

# Temperature

Validity as human test marker for alertness

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## Introduction

Biometrics of the human body are currently the best indicators of internal processes which can be measured without disturbing them. Examples of biometrics are heart rate, temperature, but also DNA and facial recognition. Tests with these markers represent different functions, including identifying an individual, but also underlying physical and mental processes. However, not all biometrics are a direct representation of a certain process. As a majority of biometric markers also interact with each other, or the underlying process of interest. Ideally, once a certain process is identified, it can eventually be classified by using a list of matching biometrics. In the main research, the internal process researched was alertness and its response to light during the daytime, in which the light is the controlled variable. As part of this research, the biometric temperature, which was initially used as biometric for alertness, will be analyzed for possible interference with other factors.

The biometrics chosen to represent alertness were *vigilance response*, *subjective alertness*, *eye blinks* and *skin temperature*. These parameters were chosen due to previous findings on alertness. Vigilance tests are the most direct means to test alertness, and contribute much to the definition of alertness as a state of attention (Oken, Salinsky, & Elsas, 2006). The subjective alertness scale, called **Karolinska sleepiness scale**, has been found to correlate with a vigilance response test and with EEG alpha power with eyes open (Kaida et al., 2006). The sleepier the subject felt, the greater the response time was and the higher the alpha power. This alpha power with eyes open has previously been found to increase with sleepiness (Akerstedt, Gillberg, 1990). Skin temperature has also been found to have a strong correlation with sleepiness (Kräuchi, 2007), either a negative or positive effect can be measured depending on the location and body part measured. To understand this difference in correlation, it is important to understand how temperature works in the human body. This understanding will help when determining whether changes in temperature correlate with alertness, or with other outside factors. So eventually, the research question of whether temperature is a good biometric for alertness can be answered.

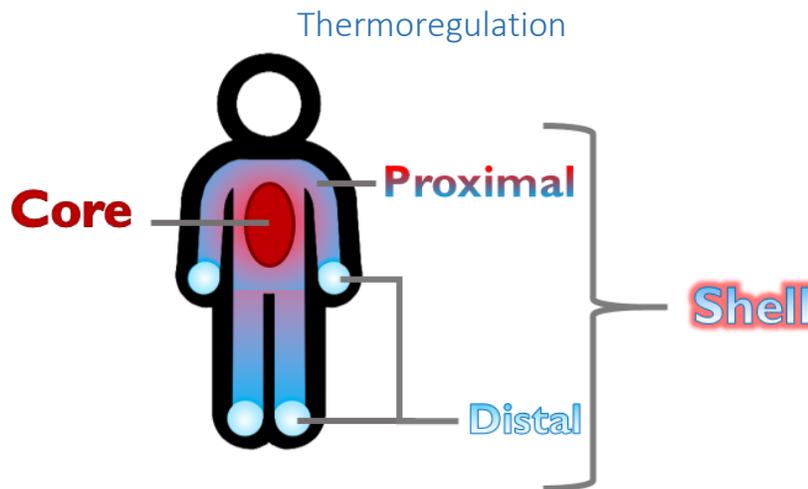


Fig. 1: Pictured here is the shell which consists of proximal and distal skin temperature, and the core that lies deeper in the body (Fronczek et al., 2008).

Human body temperature is a self-regulatory system, referred to as thermoregulation. To determine which factors of thermoregulation are relevant when dealing with skin temperature, it is important to understand how the complete temperature system works. This system has two parts: the heat-producing **core** and the

regulating **shell** (Kräuchi, 2007), (fig 1). This core is homeostatically regulated around 37 °C. It adheres to a **circadian** (24 hours) rhythm (Aschoff J., 1967), in which heat production rises during the day, starting in the morning. While the core's heat production fluctuates, it cannot cool itself down. In order for the core to cool down, heat must leave using the shell.

This poikilothermic shell comprises of the skin areas, with varying degrees of heat radiation, which makes it very dependent on environmental temperature. This means that unless environmental temperature is 37 °C or higher, the core is always producing heat as the shell is always radiating heat. Like the core, the shell contains a circadian component. Opposite to the core, the temperature of the shell decreases during the day, and starts increasing in the morning. This shell consists of proximal and distal skin regions. **Proximal** skin temperature is only regulated via slow passive blood flowing in pre-capillary arterioles and primarily influenced by core body temperature.

In the **distal** skin, this heat radiation is actively and locally controlled by structures with a shunt function called **arteriovenous anastomoses** (AVA's). These fast flowing AVA's are connections between the arteries and veins, and they are particularly rich in the distal glabrous skin area's (skin areas devoid of hair). In the hands, AVAs are located in the bed of the nails, at the fingertips, on the palm of the phalanges, and in the thenar and hypothenar (Bergersen, Hisdal, & Walløe, 1999), (fig 2).

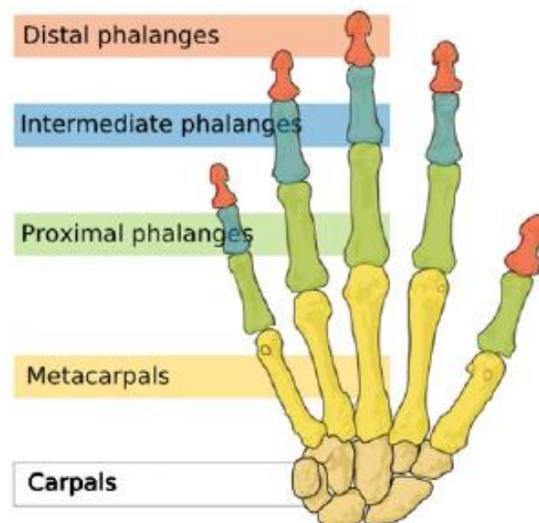


Fig. 2: Phalanges of the human hand. The palmar side is where the AVA's are located (Zieliński, 2010).

