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Research Digest

Exclusive Sneak Peek

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Table of Contents

The espresso effect: caffeine and circadian rhythm

Effects of caffeine on the human circadian clock in vivo and in vitro

Introduction

Caffeine has been a component of the human diet for centuries, primarily through the consumption of coffee and tea. Culturally, coffee and tea are two of the main beverages that tie together many human interactions. Chemically, caffeine is well-known for being an adenosine receptor antagonist that blocks the action of adenosine. Because adenosine mediates the perception of drowsiness, caffeine consumption results in alertness.

A circadian rhythm is essentially an organism's daily internal clock. It is not restricted to humans and exists in most living things, even bacteria. For us, the circadian clock follows a roughly 24-hour cycle, responding primarily to light and darkness in our environment, and acting to regulate countless bodily functions. One of the most important hormones in this context is melatonin, which is released by the pineal gland in the brain and is involved with sleepiness and sleep regulation.

Melatonin is secreted in response to darkness to help prepare the body for sleep. It is well-established that exposure to bright light suppresses melatonin production. However, the ability of a stimulus to interfere with normal circadian function depends on the time of day. A pulse of blue light before bed will indeed suppress melatonin production and delay sleep, but that same pulse of light at noon will have no effect because melatonin production is low at noon anyway.

However, one recent population-based study of adults estimated that 90% of individuals consume caffeine in the afternoon (12:00-6:00 pm) and 68.5% of people consume caffeine in the evening (6:00 pm - 12:00 am). Similarly, 37% of high schoolers who consume caffeine report not using it until after 5:00 pm. Dose-response studies in humans and animals demonstrate that increasing doses of caffeine administered at or near bedtime are associated with significant sleep disturbance. Thus, the current study attempted to test whether nighttime caffeine consumption would delay the normal circadian rhythm of melatonin production, and evaluated the cellular mediators of any potential effect.

Research has backed the commonly seen experience of caffeine late in the day disrupting sleep and hence circadian rhythms. The specific mechanisms for this, as well as impact on the sleep hormone melatonin, are not fully understood though.

Who and what was studied?

Five healthy young participants (three female and two male) who maintained regular eight-hour sleep schedules were recruited to undergo four experimental sessions to test how caffeine, bright light, or both affected their circadian rhythms. After seven days of being monitored in their daily lives to make sure they maintained a regular sleep schedule, the participants moved into a research environment that was free of external time cues for five days.

On day three of the laboratory stay, the participants consumed a pill containing rice flour (placebo) or caffeine (2.9 mg/kg, approximately equal to the caffeine found in a double espresso) in a double-blind manner about three hours before their habitual bedtime. Starting at bedtime, the participants then sat under ceiling-mounted fluorescent lamps providing either broad-spectrum white-light similar to modest sunlight (about 3000 lux) or dim-light similar to moonlight (about 1.9 lux). All procedures were repeated four times over roughly 49 days so that each participant experienced four experimental sessions: placebo + dim-light; placebo + bright-light; caffeine + dim-light; and caffeine + bright-light. Saliva, collected for melatonin assessment, was taken every 30-60 minutes to determine how each of these conditions affected the circadian rhythms of the participants.

The participants abstained from over-the-counter medications, supplements, and caffeine for two weeks, naps for one week, exercise for three days, and alcohol for two days before and throughout the experiment.

To more specifically understand the effects of caffeine at the cellular level, the circadian transcriptional rhythms were studied *in vitro* in a well-understood human cell line. This cell line expressed all of the proteins needed to assess caffeine's effects on circadian rhythms, especially adenosine receptors, phosphodiesterases, and ryanodine receptors.

