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THE EFFECT OF SLEEP DEPRIVATION ON PRION PROTEIN IN ALBINO RATS

***FO Ugwuene[1], EO Alumana[2], FC Chilaka[1], PE Joshua[2], OFC Nwodo[2]**

[1]Department of Medical Lab. Sciences, College Of Medicine, ESUT.
[2]Department of Biochemistry, University of Nigeria, Nsukka.

***Author for Correspondence**

ABSTRACT

Thirty five Wister albino rats were used in this research work. Twenty four of the albino rats were successfully sleep deprived and used as test group while eleven were not sleep deprived and used as control group. These rats were respectively sleep deprived for fourteen days using single platform sleep deprivation technique. The rats, both the sleep deprived and non sleep deprived were sacrificed by euthanization technique after the sleep deprivation period. Brain tissue of every rat was extracted by surgical dissection. Part of the brain tissues were homogenized and assayed for prion protein while a part of each brain tissue was histologically treated for the brain tissue morphological studies. The results presented evidence of the presence of prion protein (PrP) in the albino rats when compared with the commercial PrP positive control used. The results also showed a significant increase ($P<0.05$) in the PrP concentration after sleep deprivation when compared with the non sleep deprived control group but when compared with the commercial positive PrP control group, there was a significant decrease ($P<0.05$). The brain tissue micrograph showed neither abnormal cell morphology nor evidence of amyloid protein plaques. These results suggest that although there was a significant increase in prion protein concentration due to 14 days sleep deprivation, there was no abnormal protein conformation or protein misfolding due to sleep deprivation stress.

Sleep is the natural state of bodily rest observed in mammals, birds many reptiles, amphibians and fishes (Max, 2006). It could equally be said to be a state of unconsciousness from which a person or animal could be aroused (Max, 2006). It is believed to be the best state of bodily rest since at this state the brain is relatively more responsive to internal than external stimuli (Max, 2006).

Sleep, one of the most sophisticated integrative functions in higher animals, appear to be regulated by the brain in conjunction with a variety of endogenous humoral factors, called sleep substances (Inoue, 1985). These sleep substances include peptides or proteins, hormones and somnogens (Pappenleiner et al 1975; Schoenenberper and Monnier, 1977). The brain which part of its composition include the brain stem, the thalamus and the hypothalamus has nerve centres which elicit nerve signaling chemicals called neurotransmitters such as serotonin and nore-epinephrine. These neurotransmitters control whether one sleeps or keeps awake by acting on the nerve cells of relevant parts of the brain as the need arises. The thalamus is for attention and sleep while the hypothalamus, located under the thalamus promotes the type of sleep called slow wave sleep (SWS). The brain stem, a set of neural structure at the base of the brain, plays a great role in sleep and wakefulness. It is made up of the medullar, the pons and the reticular formation. While the reticular formation helps to keep the body awake and alert, the pons is involved in sleep and control of facial muscles. The neurons at the brain stem actively cause sleep by inhibiting other parts of the brain that keep the body awake (Sherwood, 1997). This physiologic response resulting to sleep is assured if all other factors such as endogenous sleep substances (Peptides or proteins, hormones and somnogens) are present (Pappenleiner, et al, 1975; Schoenenberger and Monnier, 1977). In the absence of these sleep substances, sleep may be difficult to achieve.

In an experimental design, non rapid eye movement (NREM) and SWS were reduced in prion protein null mice during a sleep recovery experiment. In another experiment, PrP null mice showed a larger degree of sleep fragmentation and latency to enter rapid eye movement (REM) and NREM sleep. This finding demonstrated that neuronal prion protein (PrP^{C}) is involved in sleep homeostasis and sleep continuity (Manuel et al, 2007).

The prion protein, designated PrP, is a special type of cell surface protein that is present in all mammals. It is encoded by a sinc gene at chromosome 20 (Dickson et al, 1968). It is anchored on the cell surface or cell memberane by a glycosyl phosphatidylinositol anchor (GPI) (Oesch et al, 1985). PrP is predorminantly expressed in the brain, spinal cord and lymphoid tissues (spleen, lymphnodes and thymus) (Choi et al, 2006). The protein can also be found in decreasing amounts in salivary glands, lungs, intestines, liver, kidney, uterus and blood (Eklund et al, 1967).

