J. Afr. Ass. Physiol. Sci. 6 (1): 38-45, July 2018

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Rapid eye movement sleep deprivation impairs hippocampus-dependent spatial memory: involvement of GABAergic receptors.

B.V. Owoyele*[1], I.I. Enaworu[1], A.A. Bayo-Olugbami[1] and G.O. Owolabi[2]

[1,2]Departments of Physiology, Faculty of Basic Medical Sciences, [1]University of Ilorin and [2]Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Keywords:
Rapid eye movement sleep, sleep deprivation, spatial memory, GABA receptors.
,

ABSTRACT

Background: This study examined the effect of rapid eye movement (REM) sleep deprivation on spatial memory in Wistar rats and the role of GABAergic receptors. **Methods:** A total of thirty-six age matched male Wistar rats weighing 170-200g were grouped into six (A-F) of six animals each. Group A served as control, Group B was sleep deprived, rats in groups C-F in addition to being sleep deprived for 72 hrs, received Diazepam (1mg/kg) (C), Baclofen (5mg/kg) (D), Phaclofen (1mg/kg) (E), Bicuculline (2mg/kg) (F) respectively. Spatial memory was accessed using Morris water maize (MWM) while motor performance (MP) and working memory were accessed using Y-Maze. Some serum biochemical parameters were also assessed. **Results:** These showed that spatial memory was decreased in the sleep deprived group compared with control ($p<0.05$). Diazepam increased, while Bicuculline decreased time latency in the MWM compared with sleep-deprived group ($p<0.05$). MP in the Y maze was significantly reduced ($p<0.05$) in all groups compared with control. Serum Creatine kinase (CK) and calcium as well as hippocampal CK were significantly ($p<0.05$) increased in the sleep-deprived group compared with control. **Conclusion:** REM sleep deprivation produced impairment in spatial memory in rats which is at least in part mediated by Gabaergic mechanisms with GABA A receptors being more implicated than the B subtype

.

INTRODUCTION

Sleep is a state of reversible unconsciousness in which the brain is less responsive to external stimuli. Although the functions of sleep are still poorly understood, the observation that sleep or, at least, an activity–inactivity cycle, is present in all species and that sleep deprivation leads to a drastic deterioration in various body functions, proves its importance. Sleep and brain functions are so intensely regulated that even a single night of sleep deprivation in humans could induce mood disturbance, fatigue, daytime lethargy and several forms of cognitive impairment (Van der Werf *et al*., 2009). Sleep loss and sleep disorders are common but appeared to be frequently overlooked. It is estimated that 50 to 70 million Americans chronically suffer from disorder of sleep and wakefulness which may hinder daily functioning and adversely affect health and longevity of the individuals. Loss of Sleep is a highly prevalent problem that continues to worsen in frequency as the age of the individuals advance. A study reported that at least 18 percent of adults suffer from insufficient sleep (Kapur *et al.,* 2002).

Available reports suggest that sleep has a key role in learning and memory, with post learning REM sleep playing a crucial role in the consolidation of newly acquired memories for long-term storage (Born *et al.,* 2006; Backhaus *et al.,* 2008). Supporting this, is the fact that the percentage of time spent in REM sleep is increased after certain learning tasks (Fu *et al.,* 2007), and REMSD after the training, produces an impairment in subsequent performance of the task thus reflecting the presence of memory deficit (Saxvig *et al.,* 2008).

Spatial memory which is a hippocampal dependent memory is the part of memory responsible for recording information about one's environment and its spatial orientation, has been shown to be impaired by sleep deprivation (Dorokhov *et al.,* 2012; Rahman *et*

*Address for correspondence:
Email: deleyele@yahoo.com
Tel: +2348035065190

al., 2013). Hippocampus-dependent memory formation and working memory are particularly sensitive to sleep deprivation as sleep-deprived mice failed to discriminate between the familiar versus novel arms of Y-maze, suggesting that hippocampus-dependent working memory was impaired (Hagewoud *et al.*, 2010).

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the Central Nervous System (CNS) and has been implicated in numerous forms of behaviour (Mohler, 2012). Administration of GABA-A agonists produces impairments in spatial memory acquisition and consolidation (Myhrer, 2003), while GABA-A antagonists and inverse agonists have been demonstrated to consistently enhance spatial memory acquisition and consolidation (Mceown & Treit, 2010). The data from investigations of GABA-B receptor function in learning and memory have however yielded conflicting results. In some studies, rats exhibited impaired acquisition and consolidation in several learning and memory tasks following the administration of baclofen (Stuchlik & Vales, 2009). However, a review of four studies investigating the effects of baclofen on the same passive avoidance task revealed that each study reported different results, including that baclofen improved, impaired, or did not alter performance. These differences may reflect the complexity of metabotropic-mediated inhibitory currents in simple learning and memory tasks (Myhrer, 2003).

