

Supporting Material

Blue-enriched morning light as a countermeasure to light at the wrong time: effects on cognition, sleepiness, sleep and circadian phase

Münch M.^{1,2}, Nowozin C.^{1,2}, Regente J.^{1,3}, Bes F.^{1,2}, de Zeeuw J.¹, Hädel S.¹, Wahnschaffe A.^{1,2}, Kunz D.^{1,2}

¹ Charité Universitätsmedizin Berlin, Sleep Research & Clinical Chronobiology, Institute of Physiology, Berlin, Germany, ² Clinic for Sleep and Chronomedicine, St. Hedwig Hospital, Grosse Hamburger-Strasse 5-11; D-10115 Berlin, ³ Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Charité Universitätsmedizin Berlin, Charitéplatz 1, 10117, Berlin, Germany

Material and Methods

Salivary Melatonin

Each participant regularly produced saliva samples for melatonin assays by using salivettes (Salivettes®, Nümbrecht, Germany). The collected saliva samples were immediately stored at a maximum temperature of 4° C and frozen at -32°C within the following 12 hours. All saliva samples were sent to an external laboratory for direct radio-immuno-assay (IBL International GmbH Hamburg, RE29301). The inter-assay variance was 14.6% - 18.2%. The intra-assay variance was 0.2-27% for low and 0.3-15.5% and high melatonin concentrations.

The RIA assays performed at IBL (Hamburg, Germany) produce higher daytime concentrations than other assays used for melatonin (detection limit IBL: 0.8pg/ml). Daytime salivary concentrations collected under strictly controlled constant routine conditions with Salivettes in 12 young males in dim light during 40 hours of extended wakefulness were on average between 5 and 10 pg/ml (unpublished data). Due to the lack of (low) daytime values we could not apply the 2-standard deviation method to calculate the DLMO (see reference [1] for details), and hence used a threshold of 10pg/ml since the lowest melatonin concentrations were in almost all samples between 5 and 10 pg/ml. Determination of the time when salivary melatonin concentrations were 10 pg/ml was performed by linear interpolation using the software from reference [2].

Sleep EEG recordings

All sleep episodes were polysomnographically recorded with five EEG derivations (C3, Cz, C4, O1, O2) referenced against mastoids (A1, A2) using a digital recording system (Monet 24-CPU, TMS International, Enschede (The Netherlands), and Sagura Polysomnograph 2000, Mühlheim (Germany) with Rembrandt 7.5 software, Medcare Automation, Amsterdam (The Netherlands), together with two electrooculograms (EOG), one electrocardiogram (ECG) and one submental electromyogram (EMG).

Supplemental Tables

Table S1: Overview on the light sources

Morning Light Sources	Correlated Color Temperature (K)	Illuminance (lx) (vertical plane at the eye)
Mixed blue-enriched polychromatic white LED/fluorescent light source (=MBL)	3537 K	750 lx
Warm-white incandescent lamp =Control lighting (CTRL)	2600 K	40 lx
Evening Light Sources	Correlated Colour Temperature (K)	Illuminance (lx) (vertical direction at the eye)
Xe-filled fluorescent light source (blue-light depleted) =Orange light (OL)	1500 K	~100 lx
Blue-enriched fluorescent light source =Blue-enriched (BL)	6500 K	~ 500 lx
polychromatic warm-white incandescent light source =Dim light (DL)	2700 K	< 7 lx

Table S1: Shows the name (and abbreviations used in this manuscript), the correlated color temperature (CCT) in K as well as illuminance levels, measured on a vertical plane at the eye. The location of the light sources was the ceiling.

