Pilot study: salivary melatonin modulates the errors on an emotional Stroop task through negative mood

The effect of melatonin on emotional Stroop task performance

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Abstract

Melatonin drugs affect sleepiness, body temperature, and cognitive performance. However, it is unclear how salivary melatonin affects cognitive performance, and how time and light separately or jointly affect melatonin levels in the body. This pilot study explored the effects of melatonin on task performance and the effects of light and time on melatonin levels. In this study, participants arrived at the lab at different time points, experienced the three light conditions, and joined cognitive tasks. It found that melatonin modulated performance on an emotional Stroop task. In particular, melatonin modulated the number of errors through negative mood. Light and time did not influence melatonin levels. Further studies on the effects of salivary melatonin on social and cognitive tasks are needed.

1 Introduction

Melatonin drug and behaviors

Melatonin is a sleep hormone that is synthesized in the pineal gland of the human brain. This secretion is controlled by the suprachiasmatic nucleus (SCN). Melatonin increases during the evening until midnight, whereas melatonin decreases during the daytime. To date, experimental research has focused on the effect of melatonin drugs on sleepiness [17] and low body temperature [4], and the behavioral impact has only been explored recently. One study indicated that melatonin drugs do not affect cognitive performance in the Stroop task [18], but another study found that melatonin drugs increase task errors in the emotional Stroop task [15]. This study suggested that this decrease in performance may manifest due to the mechanism by which melatonin disrupts the subcortical pathway from the thalamus to the amygdala. In these studies, manipulating melatonin levels by drugs has been predominant.

Salivary melatonin

Salivary melatonin measurements as well as drug administrations might also be useful in behavioral research. Salivary melatonin correlates with serum melatonin [14], plasma melatonin [24], and urinary melatonin [20]. Therefore, salivary melatonin measurement appears to be useful in behavioral research, where subjects are often reluctant to provide samples using invasive methods. Salivary melatonin levels might fluctuate depending on light [16] and time [2]. Previous studies suggested that bright light suppresses salivary melatonin, while salivary melatonin increases under dim light [16]. However, studies exploring the effect of light on melatonin levels have yielded mixed results [5], [6], [7], [16], [19]. These studies were conducted at a different time of day or manipulated melatonin levels by using lights of varying brightness. It is unclear how time and light, separately or jointly, affect salivary melatonin levels. Therefore, participants joined the Stroop task and emotional Stroop task under the three light conditions at different time periods to see how melatonin affects cognitive performance, and how light and time affect salivary melatonin in this study.

Salivary melatonin might differently affect cognitive performance from melatonin drugs. Salivary melatonin, unlike melatonin drugs, is produced in the pineal gland [1]. This is closely connected to the habenula [10], [21], which is linked to negative mood [11]. Therefore, negative mood may modulate the relationship between melatonin levels and performance in cognitive tasks.

Hence, this study aimed to explore how salivary melatonin modulated cognitive performance and determine how light and time, jointly or separately, affected melatonin levels.

2 Materials and Methods

2.1 Ethics approval

The Ethics Committee of Nagoya University approved this study.

2.2 Participants

Ten healthy participants (four males and six females) aged 18–55 years were recruited. Participants awoke between 7 am and 9 am and went to sleep between 11 pm and 1 am, and abstained from exercise, caffeine, cigarettes, and alcohol for at least 24 hours before the experimental sessions. Three participants could not complete the three sessions during the study period; therefore, their data were excluded from the analysis.

2.3 Exclusion criteria

The exclusion criteria were color blindness or weakness, retinal damage, history of seizures, neurological disorders (e.g., Parkinson's disease and epilepsy), chronic diseases (e.g., chronic liver or kidney disease), autoimmune diseases, mental disorders (e.g., depression and anxiety), and sleep disorders (e.g., insomnia and irregular sleep-wake cycle). Participants who were prescribed several medications mentioned on the eMC webpage were excluded. Participants who were currently receiving hormone replacement therapy, worked night shifts, traveled across time zones in the month before the experiment, were pregnant or may have become pregnant during the study, and were nursing were excluded. For the remainder of the night after the experimental session, participants refrained from driving a car or using machinery.

2.4 Experimental design

This study used a within-subjects design. Participants participated in three sessions, sparing the three to seven days among the three sessions.

