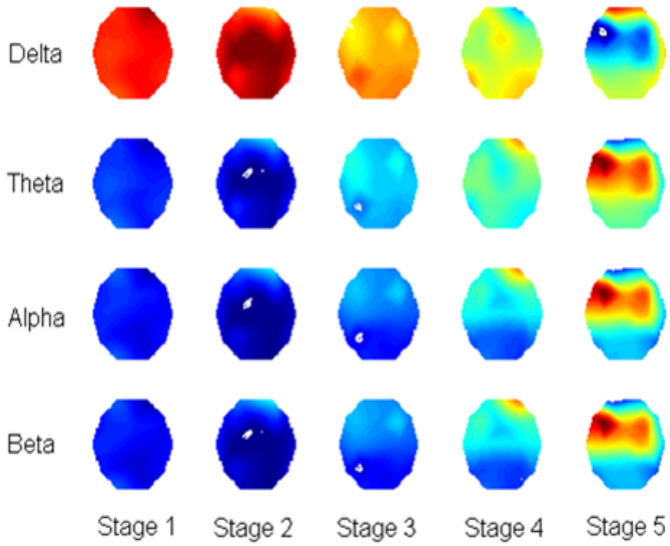

Figure 4.14: Relative energy variation of delta (δ), theta (θ), alpha (α), and beta (β) EEG bands in spatial domain (interpolated) at five stages of fatigue matched with five stages of blood biomarker analysis (Stages 0, 3, 5, 8 and 10: Refer Table 4.1).

4.2.5 Subjective Assessment

The subjective assessment of fatigue under simulated driving condition was performed using SF-36 v2 Health Scoring, which is used clinically in humans. The outcome of SF-36 v2 assessment is divided into two components i.e., Physical Component Summary (PCS) and Mental Component Summary (MCS). Each component was further sub-divided into 4 sub-components. These sub-components were evaluated based on response to a series of questionnaires listed in Appendix E and the timing of the questionnaire session

Physical Component Summary (PCS) Analysis

PF (Physical Functioning) PF accounts for capability of completing the multiple tasks assigned to the subjects. It includes motor performance like vigorous activities (running, lifting, etc.), moderate activities (moving a table) and brisk activity (walking, limbs movement during rest like driving or operating machines) at particular intervals of a task or intervention. The response based PF score at designated five stages matched with blood marker analysis decreased significantly form $51.8 \pm 0.7\%$ at Stage 0 to $25.2 \pm 0.7\%$ at 32.5 h elapse of driving task. The trend in PF score confirmed the increased disablement of the subjects out of physical exhaustion to perform the minimal possible task defined by the system.

RP (Role Physical) RP accounts for the ability of the subjects to perform the secondary tasks while giving maximum attention to the core task (driving in the current study).

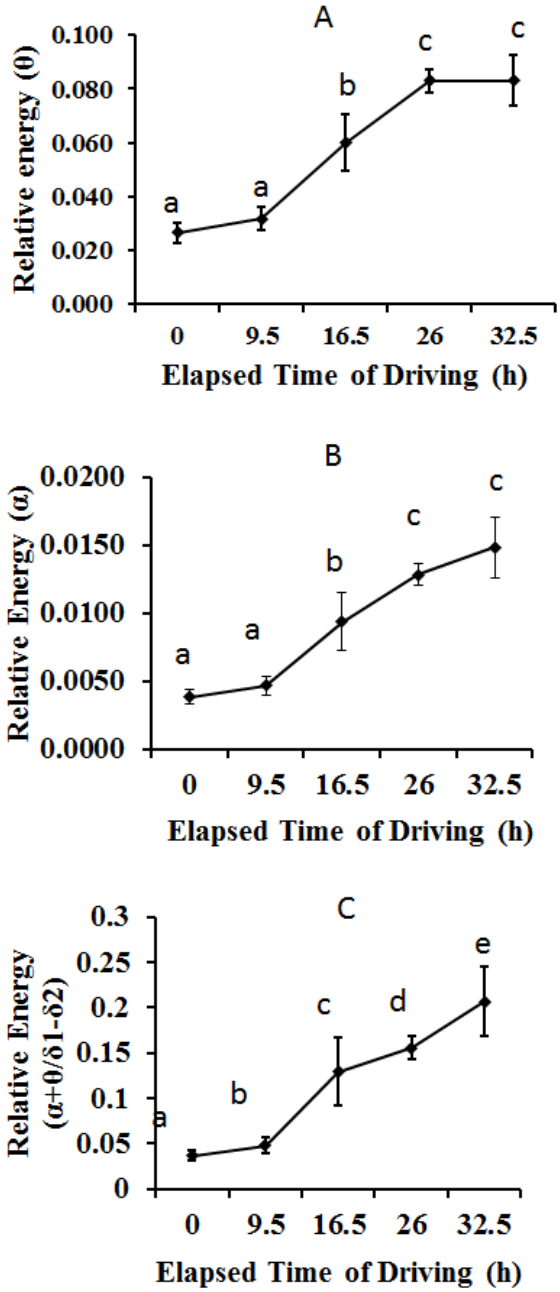

Figure 4.15: Relative energy variation of θ (A) α (B) and the derived $(\alpha+\theta)/(\delta_1-\delta_2)$ bands (C) at Cz electrode at five stages matched with five time points of blood biomarker analyses (Stages 0, 3, 5, 8 and 10: of Table 4.1). Different alphabets (a, b, c) over the curves indicate statistical difference among stages ($p<0.05$).

Additionally RP deals with accomplishment time, level of difficulty in performing the secondary work. The parameter enquired about the cut down of the amount of time from the core task while the subject hovers on secondary activity assigned to them (i.e. writing a sentence, comment upon a song). The subjects experienced more difficulties in performing the very same secondary job with the elapsed time of driving (from a score of $64.7 \pm 0.7\%$ at Stage 0 to a score of $28.4 \pm 0.7\%$ at Stage 10). The trend in RP verified the effect of

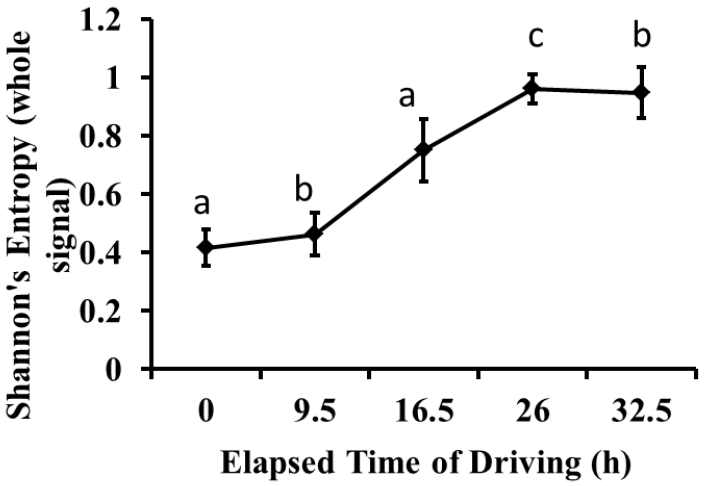

Figure 4.16: Shannon's entropy (SE) of whole signal at Cz electrode at five stages matched with five time points of blood collection (Stages 0, 3, 5, 8 and 10: Refer Table 4.1). Different alphabets (a,b,c) over the curve indicates statistical difference among stages ($p<0.05$).

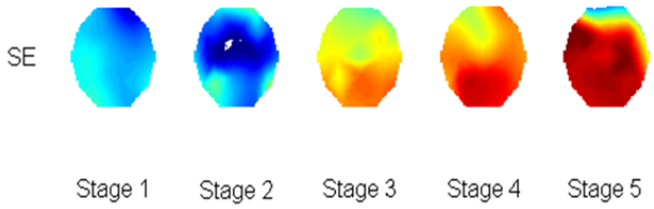

Figure 4.17: Variation in Shannon's entropy (SE) of different bands in spatial domain (interpolated) at five stages matched with five time points of blood biomarker analysis (Stages 0, 3, 5, 8 and 10: Ref Table 4.1).

progressing fatigue from simulated driving on peripheral muscles.

Bodily pain (BP) BP strictly accounts for the extent of psychosomatic pain and the interference created by it in the general regular activities during the period of experiment. It is the only component in SF36v2 tool that shows a decreased score with increased subjective feeling of bodily pain or discomfort. BP score decreased to $30.9 \pm 0.7\%$ at Stage 10 from a score of $50.8 \pm 0.7\%$ at Stage 0. It indicated the decreased tolerance to psychosomatic pain with increased hours of monotonous work like driving.

General health (GH) GH reflects general feeling of wellness at a particular instant or projecting the status after some time (feeling of getting sick and/or expecting the health to get worse in the coming hours). The average GH score in experimental subjects showed a declining trend from $68.6 \pm 0.3\%$ at Stage 0 to $30.1 \pm 0.6\%$ at Stage 10 with advancing fatigue.

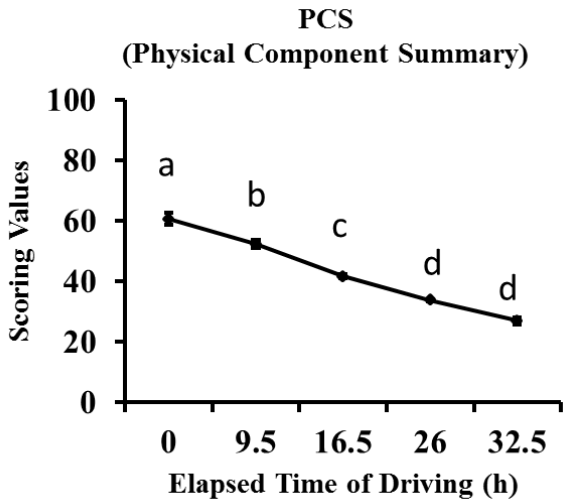

Figure 4.18: Subjective feeling of physical well-being expressed by physical component summary (PCS) measured from SF-36v2 health score tool at five stages matched with five time points of blood biomarker analysis collection (Stages 0, 3, 5, 8 and 10: Refer Table 4.1).

The scores of PF, RP, BP, and GH were analyzed in SF-36 QualiMetric software to obtain PCS score. PCS score decreased from $60.5 \pm 0.7\%$ at Stage 0 to $26.8 \pm 0.7\%$ after 32.5 h elapse of simulated driving (Figure 4.18). The trend in PCS was in accordance with the decline in the physical health condition out of physical exhaustion combined with increased hours of sleep deprivation. Thus, PCS score was considered to be reference parameter for evaluating peripheral fatigue in current study in addition to its potential to be used as an independent parameter for formulating a fatigue score for drivers.

Mental Component Summary (MCS) Analysis:

VT (Vitality) VT explains strength and liveliness. This factor is an unseen force that has contribution to both physical as well as mental health condition. The response to the questionnaires in this section revealed the subject's feeling of any nervousness regarding completing the task, down or dumbness, calm and cheerful, a sense of ineffectiveness, getting exhausted etc. The VT score decreased from $74.1 \pm 0.9\%$ at stage 0, which reflects a quite good and healthy mental status to $31.5 \pm 1.3\%$ at stage-10 that reflects a worst condition of mental exhaustion. The outcome signifies a remarkable increase in anxiety and disquiet from more than 30 hours of simulated driving under sleep deprivation.

