IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

SLEEP INDUCER USING ARDUINO

Mr.M.Manthira Lakshman*, Premkumar.G , Salman Parcey.A , Sayad Ismail.S.H
Department of Biomedical Engineering
Sri Manakula Vinagayar Engineering College, Puducherry, India.

ABSTRACT

Sleeping difficulty called insomnia, can involve difficulty in falling asleep one who has first go to bed at night, waking up too early in the morning and waking up often during night. The lack of restful sleep can affect your ability to hold out daily responsibilities. All types of insomnia can cause day time drowsiness, poor concentration, and therefore the inability to feel refreshed and rested in the morning. Magnetic flux related to the plannet is termed geo-magnetic fields. It is essentially dipolar on the earth's surface. Many of us experience sleeping well within the natural surroundings into a tent or a wooden hut. This fact is because of not only to the healthy atmosphere but also from our unconscious ability to perceive natural earth's magnetic fields. Our paper is about this sort of geo-magnetic –fields. This has been designed a circuit, which radiates an electromagnetic field which is low frequency through a radiator coil and our aim is to perceive them, in this manner our brain is surrounded by a perfect environment for a sound sleep.

1. INTRODUCTION

A large number of Indian soldiers are posted at Siachen Glacier (5,400 m) region. Apart from soldiers, many mountaineers and residents are also exposed at high altitude (HA) environment. Survive at any extreme condition is very difficult due to many environmental adverse condition. At HA, human body mainly affected by low level of oxygen in blood due to low barometric pressure (Choudhary and Choudhary 2009). The state of sub-optimal O2 availability due to reduced ambient barometric pressure is termed as hypobaric hypoxia (HH) which affects normal brain activity. Oxygen is a good acceptor of electron in the cell. Low level of oxygen causes accumulation of electrons into cells leading to increase in level of free radicals. Bakonyi and Radak 2004 have previously reported that HA exposure decrease the antioxidant in the brain. Thus imbalance of free radical and antioxidant causes oxidative stress that causes massive cells death. Decreased the accessibility of oxygen at central nervous system becomes a cause of worsen sleep quality, cognitive deficit, physiological problem, emotional changes etc. Brain is less responsive and progressively passive with outside world; however, it does not totally switch off during sleep and requires a high amount of oxygen with other nutrients for proper functioning (Mergenthaler et al., 2013).

At HA, chemoreceptors are frequently activated due to hypercapnea and hypocapnea cycle. Further, apnea is generated via activation of arousal system via chemoreceptor activation. It is well documented that sleep alteration at altitude is mostly due to respiratory disturbance emerging from the physiologic ventilatory dilemma of acute ascent, where stimulation by hypoxia alternates with inhibition by hypocapnic alkalosis (San et al., 2013). Ohi et al., 1994 noted that hypocapnia generated by voluntary hyperventilation in persons at low altitude induces sleepiness. Although researchers also found alterations of sleep regulatory enzymes and monoamines level in different brain region (Ray et al., 2011). Over all worsening of sleep quality at HA is not only due apnoea. he beneficial outcomes of good sleep quality on cognitive performances are well established. Whereas, fragmented sleep cause cognitive alterations and it adversely affects the performance in human beings (Alhola and Kantola 2007). Slow wave sleep (SWS) or non rapid eye movement (NREM) sleep is necessary for the strengthening of synaptic connections which increases the spatial memory consolidation resulting in Chapter 1: Introduction 2 improved cognitive performance by replaying the hippocampal neuronal firing (Stickgold and Walker 2007). Evidences in both animal (Bjorness et al., 2005) and human models (Varga et al., 2014) suggest that rapid eye movement (REM) sleep may also be responsible for spatial navigational memory by phosphorylation at Ser133 of CREB (cAMP response element binding) which is a key molecule for both short-term and long-term memory formation (Chen et al., 2010). Memory consolidation is a protein synthesis-dependent process which stabilizes new memories and provides resistance against traumatic and pharmacological disruptions (Hernandez et al., 2008).

Some neuropeptides naturally present in our body which have sleep initiation and maintenance effects (Richter et al., 2014). Delta sleep inducing peptide (DSIP) has been reported as an endogenous sleep promoting substances present in animals and human brain, as well as peripheral system (Nagasaki et al., 1980; Schneider-Helmert et al., 1981; Kimura et al., 1989). DSIP is an amphiphilic nonapeptide which influence HPA axis (Schoenenberger et al., 1987). It is also found in pituitary cells containing ACTH, alpha-melanotropin, in adrenal medulla, mammary gland, plasma, cerebrospinal fluid and gastrointestinal tract (Charnay et al., 1989 Iyer & McCann, 1987, Bjartell et al., 1988). Expression of DSIP positive neurons in different brain region has been reported. Generally, its level is high during initiation of sleep phase and its level changes according to circadian rhythm and seasonal variation. It is

found that during different sleep disorder DSIP level was high in human patients (Westrin et al., 1997). Furthermore, DSIP level is found decreased in obstructive sleep apnea patient when they are treated with continuous positive airway pressure (CPAP) (Becker et al., 1989). DSIP like immunoreactivity is also found to be high in major depressive disorder (Westrin et al., 1997). In control persons, DSIP level is low during SWS sleep and markedly suppressed during REM sleep (Friedman et al., 1994). Altered DSIP level may be associated with HPA axis disturbance during different stress condition. Leshch et al., 1988 found a positive correlation between DSIP-LI and serum cortisol.