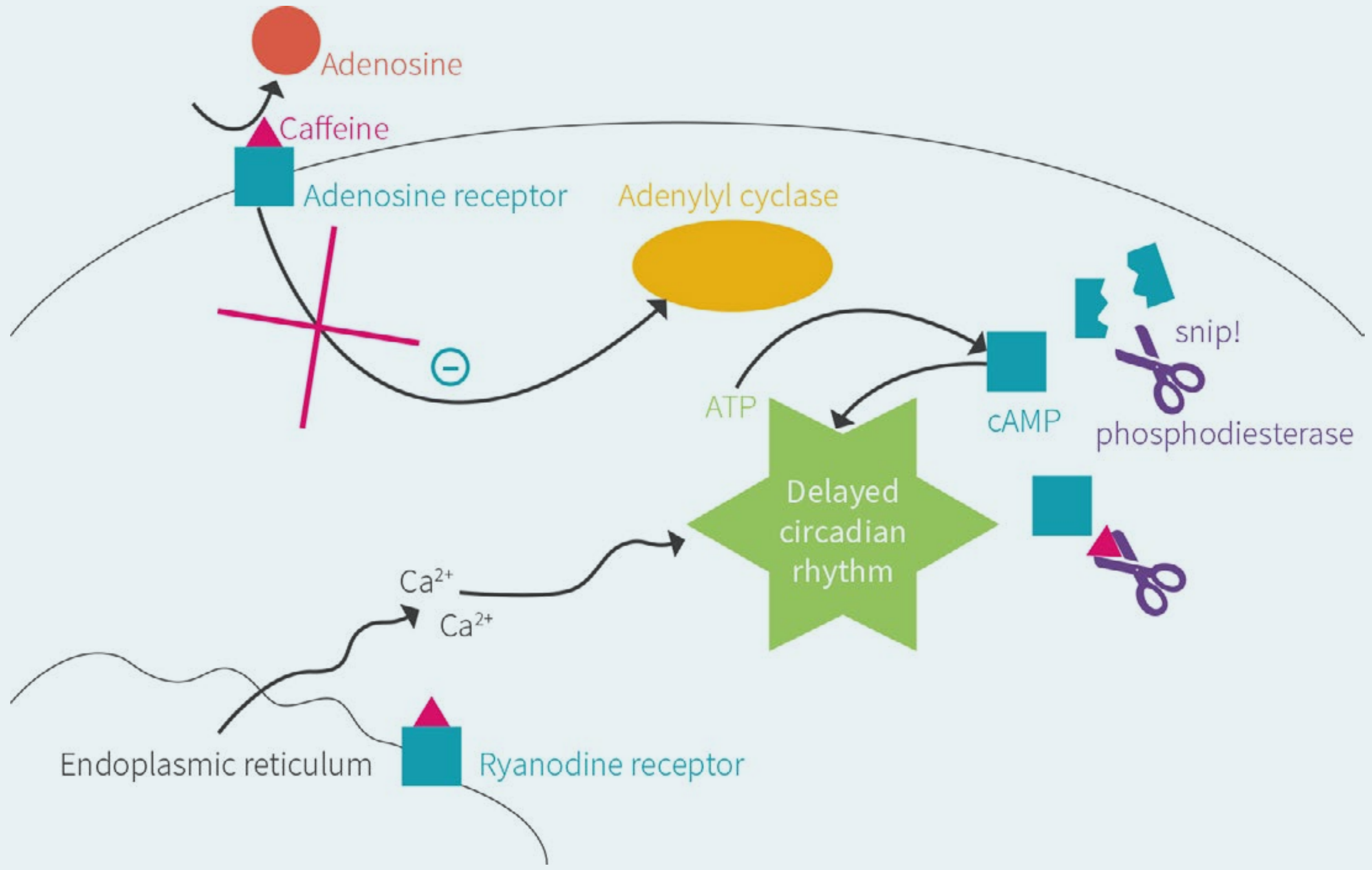
There were only five study participants, all of whom were healthy. They spent a month and a half under controlled sleep routines: taking a caffeine pill three hours before bedtime, then being exposed to either bright or dim light. Melatonin secretion was estimated, and caffeine's cellular effects were assessed in a separate *in vitro* experiment.

How exactly might caffeine screw up my circadian rhythm?

The circadian clock in humans is controlled primarily by the suprachiasmatic nucleus (SCN) within the brain. The SCN is a group of cells that sit by the optic nerve and respond to light and other stimuli that come in from the eye. Hypothetically, caffeine could affect the SCN (and other tissues throughout the body) in three ways, depicted in Figure 1.

The first is through increasing a cell's levels of cyclic AMP (cAMP), which is often created inside a cell in response to a signal. This occurs for two reasons. One reason is that caffeine blocks adenosine receptors that normally reduce cAMP levels. Secondly, caffeine binds phosphodiesterase enzymes that act to degrade cAMP, and when these enzymes are blocked, cAMP levels are raised. It's also been shown that circadian rhythms within the SCN are regulated in part by the release of calcium ions during the stimulation of ryanodine receptors, which caffeine also binds. Thus, caffeine has three primary ways in which it can interact with the SCN to influence circadian rhythms.

Figure 1: Possible ways caffeine could affect circadian rhythm



What were the findings?

