Published in final edited form as:

Sleep Med Rev. 2009 June ; 13(3): 195–204. doi:10.1016/j.smr.2009.01.001.

“Cellular Stress/The Unfolded Protein Response: Relevance to Sleep and Sleep Disorders”

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Summary

Recent transcript profiling and microarray studies are beginning to unveil some of the mysteries of sleep. One of the most important clues has been the identification of the ER resident chaperone, BiP that increases with sleep deprivation in all species studied. BiP, an ER resident chaperone is the key cellular marker and master regulator of a signaling pathway called the ER stress response or unfolded protein response. The ER stress response occurs in 3 phases. It is healthy, protective and adaptive when the ER stress is moderate. Failure of the adaptive response leads to the activation of an inflammatory response. When the ER stress burden is great and prolonged, executioner pathways are activated. Collectively this work provides new evidence that modest sleep deprivation induces cellular stress that activates an adaptive response. Aging tilts the response to sleep deprivation from one that is adaptive and protective to one that is maladaptive. Understanding the pathways activated by sleep loss and the mechanisms by which they occur will allow the development of therapies to protect the brain during prolonged wakefulness and specifically in sleep disorders including those associated with aging.

Keywords

sleep loss; sleep apnea; ER stress; unfolded protein response; BiP; apoptosis

Introduction

Sleep is a ubiquitous phenomenon that most organisms experience yet our understanding of sleep regulation and function is at the early stages. Much is known about the circuitry involved in sleep and we are beginning to get glimpses into the cellular and molecular correlates of sleep largely through subtractive hybridization, differential transcript and cDNA microarray studies carried out in several species. Recent gene expression studies in rat¹, mouse^{2, 3}, *Drosophila*^{4, 5}, and sparrow⁶ have all contributed to our growing understanding of the molecular changes that occur with sleep, wakefulness and extended wakefulness/sleep deprivation. Amongst the large number (~ 3,000) of genes that change with sleep state, change in one particular class of genes, the heat shock proteins/chaperones, is highly conserved in all species studied. One gene within this class called BiP has been shown to increase with sleep deprivation/extended wakefulness in all species examined - *Drosophila*⁷, rat cerebral cortex^{1, 8, 9}, mouse cerebral cortex^{2, 3} and in the telencephalon of the white crowned sparrow⁶. BiP (Immunoglobulin Binding Protein) is also known as GRP78 (Glucose regulated protein 78) and under the most

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recent nomenclature is known as HSP5A (Heat shock protein 5A). This chaperone serves as a sentinel for up-regulation of several signaling pathways collectively called the unfolded protein response (UPR) or the endoplasmic reticulum (ER) stress response. The UPR responds to cellular stress and the response has 3 phases. It is healthy, protective and adaptive when the ER stress is moderate. When adaptation fails, genes that mediate host defense are activated. Persistent and prolonged stress activates executioner pathways.

This review will provide an overview of how the ER as a signaling organelle responds to stress through a set of co-coordinated pathways called the unfolded protein response. The initial cytoprotective response of the unfolded protein response and the later pro-apoptotic signaling pathway will also be discussed. The review will also summarize data from recent sleep deprivation and intermittent hypoxia studies that illustrate a role for the ER stress response in sleep. Finally, the relevance of these processes to neurodegenerative diseases is discussed. Understanding the pathways activated by sleep loss and the mechanisms by which they occur will allow the development of therapies to protect the brain during prolonged wakefulness and in sleep disorders including those associated with aging.

The ER is a Multifunctional Signaling Organelle

The endoplasmic reticulum is a sub-cellular organelle comprised of a reticular membranous network that extends throughout the cytoplasm and is contiguous with the nuclear envelope. It is the site where all secretory and integral membrane proteins are folded and post-translationally modified in ATP dependent chaperone mediated processes. The ER is also the site of steroid, cholesterol and lipid biosynthesis. It is the main storage site of calcium in the cell and is the major signal-transducing organelle in the cell that continuously responds to environmental cues to release calcium¹⁰.

BiP is the Major Chaperone in the ER and is Key for Protein Folding

In addition to being the most abundant chaperone in the ER, BiP is the first chaperone that a nascent peptide, destined for the ER, encounters during protein translation when it comes off the ribosome¹¹. BiP binds to the hydrophobic domains on the peptide to prevent misfolding while the rest of the protein is being synthesized. Once the entire peptide is synthesized, it is folded with the aid of BiP and other chaperones; if properly folded it is escorted out of the ER. In contrast, if a protein is misfolded, it is refolded with the help of BiP, or if that is not possible, it is escorted by BiP to the cytosol and proteasome for degradation¹².