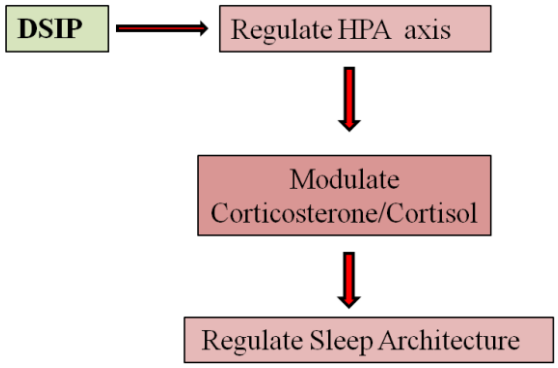

Figure 1.1: Probable schematic diagram of sleep quality regulation during stress (Westrin, Ekman and Bendz 1997)

Previous studies have reported that neuropeptide and modulators that initiate and maintain sleep show higher concentration in sleep deprived or sleep disorder patients. In these conditions DSIP might have played a proactive role in initiation of sleep (Kastin AJ 1982). During chronic simulated HA exposure sleep quality is markedly reduced. There is an immense possibility that DSIP may be responsible for poor sleep quality at HA beside other reported causes. Hence, individuals having high DSIP level as compared to others during normal condition might have better resistance during stress condition. DSIP administration continuously for one week in chronic insomniacs has shown to improve sleep architecture (Helmert 1986). Although higher level of DSIP is found in different sleep disorder and stress conditions but exogenous injection of DSIP during this period might have an effective role in sleep quality improvement without any side effect and further cognitive performance. Sleep inducing effect of DSIP was also confirmed by using μ opiate receptor and 5HT receptor antagonist (Young and key 1984). These experiments were suggested that the sleep inducing effects of DSIP was also played through these two receptors. Phosphorylated delta sleep inducing peptide (p-DSIP) is an analogue of DSIP which has six times greater effects than DSIP and has a long-lasting sleep-promoting effect on rats without having any psychological, physiological and biochemical side effects (Nakagaki et al., 1988). Injection of p-DSIP in rats increase slow wave sleep, although some studies suggest that DSIP also improves paradoxical sleep (Nagasaki et al., 1980; Schneider-Helmert et al., 1981; Kimura et al., 1989). The levels of DSIP in plasma have been found to be high in different sleep disorder patient and after sleep deprivation. This peptides help to create sleep pressure which further help initiation of sleep. DSIP has effects other than sleep inducing as it binds with NMDA, alpha1 adrenergic receptors, opioid receptors (Markus, Guido, 1987; Sudakov et al., 2004). In normal brain, it plays many important functions like a sleep promoting, antioxidant agent, anti-ageing, antihypoxic, anticonvulsion property (Nakamura et al., 1989). DSIP further possess strong anti-stress property because it inhibits HPA axis during chronic stress. It has shown to influence the activity of some mitochondrial enzymes in the rat brain [i.e. MAO A, hexokinase, creatine kinase, malate dehydrogenase] and it also inhibits lipid peroxidation which is especially pronounced under stress conditions (Khvatova et al., 1995, 2003). Presence of DSIP positive neurons in hippocampus increases the curiosity about its role in cognitive performance. Effect of DSIP on hippocampus dependent memory has not been investigated previously. Regulation of stromal stem cell adipogenic and osteogenic differentiation by DSIP indicate the possibility of DSIP in neurogenesis regulation and its neuroprotective role.

2. RELATED WORK

2.1. High altitude hypoxia

Air is a combination of gases and the principle gases are oxygen and nitrogen whose total partial pressures equivalent to the barometric pressure. With increasing altitude, the barometric pressure decreases exponentially, however the percentages of gaseous components remains unvarying. The percentage of oxygen is constant in inspired air (20.93%) at various altitudes. The decrease in atmospheric pressure at HA reduces the partial pressure of inspired oxygen and hence the driving pressure for gas exchange in the lungs (Peacock, 1998). The state of sub-optimal O2 availability due to decreased ambient barometric pressure is termed as HH which affects normal brain activity (Choudhary & Choudhary, 2009). To maintain the oxygen demand sufficient quantity of Oxygen must continuously be transported from the air to mitochondria, because of the atmospheric low pressure of PO2 at HA; gradient driving of oxygen transport at this higher point is comparatively less than at sea level. HA environments poses multiple challenges to the organism that inhabit them, including cold temperature, low humidity, ultraviolet rays and HH. A massive number of mountaineers and defence personnel reported a different type of physiological and psychological problem during chronic HA exposure.

