

SHORT COMMUNICATION

## Transcranial bright light exposure via ear canals does not suppress nocturnal melatonin in healthy adults – A single-blind, sham-controlled, crossover trial

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We investigated whether transcranial bright light (TBL) affects nocturnal melatonin and cortisol secretion in sham-controlled crossover trial. Young healthy adults were exposed in random order to 24 minutes of TBL or sham exposure via ear canals at 01:10 h. Saliva and urine samples were collected hourly between 21 h–03 h and 06 h–09 h. There were no significant differences in melatonin or cortisol concentrations between TBL and sham exposures at any sampling point indicating that TBL via ear canals does not suppress nocturnal melatonin secretion. Thus, non-visual effects of TBL are mediated via a pathway not involving melatonin suppression.

**Keywords:** Circadian rhythm, cortisol, ear light, melatonin, opsins, saliva

### INTRODUCTION

Light delivers visual or image-forming input to brain and also elicits behavioral, endocrine, circadian and physiological responses in humans, termed “non-image forming (NIF)” effects (Cajochen et al., 2005; Golden et al., 2005; West et al., 2011). These NIF effects are thought to be transmitted via an exclusive pathway, running from intrinsically photosensitive ganglion cells in the retina, containing blue-light-sensitive melanopsin (OPN4), via the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN) (Brainard et al., 2001; Provencio et al., 1998). The neural projection from the SCN to the pineal gland transfers the photic information, which determinates melatonin secretion and thus contributes to the regulation of circadian rhythms (Blackshaw & Snyder, 1999; Hannibal, 2002; Moore, 1997; Rea et al., 2005; Reiter et al., 2011).

In addition to the photosensitive melanopsin (Brainard et al., 2001; Provencio et al., 1998), the following potentially photosensitive molecules such as encephalopsin (OPN3) (Blackshaw & Snyder, 1999), neuropsin (OPN5) (Tarttelin et al., 2003), glutamatergic NMDA (Leszkiewicz et al., 2000), cryptochromes (CRYs)

(Foley et al., 2011) as well as visual opsins (Vandewalle et al., 2009) have been suggested to participate in mediating the NIF effects of light.

OPN3, OPN4, OPN5 and peropsin have been found at mRNA and/or protein levels in wide areas of the rodent and human brain and cell lines (Blackshaw & Snyder, 1999; Kojima et al., 2011; Koyanagi et al., 2013; Kumbalasiri & Provencio, 2005; Lein et al., 2007; Tarttelin et al., 2003). Furthermore, the Allen Brain Atlas describes the mRNA gene expression level of OPN3, OPN4 and OPN5 in the human brain (Anonymous, 2013). The exact physiological roles of the aforementioned brain opsins are not known yet; however, the existence of these extraretinal factors in the brain suggests that brain tissue is intrinsically sensitive to external light. There is also evidence that external light can, at least partly, penetrate into the mammalian (Ganong et al., 1963), and the human, brain (Persinger et al., 2013).

Transcranial bright light (TBL) treatment via the ear canals has recently found to ameliorate depressive symptoms in seasonal affective disorder (SAD) (Timonen et al., 2012) and affects the functional

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connectivity in neural networks of the human brain shown by functional magnetic resonance imaging (fMRI) (Starck et al., 2012). TBL through the occipital bone was recently observed to enhance quantitative electroencephalographic (QEEG) power and low-resolution electromagnetic tomography (LORETA) in the parahippocampus (Persinger et al., 2013). Taken together, these findings suggest that light is able to penetrate into the brain and affect extraretinal light-sensitive molecules. The aim of this study is to investigate the effect of nocturnal TBL on melatonin and cortisol secretion in young healthy volunteers.

## MATERIALS AND METHODS

### Subjects

Eight young healthy volunteers, three females and five males aged  $27 \pm 5$  (mean  $\pm$  SD) years participated in this randomized placebo-controlled crossover study, which was carried out in Oulu (65°N) during May and June 2011 (sunrise at 3 h and sunset at 23 h in late May). Prior to enrolling in the study, all subjects gave written informed consent according to the principles of the Declaration of Helsinki. They were also paid for participation.