What is the ER Stress Response/Unfolded Protein Response?

Because of its reticular nature the ER can sense and transmit signals that originate in any cellular sub-compartment. Perturbations that alter ER homeostasis disrupt folding of proteins and lead to the accumulation of unfolded proteins and protein aggregates, which are detrimental to cell survival. These perturbations include disturbances in calcium homeostasis, redox status, elevated secretory protein synthesis, and glucose or energy deprivation. As a consequence, the cell has evolved an adaptive coordinated response to limit accumulation of unfolded proteins in the ER and restore normal ER function. This signaling pathway is termed the ER stress response or the unfolded protein response (UPR) [for more extensive reviews see^{13–16}]. The response to ER stress has 3 stages, adaptation, alarm and apoptosis, depending on the duration and intensity of the stress (see Figure 2).

On a cellular level, the UPR triggers three kinds of adaptive, protective cellular responses: I) up-regulation of ER chaperones such as BiP/GRP78 II) attenuation of protein translation and III) degradation of misfolded proteins by the proteasome by a process called ER associated degradation (ERAD) – (see schematic in Figure 1). These three responses are protective

measures to reduce protein load and alleviate ER stress. When adaptation fails, ER initiated pathways signal alarm by activating the transcription factor, NF- κ B that induces genes expressing mediators of host defense¹⁷. Lastly, excessive and prolonged stress leads to a maladaptive response and apoptosis¹⁸. When cell protective changes mediated by the UPR fail to restore folding capacity, CHOP (C/EBP homologous protein), c-jun NH2 terminal kinase (JNK) and caspases (discussed below) have been implicated in mediating apoptosis in response to sustained ER stress (see schematic in Figure 3).

a) Activation of the UPR through BiP

BiP is a peptide-dependent ATPase and member of the heat shock 70 protein family that binds transiently to newly synthesized proteins translocated into the ER, and more permanently to underglycosylated, misfolded, or unassembled proteins. Under non-stressed conditions, BiP binds to the luminal domains of the three UPR transducers, IRE1 (Inositol Requiring 1), PERK [PKR (RNA-dependent protein kinase)-like ER kinase], and ATF6 (Activating Transcription Factor 6) to maintain them within the ER (see figure 1). Upon accumulation of unfolded proteins, BiP is sequestered from ATF6, IRE1 and PERK to chaperone the misfolded proteins thereby permitting the activation of one or more of these transducers¹⁹.

b) PERK signaling and protein translation attenuation

PERK is a type I transmembrane serine threonine kinase that appears to be present in most cells. It is held in an inactive monomeric state by binding to BiP. When this binding is disrupted, PERK homodimerizes and phosphorylates itself, thereby activating itself and initiating its eIF2 α (eukaryotic Initiation Factor 2 α) kinase activity. Phosphorylation of eIF2 α causes a general decrease in translation of most proteins. However some selected proteins such as ATF4 and BiP, with internal ribosomal entry sites (IRES), are translated more efficiently²⁰ and hence their protein levels actually increase. Other kinases including GCN2 (general control of nitrogen metabolism kinase 2), HRI (heme-regulated inhibitor kinase) and PKR also inhibit translation through eIF2 α phosphorylation, however they do so by mechanisms other than ER stress²¹.

c) ATF6 signaling and transcription of ER chaperone

ATF6 is a 90-kDa bZIP protein that is activated by posttranslational modifications. ATF6 activation as part of the UPR leads to its translocation to the Golgi and cleavage by site-1 protease (S1P) and S2P. The 50-kDa cleaved ATF6 α translocates to the cell nucleus, where it binds to the ER stress response element CCAAT(N)9CCACG²² in genes encoding ER chaperone proteins such as BiP and GRP94. GRP94 is a member of the heat shock90 family of chaperones. This results in increases in the level of these proteins and hence increasing protein folding activity in the ER^{22;23}

d) IRE-1 signaling and selective mRNA splicing

Once activated, IRE1 α gains endoribonuclease activity and splices X-box binding protein (XBP) 1 mRNA, generating a spliced variant (XBP1s) that functions as a potent transcriptional transactivator of genes involved in ER expansion, protein maturation, folding and export from the ER, as well as export and degradation of misfolded proteins^{24–28}. IRE1 α may also degrade ER-targeted mRNAs, thus providing another mechanism to decrease the production of new proteins in the organelle^{29, 30}.