SF (Social Functioning) SF refers to the extent of effect of physical health and emotional problems on social life of a being and their duration. The mean SF score in subjects declined to 30.1 ± 1.3 % after 32.5h (stage 10) of simulated driving from a score of $64.8 \pm 1.7\%$ before the starting of simulated driving task (stage 0). The declining trend in SF score precisely reflected the fall in subjects' interest in socializing with others sitting nearby (talking to

other subjects or with the examiners) which was also observed during experiment. Such a mental state arose due to annoyance and desperation to leave the task.

RE (Role Emotional) RE indicates a state of depression or anxiety and the effect of emotional problems on time-to-time life activity. RE scores (Table 3.7) also decreased from $62.5 \pm 1.3\%$ at 0 h to $19.0 \pm 1.3\%$ at 32.5 h elapse of simulated driving task consequent to advancement in fatigue. The falling score signified the increasing level of distress and apprehension for socializing with the surrounding people. RE explained the role of inducer (fatigue from driving) on the state of nervousness or downheartedness, extent of feeling peaceful or happiness in subjects.

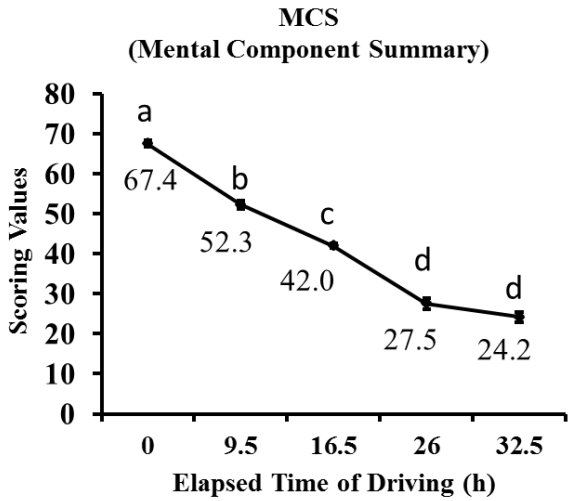

Figure 4.19: Subjective feeling of mental well-being expressed by mental component summary (MCS) measured from SF-36v2 health score tool at five stages matched with five time points of blood biomarker analysis collection (Stages 0, 3, 5, 8 and 10: Ref Table 4.1).

MH (Mental Health) MH that signifies the central integration and attentiveness in implementing a task, also decreased from $61.7 \pm 0.9\%$ at Stage 0 to $23.0 \pm 1.6\%$ after 32.5 h of simulated driving (Stage 10). The score confirmed the gradual inability of the brain to generate interest for driving further as time passed.

Aggregating the above discussed components (VT, SF, RE and MH), a cumulative score was derived using SF36 QualiMatricTM software termed as Mental Component Summary (MCS). MCS states the overall mental status of subjects and it declined (Figure 4.19) from a score of 67.4 ± 0.8 at Stage 0 to a score of $27.5 \pm 1.4\%$ at Stage 10 (32.5 h of elapse of driving). The trend in MCS was considered as reference parameter for development of central fatigue in our subjects.

Sleepiness Score

The trends in sleepiness across five stages matched with five time points of blood collection are shown in Figure 4.20. At 0 hour of elapsed time of driving (reference stage), none of the subjects felt drowsy (score 0 at stage 0). As the sleep debt increased with the duration of sleep deprived driving, the feeling of drowsiness increased and reached a score of 8.6±0.7 of 10 at stage 10. Such a score indicates a state of severe drowsiness that can lead to extreme state of inattentiveness for implementing a task. The obtained trend in sleepiness was expected and proved by sleepiness score.

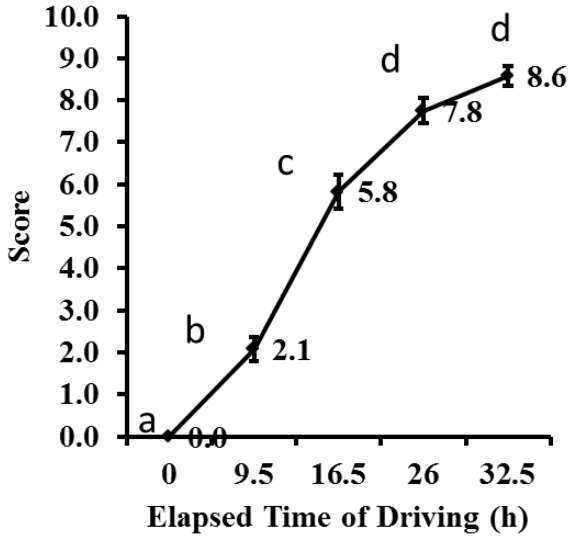

Figure 4.20: Subjective feeling of drowsiness from customized sleepiness score in experimental subjects at five stages matched with five time points of blood biomarker analysis collection (Stages 0, 3, 5, 8 and 10: Refer Table 4.1). Different alphabets (a, b, c) over the curve indicate statistical difference among stages ($p<0.05$).

4.2.6 Correlation Analysis

After real time monitoring and assessing the variation of various physiological parameters during gradual progress of physical fatigue in drivers, it was essential to deduce the significance of each parameter to be used for application in staging driver's fatigue.

To elucidate the potential of all these parameters for discriminating fatigue stages, the subjective assessment score was considered as reference and other parameters were correlated with the former. A statistical correlation analysis [173] has been carried between the following variables and are presented Tables 4.3 to 4.7:

1. The trends in EEG parameters (that reflects central manifestations of fatigue) with the subjective assessment of mental well-being (Mental Component Summary/MCS of SF-36 score) and the sleepiness score.

2. The trends in EEG parameters with that of blood biomarkers for observing their interdependency

3. The trends in blood biomarkers, HRV (power spectral densities of ECG HF components) and the spirometer data (all reflect peripheral manifestations of fatigue) with the subjective assessment of physical well-being (Physical Component Summary/PCS of SF-36 score).

4. The trends in blood biomarkers with HRV (power spectral densities of ECG HF components) and spirometer data for studying their interdependency.

For correlation study, the data at 5 stages (stages 0, 3, 5, 8 and 10) corresponding to the time points of blood sample collection and questionnaire based subjective assessment were considered. The correlation coefficients were computed between mean of all parameters (mean of all subjects) at five stages (stages 0, 3, 5, 8 and 10) of the experiment.

Table 4.3: Correlation coefficients (r) of relative energies at F_3, Fz, F_4 and Cz electrodes with MCS and three blood biomarkers (RBS, Creatinine, Urea)

	EEG electrodes			
	F3	**Fz**	**F4**	**Cz**
	Energy of [(α+θ)/(δ1-δ2)]: A derived parameter			
Subjective Assessment Score				
MCS	-0.869 (0.006)	-0.768 (0.006)	-0.746 (0.014)	-0.965 (0.007)
Sleepiness score	0.831(0.074)	0.799(0.003)	0.881(0.063)	0.973(0.005)
Blood Biomarkers				
RBS	0.726 (0.023)	0.652(0.164)	0.739(0.105)	0.730(0.162)
Creatinine	0.863(0.085)	0.822(0.043)	0.829(0.035)	0.907(0.033)
Urea	0.791(0.014)	0.890(0.069)	0.718(0.028)	0.959(0.009)

Values within the parentheses indicate the level of significance.

Table 4.4: Correlation values of Shannon's entropy(SE) of whole EEG signal at Cz, Pz, O1 and O2 electrodes with three blood biomarkers (RBS, Creatinine and Urea) and MCS

	EEG electrodes			
	Cz	**Pz**	**O1**	**O2**
	Shannon's entropy			
Subjective Assessment Score				
MCS	-0.965 (0.007)	0.327 (0.212)	0.238 (0.090)	0.671 (0.122)
Sleepiness score	0.984 (0.002)	0.327 (0.091)	0.327 (0.200)	0.727 (0.014)
Blood Biomarkers				
RBS	0.886 (0.045)	0.238 (0.178)	0.282 (0.132)	0.379 (0.142)
Creaitnine	0.907 (0.034)	0.366 (0.233)	0.331 (0.203)	0.520 (0.098)
Urea	0.912 (0.031)	0.549 (0.143)	0.527 (0.214)	0.699 (0.344)

Values within the parentheses indicate the level of significance.

Table 4.5: Correlation coefficients (r) of three blood biomarkers (RBS, Creatinine, Urea) with PCS

	Blood Biomarkers		
	RBS	Creatinine	Urea
Subjective Assessment Score			
PCS	-0.800 (0.03)	-0.951 (0.013)	-0.860 (0.061)

Values within the parentheses indicate the level of significance.

Table 4.6: Correlation coefficients (r) of power spectral density at HF component of ECG with three blood biomarkers (RBS, Creatinine, Urea) and PCS

	HRV (Power spectral density of HF components)
Subjective Assessment Score	
PCS	-0.947 (0.015)
Blood Biomarkers	
RBS	-0.741 (0.151)
Creatinine	-0.674 (0.217)
Urea	-0.839 (0.018)

Values within the parentheses indicate the level of significance.

Table 4.7: Correlation values of pulmonary parameters (FVC and FEV_1/FVC ratio) with three blood biomarkers (RBS, Creatinine and Urea) from Stage 3 onwards

	Pulmonary parameters	
	FVC	**FEV_1/FVC**
Subjective Assessment Score		
PCS	0.947 (0.211)	0.762 (0.244)
Sleepiness score	-0.911 (0.270)	-0.704 (0.502)
Blood Biomarkers		
RBS	-0.296 (0.151)	0.167 (0.223)
Creatinine	-0.824 (0.217)	0.791 (0.081)
Urea	-0.673 (0.019)	0.628 (0.182)

Values within the parentheses indicate the level of significance.

4.3 Summary and Conclusion

The objective of the discussed chapter was to make a critical analysis of drivers' fatigue during prolonged simulated driving task combined with sleep deprivation with respect to the variations of parameters that represent manifestations of fatigue. The parameters were carefully chosen to assess both central and peripheral fatigue in drivers.

With progressing time of simulated driving under sleep deprivation, both central and peripheral fatigue along with the state of drowsiness increased in severity as confirmed from the subjective assessment scores of the participants i.e., SF-36 v2 score and sleepiness score. SF-36 v2 represented both physical (PCS) and mental status (MCS) of a subject. Both PCS

and MCS declined with progressing fatigue significantly. At the same time, sleepiness score also decreased significantly across the stages reflecting continuous increment in drowsiness and inattentiveness during driving task. Thus, the PCS reflects the peripheral fatigue while MCS of SF-36 v2 tool and sleepiness score reflect central fatigue in the subjects. Considering the subjective assessment score as the exact reflection of progressing fatigue in drivers, all the physio-biochemical parameters were correlated with the former and a conclusive result was obtained. The results are outlined below:

i. The relative energies of β, α, and θ bands of EEG signal increased with severity of fatigue at F3, Fz, F4 and Cz electrodes. However, the variation across the stages was notable in θ band and significant in a derived band $(\alpha + \theta/\delta_1 - \delta_2)$ at the specified electrodes.

ii. Only the relative energies of the derived band at Cz electrode exhibited a significant and strong negative correlation with the MCS and sleepiness score (Table 4.3).

iii. Shannon's entropy (SE) of whole EEG signal increased most significantly across the stages at Cz electrode. SE at Cz also exhibited a strong correlation with MCS and sleepiness score (Table 4.4).

iv. RBS, blood urea and creatinine increased significantly with the severity of fatigue. However only serum creatinine exhibited a significant $(p = 0.013)$ and strong negative correlation $(r = -0.951)$ with PCS (Table 4.5).

v. The trend in FEV_1/FVC ratio confirmed that with the severity of peripheral fatigue, a restrictive profile of lungs function was observed.

vi. The PSD of HF components of ECG signal decreased significantly across the stages concluding that with the severity of fatigue, the parasympathetic dominance was decreased and the modulatory effect of respiratory system over the heart was increased. At the same time the PSD of LF components showed a burst at midnight that indicates sympathetic effect peaking in the heart at those time points. However, a continuous trend was not obtained along the fatigue progression.

