The Journal of Experimental Biology 215, 4034-4040

doi:10.1242/jeb.073429

RESEARCH ARTICLE

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

Lilach Ashkenazi* and Abraham Haim

The Israeli Center for Interdisciplinary Research in Chronobiology, Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, University of Haifa, Mount Carmel, Haifa 31905, Israel

*Author for correspondence (alilach@yahoo.com)

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.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/22/4034/DC1

Key words: light at night, melatonin, adaptation, stress, RT-PCR, western blot.

Received 5 April 2012; Accepted 15 August 2012

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 a long photoperiod of approximately 16–18 h of light year round, a phenomenon that alters the responses of humans and other mammals to seasonality (Wehr, 2001; Haim et al., 2005; Schernhammer and Schulmeister, 2004). Moreover, numerous studies indicate the growing impact of LAN on hormone-dependent cancer, metabolic disorders, reproduction and immune system function (Kloog et al., 2008; Haus and Smolensky, 2006; Navara and Nelson, 2007; Reddy and O'Neill, 2010; Nelson and Drazen, 2000; Vinogradova et al., 2010). Artificial LAN and LI can have serious ecological implications, especially for nocturnal animals, by increasing the risk of predation, decreasing food consumption, increasing the incidence of road-kill and altering migration patterns (Beier, 2005).

The results of experiments using different animal models show that exposure to LI during scotophase has profound effects (Haim et al., 2005). One or five minutes of light given in the middle of the night were as effective as long photoperiod acclimation for changing the pelage colour and male reproductive system function of short-photoperiod-acclimated Djungarian hamsters, *Phodopus sungorus* (Hoffmann, 1979). 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 12 h:12 h 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, HSF1 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 (SLI); and chronic AD, seven nights (MLI) and 21 nights (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 (8 h:16 h light:dark, lights on between 08:00 and 16:00 h). Light 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 manipulations, 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 00:00 h. 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 00:00 h). 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} $MgCl_2$, 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 mol 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

(Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked with 5% skim milk TBST (Tris-buffered saline, pH 7.4, 0.1% Tween) for 1 h in room temperature. Membranes were then incubated with monoclonal mouse anti-Hsp70 (Abcam, Cambridge, UK) antibody and monoclonal mouse anti-actin clone C4 (MP Biomedicals, Solon, OH, USA) antibody; the latter was used as a loading control. Protein detection was performed with peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) antibody. Membranes were visualised using enhanced chemiluminescence reagents by an LAS4000 luminescent image analyzer (Fujifilm, Tokyo, Japan). The images were analysed using QuantityOne software (BioRad Laboratories). The purity of cytosolic and nucleic fractions was estimated by blotting representative samples with anti-LaminB antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), which is a solely nucleic protein (supplementary material Fig. S1).

Total RNA extraction and reverse transcription

Total RNA was extracted from brain and hepatic tissues using an RNeasy Mini kit (Qiagen, Hilden, Germany). The concentration and quality of extracted RNA were estimated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Single-stranded cDNA was generated from 0.5 µg of total mRNA with a HighCapacity cDNA RT kit (Applied Biosystems, Foster City, CA, USA).

Real-time PCR

We used *Mus musculis* genomic sequences to assess the gene expression of *A. russatus*. The sequence similarity between these species' genomes is over 90%, as was established in our laboratory by sequencing DNA extracted from *A. russatus* tissues and performing the alignment between it and *M. musculis* sequences (supplementary material Fig. S2). Primers were designed using the *hspa1b* sequence (NM_010478.2) and the *gapdh* sequence (glyceraldehydes-3-phosphate dehydrogenase, NM_008084.2); the latter was used as reference.

We used the following RT-PCR primers: *hsp70*: forward 5′–3′ CACCAAGCAGACGCAGACC, reverse 5′–3′ CCTCGTACAC-CTGGATCAGCA; *gapdh*: forward 5′–3′ AGGTCGGTGTGA-ACGGAT TTG, reverse 5′–3′ TGTAGACCATGTAGTTG-AGGTCA (Sigma-Aldrich). Because the *hspa1b* sequence contains only one exon, samples were tested for genomic DNA contamination by running non-polymerase PCR.