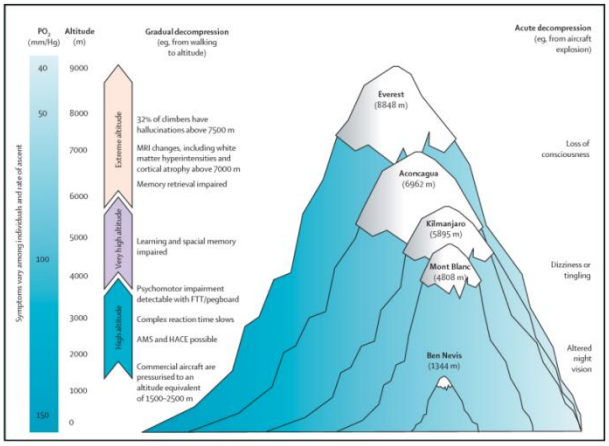

Figure 2.1: Schematic representation of different Neurological consequences at different altitude (Imraya et al., 2010).

HA is a severe risk factor which is responsible to develop acute mountain sickness (AMS), high-altitude cerebral edema (HACE), high-altitude pulmonary edema (HAPE), and other altitude-related problems. AMS is the initial symptoms of mountain illness, but HACE and HAPE are life-threatening (Imray et al., 2010, Powell & Garcia, 2000). Altitude illness mainly occurs above 2500 m but has been documented even at 1500– 2500 m. Level of adaptation occurs up to a particular limit; above 8,000 metres (26,000 ft) is called "death zone", where it is generally believed that no human body can acclimatize (Darack, 2002; Huey &Eguskitza, 2001).

2.1.1. HA hypoxia affects physiological system

Two different O2 sensors are known: Chemoreceptors involved in the ventilation and erythropoietin secreting cells (Barnard et al., 1987; Bisgard, 2000). The basic aim of these two sensors is the same, to increase the availability of O2 to the cells. The O2 sensitive events arise at different organisational levels in the body. At organism level through an increase in alveolar ventilation involving interaction of chemoreceptors, the respiratory control centres in the medulla and the respiratory muscles and the lung/chest wall systems; at tissue level, blood flow optimization done through the constriction of pulmonary vascular smooth muscle and coronary and vasodilatation of cerebral vessel; at cellular level, releases different neurotransmitters released by the glomus cells in the carotid body, erythropoietin hormone released by kidney and liver cells and secretes of vascular growth factors (VGEF) by parenchymal cells in different tissues; at molecular level there is expression/activation of an bunch of genes redirecting the metabolic and other cellular mechanisms to achieve increased cell survival during hypoxic exposure (Sarkar et al., 2003; Patinha et al., 2017). The compensatory hyperventilation, tachycardia, erythropoietin-induced polychythemia and increased cerebral blood flow can partially maintain cerebral oxygen delivery at HA. Carotid body is the primary organ which sense hypoxia and kidney is essential for physiological adaptation, when these organs are over-activated; contribute to cardiovascular disease due to positive cross-organ interactive feedback mechanisms. Signals surge from the hypoxic kidney trigger the carotid body that acts cooperatively to ensure sustained (and in the end aberrant) long term sympathoexcitation. Renin angiotensin system which is further activated in both organs in response to low blood flow/hypoxia. This chronic low blood flow/hypoxia together with the triggering of renin angiotensin system generates a non-functional positive feedback loop that leads to tissue damage. Increasing the renin angiotensin system will lead to activation of different pathways to ensure proper oxygen delivery, including hypoxia inducible factor (HIF) and erythropoiesis, that may also contribute to the dysfunctional sympathetic activation in hypertension (Figure. 2.2). It is also observed that exposure to HA induced HH can generates alteration in motor skills, mental efficiency and mood states (San et al., 2013a, 2013b; Lowe at al., 2007). Overall the exposure to HA induced HH has negative effects on brain functions and thus on the physical, mental and emotional states of individuals which ultimately reduces their work capacity and may result in an inability to work efficiently during their stay at HA.

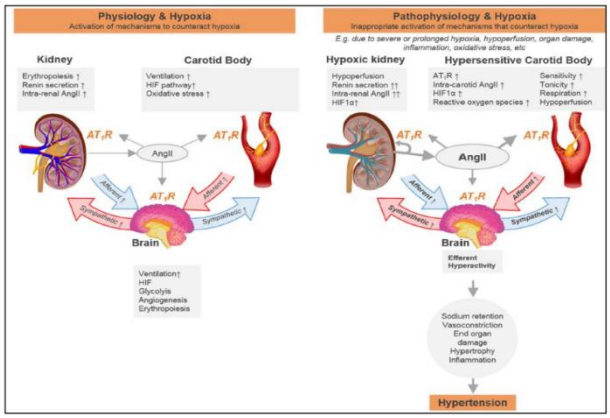

Figure 2.2: Schematic representation of the hypothesis of physiological adaptation during chronic hypoxia (Patinha et al., 2017).