The above reports on GABA and memory were not obtained in a setting of sleep deprivation. Therefore, this study explored the effects of these GABAergic drugs on memory in REMSD.

The focus on REM sleep as the sleep stage that supports memory has also been criticized by several studies revealing concomitant or even selective increases in non-REM sleep after the animal's exposure to enriched environments or other learning procedures. Non-REM sleep was even proposed as a factor that could explain REM sleep-deprivation- induced memory deficits and increases in non- REM sleep after fear conditioning correlated with the learned fear response on the next day (Barnes *et al.*,2011). In addition, in some cases learning decreased subsequent REM sleep (Mavanjii *et al*., 2003), and this could be accompanied by a concurrent increase in non-REM sleep (Malgoire and Catarelli, 2009) thereby preventing excessive sleep loss.

A series of experiments have shown that memory consolidation requiring the hippocampus is particularly sensitive to sleep deprivation. This was first shown using the Morris water maze, a task that can be designed to assess learning and memory formation that are either hippocampus-dependent (the hidden platform version of the task), or hippocampus-independent (the visible platform version of the task) (Morris *et al*.,1980). Although some studies have confirmed the finding that REM sleep deprivation impairs hippocampus-dependent learning in the Morris water maze task (Yang *et al*.,2008), others have challenged the idea that REM sleep is essential for spatial learning in the Morris water maze (Wang *et al*., 2009; Walsh *et al*., 2011).

From the on-going, it is obvious that a consensus is yet to be reached on the involvement of GABAergic receptors on the relationship between REMSD, learning and memory. Hence, the rationale behind this research.

MATERIALS AND METHODS

Drugs and Assay kits

The Cholinesterase, Calcium and Creatine kinase assay kits were products of Agappe Diagnostics, Switzerland. The GABA antagonists, Bicuculline and Phaclofen were products of Sigma Aldrich Louis, USA. The agonists, Diazepam was a product of Emzor Pharmaceuticals and Baclofen, a product of Neimeth Pharmaceuticals, Nigeria.

Experimental Animals and grouping

A total of thirty-six age matched male Wistar rats (weighing 170-200g) obtained from The Department of Biochemistry, University of Ilorin, were used in the study.

Animals were housed in cages at the animal holding of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin Nigeria under standard laboratory condition. They were maintained on a 12hour light-dark cycle, room temperature of 27°C and fed with standard rodent pelletized feed and water *ad libitum.* They were acclimatized to laboratory conditions for two weeks before the experiment. The animals were treated in accordance with the institutional ethical guidelines which agree with the internationally accepted principles for animal handling and care. Animals were randomly divided into six groups as follows:

Group A (Control): Administered tap water 2ml/kg b.wt and not sleep deprived

Group B (SDG): Administered 2ml/kg tap water and sleep deprived

Group C (SDD): Administered Diazepam 1mg/kg b.wt. (Pokk and Zharkovsky, 1995) and sleep deprived

Group D (SDB): Administered Baclofen 2.5mg/kg b.wt. (Stackman and Walsh, 1994) and sleep deprived

Group E (SDP): Administered Phaclofen1mg/kg b.wt. (Anderson and Tufik, 2004) and sleep-deprived
Group F (SDBi): Administered Bicuculline 2mg/kgb.wt. (Pokk and Zharkovsky, 1995) and sleep deprived
Where: SDG is Sleep deprived group (SDG); SDD is Sleep deprivation +Diazepam (SDD); SDB is Sleep deprivation + Baclofen (SDB); SDP is Sleep deprivation + Phaclofen (SDP); SDPi is Sleep deprivation + Bicuculline (SDBi).

After acclimatization, the rats were made to undergo spatial acquisition training using the Morris water maze before receiving the designated treatments. Following different drug administration, all the rats except the control group were immediately sleep-deprived for 72 hrs using the multiple platform method after which spatial reference memory and spontaneous behavioural tests were performed. Lastly, the rats were sacrificed humanely for assessment of some biochemical parameters.

Spatial acquisition training in MWM (Training):
Animals were trained to find a submerged platform in a Morris Water maze using extra maze cues (Morris *et al*., 1980). The water maze was a circular tank 136 cm in diameter and 60 cm tall filled with water (24°C ± 2°C) to a depth of 25 cm which was made opaque by the addition of milk. The white escape platform used for the spatial task was submerged to a depth of 1 cm from the water surface.