The results concluded that serum Cr, RBS and power distribution of HF components in ECG can be reliably used to devise a scoring system to predict peripheral fatigue in drivers while the entropy of whole EEG signal and relative energies at θ, α and the proposed derived parameter $(\alpha + \theta/\delta_1 - \delta_2)$ can be effectively used to score central fatigue in drivers.

Chapter 5

Quantifying the Inducers of Central and Physical Fatigue in Drivers during Simulated Driving Session

In the previous chapter, the genesis and progress of physical fatigue in skilled automobile drivers during sleep deprived simulated driving was investigated and the gradual progression in fatigue was assessed objectively from the variations of a series of physio-biochemical parameters. All the parameters discussed were outcome or manifestations of central as well as peripheral components of fatigue in drivers during prolonged on-road driving. However to ascertain a clear progress in fatigue genesis and progress, in addition to these outcomes of physical fatigue, it is essential to estimate those parameters that act as either direct or indirect inducers of fatigue during driving or any type of task that involves prolonged submaximal exercise.

During long distance driving that spans over day and night, the fatigue generated in the operators is not only due to various components associated with the task, but also due to a high degree of stress from sleep deprivation. The sleep deprivation with or without any task grossly disturbs the circadian rhythm that has been discussed earlier. The major biochemical marker that increased to cope with such stress is serum cortisol [174]. The release of cortisol in normal condition shows a distinctive pattern that follows the circadian rhythm and is regulated by hypothalamic pituitary axis (HPA) [175] [176]. A distorted circadian rhythm from a task induced stress can alter the rate of cortisol secretion as well as its pattern of secretion. Thus estimating the variation in cortisol level during simulated driving session can provide abundant information on the progress of fatigue.

Another parameter that is strongly linked to the stress from a task combined with sleep deprivation is blood insulin level. The level of insulin is increased during progress of fatigue through an indirect mechanism in response to high glucose concentration from glycogenolysis [177]. During prolonged and minimal task, the glycogenolysis is accelerated due to heightened cortisol level from stress [162]. Thus, excess insulin is required to establish homeostasis. However, if the fatigue is for prolonged period, the exaggerated insulin

secretion is not able to metabolize the excess glucose due to a disturbance at subcellular level [178]. Such a condition is termed insulin resistance (IR). Thus a measurement of IR can also give a clue on the genesis of fatigue in drivers while serum insulin profile can predict the level of fatigue.

In addition, neurotransmitter serotonin has been confirmed to be the key molecule that initiates the signs and symptoms of central fatigue [47]. The hypothesis that links serotonin level to central fatigue manifestations is also discussed in Section 1.3.2. During the peak of stress, serotonin production is increased from the metabolism of free tryptophan. Thus, estimation of serotonin from brain can reliably reflect the stage wise progress of central fatigue, though the method is highly invasive. But at the same time the platelets in the peripheral blood share the same machinery for serotonin synthesis with that of neuronal cells. Thus the serotonin in platelet rich plasma during fatigue development shows the same profile as that of brain [82]. Thus an estimation of serotonin in platelet rich plasma can indirectly reflect the severity of fatigue during any type of task including driving. In addition, the increased serotonin during a task also plays a role in inducing IR by acting locally on liver cells [179].

In this study, three key stress inducers i.e. cortisol, insulin and serotonin (5HT) were assessed at progressing stages of continuous simulated driving to establish the co-relation with the subjective assessment score of drivers that analyzed the psychosomatic status of subjects during fatigue.

5.1 Materials and Methods

The experiment was conducted inside the temperature controlled laboratory. The entire experiment spanned for 16 hours including the meals and call of nature (Table 4.1). The subjects were instructed for continuous virtual driving on simulator set-up to generate central, visual and physical fatigue. Just before the beginning of driving session (0 h), the blood sample was collected and each volunteer was subjected to a series of questionnaires to estimate Short Form-36 version 2.0 (SF-36 v2) health score and Beck Depression Inventory-II (BDI-II) score. The readings obtained at this state are considered as reference. The fasting blood glucose and insulin were measured in the mornings of day 1 and 2 to estimate insulin resistance before and after 16 h of simulated driving session using HOMA2 software [180]. The outcome of this method correlates reasonably well with that of “Hyperinsulinemic Euglycemic Clamp” method, the gold standard for measuring insulin resistance [181].

5.1.1 Selection of Subjects

Briefly, 14 male human volunteers from a pool of experienced heavy vehicle drivers were randomly selected for the current study. All participants were subjected to clinical examination by the registered medical practitioner and declared healthy prior to the experiments. The volunteers were strictly prohibited from tobacco and alcohol consumption 24 h before and during the experiment. A written informed consent was obtained from each subject for collection of blood samples at periodic intervals as per institutional ethical guidelines.

5.1.2 Driving Simulation Set up

The subjects were put to driving task using a pre-installed simulator set-up as described in chapter 4.

5.1.3 Blood Collection

The blood samples were collected from each subject at four instances during 16 hours driving (Day1: 18.00 h and 24:00 h, Day 2: 5.00 h and 10:00 h OR 0, 6, 11 and 16 hrs elapsed time of driving). Briefly, 5ml of blood was collected by puncturing brachial vein under strict aseptic condition and transferred to vaccutainer tubes with SST Gel and clot activator. The tubes were kept in vertical position for at least 1 hr at room temperature (RT) until the clot was formed. Centrifugation was done at 3000 rpm for 10 min at RT. The serum (supernatant) was transferred to a new sterile tube. Centrifugation was repeated at 2500 rpm for 10 min at RT in order to pellet potentially remaining on isolated serum. Collected serum was stored at -20° C as soon as possible.

Table 5.1: Experimental design to estimate the inducers of fatigue during simulated driving session in human drivers

Days	Actual Time	Elapsed Time	Serotonin	Cortisol	Insulin	Sugar (FBS)	Remarks
	10:00h	-	-	-	YES	YES	Day-1 FBS* and FBI**
Day 1	18:00h	0 hrs	YES	YES	YES		
	23:00h	**DINNER**					
	24:00h	6 hrs	YES	YES	YES		
Day 2	5:00h	11 hrs	YES	YES	YES		
	10:00h	16 hrs	YES	YES	YES	YES	Day 2 FBS and FBI

**Fasting blood sugar*, ***Fasting blood insulin*

5.1.4 Determination of Serotonin (by ELISA)

Serotonin was estimated in the platelet rich plasma (PRP) by competitive ELISA (Appendix G) using Fastrack Serotonin ELISA kit (LDN, Denmark). Plasma was collected by centrifuging the venous blood at 900 g for 15 min. at room temperature and was stored in appropriately labeled amber screw-capped micro centrifuge tubes at -20°C until analysis. Just before ELISA, plasma was thawed and centrifuged for 10 min. at room temperature at 200 g to obtain the PRP. The PRP was then acylated to remove all proteins. Briefly, 25 μL each of standards, controls, and PRP were pipetted into the respective reaction tubes and 500 μL of acylation buffer and 25 μL of acylation reagent were then added. The tubes were mixed thoroughly and were incubated for 15 min at room temperature. Next, 25 μL each of the acylated standard, control and PRP was pipetted into the wells of serotonin micro titer strips and 100 μL of serotonin antiserum was added into each well followed by incubation for 1 h at room temperature. After incubation, each well was washed with 300 μL of wash buffer (provided with kit) for 3 times. Next, 100 μL of enzyme-conjugate was added and incubated for 15 min at room temperature. Washing step was repeated after incubation and 100 μL of substrate (pNpp) was added into wells followed by incubation at room temperature for 15 min. Finally, 100 μL of 2N HCl was added to stop the reaction and the absorbance was recorded within 5 min. at 450 nm by an ELISA plate reader (ELx808, BioTek Instruments, USA). The optical signal is inversely proportional to the concentration of analyte concentration (serotonin) in sample.

5.1.5 Determination of Cortisol (by ELISA)

Cortisol level in blood samples was estimated by competitive ELISA using Fastrack Cortisol ELISA kit (LDN, Denmark) following the manufacturer's protocol. Briefly, desired numbers of wells in 96 well plate coated with appropriate capture antibody were secured in the holder. Ten μL each of standard, control and serum samples were dispensed in appropriate wells in triplicate and then, 100 μL each of cortisol-enzyme conjugate and cortisol antiserum was added into each well. The micro-titer plate was incubated for 60 min. at room temperature followed by washing. For washing, incubation mixture was removed and wells were washed with washing buffer (supplied with the kit) 5 times. Next, 100 μL of TMB solution was added into each well followed by incubation at room temperature for 30 min. After incubation, reaction was stopped by adding 50 μL stop solution (2N HCl) to each well and OD was taken at 450 nm by ELISA plate reader (ELx808, BioTek Instruments, USA). The optical signal is inversely proportional to the concentration of analyte concentration (cortisol) in sample.

5.1.6 Determination of Insulin (by ELISA)

Serum insulin level was estimated by sandwich ELISA (Appendix G) using readymade kit (Invitrogen, India) following the manufacturer's protocol. Briefly, desired numbers of wells with coated capture antibody were secured in the holder. 25 μL each of standards, controls and serum samples were pipetted into assigned wells in triplicate. 100 μL of enzyme conjugate with polyclonal detection antibody was added to each well. The reaction mixture was vortexed and incubated for 30 min at room temperature. The incubation mixture was removed and wells were rinsed 5 times with washing buffer (provided with kit). Next, 100 μL of TMB solution was dispensed into each well followed by incubation for 15 minutes at room temperature. Finally, 50 μL stop solution (2N HCl) was added to each well and OD was recorded at 450 nm within 5-min by an ELISA plate reader (ELx808, BioTek Instruments, USA).

5.1.7 Subjective Assessment

For subjective assessment, two standard protocols were followed. The first one is SF36 v2 health survey tool that detects the individual general health status (both physical and mental status) out of any external factor (task/disease/intervention). The second one is Beck Depression Inventory-II (BDI-II) consisting of a set of 21 questionnaires that estimate the degree of mood disturbance or depression (a state of detachment from the task in the subject). Both the tools were used after minor modifications to fit for a short period of study as per our experimental design (Table 5.1) and to conduct a cross-sectional evaluation instead of longitudinal evaluation.

SF-36 v2 Health Survey

The Short-Form 36 Health Survey Version 2.0 (SF-36 v2) is a multipurpose health survey that measures overall health status, functional status, and health-related quality of life at a particular instant. The detailed protocol has been described in Section 4.1.8. The details of questionnaires are outlined in Appendix E.

