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Y. A. W. de Kort, W. A. IJsselsteijn, I. M. L. C. Vogels,
M. P. J. Aarts, A. D. Tenner, & K. C. H. J. Smolders (Eds.)

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Volume Editors

Yvonne de Kort, PhD
Wijnand IJsselsteijn, PhD
Karin Smolders, MSc
Eindhoven University of Technology
IE&IS, Human-Technology Interaction
PO Box 513, 5600 MB Eindhoven, The Netherlands
E-mail: {y.a.w.d.kort, w.a.ijsselsteijn, k.c.h.j.smolders}@tue.nl

Ingrid Vogels, PhD
Visual Experiences Group
Philips Research
High Tech Campus 34, WB 3.029
5656 AE Eindhoven, The Netherlands
E-mail: ingrid.m.vogels@philips.com

Mariëlle Aarts, MSc
Eindhoven University of Technology
Department of Architecture Building and Planning
PO Box 513, VRT 6.34
5600 MB Eindhoven, The Netherlands
E-mail: M.P.J.Aarts@tue.nl

Ariadne Tenner, PhD
Independent consultant
Veldhoven, The Netherlands
E-mail: ariadne.tenner@onsmail.nl

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Evaluation of Today's Research Methods for Assessing Light-Induced Alertness

Emilia Rautkylä, Petteri Teikari, Marjukka Puolakka, and Liisa Halonen
Lighting Unit
Helsinki University of Technology
P.O. Box 3340
02015 TKK Finland
+358 50 5469255
emmi.rautkyla@tkk.fi

ABSTRACT
Daytime alertness has not established the same type of research routines as nighttime alertness. This paper evaluates today's research methods to provide help in choosing suitable methods for assessing light-induced daytime alertness in future research. The evaluation is done according to two main criteria; the method's ability to reflect alertness physiologically and its suitability for use in a lighting study. The methods under evaluation are subjective ratings, reaction tests, brain imaging, pupillometry, measurements of heart rate and skin conductance, and the use of an electro-oculogram and electroencephalogram. On the basis of a literature review and practical testing of some of the methods, the writers suggest that in low-cost studies, where detecting the effects is enough, autonomic nervous system activation should be measured. To gain broader knowledge of the mechanisms, central nervous system responses need to be studied.

Keywords
Alertness, sleepiness, central nervous system, autonomic nervous system activation, lighting research

INTRODUCTION

With growing interest in the impact of light on health [60, 70] and wellbeing [84], accelerated by the introduction of the novel photoreceptive melanopsin-containing retinal ganglion cell [10], the relation of light and alertness during nighttime hours has been the subject of considerable examination [for a review, see 17]. Daytime alertness, on the other hand, has not yet attracted the same amount of research interest. In general there are two approaches to this field of interest. One aims at detecting the effects of light [6] so that the current lighting standards used in the building business could be updated so as also to consider wellbeing instead of only the visual performance [81]. This type of research is clearly application-oriented and of high commercial value [79], which can be seen from the high level of interest in e.g. bright light treatment. The other type of research tries to reveal the mechanisms behind the detected effects [4]. This can be thought to be a more rigorous approach, trying to find out the scientific basis for the phenomenon.

The current weakness in both types of research is that the methods used for evaluating light-induced daytime alertness are not always suitable for such research. First of all, it is often forgotten what alertness is, physiologically, and hence which methods measure human alertness quantitatively. Second, it is not always considered that using light as a stimulus can create special demands for the method. Finding proper methods is challenging because in lighting research papers the problems related to the usability of the study methods chosen are reported too seldom.

The object of this paper is to provide help in choosing eligible methods for assessing light-induced daytime alertness by evaluating the research methods used today. This is done according to two main criteria: the ability of the method to reflect alertness physiologically and its suitability for use in a lighting study. Besides evaluating today's research methods theoretically, the paper also reports on the practical testing of some of the methods, namely subjective evaluation, and measuring pupil size, heart rate, and the skin's ability to conduct electricity. The methods are chosen for the practical testing on the basis of their potential value for alertness research and on the available resources.

EVALUATION CRITERIA

Physiology of Alertness

In 1949 Moruzzi and Magoun presented a theory of the activation system of the brainstem which suggests that stimuli travelling through the brainstem give rise to the level of alertness [58]. This was thought to be the missing explanation of why external stimuli increase alertness but a lack of them reduces it. Soon the concept of the "ascending reticular activating system" achieved wide currency.

Anatomically speaking, the activating system is located in the reticular formation. The reticular formation is a broad and netlike formation in the core of the brainstem running through the mid-brain, pons, and medulla oblongata. The ascending reticular activating system is connected to areas in the thalamus, hypothalamus, and cerebral cortex, while the descending reticular activating system is connected to the sensory nerves of the cerebellum. [31]

The regulation of alertness is based on the messages sent between the nuclei in the reticular formation in the brainstem and the cerebral cortex [68]. Of the nuclei in the brainstem, the locus coeruleus (LC) is the most essential one when alertness is considered, because it reacts easily to stimuli and improves alertness by increasing its noradrenalin secretion to the cortex. It has been said that a single noradrenergic neuron can innervate the entire cerebral cortex via its branches, mediating arousal and priming the brain's neurons to be activated by stimuli [6]. That supports the belief that the LC is one of the key components in light-induced alertness [4].

