**RESEARCH ARTICLE**

**Light interference as a possible stressor altering HSP70 and its gene expression levels in brain and hepatic tissues of golden spiny mice**

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**SUMMARY**

Light at night and light interference (LI) disrupt the natural light:dark cycle, causing alterations at physiological and molecular levels, partly by suppressing melatonin (MLT) secretion at night. Heat shock proteins (HSPs) can be activated in response to environmental changes. We assessed changes in gene expression and protein level of HSP70 in brain and hepatic tissues of golden spiny mice (*Acomys russatus*) acclimated to LI for two (SLI), seven (MLI) and 21 nights (LLI). The effect of MLT treatment on LI-mice was also assessed. HSP70 levels increased in brain and hepatic tissues after SLI, whereas after MLI and LLI, HSP70 decreased to control levels. Changes in HSP70 levels as a response to MLT occurred after SLI only in hepatic tissue. However, *hsp70* expression following SLI increased in brain tissue, but not in hepatic tissue. MLT treatment and SLI caused a decrease in *hsp70* levels in brain tissue and an increase in *hsp70* in hepatic tissue. SLI acclimation elicited a stress response in *A. russatus*, as expressed by increased HSP70 levels and gene expression. Longer acclimation decreases protein and gene expression to their control levels. We conclude that for brain and hepatic tissues of *A. russatus*, LI is a short-term stressor. Our results also revealed that *A. russatus* can acclimate to LI, possibly because of its circadian system plasticity, which allows it to behave both as a nocturnal and as a diurnal rodent. To the best of our knowledge, this is the first study showing the effect of LI as a stressor at the cellular level, by activating HSP70.

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**INTRODUCTION**

Light:dark cycles are a common Zeitgeber for the entrainment of the mammalian biological clock and the main cue for their seasonal acclimatization (Aschoff et al., 1982; Haim et al., 2008). Disruption of scotophase by light results in physiological and hormonal changes that activate stress (Bass and Takahashi, 2010; Zubidat et al., 2007). Light pulses during scotophase [light interference (LI)] and night illumination [light at night (LAN)] have been features of modern life for the last 130 years. Increased exposure of humans to LAN imposes long photoperiod (Gutjahr et al., 2004). Results from studies carried out at The Israeli Center for Interdisciplinary Research in Chronobiology at the University of Haifa have demonstrated that LI, apart from affecting seasonal acclimatization, can be a source of stress with a distinctive impact on the endocrine system and thermoregulation (Zubidat et al., 2007; Zubidat et al., 2011). Furthermore, when the impact of light pulses on expression of transcripts in the mouse brain was analysed by microarray, the results revealed consistent changes in more than 200 different transcripts (Ben-Shlomo et al., 2005; Ben-Shlomo and Kyriacou, 2010).

Melatonin (MLT) is a major hormone that regulates biological rhythms and seasonality and its production is prone to be affected by LI (Arendt, 2006; Reiter, 1993). The link between MLT suppression and LAN has been studied in rodents (Brainard et al., 1984) and humans (Cajochen, 2007). Exposure to light at various points of scotophase caused almost immediate suppression of plasma and pineal MLT levels in CBA mice (Kennaway et al., 2002), and human plasma MLT was suppressed by LAN of different intensities (McIntyre et al., 1989). In addition, acute LAN reduced the plasma MLT levels of common mole-rats (*Cryptomys hottentotus hottentotus*) previously acclimated to a 12h:12h light:dark photoperiod (Gutjahr et al., 2004).

One of the common cellular stress responses is the activation of heat shock proteins (HSPs). HSPs have an essential role in various kinds of stresses: hyperthermia, acidosis, energy depletion and free...
radical presence (Kregel, 2002). The primary function of HSPs is to protect proteins from structural damage that may be caused by environmental and physiologic stress and facilitate their translocation across the membranes (Morimoto and Santoro, 1998). There is evidence of circadian rhythm in hsp gene expression (Rensing and Monnerjahn, 1996; Bitting et al., 1999; Hughes et al., 2009). In chicken pinealocytes, a light pulse at the end of the subjective night stimulated the expression of 62 genes, among them were genes responsive to the heat shock and endoplasmic reticulum stress, as well as their regulatory transcription factors, HSFl and HSF2 (heat shock factors 1 and 2), (Hatori et al., 2011). As shown by Reinke and colleagues (Reinke et al., 2008), HSF1 binds to the promoter of heat shock genes in a circadian manner. Furthermore, there were many HSP genes that were found to be expressed in phase with Per2 mRNA in mice liver (Kornmann et al., 2007).