e) CHOP and other Pro-apoptotic Signaling Factors

C/EBP homologous protein (CHOP) also known as growth arrest and DNA damage 153 (GADD 153), together with Bcl-2 (B-cell lymphoma protein 2) family members [Bak (BCL-2 homologous antagonist/killer)/Bax (BCL-2-associated X)], caspase-12 and c-jun NH2 terminal

kinase (JNK) are components of the ER stress mediated apoptotic pathway³¹. CHOP is a transcription factor of the C/EBP family. Under non stress conditions the basal expression of CHOP is low. However CHOP levels have been shown to increase markedly when ER stress is not alleviated and persists³². Some of the mechanisms by which CHOP mediates apoptosis include I) the inhibition of protective anti-apoptotic factors like Bcl-2 and perturbation of cellular redox state by depletion of the anti-oxidant glutathione³³; II) promotion of apoptotic caspase activity³³ and III) the translocation of Bax from the cytosol to the mitochondria³⁴.

Insertion of Bax into the mitochondrial membrane is essential for cytochrome c release and mitochondrial mediated apoptosis³⁵. Bax translocation to the mitochondria is also downstream of JNK. The caspase family of cysteine proteases is a key mediator of programmed cell death or apoptosis. Murine caspase-12 which is a member of the interleukin-1 β converting enzyme subfamily of caspases³⁶ is an initiator caspase and the central player in ER induced apoptosis¹⁸. Once activated, caspase-12 translocates from the ER to the cytosol where it cleaves caspase-9, which induces the cleavage of caspase-3, the executioner caspase, in a cytochrome c-independent manner and activation of the rest of the apoptotic pathway³⁷.

f) Anti-apoptotic and Anti-oxidant Role of the UPR

In addition to handling protein load, the adaptive response of the UPR serves both anti-apoptotic and anti-oxidant roles. The anti-apoptotic molecules in the ER identified thus far are the chaperones BiP, calreticulin, protein disulfide isomerase (PDI), and DAD1 (Defender Against apoptotic cell Death)^{38, 39}. It has been shown that over expression of BiP leads to suppression of CHOP and CHOP mediated cell cycle arrest and apoptosis^{40–42} as well as inhibition of p38 MAPK mediated apoptosis⁴³. Other studies have also suggested that a pool of BiP serves an anti-apoptotic role by forming complexes with the caspases (caspase-7 and caspase-12) at the ER surface preventing their activation and release^{44, 45}. Also associated with the ER are the anti-apoptotic Bcl-2 family members Bcl-2 and Bcl-X_L which inhibit cytochrome c release from the mitochondria during apoptosis.

PERK-dependent signaling, independent of translational inhibition, leads to the activation of the pro-survival transcription factor NRF-2 (NF-E2-related factor-2) via site-specific phosphorylation⁴⁶. This PERK dependent phosphorylation leads to nuclear localization of NRF-2 and transcription of genes with the anti-oxidant response element. NRF-2 activation contributes to the maintenance of glutathione levels, which in turn functions as a buffer for the accumulation of reactive oxygen species during the unfolded protein response⁴⁷. NRF-2 transcript has been shown to increase with sleep deprivation consistent with the idea that there is an increase in ROS with sleep deprivation and increased energy use^{2, 48}.

Sleep deprivation and the UPR

Prolonged wakefulness over a period 6h or more does induce ER stress and the UPR in mouse cerebral cortex⁴⁹. All of the components of the unfolded protein response pathway occur with sleep deprivation. Protein levels of BiP increase with 6, 9 and 12h of prolonged wakefulness. Immunoprecipitation studies indicate that BiP is dissociated from the kinase PERK with 6h of sleep deprivation. In addition the phosphorylation of PERK and eIF2 α indicate inhibition of protein translation. Ribosome profiles from mice that have been subjected to 6h of sleep deprivation indicate that there is a disaggregation of polysomes into monosomes in the sleep deprived animals suggesting an attenuation of protein translation⁴⁹. Active translation is associated with greater amounts of polysomes as was observed in undisturbed control animals.

The concept of the unfolded protein response had been suggested by others from microarray studies that showed increased cortical mRNA expression of the UPR associated chaperones BiP and GRP94 in rats during wakefulness¹. In addition, PERK cerebellar transcript levels

were also found to be higher in wakefulness than in sleep¹. Other UPR specific transcripts that change with sleep deprivation include DNA-J which is a co-chaperone of BiP, XBP-1, calreticulin, caspase-9, ATF4 and ATF6 (see Table 1 for all genes that change with sleep deprivation).