The animals were trained in a one-day protocol as described by Frick *et al.,* (2000) which consisted of three blocks, with each block comprising four trials that were performed on the training day. Thus, each animal participated in twelve trials, organized into three blocks of four trials with 30mins in between blocks. Each trial commenced by gently dropping the rat into the water maze. The rats were allowed to swim to the hidden platform. Each animal was given 60secs to locate the platform, upon which it remained for 10secs. If the platform was not located within 60secs, the animal was placed on the platform and then removed for the next trial to start immediately. Each trial was from a different start position/quadrant but the escape platform remained at the same position throughout the trials. Animals were returned to their home cages between trial blocks.
Immediately after the third block, the animals received their designated treatments and those in the control group were returned to their cages while sleep deprivation commenced immediately for the other groups.

Sleep Deprivation
The multiple platform method of sleep deprivation was used. Based on this technique, three rats were placed in a chamber with five small platforms (~6 cm diameter) surrounded by water as described by McDermott *et al*., (2003). On entering REM sleep, muscle tone diminished causing the animals to touch the water, which arouses them. Because the animals can move from platform to platform within the multi- platform chamber, this device has been reported to produce less immobilization stress compared with the widely used single small platform technique (Coenen & Van Luijtelaar, 1985; McDermott *et al.,* 2003). All treatments lasted 72h as studies have shown that the sleep-induced impairment of long term potentiation was maximal at 72h (McDermott *et al.,* 2003*).*

Morris water maze test:
Spatial reference memory was tested 72hours after the acquisition training in the MWM (i.e., after REMSD). Time latency to locate the submerged platform was noted. The platform was located at the same position it was during training (Frick *et al*., 2000).

Spontaneous Alternation Behaviour:
SAB is a test of cognitive function and spatial memory which is known to be sensitive to hippocampal damage was also assessed. The rats were placed at the centre of the Y-maze which had three arms for 6mins. The sequence of arm entry was noted and recorded. Arm entry was said to have occurred when the hind paws had entered the arm. The spontaneous alternation was defined as successive entries into the three different arms (A, B and C) on overlapping triplet sets and expressed in percentage (Rahman *et al*., 2010). Percentage alternation was calculated as:
% Alternation = [(No. of alternations) / (Total arm entries – 2)] X 100.
Tissue and blood Preparation:
The rats were sacrificed under isofluorane anaesthesia. The brains were removed and the hippocampus dissected out following descriptions in the rat's brain atlas (Paxinos & Watson, 1982). The hippocampal tissue was then homogenised in ice-cold 0.25M sucrose solution (Swann, 1988) at 4mls per gram of tissue with a metallised mortar and pestle. The homogenate was centrifuged at 12500xg for 15mins and the supernatant aspirated for analysis. The supernatant was stored at 0°C until analysis**.** Also, blood samples collected via cardiac puncture were allowed to clot and then centrifuged to obtain clear sera for biochemical analysis. For plasma, blood was collected in EDTA sample bottles and then centrifuged, the plasma was

separated from the remaining blood cells and kept at 0°C.

Estimation of plasma calcium concentration:

Plasma calcium was estimated using the modified O-cresolphthalein complex (OCPC) reagent and the absorbance was read photometrically at 580nm (Biggs and Morehead, 1974). Calcium OCPC procedure is based on Calcium ions reacting with O-cresolphthalein complex in an alkaline solution to form an intense violet coloured complex which maximally absorbs at 578nm. The two reagents were mixed in a ratio 1:1 and incubated with the samples for 5mins at room temperature after which the absorbance was read photometrically at 580nm against the reagent blank. The calcium concentration was then calculated using the following formula.

Calcium conc. (mg/dl) = Absorbance of standard/Absorbance of sample x10

Estimation of Creatine kinase activity:

Creatine kinase activity in the serum and the hippocampus were determined kinetically using the modified International Federation for Clinical Chemistry (IFCC) methodology (Witt and Trendelenburg, 1982). The two reagents (Creatine kinase (SL) R1 and creatine kinase (SL) R2) were mixed. The mixture was added to the sample after 25secs and incubated for 2minutes at 37°c. The absorbance per minute was determined photometrically per minute at 340nm and variation in absorbance was calculated.

The Creatine kinase activity was then obtained using the following calculation;

Creatine kinase activity (U/L) = (change in absorbance/60) x 4127

Estimation of Acetylcholinesterase activity:

Acetylcholinesterase activity in the hippocampus was determined using the new DGKC method. Cholinesterase catalyses the hydrolysis of Butyrylthiocholine substrate forming butyrate and thiocholine. Thiocholine reduces hexacyanoferrate-3 to hexacyanoferrate-2. The decrease in absorbance is proportionate to cholinesterase activity.