RT-PCR was performed using SYBR Green technology. For this procedure, gene-specific primers and SYBR Green Real-Time PCR Master Mix (Applied Biosystems) were used according to the manufacturer's instructions. Relative gene expression was detected using the ABI Prism 7000 Genetic Analyzer (Applied Biosystems). *hsp70* expression was estimated relative to *gapdh* and analysed using the relative quantification (RQ) method with StepOnePlus software (Applied Biosystems).

Statistical analysis

Protein level and gene expression results for estimating the effects of the duration of LI exposure on protein level and gene expression of HSP70 were analysed using one-way ANOVA. The response to MLT treatment of different acclimation groups was estimated using two-way ANOVA. Tukey's *post hoc* test was used to determine significant differences between groups. Significant differences were considered at a level of $P<0.05$. Data were analysed with SPSS 18.0 software (IBM, Armonk, NY, USA).

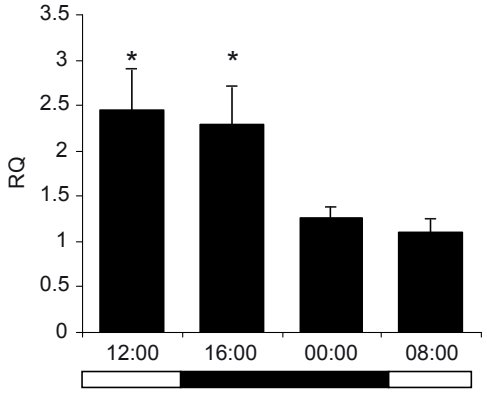

Fig. 1. Daily *hsp70* expression in the hepatic tissue of golden spiny mice, *Accomys russatus*, acclimated to a short day (SD) photoperiod (8 h:16 h light dark) and killed at 12:00, 16:00, 00:00 and 08:00 h, assayed by RT-PCR. Relative quantification (RQ) determines the changes in the levels of *hsp70* mRNA across multiple samples and expresses this relative to levels of the housekeeping control gene (*gapdh* in the present study). Scotophase (photophase) is represented by the black (white) portion of the horizontal bar. Values are means + s.e.m., $N=5$ (*$P<0.05$).

RESULTS

Daily variations in HSP70 levels and *hsp70* expression

Significant ($P<0.05$) daily variations in *hsp70* expression were observed in hepatic tissue – the values at 12:00 and 16:00 h were higher than those obtained at 00:00 and 08:00 h, and the average values of the former were ~55% (RQ from 1.3±0.26 to 2.2±0.92) higher than the latter (Fig. 1). No significant differences in protein 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 0.055±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±0.02, $P<0.05$) 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.