The AVA's consist of a thick smooth muscle wall, usually thicker than the arteries they are connected to. The AVA's are innervated by sympathetic adrenergic nerves, which enables them to contract very quickly on impulse, but dilute passively and slowly (Donadio et al., 2006). These contractions also reflexively occur in response to environmental sensory (external) and psychological (internal) stimuli, which is caused due to its connection with autonomic nervous system. When contraction of the AVA's occurs, blood is shunted back into the veins and heat-loss of the shell is lessened. Likewise, when dilution occurs, blood is able to flow into the AVA's, which enhances the heat-loss function of the shell (Walløe, 2015). In this manners AVA's may control the core's heat loss, but not the core's heat production. This is one of the reasons why AVA rich areas are not a good reflection for core body temperature, the other reason being their reaction to many other environmental stimuli. Executing the chosen tests provides the subject with either external or internal stimuli, or perhaps both. This will influence the AVA's which, in turn, influences the skin temperature measurements.

Direct influence of these tests on body temperature will always occur in research, and is inseparable from the internal process of alertness. Arousing factors, whether direct, indirect, internal or external all influence the AVA's to close rapidly, which in turn influences the skin temperature. Meaning, the expected temperature response to alertness is a quick, local drop in temperature, most notable the closer the measured area is to the AVA's.

## Materials and methods

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### Subject requirements

For this experiment, 11 males and 13 females of ages 20-30 ( $23 \pm 2$  yr) from the Netherlands were recruited to stay one night and one day at the Linnaeusborg in Groningen. Requirements for these subjects were good health, suitable chronotype and reasonable to good sleep quality, which was determined before participation of the potential subject.

To determine health, subjects were required to fill in an online questionnaire. Any subjects with a condition (i.e. asthma) or medication that potentially affected temperature or their ability to sit still were excluded. Subjects who had an eye condition besides the requirement of lenses were excluded due to the part of the experiment including eye tracking. Regular smokers were excluded, as well as subjects that drank more than 5 cups of caffeine (500 mg caffeine) per day, and/or drank more than 15 glasses of alcohol per week (150 gr alcohol), as addiction (and/or symptoms of withdrawal) could interfere with the data. Subjects were also excluded when they had traveled more than 2 time zones a month before participation, or had employment with shift hours in the last year. When subjects arrived for the experiment, they were checked for color-blindness using the Ishara's color blindness test and were excluded from participation if diagnosed as colorblind. For females, intake of hormonal birth control was required and they were not allowed to participate during their menstrual period in order to minimize hormonal variance.

**The Munich Chronotype Questionnaire (MCTQ)** was used to determine the chronotype of the applicant. Subjects of -intermediate chronotype were required for this experiment. To determine this average, 25% of the bottom and top scores found in an MCTQ database of the general population were excluded. Doing this, only applicants with test scores between timepoints 3.88 and 6.17 ( $4.6 \pm 0.6$ ) were admitted. **The Pittsburgh Sleep Quality Index (PSQI)** was used to score sleep quality. Only applicants with PSQI scores below 12 ( $4.0 \pm 2.1$ ) were admitted.

The subjects were rewarded for their participation via a monetary fee as well as travel expenses which were refunded at a standard fee per kilometer traveled.

## Protocol

The experiment took place at the Linnaeusborg in Groningen in 2016, starting in April and ending in October, as to cover the Dutch daylight saving time period. The experiment took place during weekdays, with the exception of the arrival of subjects on Sunday evenings. Individual subjects could only participate one, to prevent any anticipation of learning effect of the protocol. Each subject stayed in separate rooms (fig 3), which was completely shielded from outside light and kept at a constant 21 degrees Celsius.



*Fig. 3: Photo of the test room in which the participants stayed during the testing. Important items, from left to right: bed, nightlight, door to the bathroom, water boiler and sink, fridge containing food for the day, desk chair with desk, computer with blue light filter, and the experimental light.*

Subjects were instructed to arrive the evening before the experiment at 22:00. Upon arrival, subjects were familiarized with the protocol, a practice test session was done, and temperature sensors were applied. At 23:30 the tube lights on the ceiling were turned off and subjects were instructed to sleep. They were woken up at 7:30, at which time the ceiling lights were turned on again. The first round of tests started at 7:48 and the last session ended at 17:30, after which subjects were allowed to leave. Subjects were informed of these end and start times, but during the experiment they were not allowed access to any form of time indication. Personal devices were also not admitted, as they emit light and allowed for communication to the outside world which could also give an indication of time.

The experimental timeframe consisted out of 4 equal blocks, each of which lasting two and a half hours. Each block was divided between a dim light and experimental light situation. Once per block, during dim light, the subject was instructed to eat prepared food that consisted of an equal amount of calories every time. In both the dim light and experimental light situation, two test sessions occurred, for a total of 4 test sessions per block. One test session consisted of three tests, consecutively: The KSS, eye-tracking session and the Go/No-go. 5 minutes before entering a light block, subjects were alerted of this, and were explicitly suggested to use the bathroom if necessary.