How the function of the LC is seen in practice is through the secretion of noradrenalin, which enables the body to perform well in stressful situations [8]. Noradrenalin normally produces effects such as increased heart rate, blood pressure, and sweat gland activity, and the dilation of the pupils and of air passages in the lungs. Hence, the LC is in direct connection to the autonomic nervous system (ANS). In fact, the activation of the ANS is often used as a conceptual definition of alertness. Because the activation of the ANS increases as arousal increases, it is reasonable to claim that by observing the changes in the ANS, it is possible to see how the LC reacts to stimuli and activates the body.

This section concludes that one potential way to assess daytime alertness is by observing the brain and characterising the neural correlates of the alerting effects of light. Another option is to observe the activation of the autonomic system. Although the pathways have not yet been fully identified, there is evidence that light stimulates the ascending arousal system and eventually the cortex in order to enhance alertness [65]. It should be noted that it still remains unclear whether light induces alerting effects in daytime, when the homeostatic sleep pressure is low and there is no circadian drive for sleep. Therefore the methods need to be even more sensitive than the methods used in nighttime studies.

Demands Set by Lighting Research

In general a good method for use in a scientific study is something that can be applied both in laboratory and field studies. This permits the best further use of the results in real-life applications. To gain objective and reliable data the experiment should be repeatable and as independent of the subject and the researcher as possible. Setting a baseline or altering the testing conditions often helps in analysing the data and verifying the results.

In addition to these general guidelines, lighting research sets some special demands for the study method. The most essential demand is that the method allows light to be used as a stimulus. Often it is also important to be able to alter the lighting conditions, hence changing the exposure time, light source, spectrum of the light, irradiance etc. [62]. One crucial criterion for the method is that it presents the data in such a way that the effect of light can be distinguished from effects caused by other stimuli such as caffeinated beverages [30], indoor climate [14], and auditory stimuli [27], among others. There are two ways to do this: either eliminating other stimuli from the set-up or separating them out in the analysis.

One practical factor to consider in choosing a good method is that in research done on humans there is considerable variation between individuals [65]. Therefore the number of subjects has to be big enough to gain reliability. This results in the fact that the test cannot be too complicated to conduct. Practicalities such as the costs and availability of the method may also limit the use of some methods. In studies of long-term light exposure and its effects, it is important to make sure that the lighting conditions and the method are not too burdensome and uncomfortable for the subject.

THEORETICAL EVALUATION OF METHODS

Subjective Methods

Subjective evaluation is a commonly used research method because it is easy to conduct both in laboratory and field conditions. Sleepiness is typically assessed on a Likert-type discrete scale [i.e. 32] or a continuous visual analogue scale (VAS) anchored by word descriptors at each end [76]. Perhaps the most popular subjective measure is the Karolinska Sleepiness Scale (KSS) [88], which uses a discrete scale from 1 to 9, where 1 = very alert and 9 = very sleepy, great effort to stay awake or fighting sleep. KSS has been validated to significantly correlate with EEG and behavioural variables [39] and is therefore considered a reliable measure of alertness or sleepiness. However, as Cajochen points out [17], the precise meaning of the terms alertness and sleepiness may differ between languages and situations. Furthermore, approaching alertness through sleepiness can be inadequate because alertness is not always the inverse of sleepiness [57].

The main criticism of any type of subjective assessment arises from the fact that it relies on self-reporting, leaving it open to misinterpretation, unintended bias, and falsification for any number of reasons (e.g. the act of rating itself can affect sleepiness [42]). It is possible that the subjects may evaluate their alertness differently in light than in darkness, even though there was no real difference in their level of alertness. In fact, there is no real placebo control for light, but it can only be hoped that the subject assesses his alertness time after time following the same logic. Another weakness of subjective assessment in a study of light-induced alertness is that it can only be used to point out the changes in the subject's way of responding to light, but it does not show anything about the reasons or mechanism behind the changes. Therefore it can never give as much input to the study as objective measures that are linked to physiology.

It is also hard to be sure that the effect is caused by light and not something else. Using subjective assessments thus requires a very strictly controlled test environment where there are no other factors that could affect the person's way of answering. Finally, one major disadvantage of using

subjective assessment in lighting studies is that the data recording is not continuous. Because self-reports are produced after certain time periods, the information about the state of alertness between the measuring points is automatically lost. One could say that the data expire as soon as they are recorded. This is a big problem because it hinders one in detecting whether it is a question of a fast or slow response to light.

Objective Methods

Reaction Tests

Performance is often used to evaluate how alert a person is [85]. This is based on the assumption that alertness is involved with increased reactivity to external stimuli; thus an alert person reacts fast to stimuli. Using reaction tests to evaluate alertness is, however, not as problem-free and easy as it would at first seem. For example, the test itself can act as an activating stimulus and thus affect alertness.

The reaction tests need to be well designed in terms of the complexity of the test because the subject should be able to perform the test without too great an effort. Another important factor is how the subject manages to retain their motivation throughout the whole test. That depends on whether the subject is being rewarded after a successful test, but also on the duration of the test. For a long time it was thought that a reaction test should last no less than 10 minutes because studies indicated that shortening a performance task resulted in reduced sensitivity to changes in performance [12]. However, recently the study of Roach et al. [64] showed that a 5-minute test correlates well with a 10-minute test. They tested the psychomotor vigilance test (PVT) [83], which has been shown to be a reliable indicator of decreased alertness [19] and is commonly used for assessing neurobehavioural performance.