The two reagents were mixed at 37°c for 30secs and the sample was added to the mixture. Absorbance was measured per 30secs for 60secs at 405nm.

Cholinesterase activity was determined thus;

Acetylcholinesterase activity (U/L) = change in absorbance/60 × 22653.

Statistical analysis

Data were recorded as mean ± SEM. SPSS v.20 software was used to analyze the data. One-way analysis of variance (ANOVA) at a statistical significant level of $p \leq 0.05$ was set. Duncan post Hoc test was used to compare significant differences among groups.

RESULTS

Time latency of rats to locate platform in MWM during training:

Table 1 shows that there was no significant difference in mean time latencies to locate the escape platform between groups during training in the MWM.

Table 1: Mean time to locate escape platform during training in Morris Water Maze

Groups	Mean time latency (MTL) (secs)
Control	4.62±0.37
Sleep deprived (SD)	7.00±0.93
Sleep deprivation + Diazepam (SDD)	5.20±0.85
Sleep deprivation + Baclofen (SDB)	6.20±0.58
Sleep deprivation + Phaclofen (SDP)	5.60±0.97
Sleep deprivation + Bicuculline (SDBi)	5.80±0.92

Data are expressed as means ± SEM. n=6 rats in each group

Table 2: Effect of REMSD and GABAergic drugs on spatial memory during test in the Morris Water Maze (post training).

Groups	Time Latency (secs)
Control	6.09± 1.51
Sleep deprived (SD)	48.63±7.96[a]
Sleep deprivation + Diazepam (SDD)	66.41±7.69[a, b]
Sleep deprivation +Baclofen (SDB)	36.08±2.36[a]
Sleep deprivation +Phaclofen (SDP)	37.29±3.84[a]
Sleep deprivation +Bicuculline (SDBi)	12.33±2.95[b]

[a]$p<0.05$ is significant compared to the control. [b]$p<0.05$ is significant compared to the SD group. Data are expressed as means ± SEM. n=6 rats in each group

Table 3: Effect of REMSD and GABAergic drugs on Spontaneous Alternation Behaviour in Y maze

Groups	Spontaneous alternation (%)
Control	92.23 ± 3.36
Sleep deprived (SD)	74.52 ± 2.03[a]
Sleep deprivation+ Diazepam (SDD)	70.42 ± 3.73[a]
Sleep deprivation +Baclofen (SDB)	80.54 ± 6.42[a]
Sleep deprivation+ Phaclofen (SDP)	74.85 ± 7.29[a]
Sleep deprivation+Bicuculline (SDBi)	84.65 ± 5.40[a]

[a]$p<0.05$ is significantly different compared with the control. Data are represented as Mean± SEM. n=6 rats in each group.

Effect of REM sleep deprivation and GABAergic drugs on spatial reference memory in the MWM (post training):

As shown in Table 2 below, the mean time latencies (MTL) in all groups were significantly different ($p \leq 0.05$) from the control group. There was a

significant (p≤0.05) increase in the MTL of rats to locate the platform from (6.09±1.51) in control to (48.63±7.96) in the SDG. The MTL was also significantly increased in SDD (66.41±7.69) compared with the SDG (48.63±7.96) but it was significantly lower (p≤0.05) in SDBi (12.33±2.95) compared to SDG. Baclofen and Phaclofen both decreased the time latency to locate the platform but the difference was not significant (p>0.05).

Effect of REMSD and GABAergic drugs on SAB in the Y-maze:

In Table 3, REMSD significantly (p≤0.05) decreased the spatial working memory from (92.23±3.36) in control to (74.52±2.03) in SDG. This depicts that REMSD impaired spatial working memory in the Y-maze. All other groups were significantly (p≤0.05) different from the control group. Administration of a single dose of GABAergic drugs before the commencement of REMSD had no significant (p>0.05) effect on the sleep deprivation induced memory impairment.

Effect of REMSD and GABAergic drugs on serum and hippocampal Creatine kinase activities:

Fig 1 shows that sleep deprivation and SDD produced a significant (p≤0.05) increase in serum and hippocampal Creatine kinase activities compared with the control group. On the contrary, this activity (serum) was significantly (p≤0.05) reduced in the SDD, SDB SDP and SDBi groups compared with sleep-deprived group. Among these groups, it was lowest in the SDB and SDBi.