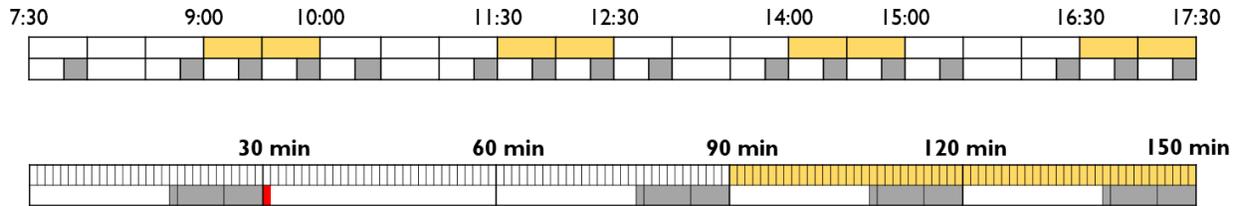


Fig. 4: The top bar displays the schedule of each test day, the bottom bar that of each test block. The white upper bar represents the time during normal light conditions, the yellow bar that of the experimental light. The grey areas below are times at which tests took place, respectively the KSS, eye-tracking session and the Go/No-Go. The red bar marks the time when the test subject could eat.

Dim light was emitted by ceiling TL light with an intensity of 10 lux, and light spectrum with minimal blue light emittance, at horizontal eye level when sitting down. As for the experimental light situation, the dim light was accompanied by a portable desktop lamp which emitted a broad white light spectrum, with some additional blue light (fig. 5) at an intensity of either 24, 74, 222, 666 or 2000 lux. The effects of each light intensity was tests one subject for either gender.

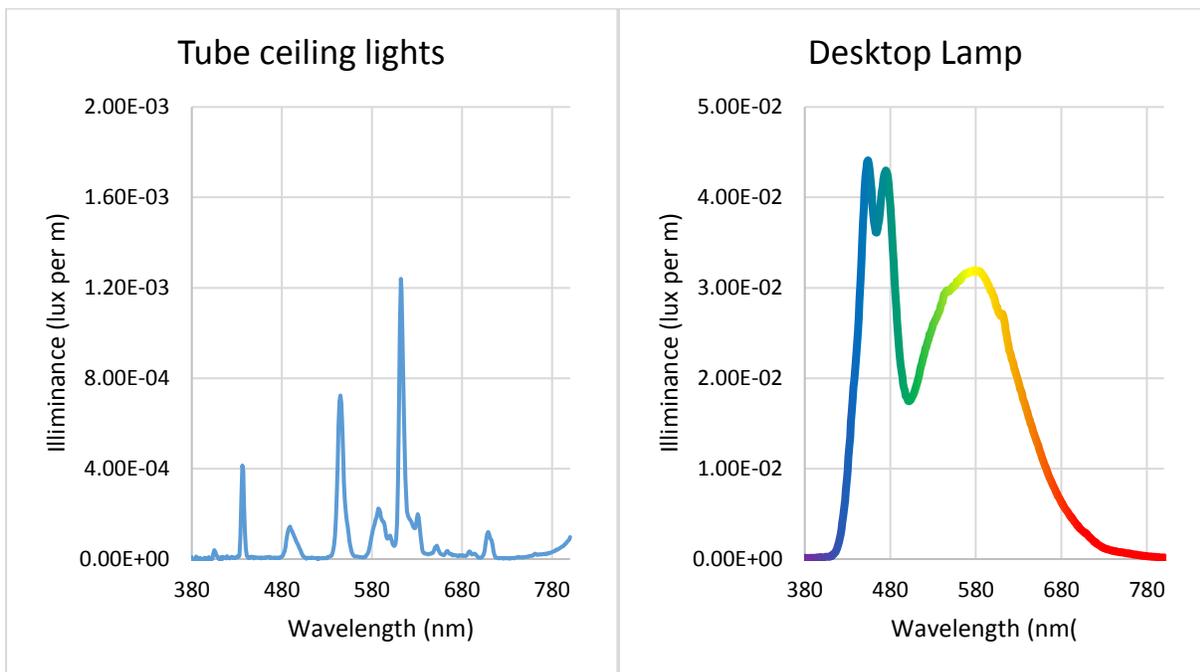


Fig. 5: Left: Average light illuminance of ceiling lights at 10 lux. Spectral peaks of the used tube lamps were as evenly distributed as possible to prevent visual color other than white, and excluded as much blue light as possible. Right: Average light illuminance at eye level in front of experimental desktop lamp at 2000 lux. For comparison to the left graph, the y-axis for illuminance has been multiplied by 20.

In order to maintain a stable light spectrum, neutral density filters were applied to the experimental desktop lamp until it reached the desired levels (24, 74, 222, 666 or 2000 lux). The intensities of the desk light were chosen so that they were equally divided over the different test subjects. Which means that every test subject got one of the five light intensities throughout their day of the experiment. At the end of the experiment, every gender, on every day of the week, and over the seasons during daylight saving time would be tested in all five light intensities (table 1).

female	Intensity					male	Intensity				
Monday	2000	74	24	666	222	Monday	222	24	2000	74	666
Tuesday	24	2000	666	222	74	Tuesday	2000	222	24	666	74
Wednesday	2000	666	74	24	222	Wednesday	24	2000	666	74	222
Thursday	222	2000	666	74	24	Thursday	74	2000	666	222	24
Friday	666	74	222	24	2000	Friday	2000	24	222	74	666

Table 1: Spread of the light intensity over the days of the week, divided by gender. As shown, subjects of both genders have been tested with all light intensities on the five different days.