The HSP70 family is one of the most conserved protein families in evolution. These are proteins with a molecular weight of ~70 kDa, present in the cell both in constitutive and inducible forms (Kiang and Tsokos, 1998). The housekeeping functions of HSP70 chaperones include the transport of proteins between cellular compartments, the degradation of unstable and misfolded proteins, and the folding and refolding of proteins (Daugaard et al., 2007).

The HSP response to MLT treatment varies and depends upon the studied species and particular HSP family. Results of an in vitro study revealed an increase in hsp60 gene expression after the cells were treated with MLT (Bonior et al., 2005). In contrast, the results of Sharman et al. (Sharman et al., 2007) showed that MLT decreased hsp70 mRNA expression in brain tissues from aged mice.

Exposure to acute or chronic stressors has different impacts on mammals, which respond in different intensities and manners (Filipović et al., 2005; Djordjevic et al., 2009; Stoney et al., 1999). Short or long duration of LAN exposure is a part of modern life in industrialized countries and it is a potential health risk factor (Davis and Mirick, 2006; Kloog et al., 2008; Schernhammer et al., 2001), the severity of which may vary as the exposure to the stressor continues.

In the present study, mice were acclimated to short day length and then subjected to LI, which probably changes seasonality (Haim et al., 2005). For this study we chose to use the term ‘acclimation’ to define experimental conditions because we kept the animals under artificial laboratory lighting. Therefore, we asked whether the duration of acclimation to LI influences the intensity of the HSP response. We intended to test the hypothesis that if HSP70 is involved in cellular stress responses, then an increase in this variable due to LI is an indication that LI is a stressor. Furthermore, if long-term exposure to LI results in decreased HSP70 levels relative to short-term exposure, then it may indicate a possible ‘adaptation’ to LI. The aim of this study was to test this hypothesis by estimating the changes in HSP70 levels and its mRNA expression in brain tissue (a central nervous system organ where environmental light signal is processed) and hepatic tissue (a peripheral organ) of short day (SD)-acclimated Acomys russatus (Wagner 1840) exposed to 30 min of LI for different acclimation durations (ADs): acute AD, two nights LI (SLI), and chronic AD, seven nights LI (MLI) and 21 nights LI (LLI).

Our animal model was the golden spiny mouse A. russatus, a nocturnal species that is a common rock-dweller in arid and hot environments of northeast Egypt, Sinai, Israel, Jordan and western parts of the Arabian Peninsula (Harrison and Bates, 1991). The ability of A. russatus to function as a diurnal species as well as a nocturnal one has been noted by several authors (Shkolnik, 1971; Haim and Rozenfeld, 1993); this biological characteristic makes it a useful animal model for the study of LI.

MATERIALS AND METHODS

Animal acclimation

Male golden spiny mice (A. russatus) 4–5 months of age were recruited from our breeding colony at the Oranim campus of the University of Haifa. Mice were placed into individual cages, with rat pellets (composed of 21% crude protein, 4% crude fat, 4% cellulose, 13% moisture, and 7% ash and non-digestible matter; Koffolk, Tel-Aviv, Israel) and fresh carrots (as a water source) provided ad libitum. Mice were kept inside a temperature- and photoperiod-controlled chamber (Shellab, Cornelius, OR, USA) at an ambient temperature of 26±1°C, under an SD photoperiod (8h:16h light:dark, lights on between 08:00 and 16:00h). Lighting was provided by a cool fluorescent illumination with a dominant wavelength of 470 nm and at an intensity of 450 lx during photophase, and constant red dim light (697 nm) of 25 lx. Prior to experimental manipulation, all mice were acclimated to SD for at least 3 weeks (Haim et al., 2008; Zubidat et al., 2011). LI was imposed by exposing the mice for 30 min of day illumination, 6 h after darkness onset, at 00:00h. All experimental procedures were approved by the University of Haifa Institutional Ethics Committee (permit number 112/08).