2.5 Methodology

2.5.1 Informed consent

Participants arrived at the laboratory with 500 lux light at different times. To investigate the effect of time on melatonin levels in this pilot study, one participant arrived at 10:00, two arrived at 13:00, one arrived at 16:00, two arrived at 19:00, and one arrived at 22:00. Participants were given an electronic copy of the consent form before arriving at the laboratory. Upon arrival, the participants read the form again and provided consent.

2.5.2 Sleepiness

Self-reported sleepiness was measured using the Stanford Sleepiness Scale [12] which comprises a 7-point Likert scale ranging from 1 (feeling active, vital, alert, or wide awake) to 7 (asleep). Measurements were taken at baseline prior to light administration, after a one-hour waiting period, and at the end of the experiment.

2.5.3 Mood

Self-reported positive and negative mood were measured using the Positive and Negative Affect Schedule [25]. Measurements were taken at baseline prior to light administration, after a one-hour waiting period, and at the very end of the experiment. The score on this scale ranges from 1 (very slightly or not at all) to 5 (extremely).

2.5.4 Impulsiveness

The Barratt Impulsiveness Scale [3] was used to measure impulsiveness. The questionnaire included 30 items on impulsiveness, rated on a scale ranging from 1 (very slightly or not at all) to 5 (extremely). Participants completed the questionnaire on arrival.

2.5.5 Circadian rhythm

This study used the Morningness-Eveningness Scale [13], which includes 19 items on circadian rhythms. Participants completed the questionnaire on arrival.

2.5.6 Light administration

Light treatments were administered to the participants in a within-subjects design. Dim light, bright light at 5000 lux, and natural light at 500 lux were administered randomly, for the duration of one hour. Participants were asked to sit in the laboratory during this hour without moving.

2.5.7 Saliva collection

Saliva was collected using passive drool. Samples were stored in a –30°C freezer until study completion. The samples were assayed in a laboratory at Nagoya University. The sampling tubes were centrifuged for 10 min, and hormone concentrations were measured using a salivary melatonin enzyme immunoassay kit (Salimetrics, State College, PA, USA). The intra-assay coefficients of variation were below 20%. Post-treatment melatonin distributions were not skewed and thus did not require transformation. All statistical analyses used raw melatonin data.

2.5.8 Stroop task

After saliva collection, the participants completed the Stroop task. The Stroop task consisted of two conditions: congruent and incongruent. In the congruent condition, “red”, “green”, "blue", and "yellow" were printed in their respective ink color. The incongruent condition included twelve different word-color pairings such as "red" printed in green, blue, or yellow, "green" printed in red, blue, or yellow, "blue" printed in red, green, or yellow, and "yellow" printed in red, green, or blue. The stimulus lasted 4000 ms with an interstimulus interval of 500 ms. Each stimulus was presented five times. The congruent and incongruent conditions comprised 20 and 60 trials, respectively. Participants were asked to press a button on the computer keyboard to indicate the ink color of the words and ignore the meaning of the word.

2.5.9 Emotional Stroop task

After completing the Stroop task, participants performed an emotional Stroop task. The emotional Stroop task comprised two conditions: mood-relevant and mood-irrelevant. Eight negative and eight neutral words were selected for the emotional Stroop task. Emotion-laden words and neutral words in four different colors (blue, red, green, and yellow) were displayed randomly. The stimulus lasted 4000 ms with an interstimulus interval of 500 ms. Each stimulus was presented five times. The mood-relevant and mood-irrelevant conditions each comprised 40 trials. Participants were asked to press a button on the computer keyboard to indicate the ink color of the words and ignore the meaning of the word.