The advantage of the PVT is that it reflects the tiredness-related reduction in performance without being confounded by the learning effect, a factor that often causes bias in the experimental data. In the traditional study protocol a visual stimulus appears on the display and the subject is instructed to press the response button as fast as possible after detecting the stimulus.

Another and more modern option is to use auditory stimuli in a psychomotor vigilance test. In the auditory PVT the subject presses a button after hearing the stimuli in the same way as in the visual PVT. By using two buttons and two different stimuli instead of one it is also possible to add complexity to the test. Today there are portable, palm-held devices that make it possible to conduct experiments in real environments such as workplaces, instead of only laboratories [83]. The Walter Reed Army Institute of Research, Maryland, offers test and analysis software for this kind of field-portable reaction time tester for free [75]. However, their PalmPVT does not allow auditory stimuli to be used.

In theory, these kinds of reaction tests can be used in lighting research in two ways. First of all, if light with the specific characteristics under study acts as the stimulus, the reaction time will show how easy it is to detect that stimulus. In practice, however, this only shows that the person reacts to light but not whether the light induces any alerting effects. Another option is to use an exogenous stimulus in the PVT to assess vigilance after being exposed to light for a certain amount of time. Following e.g. Lockley's example [44], in this kind of protocol it is better to use an auditory stimulus instead of a visual one to prevent the PVT stimulus from masking the light-induced effect under study. This has potential for revealing how the exposure to light affects the reaction times.

The biggest disadvantage of using a PVT in studies of light-induced alertness is that it measures sustained attention rather than alertness and therefore it is not a proper method to evaluate the activation system in detail. Furthermore, it does not measure the functioning of the LC or other body parts that take part in light-induced alertness but instead it exhibits the circadian and homeostatic processes that take care of the natural asleep/awake rhythm. Therefore it can be concluded that a PVT can be broadly used in chronobiology research [11] but it does not necessarily make a good assessment method for alertness in lighting research.

Pupillometry

The pupil provides control over the retinal illumination and depth of focus [50]. In addition to constricting as a response to increased light flux and vice versa, the pupil also responds e.g. to accommodative changes [41] and to anticipating effects for an instructed task [87], illustrating the wide range of confounding factors involved in pupil recordings. Given that pupil size modulates the retinal illuminance, precautions are needed to control the exact retinal illuminance. These precautions include monitoring the pupil size via a video-based infrared pupillometer [73] with or without dilating the pupil to a constant size during the recording. Additionally, one can use a Maxwellian view [82], as opposed to a free view in which the stimulus sizes are smaller than the smallest physiological pupil diameter; hence pupil size has no modulating effect on retinal illuminance.

The pupil size can be measured using a direct approach with binocular light stimulation or by a consensual approach, where only one eye is stimulated and the response of the unstimulated eye is recorded. There is no vast literature on pupillometric hardware but it should be noted that many of the approaches are similar to that used in the eye tracking literature [20]. Typical temporal resolutions range from 30 Hz in low-cost setups [43] to 6-12 kHz in more customised setups [74], with spatial resolutions going down to 0.008 mm [29] depending on the sensor resolution, quality of the optics, and the signal-to-noise ratio of the video signal. The increase in temporal resolution can be achieved by using complementary metal oxide semiconductor (CMOS) sensors instead of charge-coupled device (CCD) sensors, in which this is not possible because of technical limitations [47].

Typically, the human pupillary light reflex (PLR) exhibits roughly three phases, rapid phasic constriction in response to light onset, which is followed by a steady-state pupil, and finally, depending on the light stimulus, there can be the post-stimulus persistence of a constricted pupil even after light offset [26]. Additionally, pupil size exhibits spontaneous fluctuations called hippus or pupillary noise, which is characterised by a random noise in the frequency range of 0.05 to 0.3 Hz [67]. To avoid contamination of pupillary measurements by spontaneous fluctuations of the pupil, a continuous monitoring of the pupil is preferred. The exact origin of hippus is not fully understood, but it has been suggested that it is an indicator of the state of vigilance of a person. There is evidence that if a drowsy subject spends daytime in darkness, the 'fatigue waves' start to occur with an increasing amplitude at the frequencies 0.025–0.25 Hz [45], whereas in an alert subject pupil size remains stable for a long time, oscillating mainly with a frequency of 1 Hz.

For this reason, pupillary fluctuations have been widely exploited as an easy and non-invasive measure to track changes in autonomic nervous system activity. One example of such an approach is the Pupillary Unrest Index (PUI) which measures the cumulative changes in pupil size, typically during periods ranging from 25 seconds to 15 minutes [53] in darkness or under light. PUI was used, for example, by Szabó et al. [72] to measure the changes in the vigilance levels of subjects during bright light exposure. Among others, Nikolau et al. [59] found pupillary assessment to be a promising objective tool to detect pharmacologically induced changes in alertness. However, it should be noted that the majority of studies on pupillary fluctuations have been carried out in darkness and the relationship between fatigue and oscillations in daylight requires further validation, adding some restraints to real-life lighting studies with pupillometric alertness assessment.