Immunohistochemical studies also indicate that there is an increase in protein levels of ER chaperones GRP94, BiP/GRP78 and ERp72 during sleep deprivation in both the dorsal and lateral cortex of mouse brain⁹ again compatible with the induction of the unfolded protein response. It seems likely that the unfolded protein response with sleep deprivation might occur in other brain regions, and whether this is so will need to be addressed in future studies. Interestingly, it has recently been shown that BiP transcript levels increase in the liver of the AK mouse strain with sleep deprivation³. Further studies need to be carried out to ascertain whether sleep deprivation through the UPR affects peripheral organs. This study also demonstrated that BiP increased with sleep deprivation in the cortices of the three mice strains C57, AK and DBA studied while other UPR components, XBP-1, DNAJ and calreticulin increased only in the cortices of AK and DBA mice³.

The induction of the UPR by sleep deprivation also does not seem unique to mice since rest (sleep) deprivation in *Drosophila*, a state analogous to sleep in mammals^{7, 50, 51} also leads to up-regulation of the mRNA for BiP^{7, 51}. Further, protein levels of BiP also increase two-fold, in *Drosophila* brain, with as little as 3h of sleep deprivation⁵². While it is known that PERK and XBP-1 are found in *Drosophila* it is not known whether these molecules change with sleep deprivation in a manner similar to those in mammals. Thus, further investigation of the UPR in *Drosophila* is needed.

This recent work on sleep deprivation/sleep on the unfolded protein response provides insights into earlier studies by Nakanishi et al⁵³ and Ramm and Smith⁵⁴, that demonstrated that protein synthesis was enhanced during sleep. These earlier studies lend credence to the findings that sleep deprivation is associated with attenuation in protein translation, with likely increased protein synthesis during recovery sleep. A recent preliminary study by Ding and colleagues using SELDI mass spectrometry also indicates that protein expression is decreased with sleep deprivation⁵⁵. Thus, alteration in protein translation is a key consequence of sleep deprivation with recovery of this process during sleep.

Altering BiP, the master regulator of the UPR, affects recovery sleep in *Drosophila*

The increase in BiP expression with sleep deprivation in *Drosophila* is followed by a decline/diminution in BiP with recovery sleep. In *Drosophila*, BiP protein levels return to baseline levels over 24h with recovery sleep following an almost 3-fold increase with 6h of sleep deprivation⁵². While this occurs, these data do not indicate whether this is simply a consequence of sleep deprivation or whether alteration in BiP levels affects sleep behavior.

To address this, BiP transgenics, one with higher levels of BiP than normal and one with inactive BiP, have been used to examine the effect of BiP levels on baseline sleep and recovery sleep⁵². Altering BiP expression levels did not change the amount of baseline sleep the flies acquired. Changing the amount of functional BiP did however alter the amount of recovery sleep in flies following sleep deprivation. Flies with high levels of BiP had more recovery sleep than flies with normal BiP levels, i.e., 30% more sleep in the first 4h post deprivation. In contrast, flies with inactive BiP had less recovery sleep than flies with normal sleep, i.e., 60% less sleep in the first 4h post deprivation. Thus, altering BiP impacts recovery sleep and BiP itself may be sleep promoting. However, high levels of BiP repress the UPR as all 3 transducers of the UPR remain bound to BiP when there is excess BiP⁵⁶. These transducers need to become

free for the UPR to occur. It may be that with the protective UPR pathway repressed, more sleep is required to recover from the ill effects of sleep deprivation. In contrast, inactive BiP leads to an earlier induction of the UPR. Thus, the need for recovery sleep may be reduced.

Whether the altered amounts of recovery sleep when BiP levels are manipulated is due to BiP itself or more indirectly through the other effects on the UPR just described remains to be determined.

Interactions of ER stress signals and sleep control

In mammalian systems as well, there is evidence of an effect of these pathways on sleep/wake control. A study from the McGinty laboratory hypothesizes that sleep homeostasis may be induced by an increased demand for brain protein synthesis⁵⁷. This hypothesis builds on the model that increasing protein synthesis may be a function of sleep and that some signals reflecting the protein synthetic load affect sleep regulation. As described earlier in the review, phosphorylation of eIF2 α during ER stress leads to inhibition of protein translation. In their study Methippara et al⁵⁸ used the small molecule inhibitor salubrinal that prevents dephosphorylation of eIF2 α to test whether a build up of p-eIF2 α had any effect on sleep⁵⁸. Salubrinal administered ICV for 12h significantly increased SWS2 and suppressed active wake for the first 3h. For the remaining 9h, salubrinal did not affect sleep/wake. During the first 3h, salubrinal increased the number of SWS2 episodes and also increased delta power. The authors also reported that salubrinal treatment for 3h significantly increased the total number of p-eIF2 α labeled neurons in the basal forebrain region. In addition, they found that administration of salubrinal activated GABAergic rostral median preoptic nucleus neurons as indicated by fos expression⁵⁷. Taken together, this data suggests that ER stress signaling through phospho-eIF2 α may play a role in sleep control and suggests that sleep is part of the compensatory response to ER stress in the brain.