### Experimental protocol

The protocol consisted of 10 days' physical activity measurements (Fitbit Inc., San Francisco, CA) and two nights' (sixth and eighth night) laboratory measurements. Physical activity measurements showed that the subjects followed normal daytime rhythm during the study period. The subjects reported to the laboratory at 21 h. During the two laboratory nights, the subjects were either TBL- or sham-exposed via both ear canals in random order. The study protocol was single-blind. On both laboratory nights, all subjects were housed in the same light/dark rhythm (16 L:08 D, lights off at 23 h and on at 07 h). Blue-enriched transcranial light or sham exposure was carried at 01:10–01:34 h using a non-invasive photon application device (Valkee Ltd, Oulu,

Finland). The intensity of this light source was 8.5 lm and irradiance  $7.28 \text{ mW/m}^2$ . The spectral distribution of the light source is presented in Figure 1. The device has been approved (European Union III/2010) for experimental and medical use of TBL therapy via the ear canals.

### Saliva and urine sampling

Saliva (1.5–2 ml) and urine samples were collected into polypropylene tubes of 10 ml at the following time points: 12 (noon), 18, 21, 22, 23, 24 (midnight), 01, 02, 03, 06, 07, 08 and 09 h. During the nighttime (24–07 h), all sampling was carried out in dim red light as to not suppress melatonin. All samples were stored frozen at  $-20^\circ\text{C}$  until analyzed.

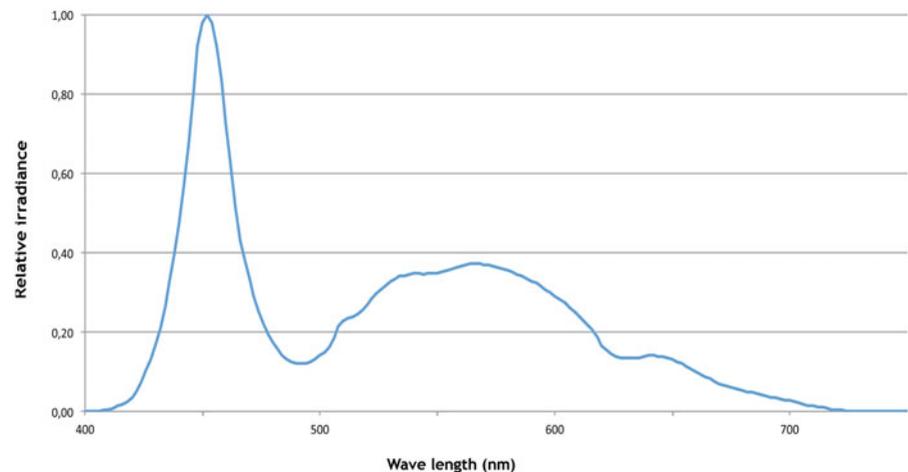
### Melatonin radioimmunoassay

Saliva and urine melatonin were assayed radioimmunologically (Vakkuri et al., 1984a) using 2-iodomelatonin as tracer (Vakkuri et al., 1984b). Briefly, all saliva and urine samples (1.5 ml) were first centrifuged ( $12\,000 \times g$  for 10 min), and then the samples of 1 ml were extracted with chloroform for melatonin. After evaporation of the chloroform phase, the residue was dissolved into phosphate-buffered saline buffer for radioimmunoassay, pipetted using melatonin-specific antiserum and  $^{125}\text{I}$ -melatonin as tracer. Assay sensitivity (calculated according to 95% binding) was 1.5 pg/ml, intra-assay and inter-assay coefficients of variation were 7.4 and 13.7%, respectively.

### Cortisol radioimmunoassay

Saliva cortisol concentration was measured according to Spectria's direct cortisol RIA kit (Orion Diagnostica, Espoo, Finland). Briefly, after centrifugation of saliva samples, 150  $\mu\text{l}$  from each was assayed for cortisol using cortisol-specific antiserum and  $^{125}\text{I}$ -labelled cortisol tracer in coated tube radioimmunoassay. Assay sensitivity (calculated according to 95% binding) was 0.8 nmol/l, intra-assay and inter-assay coefficients of variation were 1.3 and 3.7%, respectively.