PrP can be found in natural or normal state referred to as cellular prion protein and designated PrP^{c} or PrP^{sen} owing to its sensitivity to proteinase k digestion. The cellular prion protein (PrP^{c}) can be transformed to abnormal form called prion. The prion is resistant to proteinase k digestion and is therefore designated as Prp^{res}. Prions are the only ptoteinacious particles that can cause disease in vertebrates (Chessebro, 1990).

When PrP^{c} is transformed into abnormal form, it is folded into abnormal protein shape which can cause the disease called transmissible spongiform encephalopathy (TSE) (Chessebro, 1999). PrP^{c} is some time thermodynamically unstable and at some thermodynamic random occurrences or events such as stress conditions, one of the PrP^{c} molecules autocallitically follow the wrong path of conformation called misfolding, especially in the presence of protein x, a bax protein (Shyu et al, 2000). When the misfolded prion proteins (Prions) attach to the PrP^{c} molecules, further toxic conformation or misfoldings occur. Over a long time, these misfolded copies aggregate in the brain, spinal cord and lymphoid tissues and cause TSE diseases (Chessebro, 1999; Shyu et al, 2000). Prion, the abnormal PrP has more β-pleated sheets than PrP^{c} and can form insoluble plaques which deform the nervous tissues. Prion diseases generally called TSE include Bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, craitzfeld – Jacob disease (CJD) and fatal familial insomnia (FFI) in humans. Others are chronic wasting disease (CWD), kuru, transmissible mink encephalopathy (TME), variant crutzfeld Jacob disease (vCJD) to mention but a few.

Sleep deprivation is a general lack of necessary amount of sleep. This may occur as a result of sleep disorders, active choice or deliberate inducement such as interrogation, for purpose of keeping watch for security reasons, prolonged study or research and sometimes for torture. During these periods, the neurotransmitters activate the relevant parts of the brain mentioned above to keep the body awake (Sherwood, 1997). Furthermore, during sleep deprivation, there is a progressive increase in peripheral energy expenditure to nearly double normal levels, resulting to negative energy balance (Everson and Wehr, 1993). In response to this metabolic demand, an increase in serotonin and catecholamines act on both the frontal lobe of the brain stem (the reticular formation) to sustain body wakefulness (NIH Pub, 2007). Sleep deprivation affects various aspects of proteins including metabolism and translational changes involving unfolding and misfolding of proteins (Schroder and Kaufman, 2005; Cilrelli et al, 2006). Sleep deprivation promotes endoplasmic reticulum stress hormones and production of aukaryotic initiation factor 2 (elf_2) and other membrance proteins (Proud, 2005). All components of unfolded protein response (UPR) or endoplasmic reticulum (ER) response stress proteins were found after 6 hours of sleep deprivation in mouse neocortex, including increase in phosphorylated eukaryotic initiation factors 2 alpha ($P.eif_2$?) as well as free immunoglobulin binding protein (BIP) and Phosphorylated protein kinase-like ER kinase (PERK), a key kinase that phosphorylates eif_2?) (Naidoo et al, 2005). It has been identified that

endoplasmic reticulum (ER) resident chaperon, immunoglobulin binding protein (BIP) increase with sleep deprivation (Naidoo et al, 2005).

Prion Proteins, which are also membrane proteins incriminated with protein misfolding and neurodegenerative diseases of the brain are associated with alterations in sleep (Gibbs et al, 1980; Monari et al, 1994). The disease condition in humans known as fatal familia insomnia is associated with abnormal prion protein (prion) disease related to thalamic neurodegeneration (Gibbs et al, 1980; Monari et al, 1994). Mice that genetically lack the prion protein gene, demonstrated alterations in both sleep and circadian rhythms (Tobler et al, 1997). Prion protein (PrP) – null mice have low sleep pressure, leading to more frequent interruptions of sleep and reduced SWS (Tobler et al, 1997). In other words, the PrP – null mice (PrP%) show longer sleep fragmentation with an increase of slow wave activities (SWA) during NREM sleep after a short period of sleep deprivation.

Since sleep deprivation is one of the stress events and it is associated with some unusual features found in prion protein, this work therefore aims at determining the effect of prolonged sleep deprivation on prion protein in albino rats in Nigeria.