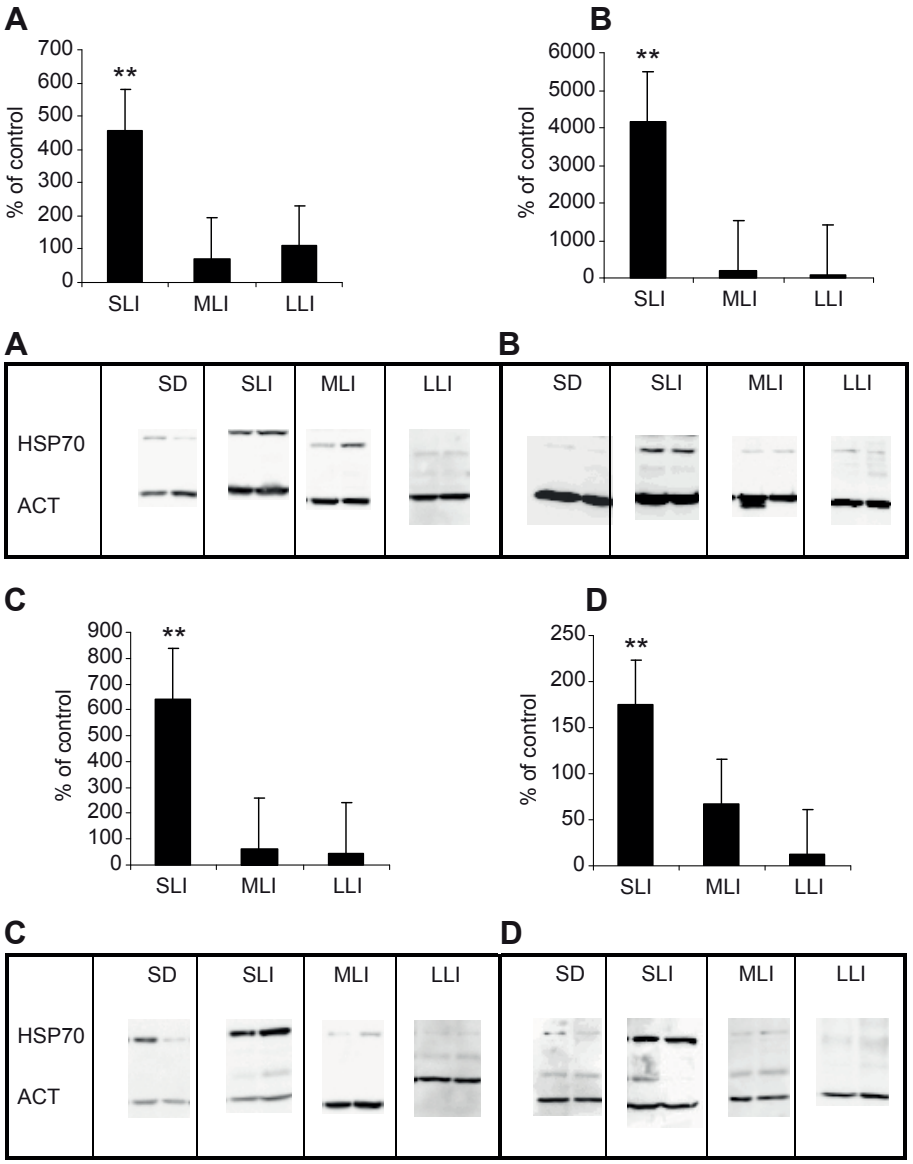

Fig. 2. Changes in HSP70 levels in brain and hepatic tissue of golden spiny mice exposed to 30 min of light interference (LI) for two (SLI), seven (MLI) and 21 nights (LLI), assayed by western blot. (A) Brain tissue, cytosolic fraction; (B) brain tissue, nuclear fraction; (C) hepatic tissue, cytosolic fraction; and (D) hepatic tissue, nuclear fraction. Representative sets of blots for the control (SD) and LI-acclimated groups are shown below each panel. Quantification of HSP70 bands are relative to actin and presented as a percent of SD. Values are means + s.e.m., N=7 or 8 (**P≤0.005).

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.

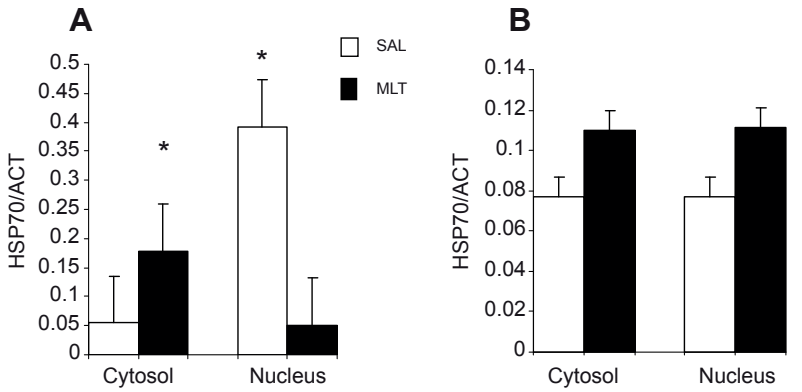

Fig. 3. Changes in HSP70 levels in (A) hepatic tissue and (B) brain tissue of SLI-acclimated golden spiny mice following melatonin (MLT) treatment relative to the control [saline (SAL)], assayed by western blot. The *y*-axis is the ratio of the densities of the HSP70 band and the actin band, which was used as a loading control. Values are means + s.e.m., *N*=7 or 8 (**P*<0.05).