FIGURE 1. The spectrum of the transcranial light. Irradiance and photon density measured in distance of 1 cm from light source were  $7.28 \text{ mW/cm}^2$  and  $1.94^{16} \text{ photons/cm}^2/\text{s}$ , respectively. See maximum at 450 nm.



### Statistics

Differences between sham (control) and TBL exposure concerning saliva and urine samples were analyzed using one-way ANOVA (repeated measures) with Tukey test. Stineman function of KaleidaGraph (Synergy Software, Reading, PA) was used to analyze circadian rhythm data (acrophase, peak duration and area under the curve; AUC). The  $p$  value  $<0.05$  was considered as statistically significant.

### Results

Saliva melatonin concentrations for the whole experiment are depicted in Figure 2(A). The daytime levels were 3–5 pg/ml both in control and TBL conditions. The rise of nocturnal melatonin began at 22–23 h with reaching the peak levels (9–10 pg/ml) at 03 h in both conditions. The melatonin onset was taking place at the same time in both conditions. Based on smoothed curve of the data, the acrophase of the rhythm was at 04 h in both conditions of the experiment. According to half-maximal saliva levels of melatonin, the duration of the nocturnal melatonin peak was 7.5 h in control and 7.0 h when exposed to ear light. The AUC analyses of the two curves gave 73 and 69 pg/ml  $\times$  h, correspondingly (Figure 2A). In statistical tests, these parameters showed no significant difference between control and TBL conditions.

The salivary cortisol profiles are presented in Figure 2(B). The daytime concentrations decreased during the afternoon and evening reaching low levels of about 1 nmol/l and stayed low until 03 h. The morning increase (cortisol awakening response; CAR) was seen from 06 h to 09 h peaking 17 nmol/l at 08 h in both control and TBL conditions. Based on half-maximal saliva levels of cortisol, the duration of the cortisol peak was 1.5 h and AUC value 22 nmol/l  $\times$  h in both sessions. No significant differences between circadian cortisol profiles could be observed.

Figure 3 presents urinary excretion of melatonin. Daytime excretion was  $<2$  ng/h. When hourly analyzed between 21 and 03 h, the excretion started to increase toward midnight peaking (about 4 ng/h) at 02–03 h both in control session and in TBL session. There was no significant difference in urinary excretion of melatonin between control and TBL sessions.

### Discussion

The aim of this study was to investigate the effects of TBL via the ear canals on nocturnal melatonin and cortisol secretions and potential phase-shifting effects of TBL. Our results show unambiguously that neither melatonin nor cortisol secretions were changed by the nocturnal TBL exposure. Circadian profiles of both melatonin and cortisol were identical in the TBL and the control conditions. This concerns all rhythm characteristics studied (acrophase, peak duration and AUC). Similarly, the circadian pattern of melatonin excretion into urine did not change following the TBL.