Beck Depression Inventory (BDI)

Beck Depression Inventory (BDI), a well-established subjective assessment tool for scoring the affective component of thought from an intervention, was used to assess the mood disturbance or depression during simulated driving. Briefly, subjects were asked to fill a questionnaire (Appendix H) that were modified for a short term assessment [182] [183], at time points matched with the time of blood collection. The total BDI score of each participant at each time point was then obtained manually as per the instructions given in the inventory. The score of first time point when each participant is assumed to be free from any type of

fatigue was considered as reference. In the inventory, the response to each question carries a highest score of 3 and a lowest score of 0 so that the total score varies between 0 (if the subject scored zero in each question) and 63 (if the subject scored 3 in all the questions).

5.1.8 Statistical Analysis

All experimental outcomes are expressed as means ± SEM. Data were analyzed by repeated measure of ANOVA using the SPSS software version 20.0 (SPSS Inc., IL, USA). Differences were considered statistically significant at $p < 0.05$. For obtaining the interdependency of various parameters, Pearson's correlation coefficients and the corresponding levels of significance were calculated.

5.2 Results and Discussion

5.2.1 Serotonin Profile in Platelet Rich Plasma (PRP)

PRP serotonin showed an increasing trend (Figure 5.1) with duration of simulated driving under sleep deprivation with a notable increment from 6 h of elapsed time. The serotonin almost doubled up from 1421.9±120 ng/mL just before the initiation of driving session to 2793.5±153 ng/mL at the end of 16h of simulated driving.

Serotonin in platelet rich plasma reliably reflects the serotonin level in brain [82]. At the same time, serotonin has been confirmed to be the key mediator in genesis and progression of central fatigue resulting from pathological or physiological stress [41]. Previous studies have shown that serotonin not only plays a direct role in inducing central fatigue, it can also generate peripheral fatigue 1) by activating HPA that secretes POMC which in turn increases the secretion of cortisol via ACTH [184], 2) by acting locally at pancreatic and hepatic level to generate insulin resistance [179] [185]. Both the events are considered as body's effort to cope with the stress that originates from serotonin induced prolonged central fatigue. Thus, PRP serotonin may be used as an additional component in scoring drivers' fatigue. No previous study has observed a gradual increase in PRP serotonin with the progression of fatigue in simulated driving condition.

5.2.2 Serum Cortisol Level

The increasing trend in plasma cortisol levels during 16 h of simulated driving has been shown in (Figure 5.2). The level increased from 56.4±9.1 mg/dL at 0h to 107.82±13.2 mg/dL at the end of 16 h of simulated driving. In normal healthy individuals, the cortisol secretion follows circadian rhythm [44] (Figure 5.3B). Plasma cortisol level peaks an hour before rising in the morning (40—50 μg/dL) and reaches the peak in the evening (5 mg/dL) in response to hypothalamic ACTH secretion as per normal 24-hour circadian cycle [88].

In the current study, plasma cortisol profile during the progression of fatigue from sleep deprived driving showed deviation from that of normal circadian cycle (compared in Figure 5.3). However, the deviation was not marked enough. At the initial point of experiment (0 hour elapsed time of driving), a low cortisol level was expected. However, the value was 56.4 μg/dL, which is above the peak value (40—50 μg/dL) found in the morning in normal circadian rhythm. Such a large cortisol level can be explained from the fact that the subjects were at stress even before the start of driving session and the stress might be due to nervousness and an apprehension of any type of intervention during experiment since all subjects were participating in a scientific experiment for the first time.

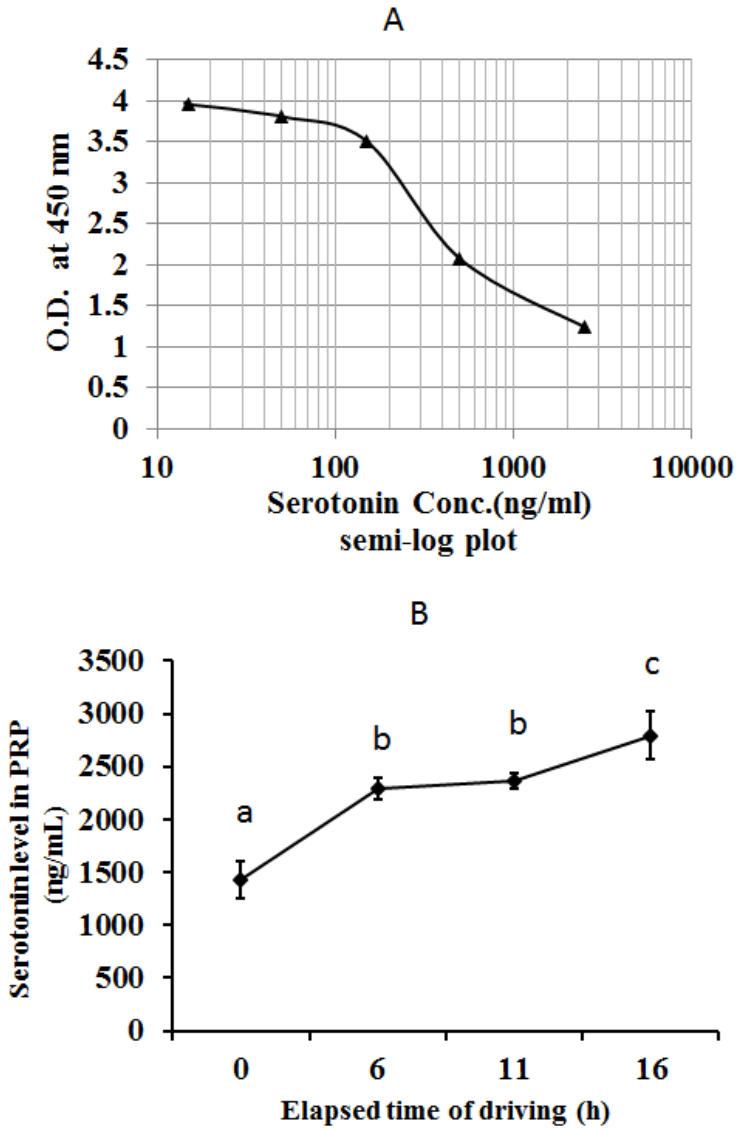

Figure 5.1: Mean serotonin level (ng/mL) in platelet rich plasma (PRP) of 14 experimental subjects (heavy vehicle drivers) at four time points (0h, 6h, 11h and 16h elapsed time of simulated driving: Refer Table 5.1. (A) Mean (±SEM) values. (B) Standard curve. Different alphabets (a, b, c) over the curve indicates statistical difference among stages ($p<0.05$)

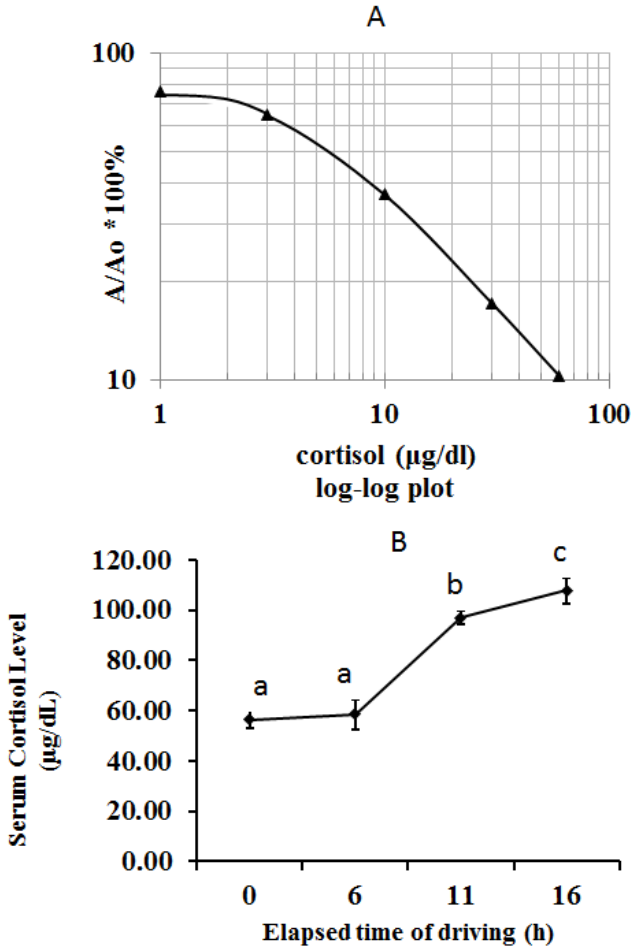

Figure 5.2: Mean serum cortisol level of 14 experimental subjects (heavy vehicle drivers) at 4 time points (0 h, 6 h, 11 h and 16 h elapsed time of simulated driving: Refer Table 5.1). (A) mean (±SEM) values. (B) Standard curve. A: OD of known samples; A0: OD of sample with no cortisol. Different alphabets (a, b, c) over the curve indicates statistical difference among stages ($p<0.05$)

The value remained high after the elapse of 11 h. In the morning, the mean plasma cortisol rose to 97.02 μg/dL following the trend of normal circadian cycle of cortisol; however, the value was almost twice that of at normal condition without any stress. From 11 h onwards, instead of decreasing as follows in normal circadian cycle, plasma cortisol value increased and reached 107.8 μg/dL at the end of 16 hours of elapsed driving . Thus, it can be concluded that though the trend in plasma cortisol level from fatigue due to simulated driving didn't show a marked deviation from that of the normal circadian cycle, but very importantly, the values of plasma cortisol remained significantly high at all-time points of simulated driving compared to the values at the same time points in normal circadian cycle. The resulting stress from sleep deprived driving was crucial in scaling up the cortisol secretion and such prolonged hours of cumulative stress ultimately culminated in fatigue.