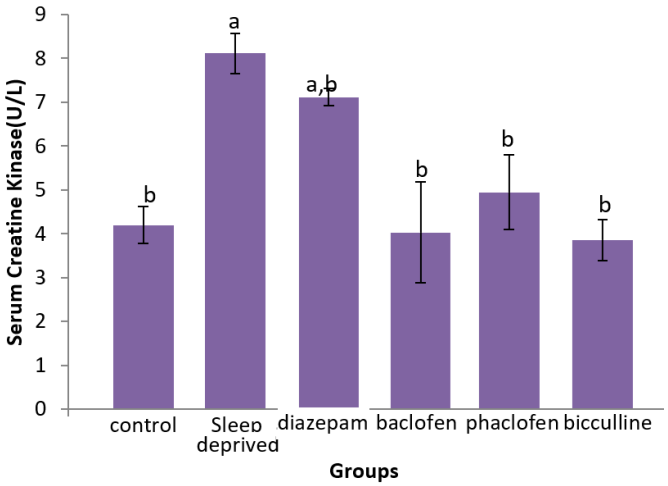

Fig 1a: Effect of REMSD and GABAergic drugs on serum Creatine kinase activity. Bars with the letter 'a' are significantly different from the control (p<0.05). Bars with the letter 'b' are significantly different from the SD group (p<0.05). n=6 rats in each group

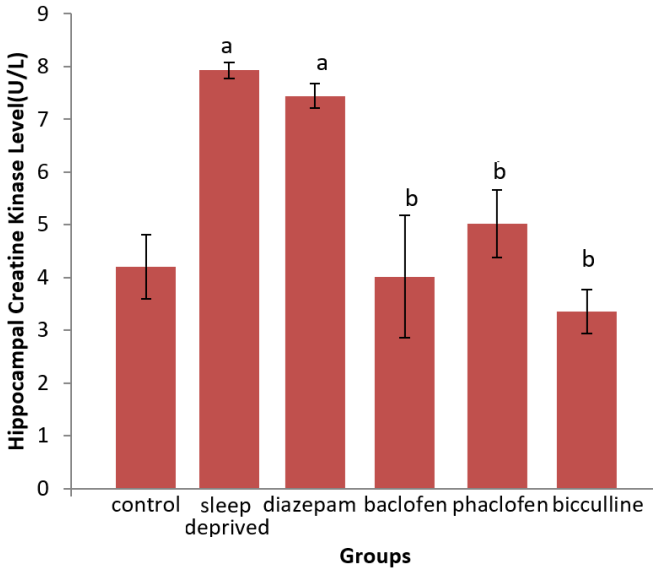

Fig 1b: Effect of REM sleep deprivation and GABAergic drugs on hippocampal Creatine kinase activities. Bars with the letter 'a' are significantly different from control (p<0.05). Bars with the letter 'b' are significantly different from the SD group (p<0.05).

Effect of REMSD and GABAergic drugs on plasma calcium levels:

Fig 2 shows that sleep deprivation produced a significant (p≤0.05) reduction in plasma calcium when compared with the control. Bicuculline and Phaclofen modulated this decrease and significantly (p≤0.05) increased Plasma calcium when compared with the sleep-deprived group. Diazepam and Baclofen both produced a significant (p≤0.05) increase in serum calcium when compared with both control and the SDG.

DISCUSSION

This study showed that post training Rapid Eye Movement Sleep Deprivation (REMSD) impairs spatial memory and this keeps with findings from previous studies (Born *et al.*, 2006; Backhaus *et al.*, 2008). This is probably due to the fact that the percentage of time spent in REM sleep is increased after certain learning tasks (Fu *et al.*, 2007), and thus REMSD after the training produced impairment in subsequent performance of the task.

The hippocampus is involved in memory consolidation and hippocampal damage has been associated with deficits in spatial memory (Pascal *et al.*, 2012). Acetylcholine (Ach) release has been shown to be increased in REM sleep, in fact previous studies have shown Ach release in REM sleep in the hippocampus is

about four times higher than the level in NREM sleep and twice the level in quiet waking (Marrosu *et al.*, 1995) and this may possibly explain why REMSD impairs memory as Acetylcholine is implicated in memory.