Considering that the pupillometer is comfortable for the subject and the protocol does not include any tasks to be performed, it might work as a good objective indicator for light-induced alertness. The method operates with a fairly delicate apparatus and requires the subject to sit still without extra blinking and head movements. There are, however, some indications that it could be used in field studies too [78]. Recent studies suggest that pupil size measurements could offer a simpler way to estimate autonomic nervous system activity than the commonly used heart rate [54]. Therefore it is reasonable to suggest that the reactivity of the pupil could well be used in lighting-related psychophysical experiments.

Heart Rate

The heart responds to psychological stress via the autonomic nervous system [52]. Over the years a correlation between heart rate and arousal caused by light exposure has been found both with rats and with humans [56,77]. Heart rate variability (HRV) has become the conventionally accepted term to describe the variations of interbeat (RR) intervals that represent autonomic nervous activity [66].

Heart rate variability is normally recorded by placing 10 electrodes on the skin on the subject's arms, legs, and chest. They measure the activity of different parts of the heart muscle and transmit it to an electrocardiogram (ECG) machine. The machine produces an ECG tracing of these cardiac electrical impulses. In clinical studies the heart rates or cycle intervals are recorded over long time periods, traditionally 24 hours, allowing more reliable calculations of the measures. Because the analysis of HRV data is more complex than generally appreciated, there is a potential for incorrect conclusions and unfounded generalisations [25]. The experimental procedures and analysis of the results should be carried out in accordance with the recommendations of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [49]. In fact, Peña et al. [61] remind that caution should be exercised concerning the use of short recording segments, a circumstance not fully considered in several studies. Therefore, although heart rate is easily measured in the presence of a light stimulus, the method does not meet the needs of detecting the effects of short-term light exposure.

From the subject's point of view, studying light-induced alertness by observing the heart rate is an easy research method because there is no task to be performed. However, real clinical equipment contains detectors and wires that can hinder the subject from conducting other things, as is often required in field studies. Fortunately, there are commercial and cost-effective heart rate monitors that can be used in studies where it is possible to reduce accuracy in order to gain mobility. There is evidence that motion does not contaminate the signal too much [36]. It should, however, be considered that in field studies the heart rate data are even more sensitive to distractions than in laboratory studies. Hence, the effects of the light stimulus are easily masked by other unintended stimuli.

Skin conductance

Because of the connection between the autonomic nervous system and locus coeruleus, arousal has long been assessed through the skin's ability to conduct electricity [28]. In fact, activation theorists long considered skin conductance to be the most appropriate measure of a generalised arousal response [21]. Skin conductance, galvanic skin response, and electrodermal response are different terms for the same physiological measure. It is known that as a person becomes more or less stressed, the skin's conductance increases or decreases proportionally [18].

The easiest way to measure electrodermal activity (EDA) is by strapping two electrodes to two fingers, namely the little finger and pointing finger of the non-dominant hand. The skin acts as a resistor whose conductance (inverse of resistance) changes with time according to the changes in hydration in the sweat glands [23]. Changes in EDA occur

with even a slight rise or decrease in the amount of sweat within the glands [69]. Therefore a typical signal recorded from a skin conductor sensor shows relatively rapid increases and slower decreases.

From the lighting research point of view measuring the skin's ability to conduct electricity can be considered a good research method for light-induced alertness because it can be used within and between light exposures with both continuous and short-term light stimuli. It has, however, not been used often in lighting studies. The recording apparatus is small and the experimental protocol does not involve any kind of task performance by the subject. One major disadvantage is that the wiring hinders its use in real-life settings.

The analysis is rather easy as long as the recordings are time-locked to specific events to allow the analyser to select the right blocks of data from the general data [40]. The analysis has the potential to show the intensity of the effect of the light on a human. However, it is important to note that changes in the signal may be elicited by external stimuli or internal events [46]. Hence, it might become hard to distinguish the effects of different stimuli from one another. Therefore, to make electrodermal activity a proper indicator of the intensity of light-induced alertness, all other emotional cues that might mask the effect of light have to be eliminated.

Electro-oculogram

Eye movements react to a decrease in alertness. The attenuation of blinking is often a marker of the fact that the person is losing interest. At the same time the duration of a blink becomes longer and the eyelids become lazier. When the eyelid closes, the eyeball makes slow roll-like horizontal movements that are called slow eye movements (SEM) [5]. From these visible neurophysiological factors it can be seen when the person is transiting from being awake to asleep. Therefore eye blink rate and SEMs are considered reliable correlates of human alertness [16].

Clinical alertness evaluation takes advantage of the knowledge that a person who is not alert finds it hard to follow targets. In the electrophysiological test called an electro-oculogram (EOG) two skin electrodes are placed as close as possible to both eyes. Moving the eyes induces a voltage between them. The voltage varies from one to several millivolts, depending on the ambient retinal illumination. The subject is instructed to look back and forth at a steady fixation rate between two fixation targets to generate consistent saccades. These saccades are amplified and registered to be considered for analysis [51]. Normally, EOG amplitude increases significantly if the eye is first kept in darkness and then in light [3]. However, it has been shown that in electro-oculogram analysis it is better to use the light-peak to dark-trough amplitude ratio instead of the actual amplitude values because the amplitude varies widely among individuals [13].