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

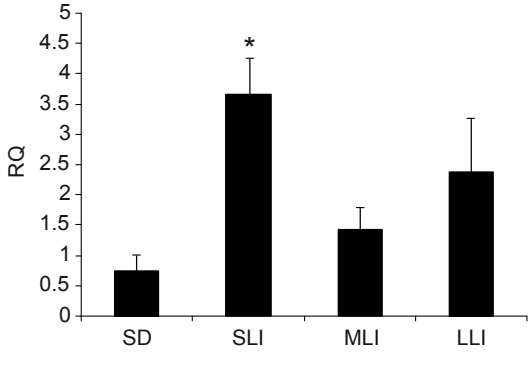

Fig. 4. Changes in *hsp70* expression, assayed by RT-PCR, in brain tissue of golden spiny mice exposed to 30 min of light interference (LI) for two (SLI), seven (MLI) and 21 nights (LLI) relative to SD-acclimated controls. Relative quantification (RQ) determines the changes in the levels of *hsp70* mRNA across multiple samples and expresses this relative to levels of the housekeeping control gene *gapdh*. Values are means + s.e.m., *N*=7 or 8 (**P*<0.05).

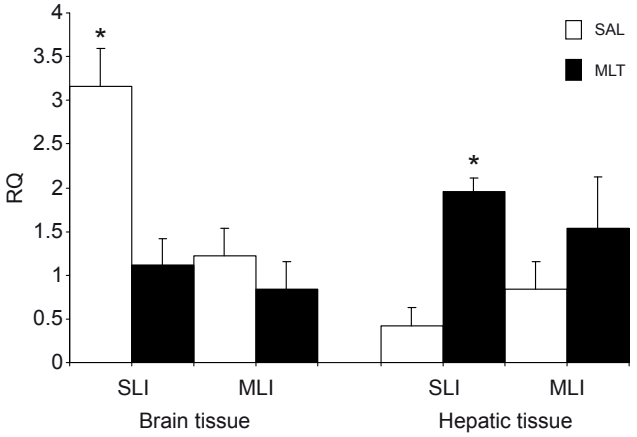

Fig. 5. Real-time PCR. Relative quantification (RQ) of *hsp70* expression in brain and hepatic tissue of golden spiny mice following light interference (LI) of 30 min and melatonin (MLT) treatment relative to saline (SAL)-treated controls, under SLI and MLI acclimation. Values are means + s.e.m., *N*=7 or 8 (**P*<0.05).

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 SLI-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. cahirinus* (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

AD	acclimation duration
HSP	heat shock protein
LAN	light at night
LI	light interference
LLI	long LI
MLI	medium LI
MLT	melatonin
RQ	relative quantification
SLI	short LI

ACKNOWLEDGEMENTS

We would like to thank two anonymous reviewers for their useful comments that contributed to the improvement of this article.

FUNDING

This research was funded by the research authorities of University of Haifa.