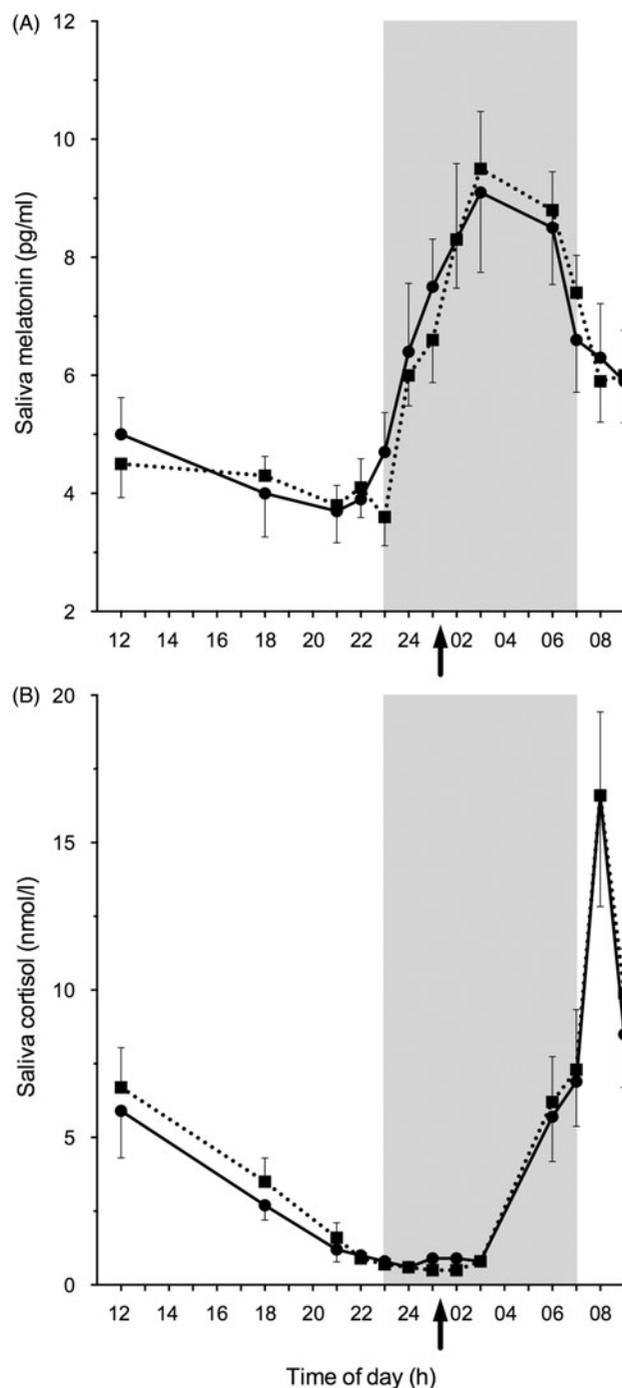
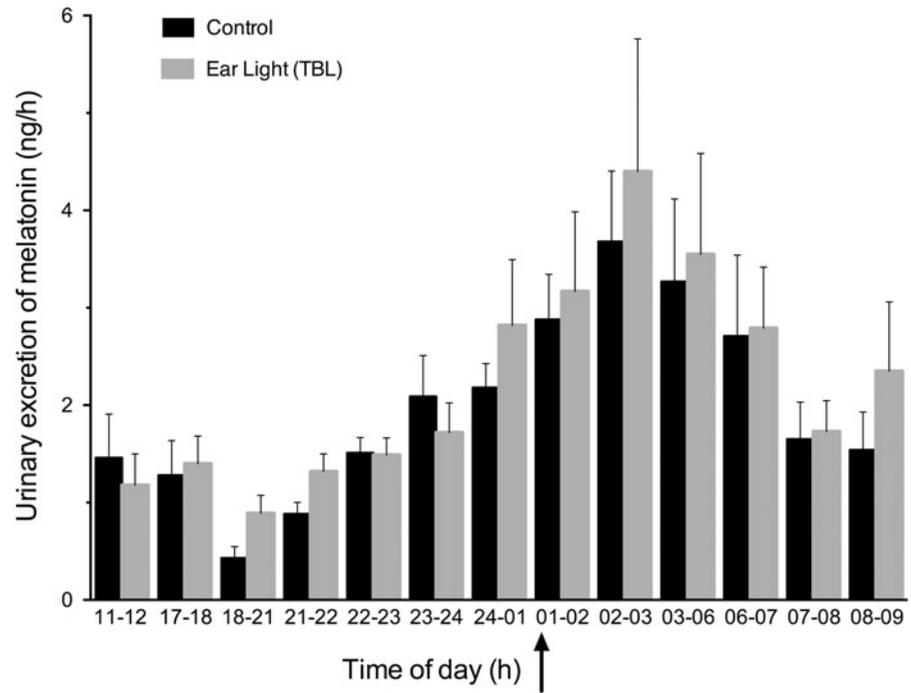


FIGURE 2. Circadian rhythm of saliva melatonin (A) and cortisol (B) of young healthy volunteers in the control session (solid line) and in ear light session (broken line). The curves are based on means  $\pm$  SEM ( $n=8$ ). The arrow indicates the time of ear light exposure, 24 min (01:10–01:34 h). Gray area shows lights off (23:00–07:00 h).