## Tests and measurements

Each test session started with the subject filling in the KSS, completing an eye-tracking session and performing the go/no-go test. Subjects were instructed via the intercom at set times to mark the start and end of each session and to provide the instructions for each test.

The **KSS** required test subjects to use the computer to fill in how sleepy or alert they were on a scale of 9 to 1, with 9 being the most. For the **eye-tracking session**, test subjects put on glasses with invisible infrared trackers that measured whether or not a subject was blinking, and for how long. In this, less blinking meant a more alert individual. Subjects were instructed to sit as still as possible while focusing on a dot on the wall (distance ~ 1 meter). **The go/no-go test** was done on the computer. Beeps of two different tones (high and low) would be played for the test subject at random intervals of time. Subjects were instructed to press a button when they heard the low tone. Failure to press (quickly) or pressing at the wrong tone resulted in an omission score, while pressing before the stimulus resulted in a commission error. This resulted in a total error score, in which a lower score meant a less alert test subject.

The body temperature of the subjects was measured by placing iButtons, which were taped to the proximal phalange of the middle finger, the infraclavicular region, and the ankles (fig. 6). Both on the left and right side of the body. The iButtons were placed on the infraclavicular region to record proximal body temperature and the iButtons on proximal phalange of the middle finger and the ankles to measure represent distal body temperature. The output of the iButtons was measured in degrees Celsius.



Fig. 6: Placing of the iButtons from left to right: proximal phalange of the middle finger, the infraclavicular region, and the ankles

## Data analysis

The recordings of temperature data was extracted from the iButtons on the same day, after subjects had left. Subjects that could not complete the full day were excluded from both the experiment and the dataset.

Due to the main question of whether or not testing affects temperature, other factors had to be excluded whenever possible. Light, food consumption and the most variation in movement of the subjects all occurred during the dim light block. Furthermore, the amount of time the subjects spent in the dim light block was less consistent in time between test sessions. Therefore, only the results of the subjects during the light block was used for the analyses, reducing the number of factors that could also influence temperature

The individual parts of the light block, which were 60 minutes in total, were divided in four parts of '*not-testing*' and '*testing*'. Since a test session consisted of 12 consecutive minutes. One minute before and after the test was added to account for outliers of slower subjects when analyzing the test sessions. This makes the total '*testing*' time 14 minutes per session, and the '*not-testing*' time 16 minutes per session.

RAW temperature data was checked for faults. Faulty or corrupted data from iButtons was removed completely, a total of 6 raw data sets. For the fingers, 2 of the left side and 3 for the right side of a subject were removed, and for the ankles 1 data set of the left side was removed. No more than one raw data set was removed per subject. The RAW data was averaged by 7, thus resulting in 3 fewer data points for each '*testing*' and '*not-testing*' section. Finally, the RAW data from the left and right side of the same body part of the same individual were averaged.

In order to analyze temperature differences over the day, and to analyze '*not-testing*' and '*testing*' and its patterns, ANOVA repeated measure multilevel regression was used. To analyze the correlation of temperature with the KSS, bivariate analysis was used to look for Pearson linear correlation.

## Results

### Light blocks

Since the testing day consisted of four dim light and four experimental light blocks (fig. 7), the first analysis contains data of the experimental light blocks, averaged as one data point. ANOVA repeated measure multilevel regression was used. The difference in temperature of the subjects between the four different light blocks were compared against each other (table 2). For all body parts, a significant difference was found when analyzing the temperature difference in tests subjects of block 1 (time period 9:00–10:00) against the values of the other blocks (with time period of 11:30–12:30, 14:00-15:00 and 16:30-17:30). Skin temperature of test subject's shoulders was lower in the morning, while temperature of the fingers and ankles was higher in the morning. Additionally, for the ankles, skin temperature of the subjects in each block was found to be different from the others, indicating consistent temperature decrease throughout the day.

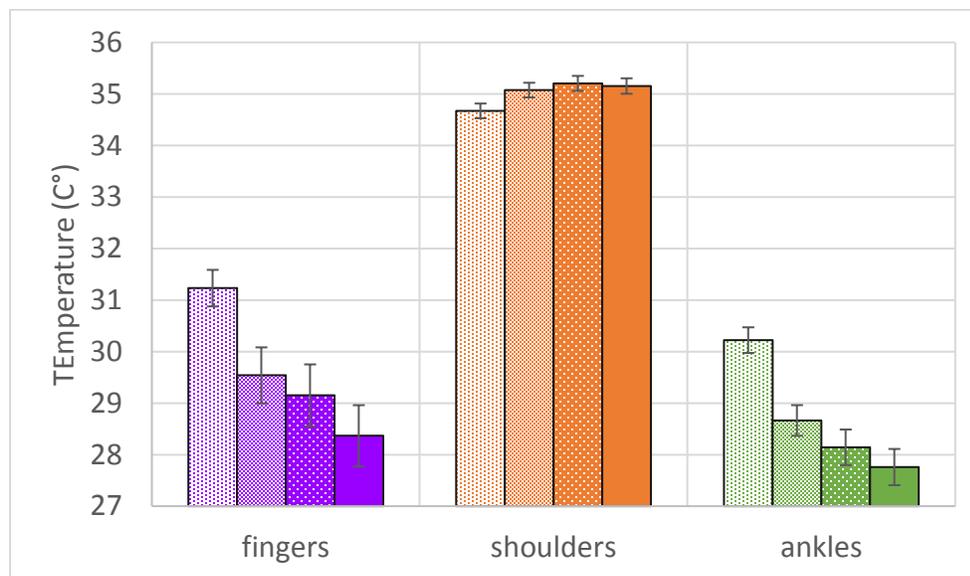


Fig. 7: Average temperature of the test subjects on different body parts of each light block ( $\pm$  SEM) of the fingers ( $n=24$ ), shoulders ( $n=24$ ) and ankles ( $n=24$ ) of all individuals. The four consecutive light blocks spread over the day are in chronological order in the graph for each body part, with the lightest shade representing the earliest block and the darkest shade the latest block.