The method is well suited to use in lighting research, both during and between light exposures. However, when designing the light stimulus, it is important to make sure that the entire visual field is evenly illuminated and that there is no direct glare on the subject that could hinder the subject from focusing on the targets [51]. As the eyes alternate direction every 1 to 2.5 seconds, the test soon becomes uncomfortable and tiresome for the subject. Therefore it is advisable to record the movements in sets and let the eyes rest between the sets. According to the international standard approved by the International Society for Clinical Electrophysiology of Vision (ISCEV), one set of 10 saccades per minute is enough to recognise the relevant peaks and troughs in the EOG data. The standard for EOG technology and protocol also offers other valuable recommendations for the recording technique. facilitating the comparability of the EOG data throughout the world.

A drawback in using an EOG to study light-induced alertness is that it does not allow the subject to concentrate on other tasks at the same time. That, and the presence of skin detectors and recording apparatus, makes the method unsuitable for real-life settings. Despite its few impracticalities, the EOG technique is quite commonly used to asses alertness objectively [34], either alone or together with brain activity measures.

Electroencephalogram

A number of observations suggest that there is a possible causal link between the activity of the locus coeruleus and electroencephalographic (EEG) activation [24]. Because the activation of the LC has been shown to induce EEG signs of cortical and hippocampal activation [9], it is reasonable to claim that by observing the forebrain EEG activity it might be possible to monitor the alerting process.

Electroencephalographic activation is a direct measure of the general cortical activation level. A set of electrodes is placed on the subject's skull to detect and amplify the small electrical voltages that are generated by brain neurons when they fire. Similarly to muscle fibres, neurons in different locations can fire at different rates. The EEG is typically described in terms of rhythmic activity and transients. The rhythmic activity is divided into bands by frequency.

Jung and Makeig state that it is possible to use the EEG power spectrum to estimate alertness [38]. The spectrum Beta band (15-20 Hz) is generally regarded as a normal rhythm, which explains why changes in Beta activity are often used to reflect different levels of arousal [7]. An decrease in Alpha activity (8-13 Hz) has also been reported to be associated with a drop in alertness and cognitive performance across the waking day [86]. This means that high levels of EEG Alpha activity could indicate a high level of alertness during an eyes-open condition [15], similarly to Beta. Theta (4-8 Hz) and Delta (2-4 Hz) activity are linked to increased drowsiness and reductions in performance [48]. However, Theta and Delta activity are rarer in awake adults.

EEG has both advantages and limitations in alertness research. One of the advantages as a correlate to human alertness is that it measures the brain's electrical activity

directly, while other methods record the responses of the autonomic system. Another advantage is that EEG is capable of detecting changes in electrical activity in the brain on a millisecond time scale. Compared to techniques such as functional magnetic resonance imaging (fMRI) that have a time resolution between seconds and minutes, EEG has a much higher temporal resolution. However, the spatial resolution of EEG is poor and therefore it is not able to indicate the location of the activity of the brain. One possibility is to use EEG simultaneously with fMRI, so that data with a high temporal resolution can be recorded at the same time as data with a high spatial resolution. However, there are technical difficulties associated with analysing the activity of the brain in exactly the same time frame. Furthermore, currents can be induced in moving EEG electrode wires as a result of the magnetic field of the MRI.

As a research method EEG is fairly comfortable for the subject, because it records spontaneous brain activity in the absence of tasks. Therefore light can easily act as a short-term or continuous stimulus. Despite the easiness of the study protocol, using it in real-life settings is complex because of the wiring and its interference-prone nature.

Brain Imaging

Brain imaging provides an opportunity to study what is really happening in a human as a result of light exposure. There are two techniques, namely functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), which provide an anatomical and a functional view of the brain and are commonly used for brain imaging [71].

fMRI measures changes in the blood flow to particular areas of the brain. Through a process called the hemodynamic response, blood releases oxygen to neurons, creating magnetic signal variation. This variation can be detected using an MRI scanner. PET, for one, detects radioactive material that is injected or inhaled. The material collects in the area of the brain being examined, where it gives off energy in the form of gamma rays. [63]

The procedure and analysis of both techniques is rather hard and requires knowledge of fields such as physics, psychology, neuroanatomy, statistics, and electrophysiology. That is why it is not within the scope of this paper to go deeper into the measurements. Instead, the most important characteristics of the two techniques from the lighting research point of view will be discussed.

With brain imaging it is rather easy to identify the precise areas that are activated in the brainstem as a result of the light. A conventional 1.5-Tesla fMRI scanner has a spatial resolution of 3 mm and higher-strength magners may decrease it down to 1 mm. PET is not as accurate; the effective spatial resolution of PET remains 8-15 mm when standard image processing procedures, such as a smoothening filter, are used. Temporal resolution is also superior with fMRI. However, compared to EEG, which has a time resolution of only a single millisecond, PET and fMRI are slow, because they can detect a new stimulus only some seconds after the first stimulus. As a matter of fact, with PET it is not at all possible to pick out neural activation patterns associated with individual stimuli measures, so event-related phenomena, such as the effect of a short exposure to light, can only be detected with fMRI. From the lighting research point of view this hinders the use of subsequent light pulses as stimuli.