2.2. Brain at HA hypoxic condition

The brain weight is less than 2% of body mass but it consumes 20% of the basal oxygen which is entirely used for the oxidation of glucose. This ability is to process large quantity of oxygen over a relatively small tissue mass which is needed to support the high amount of adenosine triphosphate (ATP) production to sustain an electrically active prone state for the continual transmission of neuronal signals (Lutz et al.,2003). In the brain, ATP concentration falls dramatically just after a minute of anoxia which can result in devastating consequences and often prove fatal. There are three compatible discoveries from years of exploration on the result of hypoxia on the brain. First, during sever level of hypoxia whole human brain oxygen utilization remains same. In order to conserve this equipoise, a correspondent accelerate in blood flow has to occur. Second, in spite of no change in oxygen consumption, hypoxia increase glucose utilization and lactate making indicate acceleration in glycolytic flux by nerve cells, but utilization is suppressed during prolonged hypoxemia while oxygen consumption remains the identical. In clinical condition of severe ischemia, the hippocampus, white matter, lateral geniculates and superior colliculus seem especially sensitive to levels of oxygen. The acceleration of brain lactate levels in prior stages of hypoxia is counter-balanced by an enhancement of bicarbonate which upshot in a near normal pH. Third, brain tissue concentrations of ATP, ADP and AMP which is the markers of the energy state of the tissue remain close to normal even during extreme hypoxia, comparable to altitude above 8000m (Dhillon, 2009). While the brain is most sensitive to a reduce in oxygen delivery, i.e. blood flow and glucose, examples from nature demonstrate some species‟ phenomenal resistance to profound hypoxia, such as the turtle (Pérez-Pinzón et al., 1992), the harbor seal (Kerem& Elsner, 1973), and high altitude birds (Faraci&Fedde, 1986; Faraci, 1986).

2.2.1. Ionic changes in brain during HA hypoxia

Altered ion homeostasis during hypoxia clearly occurs though it is still unclear whether the ionic alteration is primary, or they are due to changed oxidative or neurotransmitter metabolism. Calcium homeostasis alteration is common changes during hypoxia. For example, very low level of oxygen diminish calcium uptake at synapses. The diminish level of calcium in the endoplasmic reticulum is a critical factor in cerebral dysfunction in hypoxic environments (Paschen&Doutheil, 1999). Intracellular levels of potassium are accelerated during severe hypoxia. There is accumulation of free radicals which responsible for further damages, especially to the capillaries. Neurotransmitter metabolism is hypothesized to be sensitive to hypoxia although there is conflicting evidence about which transmitter or metabolic step is most sensitive. There is exhibit that acetylcholine synthesis by brain is oxygen-dependent as is the biosynthesis of amino acid neurotransmitters.

2.2.2. Hypoxia and vascular endothelial growth factor (VEGF)

Hypoxia-induced VEGF is known to induce fluid drain from capillaries in the brain (Fischer et al., 1999; Schoch et al., 2002), an effect known to happen with acute hypoxic exposure. The stimulus of the brain to prolonged hypoxia and ischemia is one of survival which takes the forms of angiogenesis and neuroprotection respectively. The beginning of these events depends on the stimulation of the transcription factor, HIF-1 (Semenza, 2000). HIF1α targets mRNA genes and initiate the induction of the VEGF gene which, along with the synergistic outcome of glycolytic metabolism, promotes angiogenesis in the insulted cerebral tissue (Bergeron et al., 2000; Marti et al., 2000). Several weeks exposure to hypoxia, rats exhibit an immediate and prolonged enhancement in HIF-1α (Chávez et al., 2000) which triggered VEGF. This together with other growth factors responsible for angiogenesis and vascular remodelling which further increases the capillary density and maintenance of oxygen delivery (Boero et al., 1999; Dor et al., 2001; Pichiule& LaManna, 2002; LaManna et al., 2004).

2.2.3. Hypoxia and the electroencephalogram (EEG)

Hypoxia interfere the synaptic communication in brain and membrane polarization with the normal electrical activity of the brain and alter the EEG. In cats the arterial PO2 is gradually decreased from 80 to 20mmHg, the EEG amplitude initially accelerate slightly and then slow waves and sharp spikes appear (James S Milledge, John B West, 2007). Subsequently, the slow waves decrease in amplitude and then disappear. The primary activation which is followed by depression may be the result of hypoxia on the reticular activating system. Since acute exposure to HA results in nhanced cortical activity on EEG, HA exposure may accelerate susceptibility to seizures by reducing the threshold for the initiation of epileptic discharge (Daleau et al., 2006; Basynat et al., 2000), but this theory is debated by many who suggest patients with seizure disorders who wish to go to HA.

2.2.4. Hypoxia and evoked potentials

Evoked potentials are also changed by hypoxia. Brain-stem auditory response is suppressed by decrease levels of oxygen. Visually evoked potentials are primarily enhanced and then suppressed as the level of oxygen is decreased.