Contrasting reports are found in literature on the variation of cortisol level during task

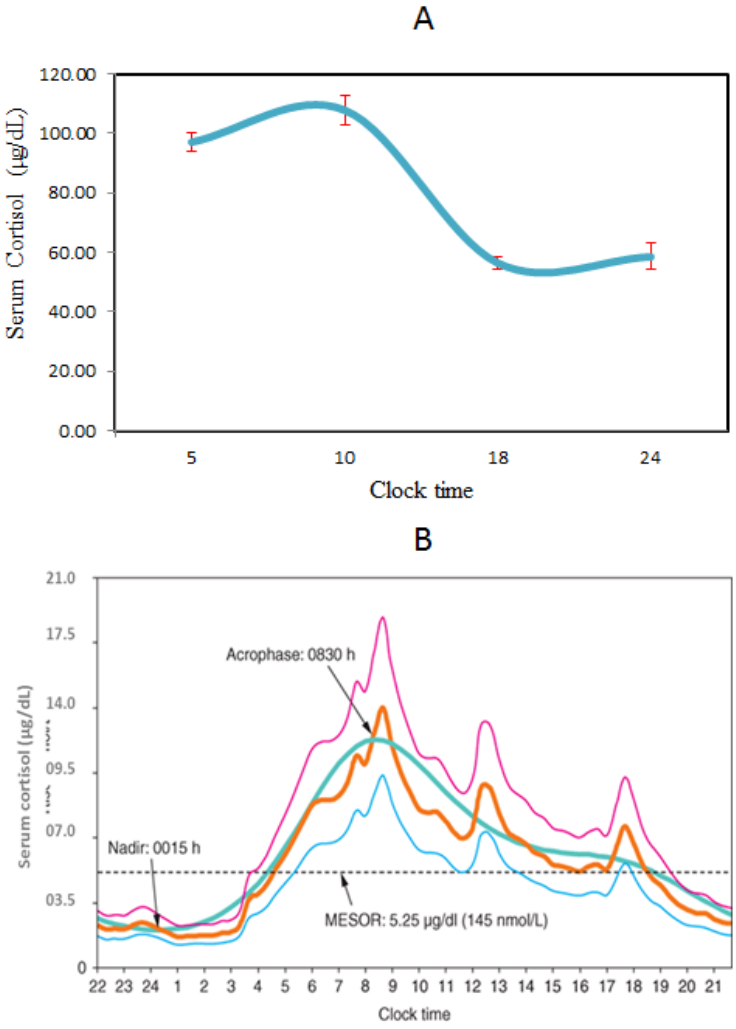

Figure 5.3: Comparison of serum cortisol level (mean ± SEM) between the experimental subjects (A) and, the normal circadian rhythm of cortisol secretion in normal healthy individuals with no stress and sleep deprivation (B). Figure (b) was adapted from [186].

that disturbs circadian rhythm. Shinkail and co-workers studied the pattern of salivary and serum cortisol level during the development of stress culminating in fatigue in shift workers where the nature of work disturbs the normal circadian rhythm due to associated sleep deprivation [122]. The saliva and blood samples were assayed for cortisol at 4-h intervals in experimental short-term shifts. They found a lowered level of both salivary and the blood cortisol at the designated time points in experimental group than the control group who were resting and with normal circadian rhythm. But, Jerjes and co-workers [123] studied pattern of cortisol including urinary adrenaline and nor adrenaline in twelve coach drivers to estimate their fatigue and discussed the effect of disturbed circadian rhythm from sleep deprived driving on those parameters. They concluded that the task that disturbed circadian rhythm affected trend in adrenaline and noradrenaline while the normal diurnal variation in urinary cortisol remained unaffected.

It was observed that cortisol level during simulated driving slightly deviated from the normal circadian rhythm. However, the level was notably increased from the normal values and therefore, cortisol level may indicate the severity of fatigue due to stress in the drivers.

5.2.3 Serum Insulin Level

The serum insulin showed a gradual increase with progressing duration of sleep-deprived driving (Figure 5.4). The mean serum insulin of 14 experimental subjects was raised to 49.4±3.2 μIU/ml by 16 h of driving stress from a low level of 34.3±1.6 μIU/ml before the driving task (Figure 5.4).

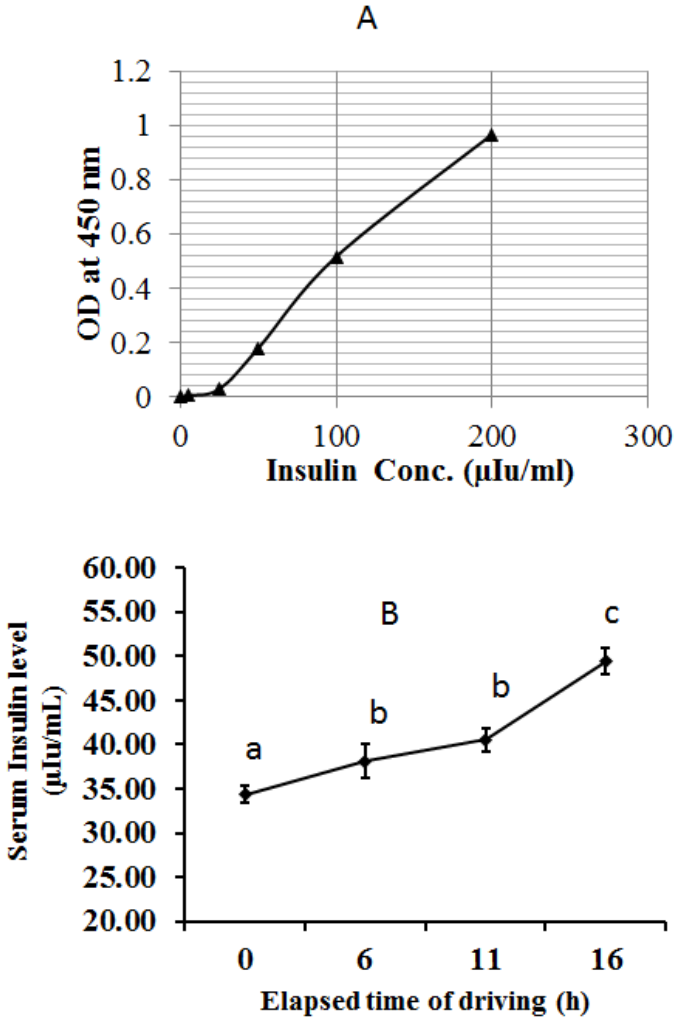

Figure 5.4: Mean serum insulin level of 14 experimental subjects (heavy vehicle drivers) at 4 time points (0 h, 6 h, 11 h and 16 h elapsed time of simulated driving: Refer Table 5.1). (a) Mean (±SEM). (b) Standard curve. Different alphabets (a, b, c) over the curve indicates statistical difference among stages ($p<0.05$)

Since the subjects had dinner between 5 and 6 hours of elapsed time of driving, a momentary rise in insulin after the meal was expected. This increased level of insulin must

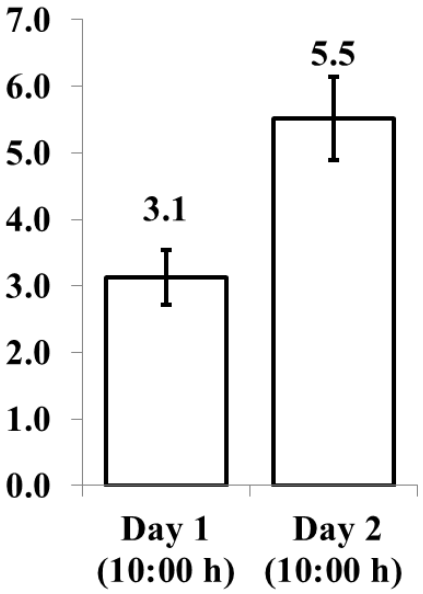

Figure 5.5: The mean (±SEM) insulin resistance (IR) score of 14 experimental subjects (heavy vehicle drivers) before (Day 1: no fatigue and no sleep debt) and after (Day 2: one night sleep deprivation) 16 h of driving task

return to normal level within 1 or 2 hours. However, even after several hours of meal, the level of insulin continued to increase which might suggest the development of "insulin resistance (IR)". Such a state leads to hyperinsulinemia as well as hyperglycemia. Previous studies have shown that corticosteroid such as cortisol can also plays a role in developing IR [178]. In the current study, RBS, insulin as well as cortisol was found to be increased under simulated driving condition and were well co-related. Indeed, Homeostatic Model Assessment-2 (HOMA-2) showed a significant rise in IR at 16 h of continuous driving with sleep deprivation in all drivers (Figure 5.5).

5.2.4 Subjective Assessment

SF36 v2 Score

The PCS value gradually decreased at five stages matched with 4 time points of blood collection (Figure 5.6A: Stage1: 60.50 ± 2.1, Stage2: 52.23 ± 1.3, Stage3: 41.52 ± 0.6, Stage4: 33.70 ± 0.3%) with the progression of fatigue with an inference that the physical health condition declines with fatigue combined with sleep deprivation.

Similarly, the average scoring values of MCS at 5 stages matched with 4 time points of blood collection are shown in Figure 5.6 (Stage1: 67.4 ± 0.8, Stage2: 52.3 ± 1.1, Stage3: 42.0 ± 0.6, Stage4: 27.5 ± 1.4%). The result clearly shows a declining trend with the progression of fatigue.

Thus, the subjective feeling of physical and mental well-being exhibited a persistent decremented profile that is well-correlated with the pattern of changes in the physiological

and blood biochemical parameters described above.

Score of Beck Depression Inventory-II (BDI-II)

The average BDI score increased from 3.16 ± 0.84 before the beginning of driving session to 16.8 ± 0.12 at the end of 16 h of simulated driving (Figure 5.7) revealing a marked mood disturbance in experimental subjects.

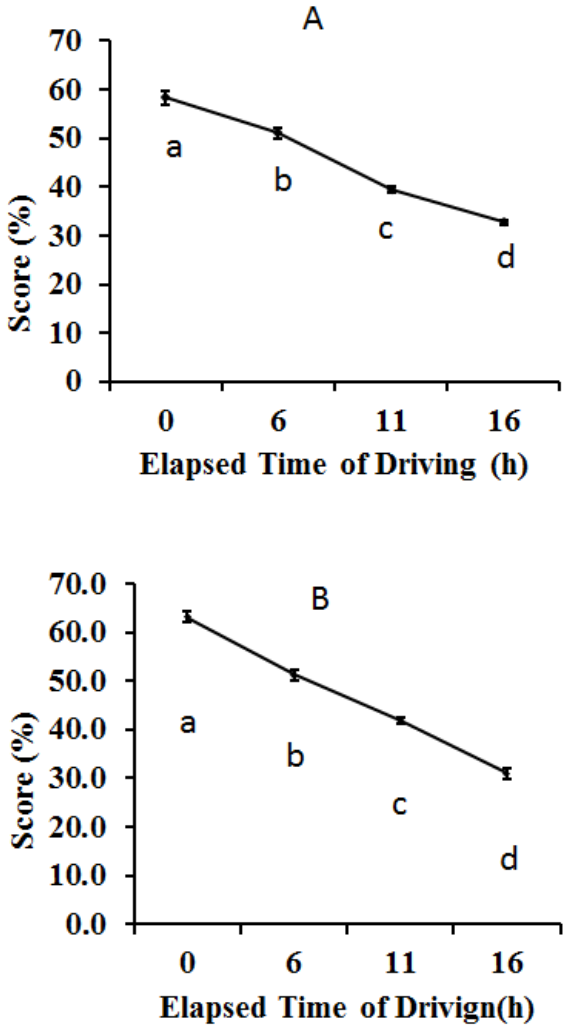

Figure 5.6: The subjective feeling of physical wellbeing and mental well-being expressed by physical component summary/PCS (A) and, mental component summary/MCS (B) measured from SF-36 Health Score Tool at 4 stages matched with 4 time points of blood collection (0h, 6h, 11h and 16h elapsed time of simulated driving: Refer Table 5.1). Different alphabets (a, b, c) over the curves indicate statistical difference among stages ($p<0.05$)

Beck Depression Inventory performs a two way psychodynamic analysis of mood disturbance, in particular depression. Depression, in specific, can have two components

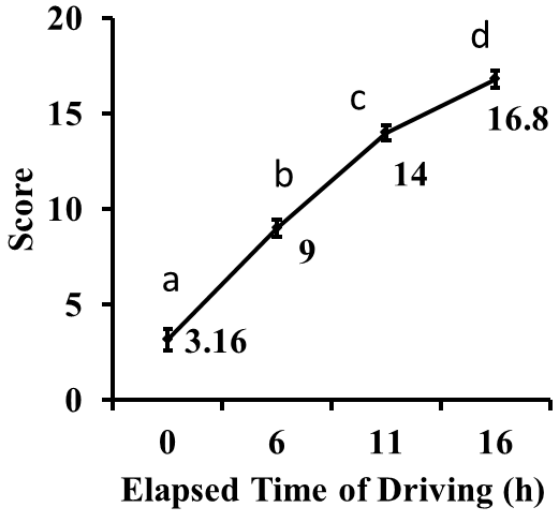

Figure 5.7: The Beck's depression inventory-II score presenting affective disturbance in thought process (vary from mood disturbance to depression) associated with central fatigue from prolonged driving task at 4 stages matched with 4 time points of blood collection (0h, 6h, 11h and 16h elapsed time of simulated driving: Refer Table 5.1). Different alphabets (a, b, c) over the curve indicates statistical difference among stages ($p<0.05$)

i.e. affective component (includes pessimism, failure, feeling of punishment, self-dislike, worthlessness; and the somatic component (includes agitation, loss of pleasure, sadness, loss of energy, loss of interest, indecisiveness, irritability, tiredness, concentration difficulties, change in sleep pattern and loss of interest) [187]. Previous study has confirmed that fatigue during prolonged driving task can induce mood disturbances in drivers and the intensity will be more if the task is combined with sleep deprivation [188]. At the same time depression can further aggravate the level of fatigue [189]. Thus, the proper assessment of mood disturbance or depression can be considered an important parameter during fatigue analysis.