Experiment 1

For estimation of the daily changes in HSP70 protein level and gene expression, SD-acclimated A. russatus males (N=20) were divided into four groups of five individuals each. Mice were killed by decapitation at four time points (08:00, 12:00, 16:00 and 00:00h). Brain and hepatic tissues were removed, flash-frozen in liquid nitrogen and kept at −80°C for protein and mRNA assays.

Experiment 2

To evaluate the impact of LI acclimation duration on HSP70 level and gene expression, SD-acclimated A. russatus males were divided into four groups (N=7 or 8) and acclimated to various durations of LI: (1) control group (SD) without LI exposure, (2) two nights LI (SLI), (3) seven nights LI (MLI) and (4) 21 nights LI (LLI).

Experiment 3

To estimate the effect of exogenous MLT treatment, SD-acclimated A. russatus males were LI-acclimated for two or seven nights and injected with 50 mg kg⁻¹ MLT (Sigma-Aldrich, St Louis, MO, USA) diluted in saline plus 0.005% ethanol, 1 h prior to lights off, while control groups were injected only with the vehicle at the same time.

At the end of experiments 2 and 3, mice were killed by decapitation 2 h after LI exposure at 02:00 h under red dim light. Brain and hepatic tissues were removed, homogenised, flash-frozen in liquid nitrogen and stored at −80°C until they were assayed.

Western blot

Protein was extracted from the cytosolic fraction with buffer containing 0.25 mol l⁻¹ sucrose, 20 mmol l⁻¹ Tris pH 7.6, 1.5 mmol l⁻¹ MgCl₂, 10% glycerol, 1 mmol l⁻¹ EDTA and Complete Protease Inhibitor Cocktail (Roche, Applied Science, Mannheim, Germany), while nucleic fractions were separated using buffer containing 20 mmol l⁻¹ Tris pH 7.6, 0.42 mmol l⁻¹ NaCl, 25% glycerol and 0.2 mmol l⁻¹ EDTA as previously described (Shein et al., 2005) with minor modifications. Protein concentrations were estimated with Bradford reagent (Sigma-Aldrich) according to the manufacturer’s instructions. Twenty micrograms of protein of each fraction were separated by SDS-PAGE and transferred to nitrocellulose membrane
levels were revealed in either brain or hepatic tissue.

Levels of HSP70 following LI and MLT treatment

A significant ($P<0.005$) increase in HSP70 in both brain and hepatic tissue cytosolic and nuclear fractions in SLI groups was noted (Fig. 2). After MLI and LLI acclimation, the levels of HSP70 were similar to those of SD-acclimated mice (control group), thus revealing a significant ($P<0.05$) decrease in protein levels relative to the SLI group. SLI mice responded to MLT treatment by a significant HSP70 increase of ~60% (from 0.05±0.55 to 0.17±0.12, $P<0.05$) in the hepatic tissue cytosolic fraction, while a significant decrease of ~70% (from 0.39±0.23 to 0.05±0.2, $P<0.05$) in the hepatic tissue nuclear fraction was revealed (Fig. 3). There was no significant impact of MLT treatment on HSP70 levels in the brain or hepatic tissue of MLI-acclimated mice.

Gene expression of hsp70 following LI and MLT treatment

There was a significant increase of ~80% (from 0.7±0.2 to 3.6±0.9, $P<0.05$) in hsp70 expression in brain tissue after SLI acclimation (Fig. 4), while following MLI and LLI, a significant ($P<0.05$) decrease in hsp70 expression of ~60% (from 3.6±0.9 to 1.4±0.35) and 40% (from 3.6±0.9 to 2.4±0.8), respectively, was observed. However, no significant effect of LI on hsp70 expression was noted in hepatic tissue samples.