Measuring melatonin and cortisol in saliva has been proven to be very reliable and highly effective while being non-invasive (Vakkuri, 1985). The linear correlation between simultaneously measured nighttime serum and saliva melatonin concentrations is as high as 0.95 (de Almeida et al., 2011; Laakso et al., 1990). Saliva cortisol has been analyzed also in many circadian

FIGURE 3. Melatonin excretion into urine in young healthy volunteers during control session (black columns) and ear light session (gray columns). Urine was collected at hourly intervals excluding two periods of three hours (18–21 h and 03–06 h). The columns represent means  $\pm$  SEM ( $n = 8$ ). For other explanations, see Figure 2.



studies in order to monitor the effect of various stress factors on circadian organization (King & Hegadoren, 2002). Usually the CAR takes place in early morning when associated with arousal. Stressful factors potentiate CAR (Chida & Steptoe, 2009; Clow et al., 2010). This effect was not seen in our study.

In humans, light-induced melatonin suppression was initially reported at higher light intensities, i.e. bright light, which means  $\geq 2000$  lx (Lewy et al., 1980; Partonen et al., 1997). This suppression has been observed to take place also through closed eyelids (Hätönen et al., 1999). Later, studies have shown that depending on the spectrum of light, also smaller light intensities suppress pineal melatonin synthesis and secretion (Brainard et al., 2001; Figueiro & Rea, 2010; Lockley et al., 2003; Trinder et al., 1996).

Direct perception of external light through extraocular photoreceptors has so far been described only in birds and lower vertebrates (Vigh et al., 2002). However, potentially light-sensitive opsins and glutamatergic NMDA have been characterized also in the rodent and human brain (Blackshaw & Snyder, 1999; Koyanagi et al., 2013; Kumbalasingi & Provencio, 2005; Leszkiewicz et al., 2000; Nissilä et al., 2012). Moreover, a recent study (Starck et al., 2012) suggests that TBL via the ear canals affects functional connectivity in neural networks of human brain as measured by fMRI. In another recent study, light exposed to the occipital area of the skull led to alterations in human brain function as measured by QEEG and LORETA (Persinger et al., 2013). TBL via the ear canals also seem to alleviate mood symptoms in patients suffering from SAD (Timonen et al., 2012).

Light-induced melatonin suppression does not always associate with psychophysiological alterations, e.g. in SAD. Evening bright light treatment (30 min for 2 weeks between 17:00 and 20:00 h) was observed to alleviate subjective sleepiness of SAD without changes in melatonin secretion or in depressive symptoms (Partonen et al., 1997). It is known that the circadian time of light exposure is one of the critical factors. Early morning light exposure has been shown effective in many SAD studies in suppressing melatonin and therefore pacing the endogenous circadian rhythms (Golden et al., 2005; Kurlansik & Ibay, 2012). On the other hand, daytime bright light exposure (at 12–17 h) had no effects on nocturnal melatonin surge but reduced sleepiness and increased vigilance (Phipps-Nelson et al., 2003). Nighttime light of 100 lx exposed to the nasal or temporal parts of the retina did suppress melatonin secretion but did not affect sleepiness or fatigue (Rüger et al., 2005). Moreover, nocturnal bright light exposure by wavelengths above 480 nm has been found to improve alertness, mood and vigilance, with no disturbing effect on nocturnal melatonin or cortisol secretion (Rahman et al., 2011).

Our results demonstrate that TBL via the ear canals has no acute suppressive or phase-shifting effect on the circadian rhythm of saliva and urine melatonin or saliva cortisol. This is in line with the recent finding showing that light via the ear canals does not suppress melatonin in the late evening (Bromundt et al., 2013). Consistently, we conclude that TBL does not acutely affect pineal melatonin secretion.

These results, together with previously observed changes in brain functions, improvements in mood after ear light exposure and existence of potentially

light-sensitive brain proteins, suggest that there might be additional routes for NIF effects besides RHT. As we and others have demonstrated that these routes do not appear to be dependent on the structures regulating melatonin secretion. Human flavoprotein CRY is expressed in the retina and has been shown to act as a light-sensitive magnetosensor (Foley et al., 2011). Yet, it is not known if TBL via the ear canals could stimulate this sensor. Further studies are needed to clarify the mechanisms associated with ear light exposure.

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## DECLARATION OF INTEREST

J. L., S. S. and O. V. have no conflicts of interests to declare. H. J. and M. R. work for Valkee Ltd., J. N. is the company founder and a shareholder and T. T. is a minor shareholder. L. H. received a grant from Valkee Ltd. for this study.

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