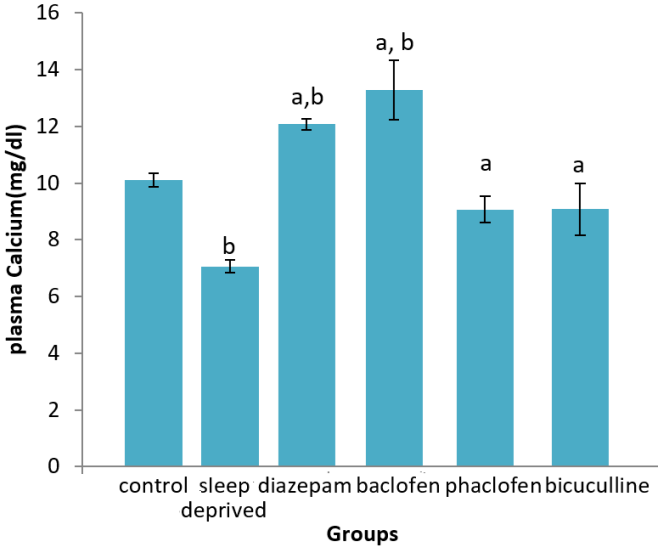

Fig 2: Effect of REMSD and GABAergic drugs on plasma calcium concentration. Bars with the letter 'a' are significantly different from the SDG ($p<0.05$). Bars with the letter 'b' are significantly different from the control ($p<0.05$)

REM sleep deprivation has been reported to impair hippocampal neurogenesis (Mirescu *et al.*, 2006) and this provides another probable explanation for its effect on consolidation of spatial memory.

GABA is the main inhibitory neurotransmitter in the central nervous system. It inhibits the release of other neurotransmitters by increasing the permeability of membrane to Chloride ions. This results in hyperpolarization and consequently reduces the generation of action potential. By this mechanism, GABA inhibits the release of acetylcholine in the CNS. It thus stands to reason that GABA agonists produce memory impairment while the antagonists enhance memory.

GABA-A agonists are used to induce sleep. At first glance it may seem contradictory that GABA agonists which induce sleep should impair memory as sleep is required for memory consolidation (Backhaus *et al.*, 2008). However, GABA-A agonists have been reported to suppress REM sleep and increase NREM sleep. Also studies showed that GABA level during NREM sleep was significantly higher than that during REM sleep and was also found to be significantly increased during prolonged periods of wakefulness (Vanini *et al.*, 2012).

The memory impairment produced by Diazepam which was significantly worse than that produced by sleep deprivation alone was probably as a result of potentiation of the effect of GABA. This is in line with findings of various studies which showed that GABA agonists produce impairment in memory (Chapouthier and Venault, 2002; Myhrer, 2003.).

GABA-A antagonists, although not in the setting of sleep deprivation have been reported to enhance memory consolidation (McNally *et al.*, 2008; McEown and Treit, 2010). Bicuculline probably enhanced memory consolidation by antagonising the effect of GABA and increasing the release of Acetylcholine thus countering the effect of sleep deprivation. Of note is the fact that Bicuculline improved memory significantly such that there was no significant difference in time latency to locate the platform when compared with the control. Reports on the effects of GABA-B agonists and antagonists on memory have been contradictory. In this study, Phaclofen and Baclofen both reduced mean time latency to locate the escape platform when compared with the sleep deprived group. However, the difference was not significant. In some studies, rats exhibited impaired acquisition and consolidation in several learning and memory tasks following the administration of Baclofen, (Myhrer, 2003; Stuchlik and Vales, 2009). However, Myhrer (2003) reviewed four studies investigating the effects of baclofen on the same passive avoidance task, and each study reported different results, including that baclofen improved (Georgiev *et al.*, 1988), impaired (Swartzwelder *et al.*, 1987), or did not alter performance (Car & Wisniewski, 1998; Kuziemka-Leska *et al.*, 1999). These differences may reflect the complexity of metabotropic-mediated inhibitory currents in simple learning and memory tasks.

The effect of Baclofen on memory is dose dependent. It impaired memory at high doses and enhance memory at low doses (Stackman and Walsh, 1994; Edward *et al.*, 2004). This probably explains why Baclofen at a dose of 2.5mg/kg in this study failed to produce memory impairment.

The increase in activity of creatine kinase in the sleep deprived group when compared with control is in consonant with the findings of (Backhaus *et al.*, 2008). Creatine kinase is required in the generation of Adenosine triphosphate from Adenosine diphosphate and the increased energy demand in a sleep-deprivation as implied by the weight loss noted despite increased intake, may explain its increased activity.

REM sleep deprivation reduced SAB in the Y maze, an observation which is in consonance with the report of Bridoux *et al.*, 2013. The apparent absence of an effect of the GABAergic drugs on SAB when compared with the sleep deprived group may be

explained by the fact that the single dose of GABAergic drugs administered prior to the commencement of sleep deprivation had an effect on consolidation of the previously acquired information from the water maze but was insufficient to have any effect on memory acquisition following 72hours REMSD. Continuous administration during or following the period of sleep deprivation may be required to investigate the effect of the GABAergic drugs on working memory in sleep deprivation.