A significant effect of both LI and MLT treatments on hsp70 expression was revealed both in brain and hepatic tissue ($P<0.05$; Fig. 5). After SLI acclimation and MLT treatment there was a significant decrease (60%, from 3.2±0.4 to 1.1±0.3, $P<0.05$) in hsp70 expression in brain tissue. A significant increase of ~70% (from 0.4±0.2 to 1.9±0.5, $P<0.05$) in hepatic tissue was noted after MLT treatment. MLT did not influence hsp70 mRNA levels after MLI acclimation, relative to the control group, in either of the tissues.
DISCUSSION

Stress was first defined by Selye (Selye, 1950) as a non-specific body response to environmental changes that endanger life, while the individual can elicit the appropriate response to bring itself back to a state of homeostasis. However, according to the latest development in stress research, it was suggested that the stress response is activated primarily by unpredictable and unanticipated events (Koolhaas et al., 2011). Moreover, it is claimed that repeated exposure to a stressor may decrease the severity of the response, thus causing an adaptation. Stress response activates molecular mechanisms that, as a consequence, alternate physiological and behavioural reactions to the stressor (de Nadal et al., 2011; Schwimmer et al., 2006).

The results of our study show that SLI acclimation of 30 min each night caused an increase in gene expression and protein levels of HSP70 in brain tissue, while in hepatic tissue only protein levels were elevated. However, chronic acclimation to MLI and LLI decreased both brain and hepatic tissue protein and gene expression to their control levels. Therefore, it can be assumed that chronic exposure to LI, in relation to the studied tissues, facilitates acclimation, meaning that these tissues can adjust to new photoperiod conditions. A similar response was shown by Martinez and co-authors, when expression of c-fos was ceased in several areas of the rat forebrain after repeated exposure to defeat as the social stressor (Martinez et al., 1998). In our experiment, sudden light exposure in the middle of the scotophase is an unpredictable event, followed by acute and quick response (Figs 2, 4) when given for a short duration, i.e. two nights. As the duration of acclimation increases to 1 week and more, it seems to become anticipated, thus minimizing the intensity of stress response. In the present study, no differences between cytosolic and nuclear fractions in HSP70 response to stress were noted, in contrast to studies that showed that HSP70 localises to the nucleus following cell stress (e.g. Knowlton and Salfity, 1996).

From the results of our study, extensive changes in HSP70 protein and gene expression levels after short-term LI acclimation are revealed, which is in agreement with the assumption that LI is a stressor that affects cellular mechanisms; this effect is not a long-lasting one, at least not in brain or hepatic tissue. Whether this pattern of response is beneficial to the organism remains an open question.
Regarding the acclimation to a stressor, various results are presented in the literature. As shown in other types of stressors, the heat shock response intensifies with the duration of the exposure to the stressor; for example, increased HSP70 levels were correlated with the longer duration of diabetes in patients (Nakhjavani et al., 2010), and HSP70 levels and its cellular location were different in acute relative to chronic stress treatments in the rat brain (Djordjevic et al., 2009; Filipović et al., 2005). The expression of brain HSP70 in rats increased significantly after chronic exposure to noise, relative to acute exposure (Samson et al., 2007). We suggest that LI is a stressor in regard to HSP70 response; however, brain and hepatic tissues became unresponsive to further LI when acclimated for a longer duration.

The changes in both HSP70 protein levels and hsp70 mRNA expression following LI show the same patterns—an increase after SLI (acute AD) and a decrease to SD-control levels after longer AD to LI. The increase in HSP70 in SLI-acclimated mice is more substantial in the nuclear fraction of brain tissue than in that of hepatic tissue; additionally, we observed a significant change in mRNA expression in brain tissue samples, but not in hepatic tissue samples. We assume that the reason can be the time lag between the transcription and translation processes (Berg et al., 2002), while the response is delayed in the hepatic tissue (peripheral tissue) relative to brain tissue (central tissue).

The consistent increase in the assayed parameters following short AD and minimal change after medium and long AD emphasises the fact that this response was caused by the treatment and not any other external factor, especially because animals were kept and killed under the same conditions.

Daily changes in hsp70 were discovered only in hepatic tissue (Fig. 1), while no significant differences in protein level were revealed. Results show that under SD conditions, gene expression was significantly higher during photophase than during scotophase.