The strong magnetic field around the functional magnetic resonance imaging scanner also causes other limitations on using light as a stimulus. The light source cannot be installed in the study room because the electricity will interfere with the magnetic field. Instead, the light stimulus has to be transmitted by an optic fibre, as was done recently by Vandewalle and his colleagues [e.g. 80]. The same problem arises when trying to measure other physiological measures during the scans to help in the interpretation of the brain imaging data. Generally speaking, it is possible to measure EEG, EOG, EMG, ECG, or skin conductance only during the scans to prevent the magnetic field from inducing a current in the electrode wires. However, several techniques are under development to deal with these issues and there are already good experiences of recording fMRI and EEG simultaneously [e.g. 55]. Positron emission tomography, for one, is free of this kind of physical limitations.

If the problems mentioned above are to be overcome there are still many practical issues that impede the use of brain imaging in a typical lighting study. First of all, there are only 100-200 MRI centres and 20-30 PET centres worldwide where the studies can be conducted. Needless to say, not only can they not be used in field studies but in laboratory studies too their use is very limited because of their huge expense, which is around $500 per session with fMRI and $1500-2000 with PET [63]. In a lighting study it is often necessary to have many subjects and run various sessions with each one, which makes the costs enormous. These two requirements can also be hard to realise for safety reasons. With fMRI the suitability of the subject for the test is very restricted (e.g. no pregnancy, tattoos, pacemaker, or claustrophobia) and with PET the repeated studies are limited by the annual permissible radiation exposure. The number or duration of fMRI tests is not limited but since the scanner is very sensitive to motion, the subject can only be expected to hold still for some hours.

PRACTICAL EVALUATION OF METHODS

Subjects

Twelve healthy young volunteers (5 women and 7 men; age range 20-28; mean age 24.4 ± 2.4 SD years) and nine healthy older volunteers (5 women and 4 men; age range 50-62; mean age 56.6 ± 3.7 SD years) participated in the study. Before the study the subjects' chronotypes were assessed using the Morningness-Eveningness questionnaire (MEQ) [33]. Extreme chronotypes that scored below 31 (Definitely Evening type) or above 69 (Definitely Morning type) were excluded from the study. The chronotypes were lower in the younger than in the older group (range, mean ±

SD: 32-56, 47.3 ± 7.6 vs. 53-63, 57.1 ± 3.3; *t* test: p = 0.001). The duration of sleep before the study did not differ significantly between the groups (young: 7:56 ± 0:59 hours vs. older: 7:36 ± 1:05 hours; mean ± SD; *t* test: p = 0.240). The subjects were instructed to avoid alcohol, coffee, energy drinks, and teas (and other drinks containing caffeine) for 3 hours prior to the study.

Protocol and Study Design

The experiment took place at the Lighting Unit of Helsinki University of Technology. The subjects were exposed to conditions of dark and lightness, the light exposure being provided by a Goldman perimeter (diameter 60 cm). One experimental session took 2 hours (15:00-17:00). As presented in Figure 1, the lighting conditions were as follows:

15:00-15:25 darkness
15:25-15:30 quasimonochromatic blue light
15:30-15:45 darkness
15:45-15:55 broadband orange-red light
15:55-16:05 darkness
16:05-16:10 quasimonochromatic blue light
16:10-16:40 minutes of darkness
16:45-16:50 quasimonochromatic blue light
16:50-17:00 minutes of darkness.

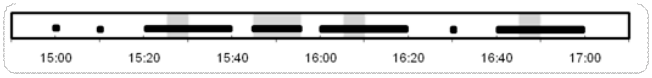

Figure 1: One 2-hour experimental session between 15:00 and 17:00. Grey = light, white = darkness, black = recording period of the pupil.

Between the recording periods the subjects were free to stretch the legs by moving around in the experimental room, which was light-proofed with dark curtains. The quasimonochromatic "standard 5 mm" blue LEDs used in the study had a peak wavelength of λ_{max} = 468 nm and a half-bandwidth of hbw = 26 nm and they provided corneal illuminance of 40 lx (corresponding to a photon density of ~1.5 x 10^{14} photons/cm^2/s). The broadband orange-red light that was used between the blue light pulses was a mixture of two types of Luxeon Star III LEDs: Red-Orange (λ_{max} = 617 nm, hbw = 20 nm) and Amber (λ_{max} = 590 nm, hbw = 14 nm). The corneal illuminance provided by this broadband red-orange light was 83 lx (corresponding to a photon density of 1014 photons/cm^2/s). All the LEDs were mounted on a Goldman perimeter providing uniform light distribution. The luminance distribution was measured using a Nikon Coolpix 8400 digital camera equipped with a Nikon FC-E9 fisheye lens. The acquired images were analysed using the PHOTOLUX 2.1 software [22] which had a calibration profile for the camera that was used.

Measurements

Pupil Size

The pupil size of the subject was recorded during the periods illustrated in Figure 1. It was recorded using a Unibrain Fire-I OEM (Unibrain Inc., San Ramon, California, USA) digital monochrome board camera with a resolution of 320 x 240 and a sample rate of 30 Hz. The camera was mounted at the back of the Goldman perimeter at a distance of 30 cm from the subject's eye. The camera was equipped with a telephoto lens and an IR bandpass. The minimum focusing distance was reduced with home-made extension tubes which, at the same time, made the depth of the field narrower.