3 Results

3.1 Primary analyses

Behavioral analyses - melatonin

To find whether salivary melatonin predicts task performance, specifically the number of errors, response times, and the Stroop/emotional Stroop effect, we conducted a regression analysis in both the Stroop and emotional Stroop tasks. We found that melatonin affects emotional Stroop task performance but not Stroop task performance. In particular, melatonin significantly predicted the number of errors and response times to mood-relevant and mood-irrelevant stimuli, and the emotional Stroop effect (B=-0.542, SE=0.249, t=-2.177, p=0.042; B<0.001, SE<0.001, t=2.562, p=0.019; B>-0.001, SE<0.001, t=-1.91, p=0.071; B<0.001, SE<0.001, t=3.163, p=0.005). We divided the data into three groups: high melatonin (+1SD), middle melatonin (mean value), and low melatonin (-1SD), performed multiple comparisons, and plotted the number of errors, response times to emotional and non-emotional stimuli and the emotional Stroop effect depending on the melatonin level. This show that the middle melatonin group and the high melatonin group marginally had a decreased number of errors compared with the low melatonin group (p=0.150, p=0.150; Fig 1). Furthermore, the middle and high melatonin group marginally showed slower responses to emotional stimuli than the low melatonin group (p=0.096, p=0.109; Fig 2). In contrast, the low melatonin group showed slower responses to non-emotional words than the middle and high melatonin group (p=0.170, p=0.170; Fig 3). An emotional Stroop effect was found in the middle melatonin groups compared with the low melatonin group (p=0.019; Fig 4). This suggests that melatonin increases the response time to emotional words, but also reduces errors in the emotional Stroop task.

Fig 1. The number of errors in the emotional Stroop task at different melatonin levels. The x-axis indicates the melatonin level, and the y-axis indicates the number of errors. The error bar represents 1 standard error.

Fig 2. The response time (mm) to emotional words at different melatonin levels. The x-axis indicates the melatonin level, and the y-axis indicates the response time to emotional words. The error bar represents 1 standard error.

Fig 3. The response time (mm) to non-emotional words at different melatonin levels. The x-axis indicates the melatonin level, and the y-axis indicates the response time to non-emotional words. The error bar represents 1 standard error.

Fig 4. Emotional Stroop effect at different melatonin levels. The x-axis indicates the melatonin level, and the y-axis indicates the emotional Stroop effect (mm). The error bar represents 1 standard error.

3.2 Exploratory analyses

Interaction with sleepiness and mood

To find the process in which melatonin affects task performance, we conducted a regression analysis of emotional Stroop task performance, with sleepiness, positive mood, and negative mood. We found a marginal interaction between melatonin and negative affect on the number of errors in the emotional Stroop task ($B=-0.116$, $SE=0.059$, $t=-1.973$, $p=0.065$). Simple slope analysis revealed that the slope of the negative affect was marginally negative for the melatonin high condition ($B=-0.586$, $SE=0.302$, $t=-1.937$, $p=0.148$), but the slope of the negative affect was positive for the melatonin low condition ($B=0.689$, $SE=0.196$, $t=3.506$, $p=0.039$). Figure 5 presents this. There were no interactions between melatonin and negative affect on the response times to emotional words and non-emotional words, and emotional Stroop effect. Negative mood modulated the relationship between melatonin and the number of errors only, but sleepiness and positive mood did not modulate the effect of melatonin on errors and other performance metrics, such as response times in the emotional Stroop task.

Fig 5. The change in the number of errors during negative mood at different melatonin levels. The x-axis indicates the severity of the negative mood, and the y-axis indicates the number of errors. The width of colors represents 95% confidence intervals.

Sleepiness, mood, and other covariates

To see if the effect of melatonin on the emotional Stroop task is observed even after controlling for sleepiness, positive mood, negative mood, impulsiveness, and circadian rhythm, we conducted the regression analysis using sleepiness, positive mood, negative mood, impulsiveness, circadian rhythm as covariates. After controlling for these variables, we still found that melatonin had a significant or marginal effect on errors ($B=-0.570$, $SE=0.272$, $t=-2.093$, $p=0.050$; $B=-0.554$, $SE=0.260$, $t=-2.131$, $p=0.047$; $B=-0.569$, $SE=0.252$, $t=-2.256$, $p=0.036$; $B=-0.567$, $SE=0.320$, $t=-1.770$, $p=0.093$; $B=-0.549$, $SE=0.256$, $t=-2.147$, $p=0.045$), response times to emotional words ($B<0.001$, $SE<0.000$, $t=2.963$, $p=0.008$; $B<0.001$, $SE<0.001$, $t=2.456$, $p=0.024$; $B<0.001$, $SE<0.001$, $t=2.640$, $p=0.016$; $B<0.001$, $SE<0.001$, $t=4.526$, $p<.001$; $B<0.001$, $SE<0.001$, $t=2.772$, $p=0.021$), response times to non-emotional words (B>-0.001, SE<0.000, t=-1.640, p=0.118; B>-0.001, SE<0.001, t=-1.788, p=0.090; B>-0.001, SE<0.001, t=-1.944, p=0.067; B>-0.001, SE<0.001, t=-1.501, p=0.151; B>-0.001, SE<0.001, t=-1.850, p=0.080) and the emotional Stroop effect (B<0.001, SE<0.001, t=3.276, p=0.004; B<0.001, SE<0.001, t=3.001, p=0.007; B<0.001, SE<0.001, t=3.338, p=0.003; B<0.001, SE<0.001, t=4.044, p<0.001; B<0.001, SE<0.001, t=3.250, p=0.004). Even after controlling for sleepiness, mood, and other variables, the effect of melatonin on emotional Stroop task performance still existed.

