A Clinical Study on Islanders to Investigate the Effect of Psychoactive Drugs and Music on Melatonin Level

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June 16, 2019

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1. Abstract

Pineal gland secretions of the hormone melatonin are known to impact sleep-wake cycles. Previous research has demonstrated that melatonin is regulated heavily by environmental cues such as light. It has also been demonstrated that nutritional factors like vitamins, minerals, vegetables and caffeine can impact melatonin levels, with caffeine causing a substantial decrease in the production of the main metabolite of melatonin, 6-sulphoxymelatonin. Our study was set up to examine the effects of central nervous system depressants similar to caffeine and environmental factors such as auditory stimuli (music) on melatonin levels. We ran a Randomized Complete Block Design with Matching experiment on virtual islanders for the intent of our study. These results should help us determine if the consumption of CNS depressants and/or listening to music play a significant role in regulating sleep-wake cycles.

2. Introduction

Melatonin is a hormone that plays a central role in regulating our sleep-wake cycles. A person's melatonin levels are highest during the night, just before bedtime, and lowest during the day. Rises in melatonin levels correspond with subsequent increases in sleep propensity (Khullar, Psychiatric Times). Changes in melatonin levels are informed by light/darkness in surroundings, which is why melatonin is known to be the "hormone of darkness" (Khullar, Psychiatric Times). However, recent literature has shown that dietary factors such vegetable and caffeine intake, as well vitamins and minerals, can affect melatonin production by modulating the availability of melatonin co-factors, activators, and tryptophan in the body (Peukuri et al). Since caffeine is known to be a Central Nervous System (CNS) stimulant, it follows that the intake of caffeine would result in a decrease in melatonin. Results from an experiment at Tel-Aviv University on the effects of caffeine on sleep quality and melatonin production demonstrated that increased intake of caffeine resulted in decreased production of a 6-sulphoxymelatonin (6-SMT), the main metabolite associated with melatonin (Peuhkuri et al). We were interested in determining if CNS depressants, such as alcohol, have the reverse effect on production of 6-SMT. Valerian is the third most commonly endorsed herbal product associated with insomnia, and acts in a similar fashion to CNS depressants (Costello). Thus, our primary research question is whether CNS depressants such as Valerian and alcohol affect levels of melatonin in the body.

Preliminary research on Alzheimer's patients has also shown that music therapy is effective in increasing melatonin levels in the body (Kumar et al). Additionally, in this study, we will also investigate whether listening to music would lead to changes in melatonin levels by manipulating the type of music played for islanders at two levels: classical and heavy metal music. We used age as a blocking factor because literature has shown that older people tend to have lower levels of melatonin (Karasek).