Acetylcholinesterase which was unchanged in the hippocampus in this study is also in line with findings from previous reports. Elevation of this enzyme was however reported in the brainstem of REM sleep deprived animals (Benedito *et al*., 2001). This is probably as a result of a higher concentration of cholinergic neurones in the brainstem.

The decrease in serum calcium observed in the sleep deprived group when compared with the control has been attributed to an increase in Neurone Specific Enolase and S100 Calcium binding proteins which are observed in sleep deprivation (Benedicto *et al*, 2014). Mallick and Gulyani in 1996 showed that REMSD reduced the concentration of free synaptosomal Calcium in rats and postulated that this might be the underlying mechanisms for the cellular expressions and behaviour of neurones in REMSD.

The increase in calcium level in rats treated with diazepam as observed in the present study may result from decrease in aldosterone and catecholamines (Glodano *et al.*, 2003). Indeed, it has been shown that diazepam administration could decrease the activity of hypothalamus pituitary adrenal axis (Mikkalsen *et al*., 2005) and secretion of aldosterone and catecholamines (Glordano *et al.*, 2003). Smaller secretion of adrenal hormone may modify the membrane permeability for electrolytes to decrease intracellular Mg^{+2}/Ca^{+2}shift and thus may decrease the levels of calcium and sodium (Ising *et al.,* 1988). Administration of diazepam could decrease the membrane permeability of catecholamines sensitive cells, which in turn reduces calcium influx into cells (Ennaceur *et al.*, 2006). The reduced availability of calcium to cells despite its high concentration is another probable explanation for the observed effect of Diazepam on memory as calcium is important in neurotransmission and hence memory.

In conclusion, this study has shown that sleep deprivation impairs consolidation of spatial memory as well as working memory. GABA-A antagonists administered after learning appear to be protective against the deficits in memory consolidation and further research into its possible therapeutic use in preventing memory loss associated with sleep deprivation should be explored.

ACKNOWLEDGMENT

The Authors thank Mr Emmanuel Areola for rendering technical assistance in the course of this research.

REFERENCES

Anderson, M and Tufik S. (2004). Inhibitory effect of GABAergic drugs in cocaine-induced genital reflexes in paradoxical sleep deprived male rats. *Pharmacol Biochem Behav*.78:301-7.

Backhaus, J., Hoeckesfeld, R., Born, J., Hohagen, F. and Junghanns, K. (2008). Immediate as well as delayed post learning sleep but not wakefulness enhances declarative memory consolidation in children. *Neurobiology of Learning & Memory*. 89:76-80.

Barnes, D., Chapuis, J., Chaudhury, D., Wilson, D. (2011). Odour fear conditioning modifies piriform cortex local field potentials both during conditioning and during post-conditioning sleep. *PLoS One* 6:18130.

Born, J., Rasch, B., and Gais, S. (2006). Sleep to remember. *Neuroscience*. 12: 410-424

Car, H. & Wisniewski, K. (1998). The effect of baclofen and AP-7 on selected behaviour in rats. *Pharmacol. Biochem. and Behaviour.* 59:685–689.

Chapouthier, G. and Venault, P. (2002). GABA-A receptor complex and memory processes. *Current Topics in Medicinal Chemistry*. 2:841–851

Coenen, A. and Van-Luijtelaar, E. (1985). Stress induced by three procedures of deprivation of paradoxical sleep. *Physiol Behav* 35:501–504

Ennaceur, S., Kovaka, M., Rensburg, R. and Chazot, D. (2006). Model of anxiety: Response of anxiety to novelty and open space. *B.BR.* 4757: 1-30

Frick, K., Stillner, Eand Berger-Sweeney, J. (2000). Mice are not little rats: species differences in a one-day water maze task. *Learning and Memory* 11:3461-3465

Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., et al., (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience*. 144:1186-1192

Georgiev, V., Yonkov, D.and Kambourova, T. (1998). Interactions between angiotensin II and baclofen in shuttle-box and passive avoidance performance. *Neuropeptides*. 12:155–158

Glordano, R., Grottoll, S., Brossa, B., Pellegrino, M., Destifenis, S., Lanfraco, F., Glanotti, L., Ghigo, E. and Arvet, E. (2003). Alprazolam (abenzodiazepine activating GABA receptor) reduces the

neuroendocrine responses to insulin-induced hypoglycemia in human. *Clin. Endocrinol*., 59(3): 314-320.

Hagewoud, R., Havekes, R., Tiba, P., Novati, A., Hogenelst, K., Weinreder, P., Van der Zee, E., Meerlo, P. (2010). Coping with **sleep** deprivation: shifts in regional brain activity and learning strategy. *Sleep.*33:1465–1473.