3.3 Melatonin, light, and time

Light reduces melatonin levels at midnight [16], [19]. Other studies have used different time points and brightness levels of light. However, it remains unclear how melatonin levels are affected by light exposure and time. We collected data at four start time slots, 10:00, 13:00, 16:00, 19:00, and 22:00, and used three light brightness levels: dim light, 500 lux light, and 5000 lux light. We performed a mixed-design ANOVA of these time slots and light conditions for the model. Light x time interaction did not affect melatonin levels (F(8,4)=0.238, p=0.960). Moreover, the main effects of light and time were not observed (F(2,4)=0.413, p=0.687; F(4,2)=0.951, p=0.570). Therefore, melatonin was not affected by the light brightness level or time points, which contrasts with previous study results [15], [17].

4 Discussion

This study found that melatonin modulated performance on emotional Stroop tasks. The high melatonin group had a reduced number of errors and a slower response to emotional stimuli compared to the low melatonin group. Particularly, these errors were reduced when melatonin induced a high negative mood. However, it increased when melatonin induced a low negative mood. Melatonin did not affect performance through sleepiness or positive mood.

In this study, melatonin reduced errors when the negative mood was high. The emotional Stroop task used negative words as emotional words. Studies have shown that a negative mood slows participants’ responses, increases response times, and reduces errors for negative words in emotional Stroop tasks [23]. Therefore, melatonin may affect negative mood [9], thereby explaining the low number of errors for negative words. This process also underlies psychophysiological changes, such as a decrease in body temperature [22] because melatonin increases in winter and lowers body temperature [8], [9]. It may also be possible that both negative mood and body temperature modulate the relationship between melatonin levels and performance. This study did not record changes in body temperature and other physiological measurements; therefore, future studies should explore whether melatonin affects cognitive performance through negative mood and psychophysiological changes, and how psychophysiological changes affect negative mood and vice versa. This study found that the number of errors was reduced in the high melatonin group, which is inconsistent with previous results using melatonin drugs [15]. We found that salivary melatonin reduced errors in the task when negative mood was high but not when negative mood was low. It is expected to find further differences in behaviors between salivary melatonin and melatonin drugs.

Unlike previous studies [16], [19], this study showed that the interaction between light and time did not affect melatonin levels, and light or time separately did not affect melatonin levels. Melatonin levels did not increase under dim light or decrease under bright light after one hour. In this study, samples were collected at an early start time, from 10:00 to 22:00.

However, existing studies report that the effect of light on melatonin was observed late at night, after 1:00 am [16]. Hence, we might not have been able to determine the effects of light and time on melatonin. Furthermore, we did not independently manipulate light and time. We may be able to determine the effect of light and time on melatonin by manipulating light and time independently and using data from a larger sample at night time.

In conclusion, melatonin modulates the cognitive performance in the emotional Stroop task. In particular, melatonin modulates the number of errors through negative mood. The high melatonin group had a reduced number of errors when negative mood increased. Furthermore, melatonin levels were unaffected by light or time. Future studies on larger samples should explore the relationship between melatonin and behavior using melatonin.

Acknowledgments

I thank Dr. Takeyuki Oba and Yumiko Suzuki for their technical support. This study has been published online in PsyArXiv.