Effect of Age on the UPR Response to Sleep Deprivation

The adaptive UPR response to sleep deprivation is attenuated in aged mice (22–24 months of age). All 3 components of the ER stress response/UPR, to sleep deprivation are compromised with aging. Specifically, ER chaperones like BiP are not increased with sleep deprivation in old animals, protein translation is not attenuated, and ER associated proteasomal degradation is reduced⁵⁹. Following 3, 6, 9, and 12h of sleep deprivation BiP levels did not increase in the cerebral cortex of aged mice in contrast to the robust increase in young animals even at baseline (i.e., without sleep deprivation). BiP levels were 30% less in aged animals compared to young (3 month old) mice suggesting that at baseline protein folding is compromised in older animals. In older animals levels of activated PERK were low and there was also no significant activation of eIF2 α by PERK with sleep deprivation. Thus, inhibition of protein translation that occurs during the UPR with sleep deprivation in young mice does not occur in the aged animals. Further, levels of GADD34 (Growth Arrest and DNA Damage-inducible protein) a protein phosphatase 1 (PP1)-interacting protein, which causes PP1 to dephosphorylate eIF2 α , and relieve the translational block imposed by eIF2 α phosphorylation^{18, 60} were increased both with age and sleep deprivation. Both this and the lack of translational inhibition suggest that protein translation proceeds in aged animals despite the ER stress and that there is most likely an increase in misfolded proteins and possibly in protein aggregation.

Finally, while baseline ubiquitin levels were higher in aged animals compared to young animals, the aged animals did not display further increases in ubiquitin with sleep deprivation, whereas they increased in the young. This suggests that misfolded proteins were not being cleared by degradation in the aged animals. In eukaryotic cells, the ubiquitin-proteasome system (UPS) is the main pathway for eliminating misfolded proteins^{61, 62}. Misfolded or

damaged proteins are tagged for UPS mediated degradation by the covalent attachment of ubiquitin molecules⁶¹.

Coupled with the absence of a robust protective UPR response, the pro-apoptotic signaling pathway alluded to earlier (see Figure 2) is turned on in older mice, even at baseline, and is much further increased by sleep deprivation (Figure 3). There was a dramatic increase in the pro-apoptotic factor CHOP with age that was further increased in older animals with prolonged waking. As described earlier, a role for CHOP in mediating apoptosis in response to ER stress is well established^{32, 63}. Increased CHOP expression results in down-regulation of BCL-2 expression, depletion of cellular glutathione, and exaggerated production of reactive oxygen species³³. Thus anti-oxidant systems are suppressed and free radical production is increased in the presence of CHOP.

The caspase cascade, that is a key mediator of programmed cell death, was also activated in aged animals. Levels of activated cleaved caspase-12 were higher in aged animals and were further increased with sleep deprivation. Caspase-12 is thought to be key for ER mediated cell death. The up-regulation of CHOP, GADD34 and caspase-12 are all indicative of sustained ER stress. When ER stress is not alleviated by the adaptive arm of UPR, the pathway switches to a mechanism that promotes cell death.

Another group of molecules called the 14-3-3 proteins which play an anti-apoptotic role also change with sleep deprivation in both young and aged animals⁶⁴. The 14-3-3 proteins prevent apoptosis through sequestration of Bax and other pro-apoptotic factors like Bad (BCL-2-antagonist of cell death) and ASK1 (Apoptosis signal-regulating kinase 1)⁶⁵. During cellular stress Bax dissociates from 14-3-3 and relocates to the mitochondria. The decrease in 14-3-3 isoforms with 6h of sleep deprivation⁶⁴ is indicative of cellular stress and an increase in pro-apoptotic signaling.

Aged animals exhibit more fragmented sleep^{59, 66, 67}. Surprisingly, recovery sleep following sleep deprivation is less in older animals than young. This has been shown in humans^{68, 69} and in rats^{67, 70}. Given that sleep deprivation in older animals is more injurious, one might have expected more, not less, recovery sleep. That older animals, who exhibit reduced recovery sleep following sleep deprivation, have no BiP response to sleep deprivation is compatible with the concept that BiP itself may play a direct role in determining the magnitude of sleep recovery, as discussed earlier.