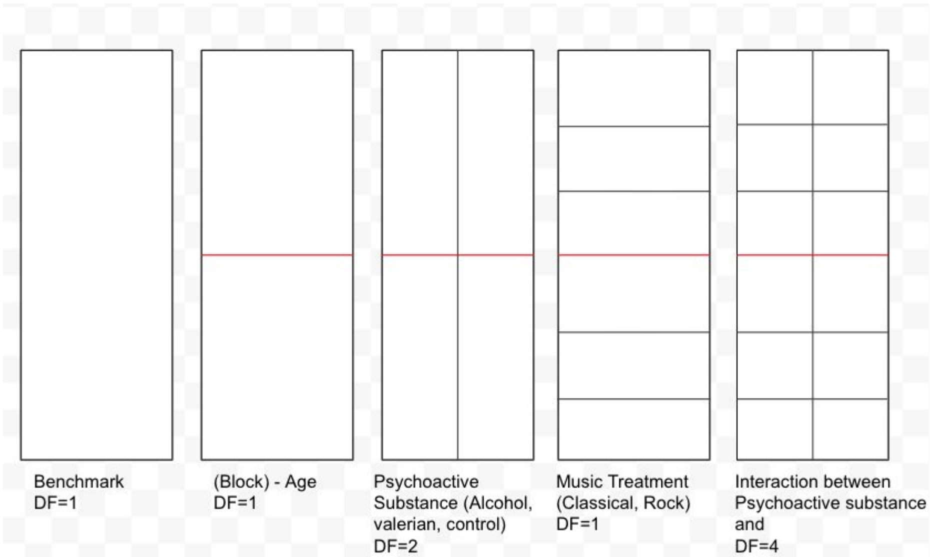
3. Methods

3.1 Design

We decided to focus on psychoactive drugs and music as the independent variables that we would manipulate, We picked two levels for music and three levels for psychoactive substances. We looked at the effect of psychoactive substances on melatonin by administering Valerian, alcohol, and a control condition where participants did not undergo any drug administration. For the alcohol level, we administered the Vodka 30mL option available on the Island. For music, we administered two types of music – classical and heavy metal. Thus, participants will be randomly assigned to one of 6 condition groups:

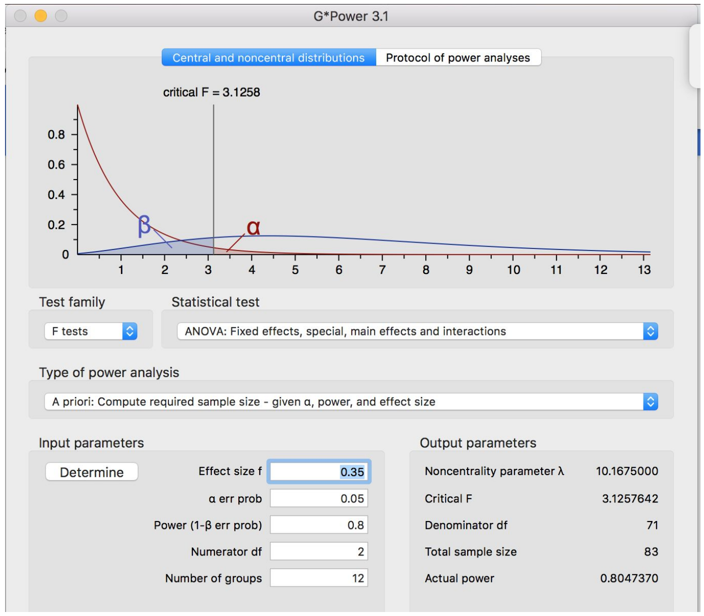
MUSIC CATEGORY	PSYCHOACTIVE SUBSTANCE ADMINISTERED		
	VALERIAN	ALCOHOL	NONE
CLASSICAL MUSIC	Condition 1	Condition 3	Condition 5
HEAVY METAL	Condition 2	Condition 4	Condition 6

In order to control for order of the treatment combinations, we will ensure randomization of order of administration within each of the 6 conditions. For example in the first condition, we will ensure that each of the participants assigned to that condition will be randomly assigned to one of the following: Valerian followed by Classical Music or Classical Music followed by Valerian. The factor diagram for the design is detailed below:



3.2 Sample Size Determination

We decided to use a power of 0.8, which means that the probability that we will correctly reject the false null hypothesis is 80%. We set alpha, which is the probability of falsely rejecting the null hypothesis, at .05. We used a moderately conservative effect size of .35, which gives us a way of quantifying the difference between groups. We utilized a two-way two-factor complete block design and used G*Power to determine that the sample size required is 83, based on the factor with the largest degree of freedom (2 for interaction). However, to ensure a balanced and representative design, we increased our sample size to 96, so that each of our 12 groups would have an equal number of males and females (4 males and 4 females per group).



3.3 Participant Selection

We chose participants aged 20-60 with no current health conditions from the town of Macondo. Out of our entire sample size, we selected 50% females and 50% males from a population of 2262 with two groups of 20-40 year olds and 40-60 year olds. We will be including all race demographics and family backgrounds. Since there was no monetary incentive we wanted to offer the participants, obtaining consent from the randomly selected islanders was the sole criteria that we had to operate within.