In all most all subjects, the score increased along with the progression of fatigue. In each subject, the somatic depression was more dominant as compared to the affective depression during and towards the completion of the whole task. The relative decrease in the last stage can be explained by the subject's feeling of assurance that the task is getting over after a prolonged binding to a task.

5.2.5 Correlation Results

A statistical correlation analysis was carried between the fatigue inducers (serotonin/5 HT, cortisol and insulin) with the Beck's depression inventory score and MCS from SF 36 tool (both indicates central fatigue state) as well as PCS from SF 36 tool (peripheral fatigue state).

Table 5.2: Correlation coefficients between blood bio-markers and subjective assessment scores

	Fatigue inducers		
	Serotonin (5 HT)	Cortisol	Insulin
Subjective score (central)			
BDI	0.955 (0.044)	0.934 (0.066)	0.909 (0.09)
MCS	-0.954 (0.045)	-0.946 (0.053)	0.96 (0.040)
Subjective score (peripheral)			
PCS	-0.912 (0.081)	-0.978 (0.023)	-0.935 (0.065)

Values within the parentheses indicate the level of significance

5.3 Summary and Conclusion

In this chapter, various stress inducers that lead to the genesis and progression of fatigue were assessed during simulated driving for 16 h under sleep-deprived condition. The results were correlated with the subjective assessment scores of physical fatigue and drowsiness. In summary, the results of the present study showed that

i. PRP serotonin level, the key inducer of central fatigue, significantly increased with the progression in central stress during simulated driving session.

- Serum cortisol, the key biochemical stressor in peripheral blood, significantly increased with increase in the duration of sleep-deprived simulated driving.

ii. The serum cortisol profile didn't show notable deviation from normal diurnal variation that follows circadian rhythm. However at each time point, the level in experimental subjects was significantly higher compared to the normal physiological values of serum cortisol at corresponding time points during circadian cycle.

iii. Insulin secretion significantly increased with increasing fatigue out of sleep-deprived driving.

iv. Insulin resistance measured at the peak of fatigue and sleep debt (10:00 AM of Day-2) increased by 350% over the value measured at non-fatigue and zero sleep debt (10:00 AM of Day-1).

v. Serotonin profile in volunteers exhibited strong and significant correlation with the MCS ($r = -0.954$, $p<0.05$) and BDI score ($r = 0.955$, $p<0.05$).

vi. Serum cortisol and insulin levels exhibited strong and significant correlation with PCS($r = -0.978$, $p<0.05$ and $r = -0.935$ and $p<0.05$ respectively) of experimental subjects.

It may be concluded that, prolonged driving with sleep deprivation can alter the blood levels of stress inducers such as serotonin, cortisol and insulin. When used in combination, they may help in identifying the level of fatigue, as indicated by clinically proven SF36v2 and BDI-II tools. Given that blood biomarkers can give an objective evaluation of fatigue, they may complement other objective tools of fatigue analysis in devising an objective fatigue scoring system.

Chapter 6

Overall Conclusion and Scope of Future Work

The study showed that the signs and symptoms of physical fatigue were more pronounced in mice models of tauopathy than in healthy mice, although the genesis and progress of fatigue followed similar trend in both the groups. Thus, a fatigue score system developed on healthy individuals might be applicable under pathological conditions such as tauopathy. A critical analysis of seasoned driver's fatigue, using SF36v2 and BDI-II tools, revealed that the severity of central and peripheral fatigue and the state of drowsiness increased with time of simulated driving under sleep deprived condition. The relative energies of β, α, and θ bands of EEG signal increased with increasing severity of fatigue at F3, Fz, F4 and Cz electrodes. However, the variation across the stages was notable in θ band and was significant in a derived band ($\alpha + \theta$ / $\delta 1 - \delta 2$) at the specified electrodes. Only the relative energies of the derived band at Cz electrode exhibited a significant and strong negative correlation with the MCS and sleepiness score. Further, SE of whole EEG signal increased significantly across the stages at Cz electrode and exhibited a strong correlation with MCS and sleepiness score. All the biochemical markers viz. RBS, blood urea and serum creatinine co-related with the EEG results. Importantly, however, serum creatinine had a strong negative correlation (r $= -0.951$) with PCS. It was also observed that, the PSD of the HF components of ECG signal decreased significantly across all the stages of fatigue analysis whereas the PSD LF component showed a burst at midnight. In another set of study, it was also observed that, PRP serotonin (the key inducer of central fatigue) and serum cortisol (the key biochemical stressor in peripheral blood) significantly increased with increase in the duration of simulated driving under sleep deprived condition. Although serum cortisol (an indicator of physical fatigue) profile did not show notable deviation from normal diurnal variation, its level was significantly high in experimental subjects at all the analyzed time points of circadian cycle. On the other hand, similar to PRP serotonin and serum cortisol, insulin (an indicator of physical fatigue) secretion significantly increased with increase in the duration of simulated driving under sleep deprived condition. Insulin resistance measured at the peak of fatigue and sleep debt (10:00 AM of Day-2) increased by 350% over the value measured at non-fatigue

and zero sleep debt (10:00 AM of Day-1). The PRP serotonin profile had a strong correlation with the MCS (r= -0.954, p<0.05) and BDI score (r= 0.955, p<0.05), which are indicators of central fatigue. On the other hand, serum cortisol and insulin levels exhibited strong correlation with PCS (r= -0.978, p<0.05 and r= -0.935 and p<0.05 respectively), which is an indicator of peripheral fatigue. An statistical analysis of these data with the clinically proven subjective tools of fatigue assessment, SF36v2, BDI-II and sleepiness score, suggest that, the entropy of whole EEG signals, relative energies of θ, α and the derived parameter ($\alpha + \theta$ / $\delta 1 - \delta 2$) and PRP serotonin can effectively indicate central fatigue while RBS, blood urea, serum creatinine, serum cortisol, serum insulin and power distribution of HF components in ECG can reliably indicate peripheral fatigue in drivers. Since these data were consistent with those of the clinically proven, but subjective, tools of fatigue assessment, they may have implications in devising an objective scoring system for the estimation of fatigue progression in drivers.

Scope of Future Work

The present study lays foundation for future studies and has several potential applications that are briefly outlined below.

- Given that fatigue and sleepiness can occur independently, a caution is suggested in interpreting and extrapolating the results of this study in the context of fatigue drivers (without sleep deprivations) and sleepy drivers (without physical fatigue). This study may lay foundation for evaluating the above parameters in subjects who are subjected to sleep deprivation but not driving fatigue, which is a technically challenging task to perform with human subjects.

- The current study was performed in controlled atmosphere of a laboratory. Although utmost care was taken to simulate the on-road driving conditions, it is impossible to completely recapitulate the unpredictability of road condition, weather, traffic condition etc. within the laboratory. Since these external factors may contribute to the development of fatigue, this study lays foundation for analyzing the developed parameters in real on-road driving condition, including night driving. Furthermore, factors such as age, experience, type of vehicle, atmosphere of the driver's cabin, frequency of tea/coffee breaks, drinking of stimulant drinks such as tea, coffee, beer etc can have influence in the genesis and progression of the fatigue. The influence of these factors could not be analyzed in the present study and should lay foundation for future study, which may require customized vehicles wherein the cabin is pre-installed with EEG, ECG, spirometer and other recording machines for implementation. In addition, real time processing of raw signals obtained from EEG and ECG would enrich the investigation.

- The current study was performed in a fixed age group of 30.5±9.5 years. Previous studies have shown that fatigue manifestations can vary significantly with advancing age [190] [191] [192] [193]. Thus, it would be interesting to analyze the robustness of the parameters, established in this study, in estimating and/or scoring the genesis and progression of fatigue in drivers of different age groups.

- A multicentric study, integrating the outcomes of both simulated and on-road driving condition, may help framing of a precise staging/scoring system for drivers' fatigue. Such staging/scoring system may be helpful in the validation of devices that are designed to detect drivers' fatigue based on non-contact features.

- It is currently unrealistic to do real-time recording of EEG or monitoring of blood profile for estimating the driver's fatigue by using currently available devices. The results of this study emphasizes the need of devices such as wireless EEG data acquisition system (e.g. wireless electrodes embedded in a cap that can be worn by the drivers and the data acquisition can be done through blue tooth mechanism by the parent device installed on dash board), nano-mosquito for blood sampling etc. Several of such devices are under development that can be tested with the data generated in the present study.

- EEG gave valuable information on the genesis and progression of central fatigue in drivers. The combination of these data with brain imaging may assist in finding the exact brain regions where fatigue is initiated and therefore, might be helpful in designing fatigue countermeasure techniques. In future, it might be valuable to perform PET (Positron Emission Tomography) scan at different stages of driving condition to obtain a precise mapping of brain regions during fatigue evolution.

- Finally, the results of the present study may serve as a basis for fatigue evaluation in other occupational groups wherein prolonged, monotonous and sub-maximal exercise is involved, e.g. shift workers, pilots, IT professionals and surgeons.