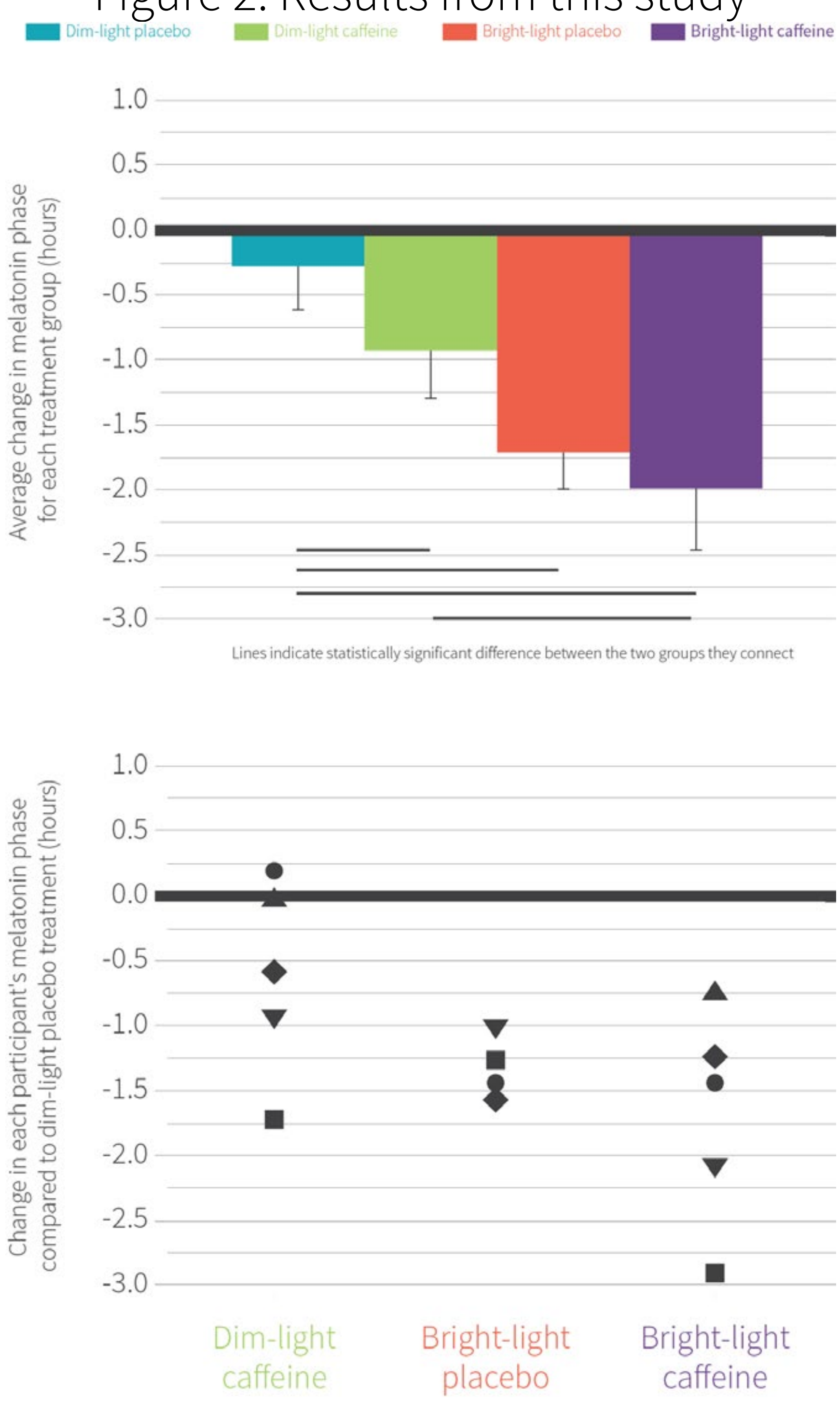
The study results are summarized in Figure 2. Bright light, caffeine, and their combination significantly delayed the onset of melatonin production compared to the dim light plus placebo condition. Specifically, dim light plus caffeine delayed production by about 40 minutes, bright light and placebo by 85 minutes, and bright light exposure plus caffeine by 105 minutes. The difference between bright light plus caffeine and dim light plus caffeine was also statistically significant, and there was a trend for bright light in combination with placebo to be greater than dim light combined with caffeine. Collectively, these results indicate that bright light and caffeine both independently interfere with the normal onset of melatonin secretion, with bright light having a stronger effect that is not significantly modified by caffeine.