MATERIALS AND METHODS

Adult wister albino rats numbering thirty five (35) were used in this study. Twenty four (24) of the rats were successfully sleep deprived and used as test subjects while eleven of them were not sleep deprived. The non sleep deprived rats were used as control subjects. The rats were fed and kept under observation for their activities and health status at the University of Nigeria, Enugu Campus animal house for five days before subjecting them to sleep deprivation.

The twenty four albino rats were subjected to sleep deprivation for fourteen days using single platform sleep deprivation technique (Rechtschaffen et al, 1999). This technique is based on the principle that if a sizeable adult rat is placed on a 6.5cm base of an inverted cylindrical pot with a dept of 7.5cm in a water proof rat cage and water poured at the floor especially in a brightly lighted non-heat producing environment (Rechtschaffen et al, 1999; Craig Lambert, 2005). During the sleep deprivation duration some vital precautions were taken. Water at the floor of the rat cage was kept relatively fresh by regular replacement on daily basis. Feed pellets were inexhaustibly supplied to the rats respectively through devices on the lid of each single plat form rat cage. Persistent glow of non heat producing light was maintained for the fourteen days duration of sleep deprivation. The control subjects were fed and kept at conducive environment for them to sleep.

At the end of the fourteen days both the test and control subjects were sacrificed by euthanization method as recommended by Canadian council for animal care. (CCAC, 1993). After sacrificing the rats, their brain tissues were respectively extracted from each of the rats by surgical dissection of the rats skull. Each rat's brain tissue was stored in 10% phosphate buffered saline (PBS) formalin for tissue protein preservation as recommended by Eric et al, 2007.

Brain tissue prion protein assay was done using Enzyme immunosorbent assay (ELISA) method using SPI-bio kit according to Grass; et al, (2000) Moujou et al, (1999) and Eliman, (1959). ELISA method using SPI-bio kit has been validated for detection of prion protein in extracts from brain tissues and other tissues as well as recombinant PrP (Grassi et al, 2000 and Moujou et al, 1999). The principle of the assay is based on double antibody sandwich on a solid phase (micro plate well) and the development of a detectable colour at 405nm wavelength with Elimans reagent. The method using SPI-bio kit is concentration dependent (Eliman, 1959).

It is necessary to note that before the brain tissues were subjected to ELISA, the organic solvent fixative was removed with 95% ethanol and PBS as recommended by Eric et al, (2007).

Brain tissue histological studies were done using Alkaline Congo-red method as recommended by Elaphetany et al, 1989). This method demonstrates amyloid protein deposits in plaques or protein plaques (prion plaques) based on the principle that abnormal protein plaques in form of amyliods, stain deep part into red in

alkaline Congo-red within 20 minutes of staining procedure. This plaque is detectable binocular tissue micrograph microscope.

Statistical analysis was done by using students t-Test and the results expressed as mean

Table 1, Fig 1: Comparison of Commercial PrP Positive Control with Non Sleep Deprived Control group.

Parameter	Commercial Positive PrP Control N=2	Non Sleep Deprived Sleep control group N= 11	t - Test
PrP(mAU)	275 ± 0	184±1	P<0.05