REFERENCES

Arendt, J. (2006). Melatonin and human rhythms. *Chronobiol. Int.* **23**, 21-37.
Aschoff, J., Daan, S. and Honma, K.-I. (1982). Zeitgebers, entrainment, and masking: some unsettled questions. In *Vertebrate Circadian Systems* (ed. J. Aschoff, S. Daan and G. A. Groos), pp. 13-24. Berlin: Springer-Verlag.
Bass, J. and Takahashi, J. S. (2010). Circadian integration of metabolism and energetics. *Science* **330**, 1349-1354.
Bedrosian, T. A., Fonken, L. K., Walton, J. C. and Nelson, R. J. (2011). Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol. Lett.* **7**, 468-471.
Beier, P. (2005) Effects of artificial lighting on terrestrial mammals. In *Ecological Consequences of Artificial Night Lighting* (ed. C. Rich and T. Longcore), pp. 19-38. Washington, DC: Island Press.
Ben-Shlomo, R. and Kyriacou, C. P. (2010). Light pulses administered during the circadian dark phase alter expression of cell cycle associated transcripts in mouse brain. *Cancer Genet. Cytogenet.* **197**, 65-70.
Ben-Shlomo, R., Akhtar, R. A., Collins, B. H., Judah, D. J., Davies, R. and Kyriacou, C. P. (2005). Light pulse-induced heme and iron-associated transcripts in mouse brain: a microarray analysis. *Chronobiol. Int.* **22**, 455-471.
Berg, J. M., Tymoczko, J. L. and Stryer, L. (ed.) (2002). Eukaryotic transcription and translation are separated in space and time. In *Biochemistry*, 5th edn, section 28.2. New York: W. H. Freeman and Company.
Bitting, L., Watson, F. L., O'Hara, B. F., Kilduff, T. S. and Heller, H. C. (1999). HSP70 expression is increased during the day in a diurnal animal, the golden-mantled ground squirrel *Spermophilus lateralis*. *Mol. Cell. Biochem.* **199**, 25-34.
Bonior, J., Jaworek, J., Konturek, S. J. and Pawlik, W. W. (2005). Increase of heat shock protein gene expression by melatonin in AR42J cells. *J. Physiol. Pharmacol.* **56**, 471-481.
Brainard, G. C., Richardson, B. A., King, T. S. and Reiter, R. J. (1984). The influence of different light spectra on the suppression of pineal melatonin content in the Syrian hamster. *Brain Res.* **294**, 333-339.
Cajochen, C. (2007). Alerting effects of light. *Sleep Med. Rev.* **11**, 453-464.
Chang, A.-M., Scheer, F. A. J. L. and Czeisler, C. A. (2011). The human circadian system adapts to prior photic history. *J. Physiol.* **589**, 1095-1102.
Cohen, R., Smale, L. and Kronfeld-Schor, N. (2009). Plasticity of circadian activity and body temperature rhythms in golden spiny mice. *Chronobiol. Int.* **26**, 430-446.
Cohen, R., Smale, L. and Kronfeld-Schor, N. (2010). Masking and temporal niche switches in spiny mice. *J. Biol. Rhythms* **25**, 47-52.
Daugaard, M., Rohde, M. and Jäättelä, M. (2007). The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* **581**, 3702-3710.
Davis, S. and Mirick, D. K. (2006). Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. *Cancer Causes Control* **17**, 539-545.
de Nadal, E., Ammerer, G. and Posas, F. (2011). Controlling gene expression in response to stress. *Nat. Rev. Genet.* **12**, 833-845.