The pupil was illuminated with infrared LEDs (Everlight HIR204/H0, λ_{max} = 850 nm, hbw = 45 nm, beam angle = 60°) positioned off-axis close to the eye. The pupil size was to be determined from a recorded uncompressed video file using an edge-based segmentation program developed by the authors under Matlab (Mathworks, USA). The corneal irradiance of the infrared LEDs was below the safety levels of 10 mW/cm^2 for chronic infrared exposure at λ = 720-1400 nm as defined by ICNIRP [35].

Heart Rate

Heart rate was monitored continuously during the whole experiment using a Polar Rs800sd heart rate monitor (Polar Electro, Vantaa, Finland). Heart rate was analysed with Kubios HRV Analysis Software [37] by dividing raw heart rate data into 5-minute bins. The mean of each bin was calculated for heart rate, low-frequency power (LF), high-frequency power (HF), and LF/HF ratio, which is considered to be a good index of cardiac activity [2].

Skin Conductance

Skin conductance was measured continuously using a ProComp Infiniti Encoder (ThoughtTechnology, Montreal, Canada). 256 samples were recorded per second with BioGraph Infiniti software [1]. Mean skin conductance was determined for the same 5-minute bins as with heart rate.

Karolinska Sleepiness Scale

Subjective sleepiness was assessed using the Karolinska Sleepiness Scale (KSS) [88] every 20 minutes during the experiment. The mean subjective sleepiness every five minutes was calculated by extrapolating the data.

Data Analysis and Statistics

For all the analysis, the Statistical Package for the Social Science (SPSS) was used. The significance level was set to 0.05 in all comparisons. To analyse the values in different lighting or recording conditions, the Student t-test for independent variables was used. This *t* test is a special case of ANOVA that assesses whether the means of two groups are different (if $p < 0.05$ the means are different). The correlations of different methods within the age group and of the same methods between the age groups were tested with Pearson's correlation coefficient (r = 0.00 = no correlation and $|r|$ = 1.00 = perfect correlation). Pearson's correlation was also used to investigate the time correlation of the measures.

Results

The mean values of normalised skin conductance, normalised heart rate, LF/HF ratio, and subjective sleepiness every 5 minutes for the young and older test groups during the 2-hour test period are illustrated in

Figure 2. Unfortunately, the pupil size values could not be calculated because of the low image quality caused by an unfocused lens, camera vibration, and the absence of a fixation point of the eye.

The subjective sleepiness increased significantly by time with both the young (r = 0.74, p = 0.000) and older (r = 0.80, p = 0.000) test groups. The LF/HF ratio decreased somewhat (young: r = -0.26, p = 0.113; older: r = -0.26, p = 0.112; not significant) and normalised heart rate values decreased significantly (young: r = -0.56, p = 0.002; older: r = -0.35, p = 0.046), corresponding to reduced arousal. In contrast, the skin conductance values supported the increase in arousal with time, but only with the young subjects (young: r = 0.47, p = 0.010; older: r = 0.07, p = 0.379).

Exposure to light (either quasimonochromatic blue or broadband orange-red) did not cause any significant effect on the values in the young group (p > 0.05 with all methods; t test). In the older group there were differences in the heart rate and skin conductance values during the light period compared to darkness (heart rate: p = 0.045; skin conductance: p = 0.021; t test). However, the effect of the recording period appeared much stronger in the values. In both age groups the heart rate, LF/HF ratio, and skin conductance were significantly higher during the periods when the subject could move freely in the dark experimental room compared to the periods when he or she was attached to the Goldman perimeter (young: p = 0.000, 0.007, 0.050; older: p = 0.000, 0.022, 0.000; p = heart rate, LH/HF ratio, skin conductance; t test). Within the recording periods there was no difference in the responses to light exposure compared to darkness in any of the methods in either of the age groups (p > 0.05 with all methods in both age groups; t test). Sleepiness acted independently and did not follow the recording or the lighting conditions. The behaviour of the skin conductance correlated negatively with the behaviour of the LF/HF ratio in both age groups (young: r = -0.50, p = 0.006; older: r = -0.27, p = 0.043). With the young subjects KSS correlated with skin conductance (r = 0.54, p = 0.003), giving conflicting information about the changes in alertness. However, the negative correlation of KSS and HR (r = -0.50, p = 0.006), implied that their alertness did indeed decrease with time. These inter-method correlations were not found significant in the older test group. However, all the measures showed corresponding behaviour in both age groups (heart rate: r = 0.73, p = 0.000; LF/HF ratio: r = 0.35, p = 0.049; skin conductance: r = 0.70, p = 0.000; KSS: r = 0.99, p = 0.000).