There is evidence of MLT influence on hsp70 expression, though not in the context of photoperiod-associated stress; MLT decreased hsp70 expression in rat brains and mouse CNS as well as in the hepatic tissue (Sharman et al., 2007; Ozacmak et al., 2009; Mathes, 2010). Here, there was a different response of hsp70 mRNA expression to MLT treatment in brain and hepatic tissues: whereas in hepatic tissue hsp70 expression was increased by MLT after SLI, in brain tissue hsp70 expression was decreased. Following MLT treatment, HSP70 protein levels decreased in the hepatic tissue nuclear fraction but were elevated in its cytosolic fraction, while no effect of MLT in the brain tissue was noted. MLT exerts its cellular effect via binding specific receptors (Dubocovich, 1995); the distribution and actions of these receptors vary between tissues (Naji et al., 2004; Sallinen et al., 2005). This may explain the difference in the effect of MLT between tissues.

In experiments with human subjects, the circadian system was found to be adaptive and maintain a high plasticity, as prior exposure to dim light was shown to influence MLT suppression following...
more intensive light stimuli (Jasser et al., 2006; Chang et al., 2011). According to our results, long AD causes the decrease in response in regard to hsp70 expression and protein levels, probably because the animal becomes used to the LI. It is possible that the high levels of HSP70 revealed in SLL-acclimated mice activated pathways that contribute to the organism’s ability to cope with LI for the longer period.

LI is considered a stressor that can alter MLT secretion (Lewy et al., 1980). Results of previous studies on various rodent species revealed that winter acclimatization of different systems was affected by exposure to LI; for instance, thermoregulatory mechanism (Haim et al., 2005; Zubidat et al., 2007), immune response (Bedrosian et al., 2011), male reproductive system and pelage (Hoffmann, 1979). Our results show that there is a direct influence of LI on HSP70 in brain and hepatic tissues, which is one of the first and most important factors in cellular stress response.

The temporal organisation of A. russatus has been studied extensively, starting with early work in the 1970s (Shkolnik, 1971). The lability of its temporal organisation expressed by daily rhythms reveals an ability to act both as a diurnal and as a nocturnal animal depending on the presence of its sibling competitor A. calhirus (Haim and Rozenfeld, 1993). Results of several studies (Zisapel et al., 1999; Cohen et al., 2009; Cohen et al., 2010) have emphasised the plasticity of its circadian system. This concurs with the rapid changes in the parameters of A. russatus assayed in our study. We suppose that this plasticity is an additional factor that influences the ability of A. russatus to acclimate to LI and regulate its cellular response to sudden changes in environmental illumination conditions.

Conclusions

The ability of LI to alter stress gene expression sheds light on the characteristics of LAN as a stressor. The mechanism and pathways that involve HSP70 activation following LI are yet to be revealed. There is evidence that LI enhances oxidative stress, because it alters MLT (known as an antioxidant) levels and rhythms (Reiter et al., 2003; Hardeland et al., 2003). This in turn can activate the heat shock response. Furthermore, future research into the heat shock response in other tissues and species, as well as the impact of LI on longevity, will be of great interest.

LIST OF ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>acclimation duration</td>
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<td>HSP</td>
<td>heat shock protein</td>
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<td>LAN</td>
<td>light at night</td>
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<td>LI</td>
<td>light interference</td>
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<td>LLI</td>
<td>long LI</td>
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<td>MLI</td>
<td>medium LI</td>
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<td>MLT</td>
<td>melatonin</td>
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<td>RQ</td>
<td>relative quantification</td>
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<td>SLI</td>
<td>short LI</td>
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FUNDING

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REFERENCES


Fig. S1. Representative western blot of cytosolic and nuclear hepatic tissue fractions blotted with anti-Lamin B antibody. C, cytosolic fraction; N, nuclear fraction.

Fig. S2. Nucleotide sequence alignment between hspa1b (NM_010478.2) of house mouse *Mus musculus* and a 300 bp PCR product of a DNA fragment extracted from golden spiny mouse *Acomys russatus* hepatic tissue. The alignment was performed with Basic Local Alignment Search Tool (BLAST) network service from the National Center for Biotechnology Information (Zhang et al., 2000). There is 94% identity between the sequences.