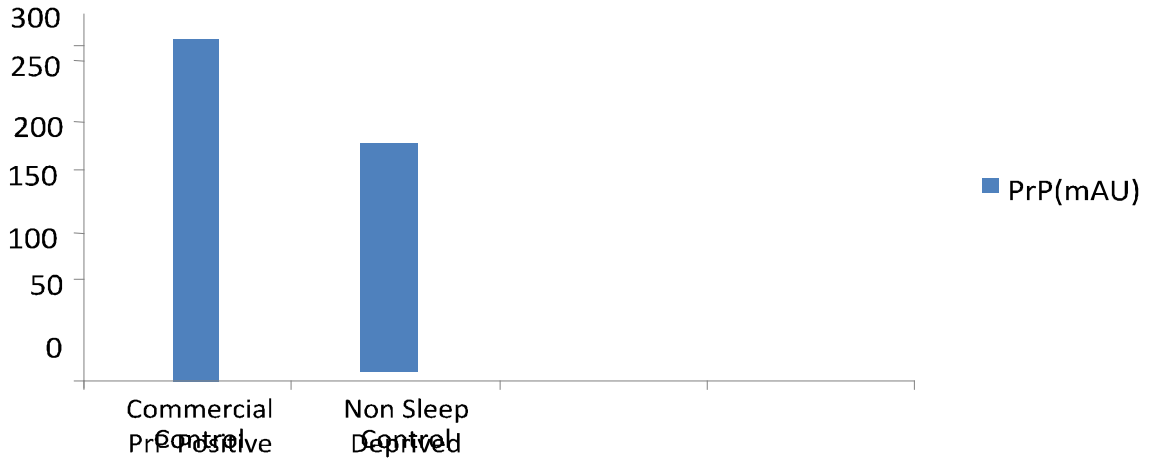


Table 2, Fig 2: Comparism of the Results of the Commercial PrP ^{c} Positive Control Group with the 14 Days Sleep Deprived Group.

Parameter	Commercial Positive Control N=2	14 Days Sleep Deprived Sleep Sample N=24	t - Test
PrP(mAU)	275±0	224±1.7	P<0.05

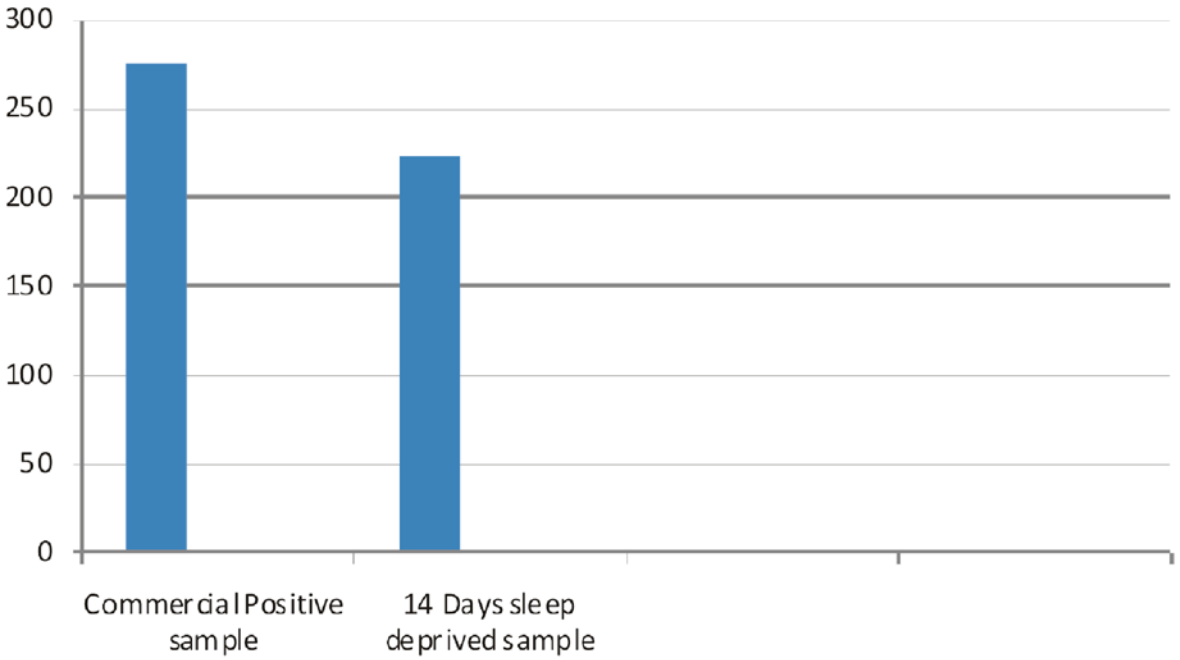
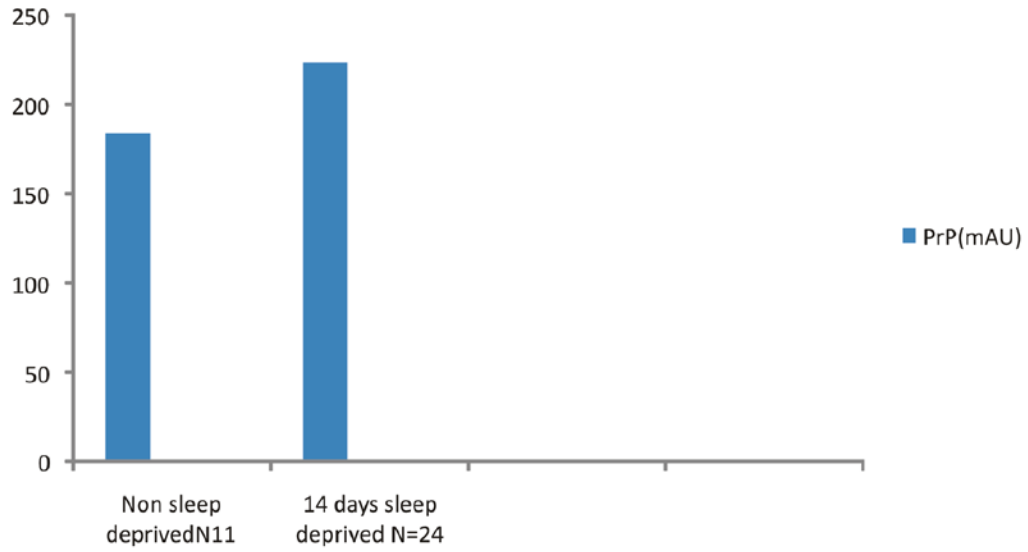