Djordjevic, A., Adzic, M., Djordjevic, J. and Radojcic, M. B. (2009). Stress type dependence of expression and cytoplasmic-nuclear partitioning of glucocorticoid receptor, hsp90 and hsp70 in Wistar rat brain. *Neuropsychobiology* **59**, 213-221.
Dubocovich, M. L. (1995). Melatonin receptors: are there multiple subtypes? *Trends Pharmacol. Sci.* **16**, 50-56.
Filipović, D., Gavrilović, L., Dronjak, S. and Radojcić, M. B. (2005). Brain glucocorticoid receptor and heat shock protein 70 levels in rats exposed to acute, chronic or combined stress. *Neuropsychobiology* **51**, 107-114.
Gutjahr, G. H., van Rensburg, L. J., Malpaux, B., Richter, T. A. and Bennett, N. C. (2004). The endogenous rhythm of plasma melatonin and its regulation by light in the highveld mole-rat (*Cryptomys hottentotus pretoriae*): a microphthalmic, seasonally breeding rodent. *J. Pineal Res.* **37**, 185-192.
Haim, A. and Rozenfeld, F. M. (1993). Temporal segregation in coexisting *Acomys* species: the role of odour. *Physiol. Behav.* **54**, 1159-1161.
Haim, A., Shanas, U., Zubidad, A. S. and Scantelbury, M. (2005). Seasonality and seasons out of time – the thermoregulatory effects of light interference. *Chronobiol. Int.* **22**, 59-66.
Haim, A., Zubidat, A. E. S. and van Aarde, R. J. (2008). Daily rhythms of body temperature and heat production of sibling *Mastomys* species from different ecosystems—the response to photoperiod manipulations. *Comp. Biochem. Physiol.* **151A**, 505-510.
Hardeland, R., Coto-Montes, A. and Poeggeler, B. (2003). Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. *Chronobiol. Int.* **20**, 921-962.
Harrison, D. L. and Bates, P. J. J. (1991). *The Mammals of Arabia*, 2nd edn. Kent, UK: Harrison Zoological Museum Publication.
Hatori, M., Hirota, T., Iitsuka, M., Kurabayashi, N., Haraguchi, S., Kokame, K., Sato, R., Nakai, A., Miyata, T., Tsutsui, K. et al. (2011). Light-dependent and circadian clock-regulated activation of sterol regulatory element-binding protein, X-box-binding protein 1, and heat shock factor pathways. *Proc. Natl. Acad. Sci. USA* **108**, 4864-4869.
Haus, E. and Smolensky, M. (2006). Biological clocks and shift work: circadian dysregulation and potential long-term effects. *Cancer Causes Control* **17**, 489-500.
Hoffmann, K. (1979). Photoperiodic effects in the Djungarian hamster: one minute of light during darktime mimics influence of long photoperiods on testicular recrudescence, body weight and pelage colour. *Experientia* **35**, 1529-1530.
Hughes, M. E., DiTacchio, L., Hayes, K. R., Vollmers, C., Pulivarthy, S., Baggs, J. E., Panda, S. and Hogenesch, J. B. (2009). Harmonics of circadian gene transcription in mammals. *PLoS Genet.* **5**, e1000442.
Jasser, S. A., Hanifin, J. P., Rollag, M. D. and Brainard, G. C. (2006). Dim light adaptation attenuates acute melatonin suppression in humans. *J. Biol. Rhythms* **21**, 394-404.
Kennaway, D. J., Voultsios, A., Varcoe, T. J. and Moyer, R. W. (2002). Melatonin in mice: rhythms, response to light, adrenergic stimulation, and metabolism. *Am. J. Physiol.* **282**, R358-R365.
Kiang, J. G. and Tsokos, G. C. (1998). Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol. Ther.* **80**, 183-201.