References

1. Arendt J. Melatonin and the mammalian pineal gland. Springer Science & Business Media. London: Chapman & Hall; 1994.

2. Aulinas A. Physiology of the pineal gland and melatonin. Endotext [Internet]. 2019 [cited 2024 January]. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK550972/

3. Barratt ES. Anxiety and impulsiveness related to psychomotor efficiency. Percept Mot Skills. 1959;9(3): 191-198.

4. Cagnacci A, Elliott JA, Yen SS. Melatonin: a major regulator of the circadian rhythm of core temperature in humans. J Clin Endocrinol Metab. 1992;75(2): 447-452.

5. Campbell SS, Dawson D. Enhancement of nighttime alertness and performance with bright ambient light. Physiol Behav. 1990;48(2): 317-320.

6. Costa G, Ghirlanda G, Minors DS, Waterhouse JM. Effect of bright light on tolerance to night work. Scand J Work Environ Health. 1993;19(6): 414-420.

7. Czeisler CA, Johnson MP, Duffy JF, Brown EN, Ronda JM, Kronauer RE. Exposure to bright light and darkness to treat physiologic maladaptation to night work. N Engl J Med. 1990;322(18): 1253-1259.

8. Dollins AB, Lynch HJ, Wurtman RJ, Deng MH, Kischka KU, Gleason RE, et al. Effect of pharmacological daytime doses of melatonin on human mood and performance. Psychopharmacology (Berl). 1993;112(4): 490-496.

9. Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc Natl Acad Sci U S A. 1994;91(5): 1824-1828.

10. Guglielmotti V, Cristino L. The interplay between the pineal complex and the habenular nuclei in lower vertebrates in the context of the evolution of cerebral asymmetry. Brain Res Bull. 2006;69(5): 475-488.

11. Hikosaka O. The habenula: from stress evasion to value-based decision-making. Nat Rev Neurosci. 2010;11(7): 503-513.

12. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. Psychophysiology. 1973;10(4): 431-436.

13. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4(2): 97-110.

14. Laakso ML, Porkka-Heiskanen T, Alila A, Stenberg D, Johansson G. Correlation between salivary and serum melatonin: dependence on serum melatonin levels. J Pineal Res. 1990;9(1): 39-50.

15. Leung SE. The Effects of Daytime Melatonin Administration on Sleep, Higher Cognitive Function, and Changes in the Brain. Master's thesis, The University of York. 2014. Available from: https://yorkspace.library.yorku.ca/items/264c0834-eb24-4c1f-9888-eba8f4c970b8.

16. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science. 1980;210(4475): 1267-1269.

17. Lieberman HR, Waldhauser F, Garfield G, Lynch HJ, Wurtman RJ. Effects of melatonin on human mood and performance. Brain Res. 1984;323(2): 201-207.

18. Liu, J, Zhong, R, Xiong, W, Liu, H, Eisenegger, C, Zhou, X. Melatonin increases reactive aggression in humans. Psychopharmacology (Berl). 2017;234: 2971-2978.

19. Morita T, Tokura H. Effects of lights of different color temperature on the nocturnal changes in core temperature and melatonin in humans. J Physiol Anthropol Appl Human Sci. 1996;15(5): 243-246.

20. Nowak R, McMillen IC, Redman J, Short RV. The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulphate excretion rates: two non-invasive techniques for monitoring human circadian rhythmicity. Clin Endocrinol (Oxf). 1987;27(4): 445-452.

21. Rønnekleiv OK, Møller M. Brain-pineal nervous connections in the rat: an ultrastructure study following habenular lesion. Exp Brain Res. 1979;37(3): 551-562.

22. Slotten HA, Krekling S. Does melatonin have an effect on cognitive performance?. Psychoneuroendocrinology. 1996;21(8): 673-680.

23. Sui J, Ohrling E, Humphreys GW. Negative mood disrupts self-and reward-biases in perceptual matching. Q J Exp Psychol. 2016;69(7): 1438-1448.

24. Voultsios A. Kennaway DJ, Dawson D. Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms. 1997;12(5): 457-466.

25. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol. 1988;54(6): 1063.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Misa Kurihara contributed to conceptualization, methodology, software, investigation, formal analysis and writing - original draft preparation and writing - review & editing. Hideki Ohira contributed to the supervision.

Funding

This research was supported by a Ph.D. student research grant from Nagoya University granted to Misa Kurihara.

Data availability

The datasets for this study can be found at osf.io/hcb2d