Table 2: ANOVA results output for significant difference in body temperature of the test subjects for the different body parts between the different light blocks. Significant difference between test subject data in their respective body part are shown with a bold font and "\*" mark.

	Block	1 VS 2	1 VS 3	1 VS 4	2 VS 3	2 VS 4	3 VS 4
<b>Bodypart</b>							
<b>Fingers</b>	p	<b>0.000*</b>	<b>0.000*</b>	<b>0.000*</b>	0.133	<b>0.000*</b>	<b>0.014*</b>
	f	31.289	29.172	41.754	2.490	15.689	7.078
<b>Shoulders</b>	p	<b>0.000*</b>	<b>0.000*</b>	<b>0.000*</b>	0.132	0.312	0.502
	f	24.183	18.716	22.380	2.435	1.068	0.466
<b>Ankles</b>	p	<b>0.000*</b>	<b>0.000*</b>	<b>0.000*</b>	<b>0.001*</b>	<b>0.000*</b>	<b>0.001*</b>
	f	87.741	63.957	70.362	13.450	24.861	13.439

## Pattern within light block

The temperature of the subjects over the light blocks were split out into four individual points, alternating 'not-testing' and 'testing' (fig. 8) to determine the pattern in skin temperature between periods of 'not-testing' and 'testing' within a light block. After which, the temperature of the subjects within each light block was corrected for the temperature pattern over time. This was done by taking the **first (F)** and **last (L)** temperature data point in the raw data of an individual light block, followed by subtracting the value of the last point from the first point. This was divided by 60, as one light block has 60 data points. Next, for every individual point, this number was multiplied times the number of the data point, which is then added to the **original (O)** data point For example, for the 20th point the formula would be  $O + ((F - L)/60) * 20 = \text{new data point}$ . These calculations were done before removing the 3 data points that account for the averaging of 7, as described in the earlier subchapter data analysis.

Using an ANOVA repeated measure multilevel regression, these four individual points of subject temperature data within a light block were compared against each other (table 3). As can be seen by both the graph and p-values, the pattern of subject's skin temperature fluctuation within a light block varies per body part. There are however, similarities showing that the fourth point is significantly lower than the third. Meaning a decrease in temperature for all body parts in subjects when moving from the last 'not-testing' to the last 'testing' of a light block.

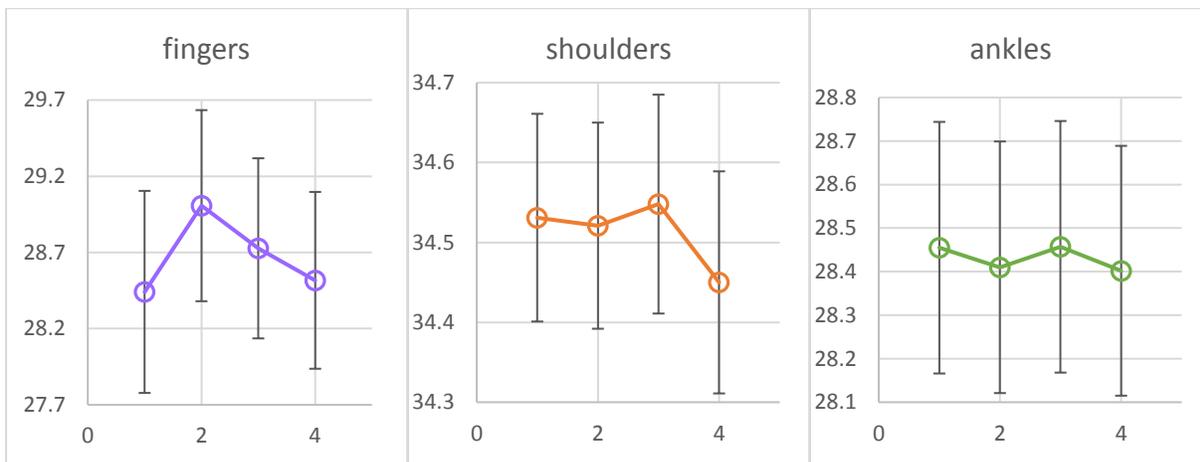


Fig. 8: Average temperature of four data points are within a light block, alternate 'no-test' and 'test', averaged for all four light blocks ( $\pm$  SEM) of the fingers (n=24), shoulders (n=24) and ankles (n=24) of all individuals. The four consecutive points are displayed chronological per body part. Corrected for time of the day.

Table 3: ANOVA results output for significant difference is body temperature of the test subjects for the different body parts between the different data points within a light block. Significant difference between test subject data in their respective body part are shown with a bold font and "\*" mark.