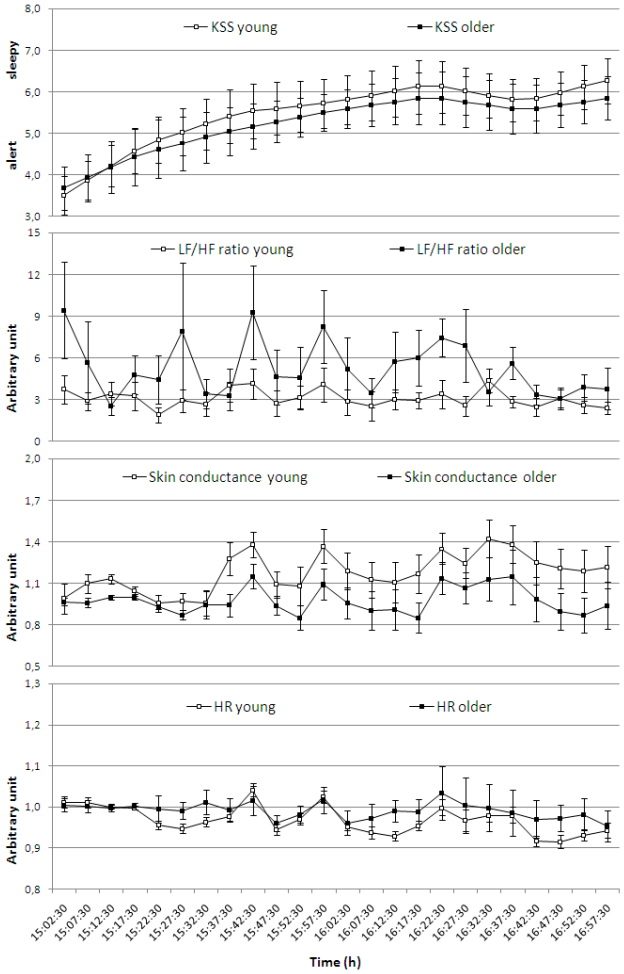

Figure 2: Time course of subjective sleepiness (top), LF/HF ratio (panel 2), normalised skin conductance (panel 3) and normalised heart rate (bottom). Mean values per 5-min bin ± SEM.

Discussion

Subjective sleepiness ratings and heart rate measures showed that the subjects became sleepier during the test. That is in conflict with the skin conductance responses, which suggest that the subjects became more aroused. It is possible that the KSS ratings reflected reduced motivation rather than alertness. However, a more likely explanation for the inconsistency is that while becoming sleepier the subjects were trying harder to fight against the desire to sleep. That appeared in the skin conductance data as arousal.

The effect of light exposure was shown in the older test group as a change in skin conductance and heart rate. However, the variations of the autonomic nervous system functions were mainly detected when the subject was able to move freely in the experimental room without having his or her head attached to the Goldman perimeter. This indicates that the presence of the pupil camera played a strong role in the ANS responses masking the effect of the light stimulus. This is a practical illustration of the sensitivity of the ANS methods to external stimuli. It shows

that more effort has to be put into the study protocol to either exclude everything that could appear as external, unwanted stimuli or choose methods that can be used in the presence of such stimuli. Apparently, sitting still in front of the Goldman perimeter eye facing towards the camera was a task that did not go together with skin conductance and heart rate measurements. Hence, in this type of study setting the measurement of pupil size cannot be used at the same time as other methods recording the activation of the autonomic nervous system. Furthermore, the recording protocol has to be designed so comfortable for the subject that no difference between the recording period and the rest of the experiment can be detected.

The biggest drawback of the study was the unsuccessful recording of the pupil size, which appeared despite the fact that the protocol had been tested in a pilot study. It was not possible to adjust the focus of the camera, so the focusing had to be done manually. In future studies one could try to adjust the distance to the eye by attaching the camera to a microrail on which the camera can move back and forth. To reduce the noise in the image, more infrared light should be applied. That is challenging because the light should be kept invisible to the subject. In this study difficulties were encountered in keeping the camera still. During some of the experimental sessions the heavy camera could not be held in a constant position, causing the eye to change its position in the image. Therefore not all the data could be read and processed with the Matlab program. This could be corrected by using a separate stand for the camera instead of attaching the camera directly to the Goldman perimeter.

CONCLUSIONS

The theoretical and practical examination of the methods showed that using subjective evaluation to assess alertness is an easy method to conduct. However, it should be kept in mind that ratings on scales such as the Karolinska Sleepiness Scale (KSS) do not necessarily indicate changes in alertness. Therefore subjective evaluation should always be used together with an objective test. Objective evaluation can be done by using either central (CNS) or autonomic nervous system (ANS) variables or, in the best case, a combination of those two. Reaction tests are also often used in lighting studies, but they measure sustained attention rather than alertness. In addition, the tasks can mask the light stimulus. For measuring ANS activity through skin conductance and heart rate, there is relatively cheap, low-tech equipment available. As the practical testing showed, it is, however, very sensitive to external stimuli, which can limit its use in lighting studies. The current study encountered some difficulties in the measurement of pupils. However, it is foreseen that with more careful study design pupillometry could be a suitable method for use in light-induced alertness research.

In the current study it was not possible to test CSN variables. Previous studies show, however, that electro-oculogram (EOG) measurements could suit lighting research if the tiresome tasks did not limit their use to short recordings. The electroencephalogram (EEG) is popular because of its high temporal resolution. However, as a result of the low spatial resolution a lot of interference can occur in the data. The authors are of the opinion that of the research methods presented in this paper, the greatest potential lies in brain imaging, because it can reveal the mechanisms behind the (hypothetical) light-induced daytime alertness by spotting the neural correlates. The protocol is expensive and hard to design because of numerous restrictions. What is common to all CNS methods is that they are not suitable for field studies.

By using validated methods and designing the experiments in accordance with the standards, the data analysis and the comparison of the results with other studies can be made easier. There is already good software available for numerous methods.

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