Kloog, I., Haim, A., Stevens, R. G., Barchana, M. and Portnov, B. A. (2008). Light at night co-distributes with incident breast but not lung cancer in the female population of Israel. *Chronobiol. Int.* **25**, 65-81.
Knowlton, A. A. and Salfity, M. (1996). Nuclear localization and the heat shock proteins. *J. Biosci.* **21**, 123-132.
Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P. et al. (2011). Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* **35**, 1291-1301.
Kornmann, B., Schaad, O., Reinke, H., Saini, C. and Schibler, U. (2007). Regulation of circadian gene expression in liver by systemic signals and hepatocyte oscillators. *Cold Spring Harb. Symp. Quant. Biol.* **72**, 319-330.
Kregel, K. C. (2002). Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J. Appl. Physiol.* **92**, 2177-2186.
Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. and Markey, S. P. (1980). Light suppresses melatonin secretion in humans. *Science* **210**, 1267-1269.
Martinez, M., Phillips, P. J. and Herbert, J. (1998). Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *Eur. J. Neurosci.* **10**, 20-33.
Mathes, A. M. (2010). Hepatoprotective actions of melatonin: possible mediation by melatonin receptors. *World J. Gastroenterol.* **16**, 6087-6097.
McIntyre, I. M., Norman, T. R., Burrows, G. D. and Armstrong, S. M. (1989). Human melatonin suppression by light is intensity dependent. *J. Pineal Res.* **6**, 149-156.
Morimoto, R. I. and Santoro, M. G. (1998). Stress-inducible responses and heat shock proteins: new pharmacologic targets for cytoprotection. *Nat. Biotechnol.* **16**, 833-838.
Naji, L., Carrillo-Vico, A., Guerrero, J. M. and Calvo, J. R. (2004). Expression of membrane and nuclear melatonin receptors in mouse peripheral organs. *Life Sci.* **74**, 2227-2236.
Nakhjavani, M., Morteza, A., Khajeali, L., Esteghamati, A., Khalilzadeh, O., Asgarani, F. and Outeiro, T. F. (2010). Increased serum HSP70 levels are associated with the duration of diabetes. *Cell Stress Chaperones* **15**, 959-964.
Navara, K. J. and Nelson, R. J. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal Res.* **43**, 215-224.
Nelson, R. J. and Drazen, D. L. (2000). Melatonin mediates seasonal changes in immune function. *Ann. N. Y. Acad. Sci.* **917**, 404-415.
Ozacmak, V. H., Barut, F. and Ozacmak, H. S. (2009). Melatonin provides neuroprotection by reducing oxidative stress and HSP70 expression during chronic cerebral hypoperfusion in ovariectomized rats. *J. Pineal Res.* **47**, 156-163.
Reddy, A. B. and O'Neill, J. S. (2010). Healthy clocks, healthy body, healthy mind. *Trends Cell Biol.* **20**, 36-44.
Reinke, H., Saini, C., Fleury-Olela, F., Dibner, C., Benjamin, I. J. and Schibler, U. (2008). Differential display of DNA-binding proteins reveals heat-shock factor 1 as a circadian transcription factor. *Genes Dev.* **22**, 331-345.
Reiter, R. J. (1993). The melatonin rhythm: both a clock and a calendar. *Experientia* **49**, 654-664.
Reiter, R. J., Tan, D.-X., Mayo, J. C., Sainz, R. M., Leon, J. and Czarnocki, Z. (2003). Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. *Acta Biochim. Pol.* **50**, 1129-1146.
Rensing, L. and Monnerjahn, C. (1996). Heat shock proteins and circadian rhythms. *Chronobiol. Int.* **13**, 239-250.
Sallinen, P., Saarela, S., Ilves, M., Vakkuri, O. and Leppäluoto, J. (2005). The expression of MT1 and MT2 melatonin receptor mRNA in several rat tissues. *Life Sci.* **76**, 1123-1134.
Samson, J., Sheeladevi, R., Ravindran, R. and Senthilvelan, M. (2007). Stress response in rat brain after different durations of noise exposure. *Neurosci. Res.* **57**, 143-147.
Schernhammer, E. S. and Schulmeister, K. (2004). Melatonin and cancer risk: does light at night compromise physiologic cancer protection by lowering serum melatonin levels? *Br. J. Cancer* **90**, 941-943.
Schernhammer, E. S., Laden, F., Speizer, F. E., Willett, W. C., Hunter, D. J., Kawachi, I. and Colditz, G. A. (2001). Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl. Cancer Inst.* **93**, 1563-1568.
Schwimmer, H., Eli-Berchoer, L. and Horowitz, M. (2006). Acclimatory-phase specificity of gene expression during the course of heat acclimation and superimposed hypohydration in the rat hypothalamus. *J. Appl. Physiol.* **100**, 1992-2003.
Selye, H. (1950). Stress and the general adaptation syndrome. *BMJ* **1**, 1383-1392.
Sharman, E. H., Bondy, S. C., Sharman, K. G., Lahiri, D., Cotman, C. W. and Perreau, V. M. (2007). Effects of melatonin and age on gene expression in mouse CNS using microarray analysis. *Neurochem. Int.* **50**, 336-344.
Shein, N. A., Horowitz, M., Alexandrovich, A. G., Tsenter, J. and Shohami, E. (2005). Heat acclimation increases hypoxia-inducible factor 1alpha and erythropoietin receptor expression: implication for neuroprotection after closed head injury in mice. *J. Cereb. Blood Flow Metab.* **25**, 1456-1465.
Shkolnik, A. (1971). Diurnal activity in a small desert rodent. *Int. J. Biometeorol.* **15**, 115-120.
Stoney, C. M., Niaura, R., Bausserman, L. and Matacin, M. (1999). Lipid reactivity to stress: I. Comparison of chronic and acute stress responses in middle-aged airline pilots. *Health Psychol.* **18**, 241-250.
Vinogradova, I. A., Anisimov, V. N., Bukalev, A. V., Ilyukha, V. A., Khizhkin, E. A., Lotosh, T. A., Semenchenko, A. V. and Zabezhinski, M. A. (2010). Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in young but not in old rats. *Aging* **2**, 82-92.
Wehr, T. A. (2001). Photoperiodism in humans and other primates: evidence and implications. *J. Biol. Rhythms* **16**, 348-364.
Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **7**, 203-214.
Zisapel, N., Barnea, E., Izhaki, I., Anis, Y. and Haim, A. (1999). Daily scheduling of the golden spiny mouse under photoperiodic and social cues. *J. Exp. Zool.* **284**, 100-106.
Zubidat, A. E., Ben-Shlomo, R. and Haim, A. (2007). Thermoregulatory and endocrine responses to light pulses in short-day acclimated social voles (*Microtus socialis). Chronobiol. Int.* **24**, 269-288.
Zubidat, A. E., Nelson, R. J. and Haim, A. (2011). Spectral and duration sensitivity to light-at-night in 'blind' and sighted rodent species. *J. Exp. Biol.* **214**, 3206-3217.