2.3. Sleep

Sleep is an auto-regulatory global phenomenon. Experiments on sleep in drosophila indicate that sleep is a whole-organism phenomenon assist by global changes in neural activity (Wu et al., 2010). Globally in the sense that sleep and vigilance in a control animal seems to appear from the dynamic interaction of neuronal network all over the brain. Theories regarding sleep in day to day life started with the common thinking that the brain is active during awake period and it gets tired at the end of the day and then goes to sleep. According to passive theory, sleeping sickness is the basic concept which reported by Economo in 1916. Discovery of new phase of sleep or REM sleep changes the era of passive theory in the year of 1953. When it was realized that brain function is only slightly decreased during sleep, the passive theory of sleep was replaced by the active sleep genesis concept. Kumar, 2010 gave

his opinion that Sleep is neither the results of an active mechanism nor the product of a passive process. The propensity for sleep-wake oscillation is derived from a collective feedback of the several neurons with oscillating membrane potential. Normal alteration and expression of sleep-wakefulness are influenced by several factors including external input and internal feedback.

3.METHODOLOGY

Faradays law of electromagnetic induction is the main principle of this device. Which is a introductory law of electromagnetism prognosticating how a glamorous field will interact with an electric circuit to produce an electromotive force(EMF) a miracle called electromagneticinduction. The brain is always generating a pattern of internal neural frequentness, so called nascence, theta, delta, and beta; names for different ranges of frequentness, plus others, some of which are altered by the patterns of electromagnetism in our terrain. Radio swells, cell phone broilers, television, and general noise from electric circuits also induce electromagnetic frequentness. The nanosecond electromagnetic patterns of the Earth are also a part of the terrain The world is girdled by glamorous fields some generated by the earth's captivation, others generated by the brain is always generating a pattern of internalneural frequentness, so called nascence, theta, delta, andbeta; names for different ranges of frequentness, plusothers, some of which are altered by the patterns ofelectromagnetism in our terrain. Radio swells,cell phone broilers, television, and general noise fromelectric circuits also induce electromagneticfrequentness. The nanosecond electromagnetic patterns ofthe Earth are also a part of the terrainThe world is girdled by glamorous fields some generated by the earth's captivation, others generatedby solar storms and changes in rainfall. Glamorousfields are also created by electrical bias(e.g.motors, boxes, office outfit, computers,microwave oven ranges, electrical wiring in homes, powerlines). Indeed the mortal body produces a subtleglamorous fields generated by chemical response withincells and ionic currents of the nervous system. Anelectromagnetic field(EMF) is composed of both anelectric and a glamorous field. The electric field is dueto the presence of charged patches(similar as electrons)and the glamorous field is due to the movement of thecharged patches(similar as an electron current).lately, scientists discover that external glamorousfields affect the body's functioning in both differentways.

PROCEDURE

- elect atiming option by means of the rotary switch SW1.
- Choose 15, 30 or 60 minutes'operation.
- Select " Stop " or " Alternate " mode operation by means ofSW2.
- With SW2 closed(Stop mode operation) the electromagnetic radiation stops after the preset time is elapsed.
- With SW2 opened(Alternate mode operation) the device operates for the pre-set time, also pauses for the same quantum of time, this cycle repeats indefinitely.

The cited gadget above is the use of a pulse oximeter to realize whether sleep apnea incident is it happens, in case it befell the form and peak of the pillow will be mechanically adjusted to mitigate the sleep apnea incident. Unlike the proposed solution, the proposed answer is detecting person snoozing sample and every other element that may have an effect on person sleep quality. From the data that collects via the sensor node, this records will be analysed and radically change to drowsing chart and some of the advice that can be let person enhance their dozing high-quality the usage of clever pillow software on their smartphone. The Mimo recorder that let mother and father of the new child infant make a recording of their heartbeat and it is a white cubical box. A heartbeat photoplethysmograph (PPG) is connected to the facet of the box. The the front of the container contained a quantity of LEDs that indicated the fame of the recording procedure and there have a button to begin up the recording process. The core of the Mimo recorder is an Arduino Uno microcontroller, and it's supplied by way of a 9-V battery. The Arduino Uno has carried out almost the complete recording stages, from sensing from parent's coronary heart fee and switch to transmission of the heartbeat to the pillow. To make sure the intense portability to mother and father for their determination of a recording environment, it used to be purposely chosen to use a battery as a substitute than a constant strength supply. The technique of the usage of Mimo recorder is the usage of heartbeats of the moms of a new child child had been recorded into the Mimo recorder. The specific sign was once uploaded to the Mimo pillow. After that, change in the Mimo pillow it will be felt rhythmic vibrations on the floor of the Mimo pillow. After infant diaper exchange earlier than their feeding guardian can use Mimo pillow positioned on the chest of the babe.

3.6. Sleep recording and analysis

After 21 days of post-surgical recovery sleep recording was conducted from telemetry implanted rats during normobaricnormoxic condition. After seven days recording of Chapter 5: Materials and Methods 41 sleep pattern during normoxia rats were exposed at HA. Hypoxia chamber was made as per compatible with EEG recording by help of technicians. EEG and EMG were recorded from freely moving rat during chronic HA condition. Neuroscore 2.0 CNS analysis software (DSI, USA) was used form analyzing the acquired EEG and EMG signals. Different sleep stages i.e wake, NREM 1, NREM 2 and REM sleep were scored by Neuroscore (DSI, USA) automated sleep scoring module and further corrected manually using standard guidelines for every 10 Sec epoch (Mallick et al., 2001).The relative power of delta (0.5-4Hz), theta (4-8Hz), alpha (8-12Hz) and beta (15-20 Hz) wave from frontal cortex in every epoch was extracted in MS Excel shit and did further analysis.