Chronic Sleep Loss/Long term Sleep Derivation

While acute sleep deprivation leads to induction of the UPR, it appears that chronic sleep loss or long term sleep deprivation as assessed by an increase in BiP transcript levels does not. A study by Cirelli et al⁷¹ showed that rat cerebral cortex BiP mRNA levels do not increase after long-term (7 days) sleep deprivation as much as after short-term (8h) sleep deprivation. It is likely that 7 days of sleep deprivation results in sustained ER stress much like that experienced by aged animals which results in a shutdown of the adaptive ER stress response. It is likely that Thus, it is likely to be more injurious. Further studies are needed to address this.

A more recent study indicates that long term REM sleep loss (6–10 days) does lead to increased apoptosis in several regions of rat brain⁷². Using amino cupric staining as a marker of neuronal degeneration, TUNEL (TdT-mediated dUPT nick end labeling) assays and the ratio of Bcl-2 to Bax as indices of apoptosis this study demonstrates that neurons in locus coeruleus (LC), laterodorsal tegmentum (LDT), medial preoptic area (MPO) but not in the lateral septum undergo degeneration with REM sleep loss. The increase in Bax positive neurons over BCL-2 positive neurons observed in this study suggests that the last apoptotic phase of the UPR is activated in the REM sleep deprived animals. The absence of any apoptotic factors in the lateral

septum following REM sleep loss illustrates nicely that there is differential vulnerability between neuronal groups to stress.

ER stress and sleep apnea

Producing exposure to intermittent cyclical hypoxia/reoxygenation similar to what occurs in obstructive sleep apnea, results in ER stress and injury in select brainstem motor neurons⁷³. Many motor-neurons process large amounts of secretory and membrane proteins that must be properly folded within the ER⁷⁴, and as such are prone to experiencing ER stress and the UPR. Short term intermittent hypoxia (3 days) selectively activates the PERK pathway of the UPR in some motor nuclei including hypoglossal and facial but not the motor trigeminal. Longer term intermittent hypoxia for 8 weeks results in similar PERK activation by phosphorylation in both the hypoglossal and facial motor neurons. In addition to PERK activation, however, the pro-apoptotic proteins, CHOP, GADD34, cleaved caspase-7 and caspase-3, are all increased with longer term intermittent hypoxia in these motor neurons. As a result these motor neurons undergo UPR even at baseline that is exacerbated by long-term intermittent hypoxia. The presence of CHOP and subsequently GADD34 lead to dephosphorylation of eIF2 α , increased protein synthesis and greater ER stress in these neurons. The inability of these neurons to relieve the ER stress leads to neural injury that is observed at the ultrastructural level. Specifically, the ER is swollen and distorted and there is disaggregation of ribosomes and degranulation of rough ER⁷³. In contrast, the trigeminal neurons in which eIF2 α is phosphorylated and the adaptive response of the UPR is turned on have no such injury. Use of salubrinal, a small molecule inhibitor of eIF2 α dephosphorylation, protected the susceptible motor neurons against neural injury. With this protein, translation is inhibited. Salubrinal treatment also decreased the amount of the pro-apoptotic factor CHOP. Thus, there is a differential response and ability to handle ER stress by different motor neurons. Neurons with low levels of baseline CHOP are able to mount a UPR that confers protection to intermittent hypoxia, while those motor neurons that experience sustained stress as evidenced by increased CHOP expression are not able to mount an adaptive UPR, and subsequently are more susceptible to ER stress and injury (See model in [Figure 4](#)). This effect of cyclical intermittent hypoxia may occur in other tissues and this is an area of future study.

Neurodegenerative diseases, ER stress and Sleep disorders

Misfolded proteins and the associated ER stress are emerging as common features of neurodegenerative diseases (see reviews^{39, 75}). Neuronal loss in both familial and sporadic forms of neurodegenerative disorders is often accompanied by aggregation of misfolded proteins⁷⁶. Accumulating evidence suggests that ER dysfunction and aberrant protein degradation play a role in dopamine neuron loss in Parkinson's disease⁷⁷. The ER stress response has also been implicated in Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease and spinocerebellar ataxias^{39, 75, 77}. Accumulation of misfolded proteins that lead to alterations in organelle structure including the ER has been described in transgenic models of ALS, Alzheimer's and Huntington's disease^{39; 78}. Neurons over expressing mutant presenilin 1 have been shown to be more sensitive to ER stress induced apoptosis⁷⁹. Whether or not the mutation in presenilin 1 down-regulates BiP and induces CHOP is still a matter of debate. It has also been shown that extended polyglutamine tracts also stimulate ER stress induced cell death^{80, 81}. These diseases usually appear late in life and are associated with aging. It is well documented that patients with neurodegenerative diseases like Parkinson's and Alzheimer's experience sleep fragmentation^{82, 83 84}. Sleep fragmentation could place an additional burden on an already stressed protein folding and degradation system and could exacerbate protein aggregation acting as a positive feedback in these conditions. Thus, one needs to question whether sleep fragmentation in neurodegenerative disorders accelerates their

progress based on the mechanism described in this review. This is, I believe, a topic worthy of further study.