Table 3 and Fig 3: Comparison of PrP of non Sleep Deprived (Control Group) with Sleep Deprived (Test Group)

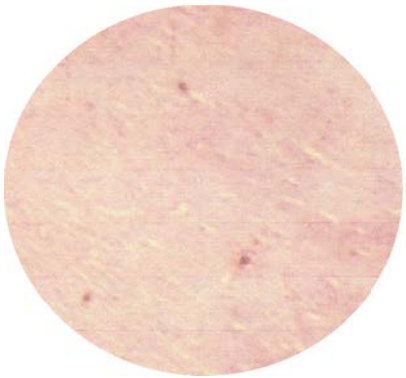
Parameter	Non Sleep Deprived N=11	14 Days Sleep Deprived N=24	t-Test
PrP(mAU)	184±1	224±1.7	P<0.05



Brain Tissue Morphology

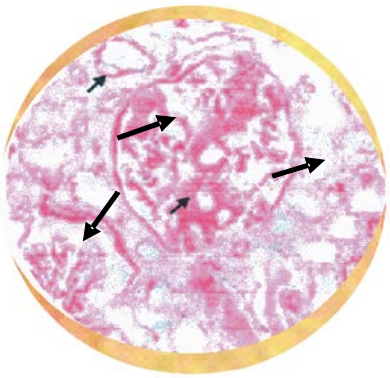
The brain tissue morphology as demonstrated in plate 1 of Brain Tissue micrograph showed neither abnormal cell morphology, nor amyloid protein deposits after 14 days sleep deprivation compared with non-sleep deprived control group (plate 3) and brain tissue micrograph of Transmissible Spongyform Encephalopathy (TSE) (plate 2).

Plate 1



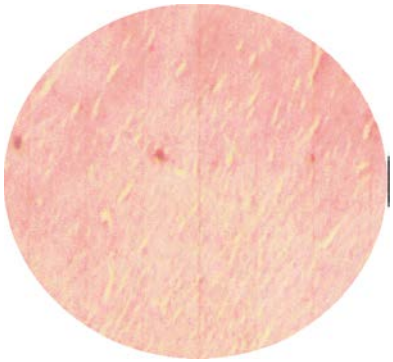
Magnification: X 10
Brain Tissue micrograph of 14 days sleep deprived rats (test group), showing intact neuronal parenchyma, cortical and perineural cells and white matters.
No cell vacuolation
No amyloid plaques.

Plate 2



Magnification: X 10
Brain Tis sue micrograph of TSE, showing vacuolation of nerve cell bodies and perivascular cells, spongy parenclyma and amyloid plaques.(Jin *et al*,2003)

Plate 3



Magnification: X 10
Brain Tissue micrograph of non -sleep deprived rats (control group), showing intact neuronal parenchyma, cortical and perineural cells and white matters.
No cell vaculation.
No amyloid plaques.