3.4 Procedure and Measurement

1. We randomly assigned 4 males and 4 females to each treatment group, so that each condition consists of 8 unique individuals.
2. We conducted two blood tests to measure the blood melatonin level of our participants. We conducted the initial screen before the assignment of any treatment to get a pre-treatment melatonin reading at a uniform time (late afternoon) during the day.
3. We then administered treatments based on the participants assigned group. The treatment intervals varied depending on the random order of treatment level

administration. This helped us ensure that the effects of treatments occur concurrently.

4. Finally, we measured post-treatment melatonin and set forth on our analysis of how psychoactive drugs and music affect blood melatonin levels. Since studies have shown that valerian and alcohol takes anywhere between 30 minutes to two hours to take effect (Cafasso) we conducted our post-treatment blood test between 30-120 minutes after administering the combination of treatment levels. Since the literature around music is a little murkier, we were not able to factor in an alternative time window for music to have its optimal impact.

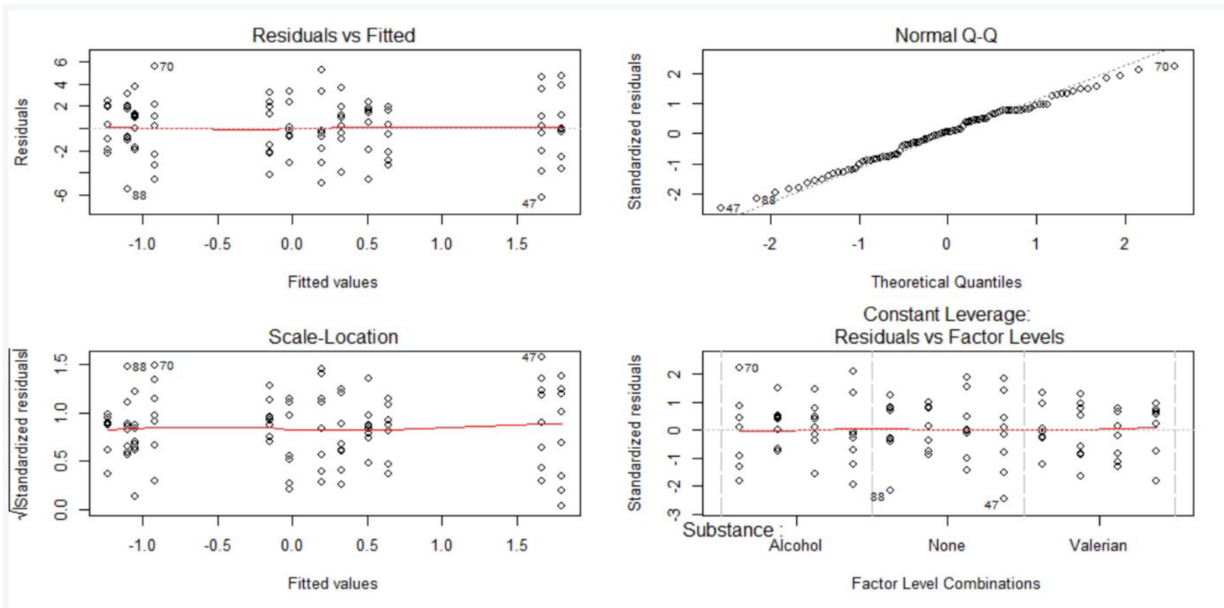
4. Data Analysis

The measurements we obtained for blood melatonin content were in pg/mL units. Since we measured melatonin before administering treatment and after administering treatment, we calculated the difference between the two melatonin measurements. Therefore, our data consists of difference measures with the unit pg/mL for each participant. And each participant was only assigned to one condition and therefore has only one datapoint: their difference score. To analyze this data, we ran ANOVA to compare the variances between treatment conditions. The F-tests revealed whether there was a significant difference between blocks as well as between the different levels of our treatment groups, and if the interaction between our two factors was significant.