There were marked inter-individual differences in the response to caffeine ingestion. When caffeine was ingested under dim light conditions, two of the five participants experienced no delay of melatonin secretion, two were delayed by 30-60 minutes, and one was delayed by nearly two hours. This variability was also observed under the bright light caffeine condition, with phase delays ranging from 45-180 minutes. In contrast, the effect of bright light only was more clustered around phase delays of about 60-100 minutes. This variability could be the result of individual differences in caffeine metabolism, but this study didn't evaluate such differences.

There was also a significant positive linear association between the phase shifts induced by dim light with caffeine and bright light with caffeine as well as dim light placebo and bright light placebo. This suggests that the gradual phase shift that occurs naturally, due to the fact that many people's circadian rhythm is longer than 24 hours, correlates to the strength of the light that affected their sleep cycles. In other words, the more a person's sleep cycle drifts naturally, the more light affected it.

Finally, experiments in human cells showed that caffeine delays the circadian rhythm by acting on the A1-type adenosine receptor to increase cellular levels of cAMP rather than affecting phosphodiesterases and ryanodine receptors. Therefore, this may be the mechanism responsible for the phase delay observed in the study participants.

Figure 2: Results from this study



Consuming caffeine three hours before bedtime delays the circadian clock by blocking adenosine receptors. The magnitude of the effect is about half that observed with bright light alone, and combining caffeine with bright light showed a larger delay than either alone, although the difference wasn't statistically significant. There were marked individual differences in the response to caffeine ingestion, while the effect of bright light exposure was similar in all participants.

What does the study really tell us?

This study had three main takeaways: caffeine delays melatonin secretion when consumed three hours before sleep, there are marked individual differences in the magnitude of this effect, and caffeine delays the cellular clock through the blocking of adenosine receptors that subsequently increased cAMP in the cell.

The dose and timing of caffeine in the study are two limitations, especially in light of the individual differences in response to caffeine. The amount of caffeine used in this study was 2.9 milligrams per kilogram of bodyweight, which equates to roughly 240 milligrams for a 180 pound person. Black and green teas contain 14-70 milligrams of caffeine per eight fluid ounces (one cup) and brewed coffee contains 95-200 milligrams. Thus, unless someone is used to downing energy drinks or having a double espresso for dessert, the dose of caffeine used in this study was relatively high compared to real life scenarios. Future research could test smaller doses to determine if a threshold level for intake exists. Even so, two of the five participants showed no phase delay with the current dose, suggesting that the effects of caffeine may ultimately depend on the individual.

As mentioned in the introduction, the ability of a stimulus to interfere with normal circadian function depends on the time of day. The caffeine in this study was consumed three hours before habitual bedtimes in an environment that was free of light cues. It stands to reason that caffeine may not be as detrimental when consumed later in the day during the summer season, compared to the winter season, as there is more sunlight at later times of day. Still, even during winter, the advent of modern technology and blue light may very well trick our bodies into believing it is sunny out. How caffeine interacts with environmental cues remains to be elucidated.

In a study using a more real-life research setting, healthy day workers popped a 400 milligram caffeine pill zero, three, or six hours before their typical bedtime. This study showed that timing does not differentially impact overall sleep disruption. Caffeine is metabolized mostly by the P450 enzyme system within the body (specifically the CYP1A enzymes). This is the bottleneck step in clearing caffeine from the blood and it is subject to genetic variance, which could explain the variability observed in the current study and possibly the results of this aforementioned study as well. People who are "fast" caffeine metabolizers may be able to consume caffeine closer to bedtime without influencing their circadian rhythms, compared to "slow" metabolizers. If you're curious, data from commercially available genetic tests can tell you what kind of caffeine metabolizer you are.

The finding that caffeine acts on a cellular level via the adenosine receptor to activate cAMP lends support to the hypothesis that dynamic changes in cAMP signaling regulate circadian rhythms. The current study therefore suggests that caffeine and other compounds that bind and inhibit adenosine A1 receptors would have similar effects. Other mechanisms for caffeine's effect cannot be ruled out, however.

The human portion of this experiment used a very tightly controlled design that may have substantially limited the number of participants, which came in at a meager five people. The laborious nature of sleep studies makes this type of research difficult. Nonetheless, future trials with larger sample sizes will be needed to confirm these findings, as well as extend the data from just melatonin to actual sleep onset and wake times.