3.7. Single cell preparation from different brain tissues

Experimental animals were euthanized by a standard dose of urethane (1.2 g/kg body weight). Whole brain was extract from skull and collected into ice chilled PBS with 2% BSA solution. Hippocampus and brain stem were isolated by help of brain matrix and stored in ice chilled PBS with 2% BSA solution.

Single cells were prepared from rat brain by using trypsin based neural tissue dissociation kit (Miltenyi Biotech, Germany) (Pennartz et al., 2009). Brain tissue was collected into HBSS buffer and transferred into tube which contained enzyme mix solution 1. The tube was placed onto the gentle MACS dissociator (Miltenyi biotech, Germany) and incubated with rotation (10rpm) at 37°C for 15min. Then, added the enzyme mix solution 2, and incubated at 37°C for 10min. After centrifugation, samples were collected from the bottom of the tube and passed through 30µM cell strainer. Cells were washed twice with 1X PBS (containing 2% BSA) and subjected for staining (Ghosh et al., 2017; Das et al., 2018).

3.8. Flow cytometry

Hippocampus and brain stem were isolated from experimental animals and subjected to single cell isolation according to the manufacturer‟s instructions (MiltenyiBiotec). Single cells were fixed in ice cold 4% PFA for 20mins followed by two washings. Then cells were again suspended in the binding buffer (1× PBST+2%BSA) and incubated times washing with PBS (3000rpm for 7 min at 4 ºC) and the cells were re-suspended in PBS. According the host of primary antibody, secondary antibodies were selected and incubate for two hr at room temperature. Unbounded secondary antibody was washed by follow the same washing procedure (3000rpm for 7 min at 4 ºC). After washing, 50µg/ml Propidium Iodide was added and analyzed using BD Accuri C6 software and analysed (1, 00000 events) using a flow cytometer (BD FACS Calibur) with Cell Quest Pro software.

3.9. DSIP level measurement from rat tissue and plasma by ELISA

DSIP level was measured from plasma and different brain region of rat by using DSIP ELISA kit from Sincere company (Cat No.E12410725, 96T).This ELISA kit is used for the vitro quantitative determination of rat DSIP in plasma and tissue lysates. The detection range of this kit is 10ng/ml-0.156ng/ml and sensitivity is

3.10. Neuronal pyknosis

Nissle staining was performed using Cresyl Violet staining used for detecting of pyknotic neurons. Nissl granules are the extra nuclear RNA. Cresyl violet is an aniline dye used to stain RNA and highlight the neurons. Due to staining of ribosomal RNA by Cresyl Violet, a nissle substation (rough endoplasmic reticulum) appears the dark blue colour. Cresyl Violet staining was prepared by adding 0.1 g Cresyl Violet in 100 ml of water. Few droplets of acetic acid were added in water with continuous heating for batter dissolving of the Cresyl Violet and then dissolving solution was filtered by whatman filter paper. After taking the section on slide it was washed by distilled water for three times and then stained in 0.1% Cresyl Violet (Sigma, USA) for 2-3 minutes. Sections were thoroughly washed by distilled water and kept it in for air dry. After completely dried, the sections were dehydrate by graded alcohol i.e 50%, 70%, 90% and 100% ethanol alcohol for 3 minutes. D.P.X was used to mounted the sections and stored it in room temperature until observe under bright filed microscope.

3.11. Pharmacological intervention

p-DSIP (Biochain, USA) and naloxone methiodide (Sigma-Aldrich, USA) were freshly dissolved in sterile physiological saline solution (0.9%). p-DSIP (10µg/kg bw) was administered intra-peritoneally during HH exposure at around 18.30h daily. Naloxone (Nal) was given intraperitoneally in HH+p-DSIP+Nal group 10 mins prior to injecting p-DSIP.

3.12 Statistical analysis

All experimental results were represented as mean ± SEM. Statistical analysis was done using one-way repeated measures Analysis of Variance (ANOVA) with Bonferroni post hoc analysis. To analysis the results of incorporation of mesenchymal stem cells (MSCs) into Functional neuronal network, DSIP level measurement in two groups and neurodegeneration study in two different groups unpaired Student t-test was used. A Pearson‟s correlation study was also done. A value of $p \leq 0.05$ was considered statistically significant. GraphPad Prism for windows 10 was used for all statistical analysis (GraphPad Software, San Diego, California, USA).

4. CONCLUSION AND FUTURE WORK

Good quality of sleep is very necessary for healthy life. Different stress conditions alter the sleep architecture and cognitive performance. Better acclimatization procedure or rapid acclimatization helps to minimize the stress intensity. The exact cause of worsening sleep quality is still unknown. Increase arousal activation and alteration of sleep regulatory monoamines also play important role in altered sleep quality. Different probable cause of cognitive performance alteration has been reported by many researchers but whether sleep also plays any role in maintaining cognitive performance is still enigma. By using of some benzodiazepine and non-benzodiazepine drug at high altitude slight improvement in sleep quality has been found but these drugs failed to improve cognitive performance.