Appendix A

Non-contact Feature Based Fatigue Detecting Devices

Table A.1: Important patents on devices for detecting fatigue in drivers

Inventor	Company	Patent Title	Patent Number Country
LV Jian, Chen Zhekang	Nanjing Aquapel Co Ltd	Driver fatigue detection warning device	CN102867394A China
Wang Weidong	-	Auxiliary driving adaptive cruise control system for drivers	CN201220217703 China
Wentao Wang, Yang Liu	Geely Automobile	Anti-fatigue driving system for electric automobile	CN104574816A China
Zutao Zhang, Xiaopei Li	-	Wearable electro-encephalogram signal collection equipment for high-speed train drivers	CN102697494A China
Chen Tai-Liang	-	Vehicle anti-doze system and method thereof	TW20100128083 Taiwan
You Song, Yunfeng Luo	-	Driver fatigue detecting method and system based on vision	CN102201148A China
Zhenyu Liu, Zhentian Li	-	Multimodal driver fatigue detection method and special equipment thereof	CN102073857A China
Huanbin Chen, Yanze Li	Shenzhen Feiruisi Technology Co Ltd	Drowsy driving warning system	CN20102188359U China
Shaobin Wu, Li Gao	-	Semi-physical driving fatigue vision simulation system platform	CN2009179326 China
Shengqian Liao, Liming Wang	Shanghai East Container	Reversing safety monitoring system for container lift truck	CN201237643Y China
Yong Chen, Qi Huang	-	Method for monitoring vehicle driver's status and system thereof	CN101224113A China
Yanai Tatsumi	Nissan Motor	Mental fatigue level judgment device for vehicle	JP19970334848 Japan
Yanai Tatsumi	Nissan Motor	Driver state measuring instrument for vehicle	JP19970333749 Japan
Hu Shengyi, Huang Yuping	-	Long-range monitor of fatigue drive and overload transportation for bus	CN2754929Y China
Callahan Thomas, Edmunds Lyle	-	Road fox	CA2526299A1 Canada

Table A.2: Current status of selected wearable devices for fatigue in drivers based on non-contact features

Device	Manufacturer	Current Status	Parameters Analyzed
DFM (Driver Fatigue Monitor)	Attention Technology, Inc., Pittsburgh, USA	Under field trial	Slow eyelid closure and drowsiness level
FaceLAB	Seeing Machines, Canberra, Australia	Commercialized	Blink and PERCLOS
DSM (Driver State Monitor)	Delphi Electronics, Kokomo, USA	Commercialized	Eye closures and the forward attention level
Smart Eye Pro 3.0	Smart Eye AB, Göteborg, Sweden	Commercialized	Individual facial features and a three-dimensional (3D) head model
InSight	SensoMotoric Instruments GmbH, Germany	Commercialized	Head orientation, gaze direction, eyelid opening, and pupil position and diameter.
ETS-PC II	Applied Science Laboratories, Bedford, USA	Under field trial	Eye movement and line of sight over full 90° horizontal and 45° vertical field of view
DEM (Driver Eye Monitor)	LC Tech, Inc., Cleveland, USA	Commercialized	Eye motion and related eye data
DDDS (Drowsy Driver Detection System)	Applied Physics Laboratory, Johns Hopkins University, USA	Under field trial	Speed, frequency, and duration of eyelid closure, rate of heartbeat, respiration and, pulse.
Fatigue Management System (FSM)	ARRB Transport Research, Vermont South, Australia	Commercialized	Mental reaction time
Sleep Control Helmet System	Sleep Electronics System, USA	Commercialized	Head nodding detection
NOV alert	Atlas Research Ltd., Washington DC, USA	Commercialized	Muscle tone

Appendix B

Microprocessor Based Behavioral Equipment for Mice

Rotarod

The rotarod test is conducted using an accelerating rotarod apparatus that consists of 2-6 rotating drums (3 cm ϕ).

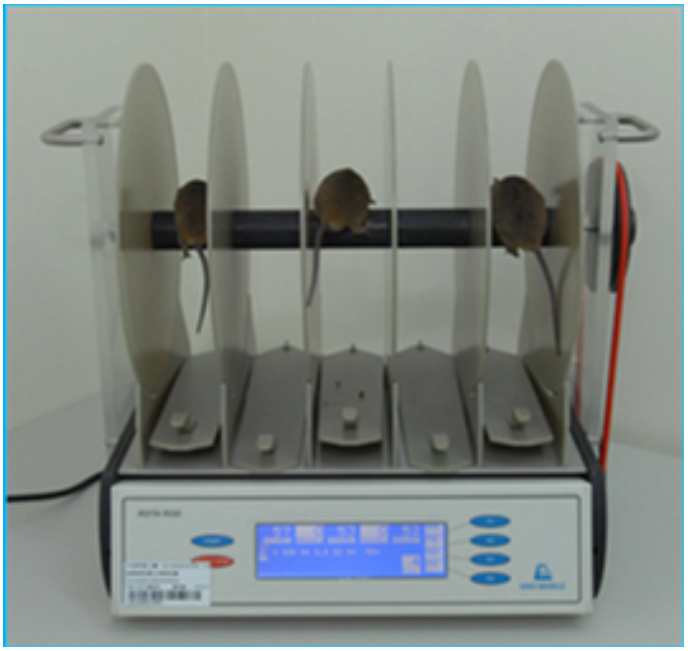

Figure B.1: Apparatus for conducting rotarod test on mice

Open Field Apparatus

Open field observations are carried out in a 100×100 cm Plexiglas open field divided into 9×9 cm squares. It is illuminated by a 150 W electric bulb 100 cm above the open field floor.

Figure B.2: Apparatus for conducting Open Field Test on mice

Figure B.3: Apparatus for conducting elevated plus maze test on mice

Principle: The light dark (LD) test is used to evaluate the relative anxiety status of mice. The light dark paradigm in mice is based on a conflict between the innate aversion to brightly illuminated areas and the spontaneous exploratory activity.

Elevated Plus Maze Apparatus

The maze consists of two open arms (25×5 cm), two enclosed arms (25×5×30 cm), arranged so that the two arms of each type were opposite each other and extended from a center platform (5×5 cm). The floor and side-walls of maze are constructed from gray opaque Plexiglas material. The maze is elevated to a height of 50 cm.

Principle: Non-anxiety state in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms), and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). Total number of arm entries and number of closed-arm entries are usually employed as measures of general activity.

Light and Dark Transition Test Box

The apparatus used for the light/dark transition test consists of a cage (21 cm×42 cm×25 cm) divided into two sections of equal size by a partition containing a door. One chamber is brightly illuminated (390 lux), whereas the other chamber is dark (2 lux).

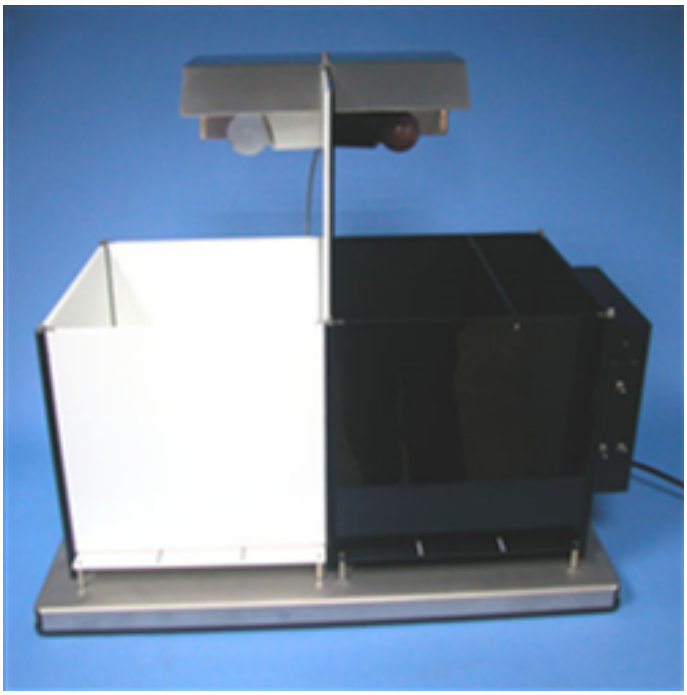

Figure B.4: Apparatus for conducting light and dark test on mice

Principle: The light dark (LD) test is used to evaluate the relative anxiety status of mice. The light dark paradigm in mice is based on a conflict between the innate aversion to brightly illuminated areas and the spontaneous exploratory activity.

Porsolt Forced Swimming Apparatus

Figure B.5: Apparatus for conducting porsolt test on mice

Principle: The light dark (LD) test is used to evaluate the relative anxiety status of mice. The light dark paradigm in mice is based on a conflict between the innate aversion to brightly illuminated areas and the spontaneous exploratory activity.

Appendix C

Simulated Driving Setup

The driving simulator set up used in the current study had a driving module and an audiovisual component. The driving module consisted of

- Driving simulator software (Euro Truck Simulator, SCS software, Denmark).
- A software compatible steering wheel (capable of 90° rotation) with a vibration leg pad and hydraulic brake system (DriveForce^{TM} GT, Logitech, India).

The audiovisual component comprised of

- An LCD projector with screen and
- A high definition sound system (Inspire-M 4500^{TM}, Creative India Ltd.) to recreate an environment of highway driving.

The driving module and the audiovisual unit were installed in a PC (HP desktop, Intel Dual Core, Microsoft 7 OS, 4 GB RAM and Nvidia Graphic card).

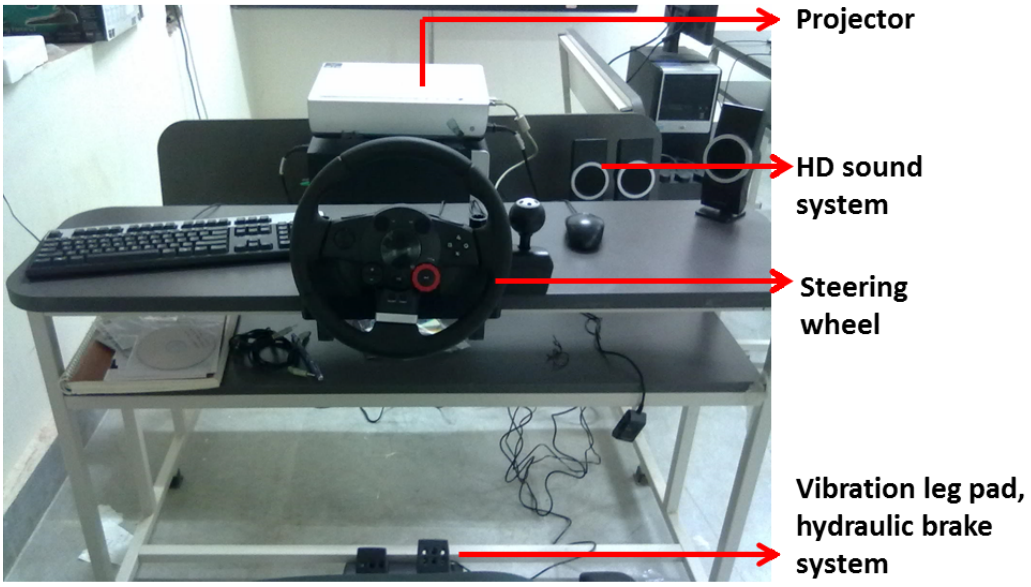

Figure C.1: Driving simulation set up used in the current study

Appendix D

Wavelet Decomposition

Wavelets are small wave-like oscillating functions that are localized in time and frequencies [194] [195]. In discrete domain, any finite energy time domain signal can be decomposed and expressed in terms of scaled and shifted versions of a mother wavelet $\Psi_{j,k}(t)$ and a corresponding scaling function $\Phi(t)$. The scaled and shifted version of the mother wavelet and the corresponding scaling function $\Phi_{j,k}(t)$ are mathematically represented as:

$$\Psi_{j,k}(t) = 2^{\frac{j}{2}} \Psi\left(2^j t - k\right), \quad j,k \in Z \tag{D.1}$$

$$\Phi_{j,k}(t) = 2^{\frac{j}{2}} \Phi\left(2^j t - k\right), \quad j,k \in Z \tag{D.2}$$

A signal $S(t)$ can be expressed mathematically in terms of the above wavelet $\Psi_{j,k}(t)$ and the corresponding scaling function $\Phi_{j,k}(t)$ at level j as:

$$S\left(t\right) = \sum_{k} s_j\left(k\right) \Psi_{j,k}\left(t\right) + \sum_{k} d_j\left(k\right) \Phi_{j,k}\left(t\right) \tag{D.3}$$

where $s_j\left(k\right)$ and $d_j\left(k\right)$ are the approximate and detailed coefficients at j^{th} level.