The variance in caffeine metabolism shown in previous research, specifically due to the CYP1A enzymes, may explain why individual results varied so much in this experiment. This study also suggests that out of the ways in which caffeine may impact circadian rhythms on a cellular level, acting on the adenosine receptor may be the most significant.

The big picture

Sleep is a fundamental biological process for most organisms. Humans spend, on average, about one-third to one-fourth of their lives asleep. There are many hypotheses for the purpose of sleep, but it is clear that sleep deprivation and a dysregulation of normal circadian rhythms are linked to disease: cancer, metabolic syndrome, type-2 diabetes, and even death. In fact, shift work that involves circadian disruption has been labeled as a probable carcinogen by the International Agency for Research on Cancer.

Very rarely, such as in the laboratory, would we be deprived of environmental time cues that entrain our circadian rhythm. However, the modern era has brought with it wildly altered zeitgebers (German for "time giver"; the term used to denote circadian rhythm cues) that may interrupt the natural light/dark cycle, the most notorious being devices that emit blue light. This includes smartphones, laptops, televisions, and most modern light bulbs. In ERD Issue #4 (Feb, 2015), *The iPad Hangover* discussed research that showed that people using iPads before bed took about ten minutes longer to fall asleep, experienced less REM sleep, and found it much harder to feel fully awake in the morning than people that read print books before bed. Importantly, it appears that bright light exposure duration is a more influential variable on the human clock than bright light intensity, at least at bright light levels replicating various daytime conditions.

The current study covers another zeitgeber – caffeine, and shows that it can influence the circadian clocks of

> "[...] people using iPads before bed took about ten minutes longer to fall asleep, experienced less REM sleep, and found it much harder to feel fully awake in the morning than people that read print books before bed."

individuals when consumed three hours before bed. Moreover, the study shows that these effects occur on a cellular level but are subject to individual differences in caffeine metabolism. Other research has supported these findings by showing that caffeinated tea and coffee beverages consumed throughout the day had a dose-dependent negative effect on the time it takes to fall asleep, the time spent sleeping, and sleep quality.

Caffeine and bright light exposure are not the only lifestyle and environmental variables to affect the human clock. It is not uncommon for many people to exercise after work and several hours before going to bed. Yet, one small study in young, fit males showed that exercise delays the onset of melatonin production just like caffeine and bright light do, and the delay is more pronounced the closer to bedtime the exercise is performed. This may be due to the stress response of exercise and rise in cortisol levels that appear to precede the suppression in melatonin. On the other hand, exercising in the morning with sun exposure has been shown to have positive effects on sleep-related hormonal responses, sleep habits, and quality of sleep.

Finally, some research suggests that drinking alcohol at night can suppress melatonin production with doses as little as 0.5 grams per kilogram of bodyweight, or about 2.5 cans of beer for a 160 pound person. Smoking may also reduce melatonin levels, albeit only in response to melatonin supplementation (which is not uncommon) rather than natural production.

Poor sleep is linked to most of the major causes of death, so normalizing one's circadian rhythm may be one of the highest-return health habits possible. While this study focused on two specific zeitgebers, light and caffeine, others such as exercise (specifically late in the day), alcohol, and smoking can also impact circadian rhythms.

"Caffeine and bright light exposure are not the only lifestyle and environmental variables to affect the human clock. It is not uncommon for many people to exercise after work and several hours before going to bed."

Frequently asked questions

How can I maximize my sleep quality?

As we just learned, avoid blue light and caffeine close to bedtime to maximize sleep quality. This sounds simpler than it is in reality, as few people turn off all computers, tablets, and light bulbs at night (the light output of some of these sources is shown in Figure 3). If you have a dimmer for a light switch, that can come in handy. If you really, really want to reduce or eliminate blue light exposure and improve circadian rhythms, blue light blocking glasses are an option with research backing.

A quiet environment also helps, as loud noises can disrupt sleep even if the sleeper doesn't wake up. Establishing a habitual bedtime can reduce the time it takes to fall asleep. As a last resort, there are also supplemental options. Melatonin is probably the most popular option and is safe for daily use. Lemon balm is another option.