Ising, H., Bertschat, F., Stoboy, V., Goossen, C.and Hengsy, G. (1986). Stress-induced Ca/Mg shifts and vascular response in animals and men: Comparison to electrolyte alteration in myocardial infarction patients. *Magnes. Bull.* 8: 95- 103.

Kapur, V., Strohl, K., Redline, S., Iber, C., O'Connor, G.and Nieto, J. (2002). Under-diagnosis of sleep apnoea syndrome in U.S. communities. *Sleep and Breathing* 6:49–54.

Kuziemka-Leska, M., Car, H.and Wisniewski, K. (1999). Baclofen and AII 3-7 on learning and memory processes in rats chronically treated with ethanol. *Pharmacology, Biochemistry, and Behaviour*. 62:39–43

Magloire, V., and Cattarelli, M. (2009). Delayed changes of sleep duration after rewarded olfactory discrimination learning in the rat. *Behav Brain Res* 205: 568–571.

Marrosu, F., PortasGian, C and Gessa, L. (1995). Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Research*. 671:329–332.

Mavanji, V., Siwek, D., Patterson, E., Spoley, E.and Datta, S. (2003). Effects of passive-avoidance training on sleep-wake state-specific activity in the basolateral and central nuclei of the amygdala. *Behav Neurosci* 117: 751–759

McDermott, C., LaHoste, G., Chen, C., Musto, A., Bazan, N., and Magee, J. (2003). Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J Neurosci*. 23:9687–9695

McEown, K. and Treit, D. (2010). Inactivation of the dorsal or ventral hippocampus with muscimol differentially affects fear and memory. *Brain Research*. 1353:145–151

McNally, G., Augustyn, K.and Richardson, R. (2008). GABA-A receptors determine the temporal dynamics of memory retention. *Learning & Memory*. 15:106–111

Mirescu, C., Peters, J., Noiman, L. and Gould, E. (2006). Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. *PNAS*. 103:19170–19175

Mohler, H. (2012). The GABA system in anxiety and depression and its therapeutic potential. *Neuropharmacology*. 62:42–53.

Myhrer, T. (2003). Neurotransmitter systems involved in learning and memory in rat: A meta-analysis based on studies of four behavioural tasks. *Brain Research Reviews*, 41:268–287.

Paxinos G. and Watson C. (1982). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney.

Pokk, P. and Zharkovsky, A. (1995). The effects of drugs working at the GABA- Benzodiazepine – Barbiturate complex on the behaviour of sleep deprived rats. *Pharmacology and Toxicology* 76:23-28.

Rahman, A., Languille, S., Lamberty, Y., Babiloni, C., Perret, M. (2013). Sleep Deprivation Impairs Spatial Retrieval but Not Spatial Learning in the Non-Human Primate Grey Mouse Lemur. PLoS ONE 8(5): e64493. doi:10.1371.

Saxvig, I., Lundervold, A., Grønli, J., Ursin, R., Bjorvatn, B. and Portas, C. (2008). The effect of REM sleep deprivation procedure on different aspects of memory function in humans. *Psychophysiology*. 45:309-317.

Stuchlik, A. and Vales, K. (2009). Baclofen dose-dependently disrupts learning in a place avoidance task requiring cognitive coordination. *Physiology & Behaviour.* 97:507–511.

Swartzwelder, H., Tilson, H., McLamb, R and Wilson, W. (1987). Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology*. 92:398–401.

VanderWerf, Y., Altena, E., Schoonheim, M., Sanz-Arigita, E.and Vis, J. (2009). Sleep benefits subsequent hippocampal functioning. *Nat Neurosci* 294:1048–1052.

Xu R, Xia H, He W, Li Z, Zhao J, Liu B, Wang Y, Lei Q, Kong Y, Bai Y, Yao Z, Yan R, Li H, Zhan R, Yang S, Luo G and Wu J(2016). Controlled water vapor transmission rate promotes wound-healing via wound re-epithelialization and contraction enhancement. *Sci Rep*.; 6: 24596.

You H-J and HanS-K(2014). Cell Therapy for Wound Healing. *J Korean Med Sci* 2014; 29: 311-319

Yulin A., Wei W., Huan J., Leiguo M., Shiyu L. and Yan J. (2015). Bone marrow mesenchymal stem cell aggregate: an optimal cell therapy for full-layer cutaneous wound vascularization and regeneration.*Scientific Reports*; 46(4-5):431–438.

Zheng Z., Yin W. and Zaraetal J.N. (2010). The use of BMP-2coupled— nanosilver-PLGA composite grafts to induce bone repair in grossly infected segmental defects. *Biomaterials*; 31(35):9293–9300.