	Point	1 VS 2	1 VS 3	1 VS 4	2 VS 3	2 VS 4	3 VS 4
<b>Bodypart</b>							
<b>Fingers</b>	p	<b>0.000*</b>	<b>0.034*</b>	0.515	<b>0.002*</b>	<b>0.000*</b>	<b>0.012*</b>
	f	42.911	5.096	0.440	12.17	32.627	7.541
<b>Shoulders</b>	p	0.562	0.380	<b>0.005*</b>	0.317	<b>0.008*</b>	<b>0.002*</b>
	f	0.347	0.801	9.876	1.045	8.535	12.046
<b>Ankles</b>	p	0.151	0.938	<b>0.013*</b>	0.133	0.794	<b>0.005*</b>
	f	2.206	0.006	7.172	2.43	0.07	9.451

### Half light-blocks

To determine if a pair of data points for subject's skin temperature between 'not-testing' and 'testing' have a similar pattern, temperature of the subjects within a light block was split in half. Because of this split, each half had two temperature data points ('not-testing' and 'testing') on one half, and the next two data points on the second half (fig. 9). Next, an ANOVA repeated measure multilevel regression was used to look for a similar pattern of skin temperature of the subjects between 'not-testing' and 'testing'.

The temperature of the test subjects for the first and second half of the light block were found to be significantly different for the fingers ( $F=34.892$ ,  $p < 0.05$ ), and shoulders ( $F=11.355$ ,  $p < 0.05$ ). For the ankles, no significant different was found, meaning both half blocks display the same trend: decreasing subject's skin temperature when going from 'not test' to 'test'.



Fig. 9: Average skin temperature of the test subjects within the two halves of the four light blocks ( $\pm$  SEM) of the fingers ( $n=24$ ), shoulders ( $n=24$ ) and ankles ( $n=24$ ) of all light blocks and all individuals. The four data points represent data within a light block, and alternate 'no-test' and 'test'. First two data points are the first half of the block, second two data points the second half of the block. The data points shown are corrected for time of the day.

## Not testing VS testing

When comparing the temperature of the subjects in 'not-testing' and 'testing' sessions (fig. 10), the influence of time of day was subtracted first, with the same formula as shown above.

The 'not-testing' and 'testing' periods were found to be significantly different for the fingers ( $F=13.507$ ,  $p < 0.05$ ), shoulders ( $F=7.558$ ,  $p < 0.05$ ) and ankles ( $F=9.292$ ,  $p < 0.05$ ) of the subjects. In these findings,, the temperature was lower for the fingers during the 'not-testing' period, and higher for the shoulders and ankles.

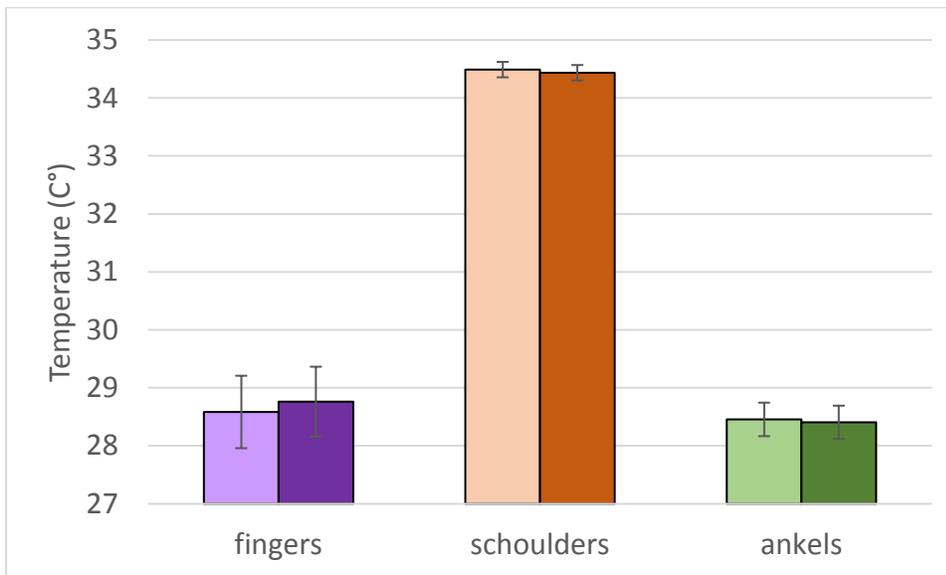


Fig. 10: Average temperature of 'no-test' vs 'test' ( $\pm$  SEM) of the fingers ( $n=24$ ), shoulders ( $n=24$ ) and ankles ( $n=24$ ) of all light blocks and all individuals. The data is corrected for time of the day. The lighter colors represent the 'no-test' period, the darker colors represent the data 'test' period.

## Karolinska Sleepiness Scale and temperature

Finally, the skin temperature of tests subjects was compared with sleepiness scores rated on the Karolinska Sleepiness Scale. This was done in order to determine if skin temperature measurements of the different body parts correlated with subjective sleepiness. For this analysis, skin temperature data of the subjects was not corrected for the time of the day as it was needed for this analysis. Both KSS and temperature data of the subjects were standardized to be comparable on an equal scale, using the excel (=STANDARDIZE) function. As the KSS test only occurred during 'testing' time periods, this resulted in two temperature data points per block per body part instead of four. The delta of these two points were taken, so a possible change in skin temperature of the subject could be correlated with a change in KSS score. This resulted in one data point per block per subject for each body part. For this analysis 4 subjects had to be excluded due to data that could not be standardized. Bivariate analysis was used to look for Pearson linear correlation.