Since we noticed a significant difference, we ran a post-hoc test (Tukey HSD) to see which groups specifically had a significant difference. We also visualized the results using the 'ggplot' package in RStudio to understand the results better. The analyses allowed us to interpret the results in context. Depending on the results, we were able to conclude if there is a main effect of psychoactive substances or a main effect of listening to music on blood melatonin levels. A significant interaction would allow us to conclude that the levels of one factor influence the effect that the other factor has on blood melatonin levels.

5. Results

5.1 Testing the Validity of the Model



From the diagnostic plots we note that the linearity, constant variance and normality assumptions seem to be valid. In addition, we do not have any bad leverage points and as a result our model seems to be valid.

5.2 ANOVA tables

	Df	*Sum of Squares*	*Mean Sq*	*F-value*	*P-value*
Substance	2	8.39	4.197	0.6077	0.546842
Music	1	61.76	61.76	8.94	0.0036**
Age.Category	1	0.43	0.427	0.0618	0.804277
Substance:Music	2	21.70	10.848	1.5708	0.213590
Residuals	89	614.66	6.906		

Table 1: *Anova Analysis of of our Full Model -*

Model: Difference ~ Music + Age + Substance + Substance:Music

	Df	*Sum of Squares*	*Mean Sq*	*F-value*	*P-value*
Condition	5	91.85	18.37	2.688	0.02603
Residuals	90	615.08	6.8342		

Table 2: *Anova Analysis of Change in Melatonin levels across the 6 condition groups*

We ran two ANOVA analyses to investigate to first check whether condition at all had a significant difference on the response variable and then more specifically whether Music (factor 1), Age (blocking factor), Substance (factor 2), or the interaction of the two factors is significant. In context, we ran the analysis to check if either or any of these contributed to the response variable (difference scores of blood melatonin levels before and after). The first table shows that treatment condition was significant (p-value of 0.02603). And the second table shows that the Substance factor itself and the interaction do not have a significant effect on the difference score given the p-values of 0.546842 and 0.213590, respectively. The music factor, however, does have a significant p-value of 0.0036. This analysis also shows that our blocking factor (age brackets) was not significant.

5.3 Tukey HSD

	Difference	***Lower***	***Upper***	***P-value***
2-1	0.66250	-2.02901284	3.3540128	0.9794406
3-1	-0.90000	-3.59151284	1.7915128	0.9250913
4-1	0.34375	-2.34776284	3.0352628	0.9990423
5-1	-1.08750	-3.77901284	1.6040128	0.8468150
6-1	1.81875	-0.87276284	4.5102628	0.3688899
3-2	-1.56250	-4.25401284	1.1290128	0.5415982
4-2	-0.31875	-3.01026284	2.3727628	0.9993360
5-2	-1.75000	-4.44151284	0.9415128	0.4129381

6-2	1.15625	-1.53526284	3.8477628	0.8103463
4-3	1.24375	-1.44776284	3.9352628	0.7587565
6-3	2.71875	0.02723716	5.4102628	0.0462680
5-4	-1.43125	-4.12276284	1.2602628	0.6341102
6-5	2.90625	0.21473716	5.5977628	0.0265388

Table 3: *Tukey Post Hoc HSD Analysis of Change in Melatonin Levels across the Different groups*

Since the ANOVA analysis revealed that Condition was a significant contributor to the response variable, we ran a Tukey Honestly Significant Difference test to focus on Condition and find out which specific treatment conditions were significantly different. The results of the test show that conditions 6 and 3 as well as conditions 6 and 5 varied significantly given the p-values of 0.0462 and 0.0265, respectively. Condition 6 was the combination of Heavy Metal music and no substance. Condition 3 was the combination of Classical music and Alcohol. Condition 5 was the combination of Classical Music and no substance.