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

```
>
ref|NM_010478.2| Mus musculus heat shock protein 1B (Hspa1b), mRNA
Length=2810

 GENE ID: 15511 Hspa1b | heat shock protein 1B [Mus musculus]
(Over 100 PubMed links)


 Score =  411 bits (222),  Expect = 1e-112
 Identities = 256/273 (94%), Gaps = 2/273 (1%)
 Strand=Plus/Minus

Query  1    ACC-GCCGGTTGTTTTANGTCCTCGCCGCCCAGGTGCGTGTCGCCCGCCGTGGCCTTCAC  59
            ||| |||||||| |  | |||||| || ||||||||||||||||||||||||||||||||
Sbjct  944  ACCAGCCGGTTG-TCGAAGTCCTCCCCTCCCAGGTGCGTGTCGCCCGCCGTGGCCTTCAC  886

Query  60   CTCGAAGATGCCGTCGTCGATGGTCAGGATGGACACGTCGAACGTGCCGCCCCCCAGGTC  119
            |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  885  CTCGAAGATGCCGTCGTCGATCGTCAGGATGGACACGTCGAACGTGCCGCCCCCCAGGTC  826

Query  120  GAAGATGAGCACGTTGCGCTCCCCCTTGCCAGAGCGGTCCAGCCCGTAGGCGATGGCGGC  179
            |||||||||||||||||||| |||||||| |  ||||||||||||||||||||||||||
Sbjct  825  GAAGATGAGCACGTTGCGCTCGCCCTTGCCGGTCCGGTCCAGCCCGTAGGCGATGGCGGC  766

Query  180  CGCCGTGGGCTCGTTGATGATGCGCAGCACGTTGAGTCCCGCGATCACGCCCGCGTCTTT  239
            |||||||||||||||||||| |||||||||| || || |||||||||||||||| ||
Sbjct  765  CGCCGTGGGCTCGTTGATGATCCGCAGCACGTTTAGACCGGCGATCACGCCCGCGTCCTT  706

Query  240  GGTGGCCTGCCGCTGAGAGTCGTTGAAGTAGGC  272
            |||||||||||||||||||||||||||||||||
Sbjct  705  GGTGGCCTGCCGCTGAGAGTCGTTGAAGTAGGC  673
```

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.