Supplemental Table S2: EEG sleep stages during adaptation nights

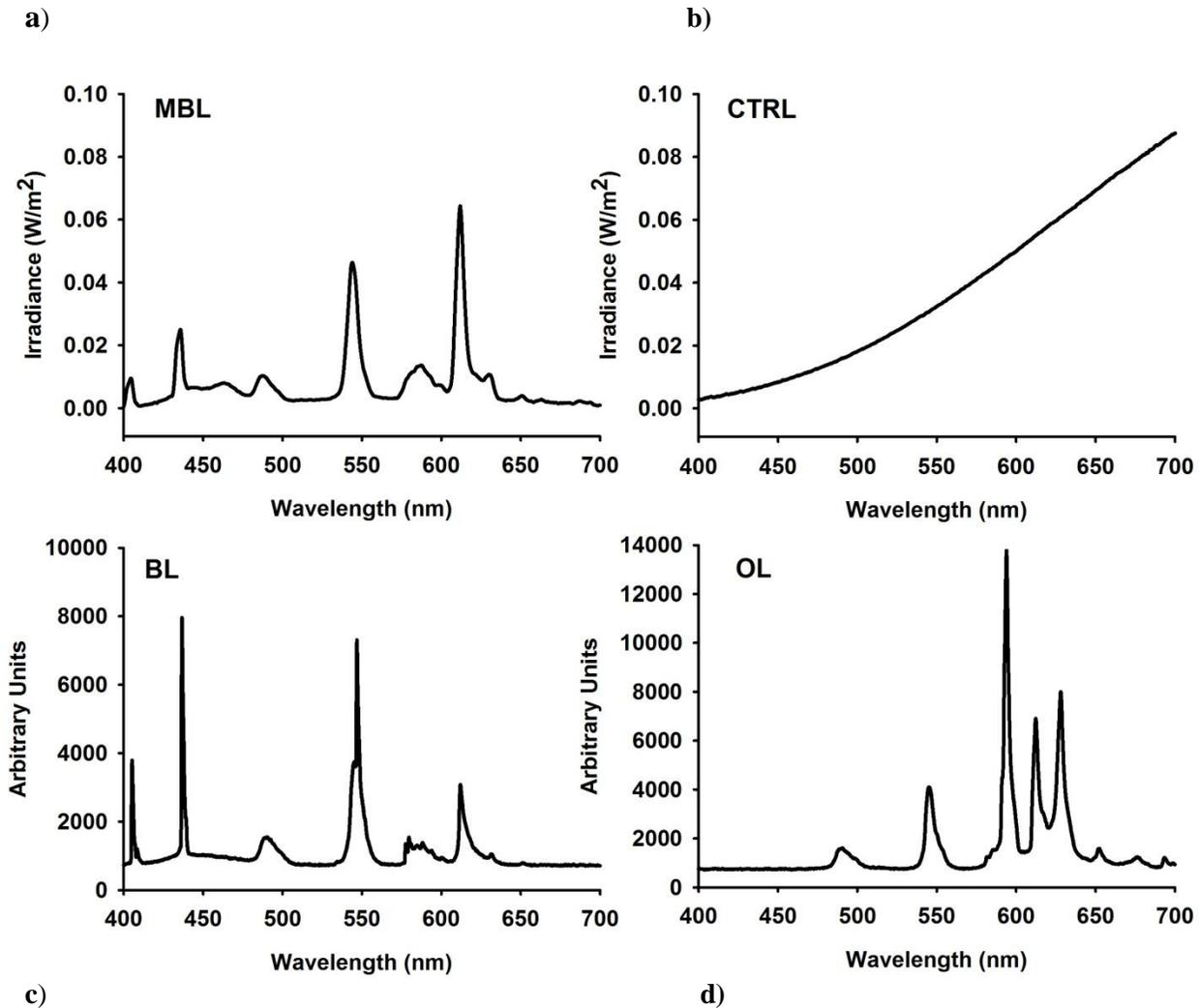
Variable		Adaptation 1 (=AN1; \pm SD)	Adaptation 2 (=AN2; \pm SD)	One-way ANOVA (AN1 vs. AN2)
Time in Bed (TIB)	min	500.56 (25.29)	508.28 (20.56)	ns
Total Sleep Time (TST)	min	457.11 (37.90)	483.92 (22.07)	*AN2>AN1
Sleep Period Time (SPT)	min	480.14 (28.37)	495.72 (21.69)	*AN2>AN1
Sleep Efficiency (SE)	%	91.32 (5.90)	95.22 (2.54)	*AN2>AN1
Sleep Latency (SL)	min	16.36 (15.76)	11.39 (8.17)	ns
REM Latency (RL)	min	89.58 (34.83)	72.81 (23.75)	ns
Wake Time (during TIB)	min	42.33 (28.66)	22.14 (11.22)	*AN1>AN2
Wake after sleep onset	min	29.81 (24.79)	13.44 (8.54)	*AN1>AN2
Wake after sleep end	min	3.86 (6.32)	1.06 (3.74)	ns
REM-Episodes	count	4.78 (0.88)	5.17 (0.99)	ns
Wake time (during SPT; min)	min	25.94 (25.21)	12.39 (7.93)	*AN1>AN2
Wake (%)	% SPT	5.43 (5.24)	2.50 (1.59)	*AN1>AN2
Stage N1	min	36.97 (10.34)	35.19 (11.99)	ns
Stage N1 (%)	% SPT	7.00 (2.18)	6.51 (2.49)	ns
Stage N2	min	211.44 (37.20)	224.50 (20.61)	ns
Stage N2 (%)	% SPT	43.95 (6.78)	45.27 (3.42)	ns
Stage N3	min	104.89 (19.07)	102.94 (18.33)	ns
Stage N3 (%)	%SPT	21.94 (4.27)	20.85 (4.07)	ns
NREM	min	353.31 (31.74)	362.64 (20.05)	ns
NREM (%)	% SPT	72.89 (4.85)	72.64 (3.58)	ns
REM	min	103.81 (23.73)	121.28 (18.93)	*AN2>AN1
REM (%)	% SPT	21.62 (4.68)	24.42 (3.40)	*AN2>AN1

Table S2: Sleep variables of the two polysomnographically recorded adaptation nights. TIB=time in bed (time between lights off and lights on); TST=cumulated time in stages N1-N3 and rapid eye movement (REM) sleep; SPT= sleep period time (time between sleep onset and end of sleep); AN= adaptation night; SE= sleep efficiency (TST/TIB); sleep latency: time between lights off and first occurrence of N2 sleep; REM latency: time between sleep onset and first occurrence of REM sleep; NREM=Non-REM sleep (stages N1-N3); n=18; * = $p < 0.05$; ns=not significant; means (\pm SD).

Supplemental Figure

Supplemental Figure S1a-d:

Different spectral power distributions for the light sources



Supplemental Figures 1a-d The spectral power distributions for both morning light exposures are shown in (a) for the mixed blue-enriched polychromatic white light source (MBL); and in (b) for the control lighting (CTRL=warm-white incandescent polychromatic white light source). In the evening, participants were exposed either to (c) a blue-enriched light source (=BL), or (d) a bright orange lighting (=OL; blue-light depleted light source; or to dim light (=DL; polychromatic white light source <7 lx; not shown). Figures 1a-b are shown as absolute power distributions (W/m²) and Figures 1c-d are depicted in arbitrary (relative) units.

Supplemental References

- 1 Benloucif S, Burgess H, Klerman E, Lewy A, Middleton B, Murphy P, Parry B, Revell V: Measuring melatonin in humans. *Journal of Clinical Sleep Medicine* 2008; 15:66-69.
- 2 Danilenko K, Verevkin E, Antyufeev V, Wirz-Justice A, Cajochen C: The hockey-stick method to estimate evening dim light melatonin onset (DLMO) in humans. *Chronobiology International* 2014; 31:349-355.