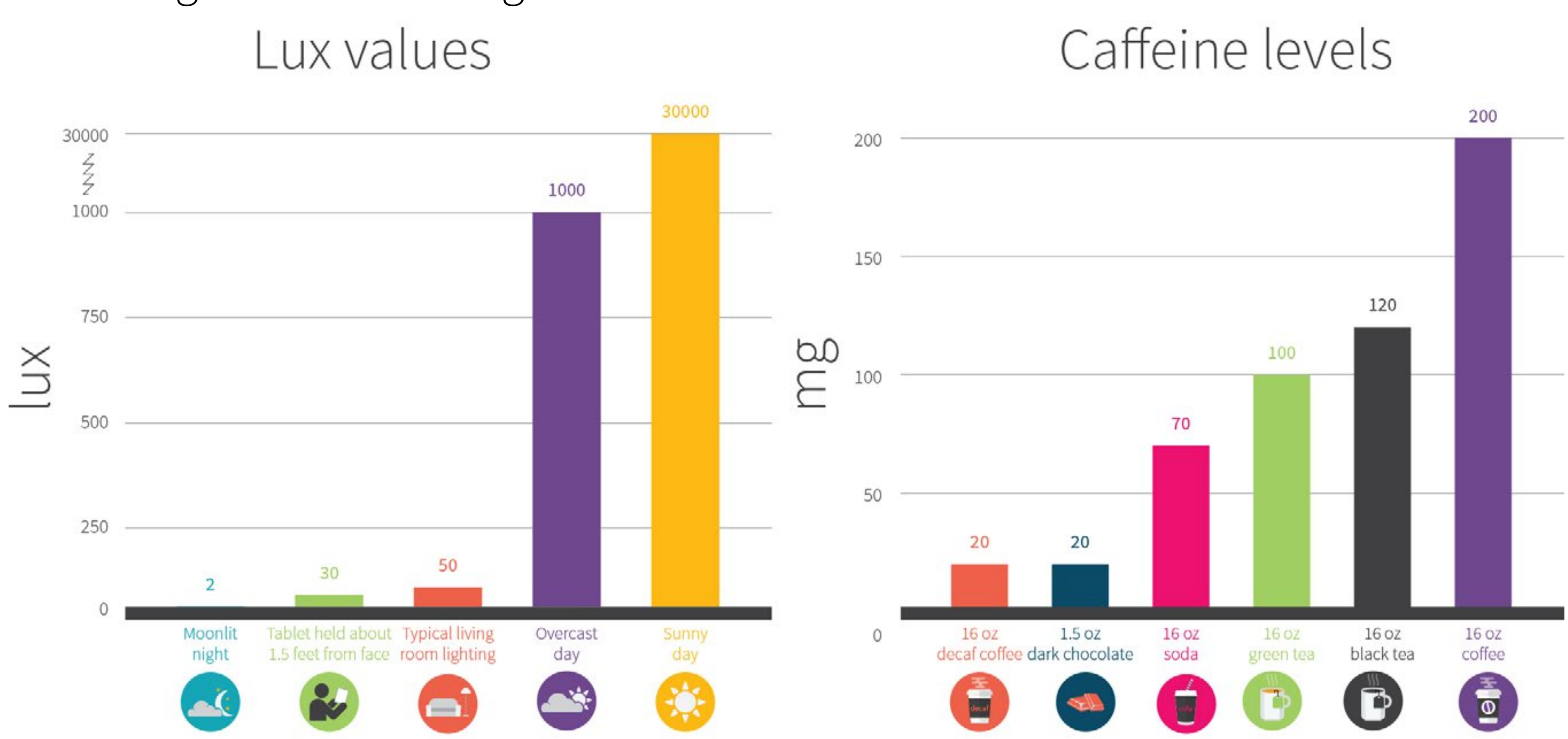
What should I know?

Caffeine isn't just used a morning pick-me-up. Many people drink caffeine late into the afternoon and into the evening, which this trial shows is a recipe for disrupted melatonin synthesis. The study also showed some additive effect of consuming caffeine late in the day while being exposed to bright light at night, the latter of which is quite common in modern society.

Luckily, the takeaway isn't complex: don't expose yourself to bright light or consume caffeine close to bedtime. Though specific responses to caffeine can be highly variable, due to individual genetic differences in its metabolism, sleep disruption has been strongly linked with so many diseases that when in doubt, go decaf. ◆

If you're reading this late at night, shame on you. But knowing is half the battle, so get some sleep before heading to the ERD Facebook forum to discuss the world of zeitgebers and circadian rhythms.

Figure 3: Some rough estimates of lux values and caffeine amounts



Note: Caffeine content varies quite a bit based on brand, preparation, and more.
Lux values also vary quite a bit based on the specifics, such as distance from light source, type of light source, and more.
The above values are only rough approximations.