Some neurotransmitter and neuropeptide regulate different stages of sleep. Neuropeptide help in initiation and maintenance of different stages of sleep. DSIP, a neuropeptide induces deep sleep and helps in initiation of sleep. This peptide has strong anti-stress and anti-hypoxic action without any side effect. Previous studies using DSIP report that during sleep problem and stress conditions this peptide has been found to be increased suggesting that exogenous injection of DSIP plays an antistress role. This study explores the possible effect of sleep on spatial navigational memory improvement at HA and role played by p-DSIP as a countermeasure against the effect of chronic HA exposure on altered sleep quality and cognitive performance.

Major findings of the present study are as follows:

- Different stages of sleep (NREM2 and REM) were severely affected with decrement in percentage of total sleep time during chronic exposure to simulate HA, although NREM1 was increased as compared to the control group. Plasma DSIP was found to be significantly increased after chronic HH. In different brain regions DSIP levels were differentially altered during chronic HH stress. In BS region DSIP level was significantly decreased. The same pattern was observed in hippocampus although it was not significant. Hypothalamus showed increased level of DSIP, although in cortex we did not notice any significant alteration. Brain biogenic amines were altered in hypothalamus and brain stem region which are very essential for regulation of sleep. Simultaneously, hippocampus dependent memory was affected after chronic HA exposure with decrease of CREB phosphorylation, which is necessary for new memory formation. More number of pyknotic cells and Caspase 3 expressed neurons were found in different region of hippocampus (DG, CA3, CA1) in exposed group compared to NN condition. Overall, chronic HH exposure causes poor sleep quality and DSIP level alteration in sleep promoting areas of brain. Alteration of spatial navigational memory was found which may be due to decrement of phosphorylation of CREB due to poor sleep quality.

- Exogenously injected p-DSIP (10µg/kg bw) was found effective in improving sleep architecture and spatial memory with partial restoration of brain biogenic amines and sleep regulatory enzymes, as well as normalized the DSIP level in sleep inducing area. Exogenously injected p-DSIP may fulfil the demand of p-DSIP level for initiation of sleep at chronic stress condition. Improved sleep quality restores the spatial navigational memory and increase of CREB phophorylation in hippocampus. Increase level of DSIP in hippocampus was found after p-DSIP treatment which may responsible to restores neurodegeneration during chronic HH.

- It was observed that when sleep inducing effect of DSIP was inhibited by naloxone, spatial navigational memory task performance was also impaired. p-DSIP may help to improve spatial navigational memory task performance at simulated HA by improving sleep quality, although other anti-stress function of DSIP also play important role to maintaining sleep and behavioural function.

- Synthesis of synaptic proteins is necessary for maintaining the newly formed memory. Decrement of pre and post synaptic proteins level was observed. BDNF level was also found to be decreased after chronic HA exposure. With p-DSIP, the levels of pre and post synaptic proteins and BDNF restored which may also be the reason for improvement of navigational memory task performance. p-DSIP also restored neurogenesis which was found altered during chronic HA exposure. Formation of new neurons are severely affected in any type of chronic stress but

- The reason for alteration of neurogenesis at HA was also investigated. Formation of new neurons depends on neuronal stem cells progenitors and MSCs incorporation in neurogenesis during chronic stress or in case of brain injury. Result show that CFSE tagged cells cross the BBB and enter into the hippocampus during chronic HA. It was confirmed that MSCs enter in to the hippocampus and participate in neurogenesis. Less number of MSCs homing and differentiation into hippocampus in p-DSIP treated group compared to only stress group was observed as a novel finding. Number of MSCs homing is dependent upon intensity of injury. Hence, this may be a cause of alteration of neurogenesis at chronic HA. p-DSIP with BMT significantly restored neurodegeneration in hippocampus compared to saline with BMT group, which indicates that p-DSIP decrease the neurodegeneration which may be the cause of less number of MSCs entry in hippocampus during chronic HA exposure.