DISCUSSION

The results of PrP assay of control groups and test subjects shown in Tables 1 and Figure 1 presented evidence of the presence of PrP in Wister albino rats. This detection of PrP in albino rats in this part of the world is important as it serves as a reference point for certainty of further research work in prion protein in this part of the world, using mammals as research subject. Interest and research work had been on prion protein in cattles, sheep and other mammals in United Kingdom and United States of America, rather than Nigeria. (Prusiner et al, 1982; Chesebro, 1990; Hope et al, 1999).

The result of PrP assay of the test subjects and control groups shown in Tables 3 Figures 3 presented a significant increase ($P<0.05$) in the PrP^{c} concentration after sleep deprivation when compared with the non sleep deprived (control) group but when compared with the commercial PrP^{c} positive control as shown in Table 2 and Figure 2 there was a significant decrease ($P<0.05$). The findings suggest that there was no conformational change from PrP^{c} to PrP^{res} in the brain cells of the rats during 14 days sleep deprivation. This is because the ELISA method of SP-bio kit used in this research work was specific for prion protein assay and Eliman's reagent applied is a chemical used to quantify the concentration of thiol group of proteins in a sample (Eliman, 1959; Reiner et al, 2002). Following its principle in ELISA technique used in this work, the density of colour developed is directly proportional to the concentration of thiol group of prion protein in the specimen. The comparison of conformational change from PrP^{c}

to PrP^{res} using Eliman's reagent is concentration dependent. It could therefore be deduced that there was no conformational change from PrP^{c} to PrP^{res} having obtained a significantly lower PrP^{c} concentration from sleep deprive tissue extracts than the PrP^{c} positive commercial controls. This is because following a report from heterodimer model, the PrP^{res} formed by a spontaneous conformational change from PrP^{c} has a much higher -sheet secondary structure composition and more protein concentration than PrP^{c}, yet much of the -helical content remains intact. The Prp^{res} becomes bulkier because of more concentration of -sheets and assembles into amorphous aggregates or amyloid fibril structures (Caughey, 1991; Merz et al, 1981; Will et al, 1996). These features confer on PrP^{res} greater protein concentration than PrP^{c} and therefore, more thiol groups in the sample. If there was a conformational change, the prion protein concentration detected in the brain tissue extracts of the sleep deprived rats would have been greater than that of positive commercial PrP^{c} *controls* provided. Furthermore, the absence of amyloid proteins in the brain tissues of the sleep deprived rats as demonstrated in the brain tissue micrograph confirms that there was no conformational change from PrP^{c} to PrP^{res}.

On the other hand, the significant increase in PrP^{c} concentration after sleep deprivation when compared with the non sleep deprived (control) group, could be attributed to more PrP^{c} induction in the brain due to sleep deprivation stress. It was suggested after a research work that sustained sleep loss could trigger a generalized inflammatory and stress response in the brain. (Cirelli et al, 2006). This stress response is carried out by the endoplasmic reticulum (ER). It was also reported that all components of unfold protein response or ER stress response were found after six hours of sleep deprivation in mouse neocortex including increased immunoglobulin binding protein (BIP), phosphorelated eukaryotic initiation factor 2 ($p\text{-}eif_2$) and phosphorylated protein kinase-like ER (PERK) and chaperons. The same researcher also remarked that ER is the key marker and master regulator of signaling pathway called ER stress response (Naidoo et al, 2005).

Since this endoplasmic reticulum, whose proteins increase during sleep deprivation, is strongly associated with PrP^{c} for its neuronal signaling process and signal transduction in neuronal cells (Spiechauper and Schatzl, 2001; Monillet-Richard et al, 2000), PrP increase as seen in Table 3 must have been induced along with the other ER proteins by sleep deprivation and it is likely a contribution of PrP^{c} which is said to be associated with neuronal cell signal transduction. (Naidoo et al, 2005; Spiechauper and Schatzl, 2001; Monillet-Richard, et al, 2000). It was also reported that sleep deprivation promotes the production of $p\text{-}eif_2$ and membrane proteins of which prion protein is one. (Proud, 2006).

It is equally note worthy that the report by Esiri and Colleagues which suggested after some studies that expression of PrP^{c} may be upregulated in certain neurodegenerative conditions lends a support to the increased PrP found after 14 days sleep deprivation stress/sleep disorder (Esiri et al 2000; Voigtlander et al, 2001).

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