Conclusions

Both sleep deprivation and the cyclical intermittent hypoxia that occurs in sleep apnea lead to ER stress. During ER stress the protein production machinery is compromised and protein misfolding occurs. The ER responds to this stress by up-regulating a series of coordinated cellular protective signaling pathways called the unfolded protein response. This protects the cell from the effects of misfolded proteins that can form toxic protein aggregates. This response appears to be highly conserved as sleep deprivation up-regulates BiP, the sentinel marker of the UPR in all species studied thus far, including fruit flies, birds and rodents. The protective response can be overwhelmed by additional stress and if the cumulative burden is too great then pro-apoptotic signaling is activated. This occurs in older animals with short-term sleep deprivation, in certain motor neurons with cyclical intermittent hypoxia and with prolonged sleep deprivation even in young animals. These are the beginning of pharmacological approaches to modify these pathways and this will be an area of further study.

Practice Points

1. Acute sleep deprivation leads to the up-regulation of ER stress response genes and proteins, specifically the chaperone BiP, in the brains of all species studied. Thus, sleep deprivation is injurious to cells.
2. The ER stress response/Unfolded Protein Response is cytoprotective and helps the brain deal with the adverse consequences of sleep deprivation.
3. This protective unfolded protein response is impaired with aging, and sleep deprivation in older animals has more profound injurious effects on cells.
4. Phosphorylated eIF2 α which indicates stalled protein synthesis may serve as a signal, thereby allowing protein synthesis to occur.
5. Long term cyclical intermittent hypoxia, akin to that which occurs in obstructive sleep apnea, leads to ER stress and can result in neural injury.

Research Agenda

1. Understand the mechanism by which sleep deprivation and sleep fragmentation perturb ER homeostasis and what this means to neuronal function.
2. Understand how intermittent hypoxia similar to that in severe sleep apnea disturbs ER homeostasis and can promote neuronal death.
3. Explore potential therapeutic avenues to augment ER homeostasis in various sleep disorders.

Acknowledgments

I am grateful to Drs Allan Pack and Sigrid Veasey for their helpful comments during the writing of this manuscript. I would like to thank Ms. Jennifer Montoya for the illustrations and Mr. Daniel Barrett for editing. This study was supported by NIA grants AG025353 and AG17628.

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Figure 1.

Schematic illustrating the 3 stages of the ER Stress Response (ESRS). ER stress signals first activate the adaptive response through the PERK, ATF and IRE-1 pathways, when adaptation fails ER initiated pathways activate NF-KB mediated pathways. Lastly when ER stress is prolonged and remains unresolved apoptotic signaling is initiated.

Figure 2.

Schematic showing the 3 major components of the unfolded protein response. Misfolded or unfolded proteins titrate BiP away from the 3 transducers of ER stress: PERK, IRE1 and ATF6. Activated PERK phosphorylates eIF2α to attenuate protein translation and phosphorylates NRF-2 to up-regulate the anti-oxidant response. Cleaved activated ATF6 leads to induction of molecular chaperones BiP, PDI, GRP94 etc. IRE1 activation leads to XBP-1 splicing, transcriptional activation of chaperones and stimulation of protein degradation. The various ER chaperones, such as BiP and GRP94 are protective and control protein folding and components of the UPR. Modified with permission from Naidoo et al, *Journal of Neuroscience* 2008 28(26):6539–6548

Figure 3.

Sustained ER stress leads to pro-apoptotic signaling.

Prolonged UPR activation leads ER calcium release and to cell death signaling. Activated IRE-1 acting on downstream factors activates JNK and caspases. ATF4 dependent transcription leads to increases in CHOP. CHOP is a pro-apoptotic transcription factor. CHOP inhibits Bcl-2 leading to calcium release; higher calcium levels sensitize mitochondria to other insults inducing cell death. Bcl-2 exerts an anti-apoptotic function in the ER. CHOP and JNK also promote the translocation of Bax to the mitochondria where it facilitates the release of cytochrome c required for caspase activation. ER specific caspases, such as the caspase-12, are thought to directly induce cell death through activation of caspases 9 and 3. Modified with permission from Naidoo et al, *Journal of Neuroscience*, 28(26):6539–6548, 2008

Figure 4.