These coefficients are computed using the filter bank approach as proposed by Rioul and Vetterli [196].The original signal $S(t)$is first decomposed into high-frequency and low-frequency components. The low-frequency component approximates the signal while the high-frequency components represent the residuals between the original and approximate signals. At successive levels, the approximate component is decomposed further. After each stage of filtering, the output time series is down-sampled by two and then fed into the next level of input. The features extracted from wavelet decomposition depend primarily on the type of the mother wavelet chosen. A rule of thumb says that the best results are obtained when there is a close resemblance between the signal and the mother wavelet. The Daubechies family of wavelets has a compact support with a relatively greater number of vanishing moments [195]. This makes it a suitable candidate for signal compression and characterization.

Appendix E

SF36v2 Health Survey Scoring

This survey asks for a subject's views about his/her health. This information help keep track of how he/she feels and how well he/she is able to do usual activities in response to an intervention/ medication/ diseases. Each participant was asked to answer every question by selecting the answer as indicated. If he/she was unsure about how to answer a question, he/she was instructed to give the best answer he/she can. The questionnaires are (after some minor modifications for our requirement):

1. In general, would you say your health is now ?

Excellent	Very Good	Good	Fair	Poor

2. Compared to 0/4/8/12/16 hours (depending upon the stage of driving) ago, how would you rate your health in general now?

Much better now than 0/4/8/12/16 hours ago	Somewhat better now than 0/4/8/12/16 hours ago	About the same as 0/4/8/12/16 hours ago	Somewhat worse now than 0/4/8/12/16 hours ago	Much worse now than 0/4/8/12/16 hours ago

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Vigorous Activities, such as running, lifting heavy objects, participating in strenuous sports			
b. Moderate Activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf			
c. Lifting or carrying groceries			
d. Climbing several flights of stairs			
e. Climbing one flight of stairs			
f. Bending, kneeling, or stooping			
h. Walking several hundred yards			
i. Walking one hundred yards			
j. Bathing or dressing yourself			

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities					
b.Accomplished less than you would like					
c. Were limited in the kind of work or other activities					
d. Had difficulty performing the work or other activities (for example, it took extra effort)					

5. During the past 0/4/8/12/16 hours (depending upon the stage of driving), how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities					
b.Accomplished less than you would like					
c. Did work or activities less carefully than usual					

6. During the past 0/4/8/12/16 hours (depending upon the stage of driving), to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely

7. How much bodily pain have you had during the past 0/4/8/12/16 hours (depending upon the stage of driving)?

None	Very Mild	Mild	Moderate	Severe	Very Severe

8. During the past 0/4/8/12/16 hours (depending upon the stage of driving), how much did pain interfere with your normal work?

Not at all	A little bit	Moderately	Quite a bit	Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 0/4/8/12/16 hours (depending upon the stage of driving).

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?					
b. Have you been very nervous?					
c. Have you felt so down in the dumps that nothing could cheer you up?					
d. Have you felt calm and peaceful					
e. Did you have a lot of energy?					
f. Have you felt downhearted and depressed?					
g. Did you feel worn out?					
h. Have you been happy?					
i. Did you feel tired?					

10. During the past 0/4/8/12/16 hours (depending upon the stage of driving), how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't Know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people					
b. I am as healthy as anybody I know					
c. I expect my health to get worse					
d. My health is excellent					

Appendix F

Sleepiness Score

Sleepiness score gives the state of drowsiness of an individual. Such scores are used clinically for estimating the sleepiness in the subjects in response to a medication/intervention/task. In the current study, a customized questionnaire based sleepiness score was used based upon three established sleepiness scores i.e. Stanford Sleepiness scale, Piper's scale and Epworth Sleepiness scale. The questionnaires are presented below:

1. Are you fatigue now? If yes, to what degree you are feeling fatigue? (Scale: 1–10) ?

2. How long have you been feeling fatigue?

15 min	15 min	1 hour	1.5 hour	2 hour	3 hour	4 hour	More

3. To what degree your fatigue may affect your ability to work? (Scale: 1–10)?

4. To what degree you are feeling sleepy now? (Scale: 1–10)

5. To what degree you are feeling able to walk normal? (Scale: 1–10)?

6. To what degree you are feeling energetic? (Scale: 1–10)?

7. To what degree you are feeling able to concentrate? (Scale: 1–10)

8. To what degree you are feeling able to think clearly? (Scale: 1–10)?

9. What do you think is the main cause of your fatigue?

Sleep deprivation	Long time	Monotonous road	Traffic	Mood	Others (mention)

10. What do you think is the best thing that can relieve your fatigue?

Music	Caffeine	Water	Rest	Other

11. Are you experiencing any other symptoms right now?

Head ache	Body ache	Head reeling	Others (mention)

12. Chance of dozing:

(a) If allowed to read a newspaper (Scale: 0–4).

(b) If allowed to lie down for rest (Scale: 0–4).

(c) If allowed to listen to music (Scale: 0–4).

(d) If allowed to drive in a long and monotonous road (Scale: 0–4).

Appendix G

Enzyme Linked Immunosorbent Assay

ELISAs are a group of immunoassay methods utilized to detect and quantify substances such as peptides, proteins, antibodies and hormones. In an ELISA, an antigen is immobilized to a solid surface. The antigen is then complexed with an antibody conjugated to an enzyme. Next, a substrate specific to the enzyme is added so that it is metabolized and a color product is formed. The intensity of the color is directly proportional to the analyte concentration (antigen). The most crucial element of the detection strategy is a highly specific antibody-antigen interaction. There are many variants of ELISA that are used in clinical set up for diagnostic purpose. In the current study, two variants were used i.e. Competitive ELISA (for serotonin and cortisol estimation) and sandwich ELISA (for Insulin estimation).

Competitive ELISA: The detection of analyte in this ELISA method relies on competitive binding of original antigen (sample/analyte) and add-in antigen. The procedure is highly sensitive for analyte that is mixed with other proteins (like blood /serum) and doesn't require the analyte to be purified. The primary antibody (capture antibody) is immobilized on a substratum. The sample antigen is added along with the antigen conjugated with an enzyme. Both the antigens (in sample and conjugated one) compete to bind with capture antibody. Next, after proper wash, the substrate specific for the enzyme is added so that it is metabolized to give a colored product. The optical signal of final product is inversely proportional to the analyte concentration.

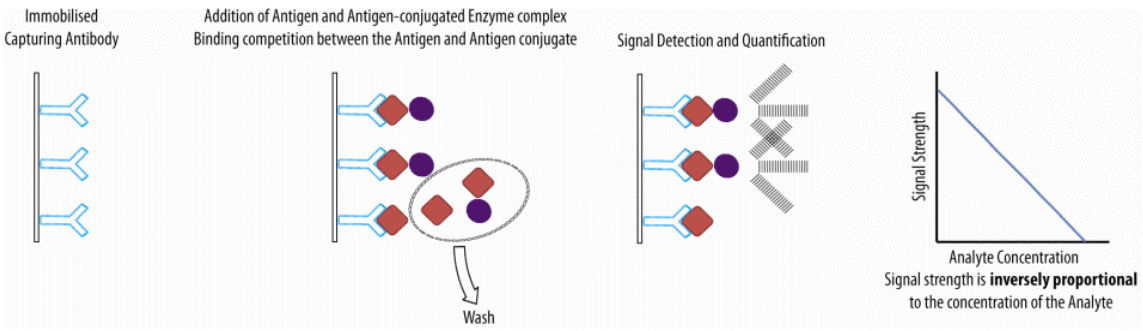

Figure G.1: The principle of competitive ELISA (Enzyme Linked Immunosorbent Assay) [197]

Sandwich ELISA: This ELISA format is also highly sensitive for the analyte which is mixed with other proteins (i.e. blood /serum) and doesn't require the analyte to be purified. The primary antibody (capture antibody) is immobilized on a substratum. The sample antigen is added and allowed to bind with capture antibody. Next, after proper wash, the secondary antibody (detection antibody) labeled with an enzyme is added.

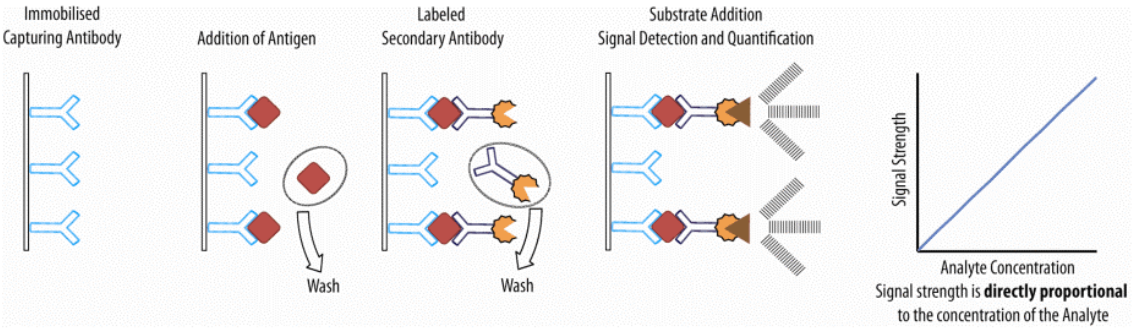

Figure G.2: The principle of sandwich ELISA (Enzyme Linked Immunosorbent Assay) [197]

Appendix H

Beck's Depression Inventory-II (BDI-II)

The scoring system in BDI-II that indicates low to severe mood disturbance is given below.

Classification	Total Score	Level of Depression
LOW	1-10	These ups and downs are considered normal
	11-16	Mild mood disturbance
MODERATE	17-20	Borderline clinical depression
	21-30	Moderate depression
SEVERE	31-40	Severe depression
	Over 40	Extreme depression

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Dissemination

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3. B.P. Nayak, G.R. Satpathy, and B Maharathi. Detecting the genesis of fatigue in trained drivers by studying the variation of blood biochemical parameters under simulated driving condition. *Int. J Biol Sci. Engg.*, 2(2): 323–326, 2011.

4. B.P. Nayak, A. Routray, and M.K. Gupta. Targeting serotonin linked pathways might abate the development of fatigue in prolonged monotonous tasks like long distance driving. *J Sports Med Phys Fitness* (communicated).

Conferences

1. B.P. Nayak, S. Kar, A. Routray, and A.K. Padhi. A biomedical approach to retrieve information on driver's fatigue by integrating EEG, ECG and blood biomarkers during simulated driving session. *IEEE International Conference on Intelligent Human Computer Interaction*, (IHCI-2012), Kharagpur, India. December 2012.

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