5. REFERENCE

1. Abel, T., Havekes, R., Saletin, J. M., & Walker, M. P. (2013). Sleep, plasticity and memory from molecules to whole-brain networks. Current biology : CB, 23(17), R774-88.
2. Abel, T., & Nguyen, P. V. (2008). Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. Progress in brain research, 169, 97–115.
3. Abrahám, I. M., Meerlo, P., & Luiten, P. G. M. (2006). Concentration dependent actions of glucocorticoids on neuronal viability and survival. Dose-response : a publication of International Hormesis Society, 4(1), 38–54.
4. Ben Achour, S., & Pascual, O. (2010). Glia: The many ways to modulate synaptic plasticity. Neurochemistry International, 57(4), 440–445.
5. Adam, K., & Oswald, I. (1977). Sleep is for tissue restoration. Journal of the Royal College of Physicians of London, 11(4), 376–88.
6. Ahrberg, K., Dresler, M., Niedermaier, S., Steiger, A., & Genzel, L. (2012). The interaction between sleep quality and academic performance. Journal of Psychiatric Research, 46(12), 1618–1622.
7. Ahuja, S., Chen, R. K., Kam, K., Pettibone, W. D., Osorio, R. S., &Varga, A. W. (2018). Role of normal sleep and sleep apnea in human memory processing. Nature and science of sleep, 10, 255–269.
8. Alam S, Ray K, Jain V, Wadhwa M, Kumari P, Roy K, Chauhan G, Kishore K, Panjawani U, S. S. (2019). Reduced expression of Kalirin-7 contributes to working memory deficit during chronic hypobaric hypoxia exposure. Behav Brain Res.

9. Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. Physiological reviews, 89(1), 121–45.

10. Alberini, C. M., & Kandel, E. R. (2014). The regulation of transcription in memory consolidation. Cold Spring Harbor perspectives in biology, 7(1), a021741.

11. Alberini, C. M., & Kandel, E. R. (2015). The Regulation of Transcription in Memory Consolidation. Cold Spring Harbor Perspectives in Biology, 7(1), a021741.

12. Alhola, P., & Polo-Kantola, P. (2007). Sleep deprivation: Impact on cognitive performance. Neuropsychiatric disease and treatment, 3(5), 553–67.

13. Alkadhi, K., Zagaar, M., Alhaider, I., Salim, S., &Aleisa, A. (2013). Neurobiological consequences of sleep deprivation. Current neuropharmacology, 11(3), 231–49.

14. Anholm, J. D., Powles, A. C., Downey, R., Houston, C. S., Sutton, J. R., Bonnet, M. H., &Cymerman, A. (1992). Operation Everest II: arterial oxygen saturation and sleep at extreme simulated altitude. The American review of respiratory disease, 145(4 Pt 1), 817–26.

15. Anokhin, K. V, Ryabinin, A. E., & Sudakov, K. V. (2001). Expression of the c-fos gene in the mouse brain during the acquisition of defensive behavior habits. Neuroscience and behavioral physiology, 31(2), 139–43.

16. Aquino Lemos, V., Antunes, H. K. M., Santos, R. V. T., Lira, F. S., Tufik, S., &Mello, M. T. (2012). High altitude exposure impairs sleep patterns, mood, and cognitive functions. Psychophysiology, 49(9), 1298–1306.

17. Atherton, L. A., Dupret, D., & Mellor, J. R. (2015). Memory trace replay: the shaping of memory consolidation by neuromodulation. Trends in Neurosciences, 38(9), 560–570.

18. Bailey, C. H., Kandel, E. R., & Harris, K. M. (2015). Structural Components of Synaptic Plasticity and Memory Consolidation. Cold Spring Harbor perspectives in biology, 7(7), a021758.

19. Bakonyi, T., &Radak, Z. (2004). High altitude and free radicals. Journal of sports science & medicine, 3(2), 64–9.

20. Bakos, J., Zatkova, M., Bacova, Z., &Ostatnikova, D. (2016). The Role of Hypothalamic Neuropeptides in Neurogenesis and Neuritogenesis. Neural Plasticity, 2016, 1–10

21. Barnard, P., Andronikou, S., Pokorski, M., Smatresk, N., Mokashi, A., & Lahiri, S. (1987). Time-dependent effect of hypoxia on carotid body chemosensory function. Journal of applied physiology (Bethesda, Md. : 1985), 63(2), 685–91.

22. Basnyat, B., Sleggs, J., &Spinger, M. (2000). Seizures and delirium in a trekker: the consequences of excessive water drinking? Wilderness & environmental medicine, 11(1), 69–70.

23. Baud, M. O., Parafita, J., Nguyen, A., Magistretti, P. J., & Petit, J.-M. (2016). Sleep fragmentation alters brain energy metabolism without modifying hippocampal electrophysiological response to novelty exposure. Journal of sleep research, 25(5), 583–590.

24. Baumgartner, R. W., Spyridopoulos, I., Bärtsch, P., Maggiorini, M., &Oelz, O. (1999). Acute mountain sickness is not related to cerebral blood flow: a decompression chamber study. Journal of applied physiology (Bethesda, Md. : 1985), 86(5), 1578–82.

25. Bear, M. F. (1995). Mechanism for a sliding synaptic modification threshold. Neuron, 15(1), 1–4.

26. Beaumont, M., Batéjat, D., Piérard, C., Van Beers, P., Philippe, M., Léger, D., Savourey, G., et al. (2007). Zaleplon and zolpidem objectively alleviate sleep disturbances in mountaineers at a 3,613 meter altitude. Sleep, 30(11), 1527–33.

27. Becker, P. M.; Fuchs, E.; Jamieson, A. 0.; Brown, W. D. . R. H. P. (1989). Delta sleepinducing peptide in obstructive sleep apnea: Changes with nasal CPAP treatment-a pilot study. Sleep Res, (18:85).