Model of hypoxia/reoxygenation ER injury and protection.

Motor neurons may be considered susceptible or resistant to injury from hypoxia/reoxygenation. Susceptible neurons have at baseline an unfolded protein response and activation of CHOP, a proapoptotic protein. Under conditions of hypoxia/reoxygenation, CHOP is markedly increased and activated in susceptible motor neurons. There is in parallel an increase in activation of caspase-7 (ER specific) and caspase-3. In contrast, resistant motor neurons are able to phosphorylate eIF-2 α and circumvent activation of CHOP or caspases. Salubrinal, a specific protein phosphatase inhibitor, maintains phosphorylation of eIF-2 α in susceptible motor neurons and converts the phenotype from susceptible to resistant to hypoxia/reoxygenation injury. Reproduced with permission from Zhu et al, *J. Neuroscience*, 28: 2168–2178, 2008

Table 1**ER Stress Response Associated Genes that Change with Sleep Deprivation**

Gene name	Function	References
BiP	ER chaperone, folding, ERAD, ATPase activity, anti-apoptotic	Shaw et al, 2000 ⁷ , Cirelli et al, 2004 ¹ , Terao et al, 2003 ⁹ , Mackiewicz et al, 2007 ² , Jones et al, 2007 ⁶ , Maret et al, 2007 ³
GRP94	Heat shock 90 family, ER chaperone, folding	Cirelli and Tononi, 2000 ⁸² , Terao et al, 2003 ⁹
CHOP	Apoptotic signaling	Cirelli and Tononi, 2000 ⁸²
ERP72	ER chaperone, folding	Cirelli and Tononi, 2000 ⁸²
PERK	kinase, protein translation inhibition, antioxidant response	Cirelli et al, 2004 ¹
Calcineurin	Calcium activated phosphatase	Cirelli et al, 2004 ¹
FK506 binding protein12	peptidyl propyl cis/trans isomerases, conformational change, translational control	Cirelli et al, 2004 ¹
IP3 receptor	Calcium receptor signaling	Cirelli et al, 2004 ¹
XBP-1	Transcription factor, ER chaperone induction	Mackiewicz et al, 2007 ² , Maret et al, 2007 ³
Calreticulin	Calcium binding, ER chaperone of glycoproteins	Mackiewicz et al, 2007 ² , Maret et al, 2007 ³
eIF4B	Translation initiation factor	Mackiewicz et al, 2007 ²
Ribosomal S6 kinase	Protein Translation	Mackiewicz et al, 2007 ²
Dnajc1	DnaJ (Hsp40) homolog, ER chaperone) – protein synthesis and folding – ERAD, translocation, HSP70 assistance, ATPase activity	Mackiewicz et al, 2007 ²
Dnajb5	HSP40; binds unfolded ER proteins	Mackiewicz et al, 2007 ²
Gadd45a	(demethylation – DNA repair)	Mackiewicz et al, 2007 ²
Calpain 5	ER protease, Caspase cleavage	Mackiewicz et al, 2007 ²
mannosidase 2, alpha B1	ER degradation enhancing – EDEM / ERAD	Mackiewicz et al, 2007 ²
BCI-associated death promoter	ER associated Apoptosis	www.Sleepgene.org ⁸³
BCI-2-like 11 (apoptosis facilitator)	ER associated Apoptosis	www.Sleepgene.org ⁸³
Caspase 9	Apoptotic factor	www.Sleepgene.org ⁸³
Herpud1	homocysteine-inducible, ER stress-inducible, ubiquitin-like domain member, ERAD	www.Sleepgene.org ⁸³
NRF-2 (Gabpa)	Transcription factor regulating antioxidant response	www.Sleepgene.org ⁸³ , Nikonova et al, 2008 ⁴⁶
ATF6	Binds to ATF/CRE element, and to ER stress response elements I and II. Targets ANF(+), BiP(-/+), CHOP(+), ER chaperones, Herp(+), p58 ^{IPK} (+), XBP-1(+)	www.Sleepgene.org
ATF4	Required for expression of genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress. Activates ATF3, CHOP, GADD34	www.Sleepgene.org ⁸³
Caspase	Apoptosis, necrosis, and inflammation	www.Sleepgene.org ⁸³

Gene name	Function	References
IRE	Chaperone induction, ERAD, and expansion of ER in response to ER stress	www.Sleepgene.org ⁸³
TRAF	TRAF-2: promotes clustering of and is released from procaspase-12 upon ER stress by sequestering IRE1	www.Sleepgene.org ⁸³