The analysis was done for the subject's data for each of the four light blocks separately, and averaged of all four of the blocks per body part. (table 4) For the skin temperature data of subjects per separate light blocks, only the temperature of the fingers of block 3 showed a significant positive linear correlation with the KSS. This means that the sleepier the subject felt, the higher their temperature was. No other data regarding separate blocks or other body parts tested in subjects, showed a significant linear correlation. When averaging all light blocks (fig. 11), again a significant positive linear correlation with the KSS was found for finger temperature. Other body parts showed no correlation.

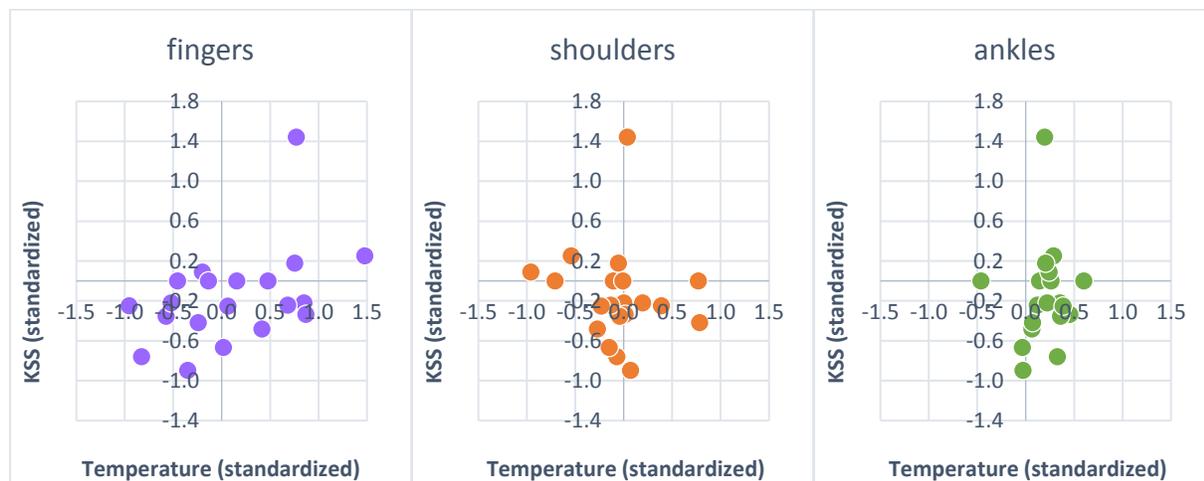


Fig. 11: Average standardized delta of the KSS and temperature ( $\pm$  SEM) of the fingers ( $n=20$ ), shoulders ( $n=20$ ) and ankles ( $n=20$ ) of all light blocks. One data point per subject.

Table 4: P-values and slope for the linear correlation of temperature and KSS, separated for body part and light blocks.

	Block	1	2	3	4	Avg all 4
<b>Bodypart</b>						
<b>Fingers</b>	p	0.295	0.777	<b>0.019*</b>	0.194	<b>0.028*</b>
	slope	0.247	0.068	0.519	0.303	0.491
<b>Shoulders</b>	p	0.234	0.108	0.737	0.201	0.513
	slope	0.279	-0.371	0.080	0.299	-0.155
<b>Ankles</b>	p	0.765	0.057	0.058	0.812	0.784
	slope	0.071	0.433	0.430	-0.057	0.065

## Conclusion & discussion

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The main research investigated the effect of light on alertness during the daytime. As part of this research, skin temperature was investigated for its validity as biometric for alertness. First, the differences in results of the data between test-sessions were investigated as possible disturbing factor. Secondly, the correlation between skin temperature and subjective sleepiness of the subjects were analyzed.

The **first data analysis** was done to investigate the pattern of temperature over the day. This was done by looking at the temperature of each body part, averaged per individual, per light block. These light blocks occurred four times over the day, at the following times: 9:00-10:00, 11:30-12:30, 14:00-15:00 and 16:30-17:30. For the shoulders of the subjects, more precisely the infraclavicular region, this temperature was lower in the morning. The fingers and ankles of the subjects showed a higher temperature in the morning. These results are consistent with the circadian model of skin temperature found in literature (Aschoff J., 1967). In this model, the fingers and ankles represent the distal temperature, and the shoulders the proximal. Another notable result is that between all four light blocks of subject's temperature, the ankles differ from each other, decreasing over time.

The **second analysis** was done in order to investigate the whole data pattern of the subject's body temperature over one light block, which was the average of all four blocks. One such block consists of four data points, alternating '*not-testing*' and '*testing*'. Since the first analyses showed that a temperature pattern during the day does exist, for this analysis temperature of each light block was first corrected for this temperature pattern over time, using the formula in the results section. The consistent significance was when skin temperature for all body parts decreased from the last '*not-testing*' of the block to the last '*testing*' of the light block. No other similar patterns were found for any body parts. However, interesting to note is that for the fingers, all data points differ from each other, except the first and fourth data point, thus showing the most fluctuation in temperature of the subjects.

The third and fourth analysis investigated the tests themselves. The **third analysis** was done to determine whether this drop in subject's body temperature could still be caused by a pattern of '*not-testing*' and '*testing*'. The light block that was already split into four data points, was grouped in two. This was done by testing one half of the block (containing two data points, respectively '*not-testing*' and '*testing*'), against the second half of the block. For temperature in the fingers and shoulders of the subjects, these patterns were significantly different, meaning that the first half of the block did not show the significant temperature decrease in skin temperature that the first half showed. Thus, due to the difference in pattern, the temperature drop found could not be due to testing. However, for results in the temperature of the subject's ankles this significant difference could not be found.

The fourth analysis compared all '*not-testing*' data points against '*testing*' data points. In this analysis, skin temperature of all body parts of the subjects showed a significant difference. This should mean that '*not-testing*' and '*testing*' data points do differ from each other. However, since the previous analysis indicated that the pattern of '*not-testing*' and '*testing*' was significantly different, depending on the half of the block; this significance in data is not valid for the fingers and shoulders. For the ankles however, this indicates that '*not-testing*' and '*testing*' does indeed differ.

Finally, temperature data was examined for linear correlation with the **Karolinska Sleepiness Scale**, which is has been validated for correlation with sleepiness (Kaida et al., 2006). This was done to determine if skin temperature of humans was a good biometric for alertness in humans, and if so, if this would be similar to other literature. This standardized data of both the KSS and temperature showed a significant positive linear correlation for the skin temperature of the subject's the fingers for block 3 and the average of all light blocks.

In this, it showed that increased temperature correlates with a higher KSS, which meant a sleepier subject. Since the finger represents the distal skin temperature, this data is similar to what was previously found in literature (Kräuchi, Cajochen, Werth, & Wirz-Justice, 1999).

In this research, the **circadian component** of skin temperature of all body parts is what is closest to previous literary findings (Aschoff J., 1967). When looking at the complete pattern of four data points within one light block, a similar decrease in skin temperature appears when going from the last *'not-testing'* to the last *'testing'*. Since skin temperature decrease occurs for all body parts, it is not caused by the circadian component over time. What causes skin this temperature drop in subjects could not be analyzed with the current data. This is because any recurring event over a whole block, both dim and experimental light, may have caused the skin temperature of the subjects to decrease.

Other **factors**, like the subject mentally preparing for the end of the light block (since this is fairly predictive), may be influencing the decrease in skin temperature at the end of a light block. However, while these internal psychological factors do influence the sensitive AVA's present in the body, these skin temperature decreases in subjects also occur in the shoulders of the subjects, where AVA's are not present. Another recurring factor within an entire block of two and a half hours, is the time the test subject has to eat their prepared food. At the end of the test block, the subject has not eaten for two hours. Also, the most consistent occurrence in a whole two and a half hour block, is the start of the light block. Thus, this includes the possibility of a delayed reaction to the experimental light, or saturation of the reactions that occur in reaction to the experimental light. But this is unfortunately undistinguishable whether this temperature change is from the reaction of alertness to light, or from a direct reaction of temperature to light.

But, it is the distal skin temperature of the subjects shows the most interaction with alertness, albeit in different ways depending on the body part. The temperature difference of fingers in particular show the biggest difference in temperature within one light block. The largest difference in temperature seems to be a temperature increase of 0.566 degrees, between the first *'not testing'* and the first *'testing'* of the block. The skin temperature changes of the fingers of the subjects are also the only body part that correlates with alertness. These results can be explained by the influence of the AVA's. In the introduction, AVA's were explained as shunting vessels between arteries and veins. They are sensitive to environmental stimulation and psychological stimuli (Walløe, 2015). Logically, AVA's should correlate with alertness, as environmental stimuli can induce alertness, and alertness can be classified as psychological stimuli. As closing the AVA's leads to blood being shunted back to the body, this cools down the location of the AVA's. Meaning, the more alert the subject is, the colder their fingers are, which is exactly what was found when analyzing the data as linear correlate of the KSS.

While testing has been found not to significantly influence alertness in subjects based on looking at the temperature section in which time directly correlates with the tests being done, it cannot be excluded that doing tests influences temperature. Since AVA's work fast, and the test subject is aroused 5 minutes before starting the light block, this initial point might be where the AVA reaction happens, actually relaxing again during the light block.

To my knowledge, it is not known precisely how long constriction of the AVA's can hold, and if the adrenergic nerves may eventually be satiated on (specific) stimuli, thus releasing construction. Looking at the tests chosen for this research reveals some stimuli that, when specifically tested and looked for, may help determine the AVA's reaction to tests. The first test, the Karolinska sleepiness scale or KSS, provided the subject with external visual stimuli, by means of a computer screen. Next, internal psychological stimuli were present in the subject (however briefly), as they had to determine their subjective sleepiness. The second test

was the eye-blink test. This consisted of the subject sitting as still as possible, being exposed to the experimental light while looking at a dot at the wall. Seemingly a passive activity to the observer, the subject had to consider their stature multiple times, and had to tense their muscles intentionally, affecting the AVA's (Bruin, Wijers, & Staveren, 2001). This results in both psychological and physiological internal stimuli. All while doing this, they were subjected to a constant external visual stimuli. Finally, vigilance test called the Go/No-go provided the subjects with an auditory influence, which has been found to affect the AVA's (Walløe, 2015). However, unlike a constant single experimental light, these auditory were two-fold, and not continuous.

It can be argued that the subject's AVA's reacting to these stimuli, physically or psychologically, is exactly what alertness entails. And that these tests cover a wide range of stimuli, from psychological to physical, from internal to external, and even across different sensory systems is exactly what is needed to properly measure alertness. However, exactly because the process of alertness appears to be so complex, the system behind it should also be dissected. For the KSS, this has already been done, being validated against certain brain waves and a vigilance test (Kaida et al., 2006). Although, all tests should be validated against the subject of interest of a research, before taking the test results as objective truth. If this is not (yet), possible, biometrics of tests should be validated against the next best biometric.

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