5.4 Interaction Plot & Graphs

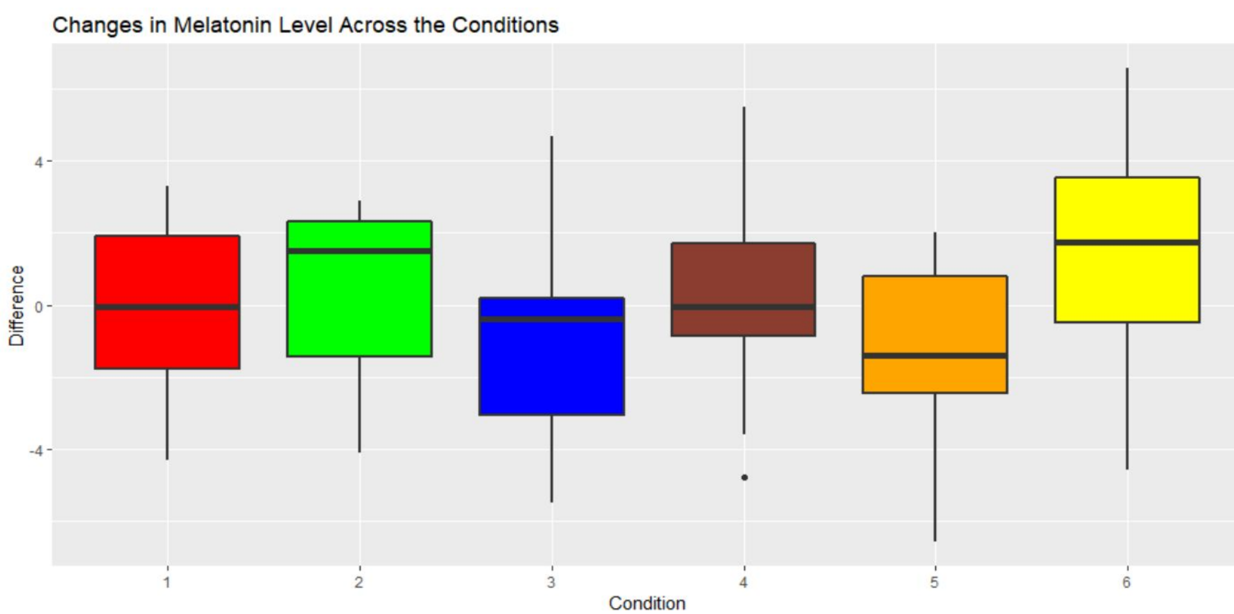


Diagram 1: *Boxplots showing difference in melatonin levels across the 6 condition groups*

The boxplot above highlights that the means of each treatment group varied above and below zero. The groups with the mean below zero indicate that those treatment conditions were associated with a decrease in melatonin levels. Conversely, the groups with the mean above zero indicate that those treatment conditions were associated with an increase in melatonin levels.

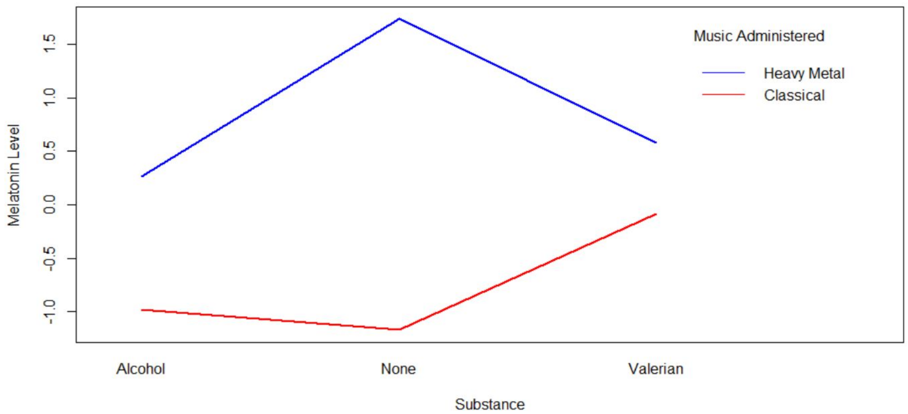


Diagram 2: *Interaction plot showing relationship between our two factors, Music and Psychoactive substance Administered, on the response*

The plotted lines in the interaction plot between our Substance and Music factors seems to suggest that there is an interaction. However, as we noted from the anova table, there is nothing more than a spurious relationship between the two factors.

5.5 T-test

	t	*Df*	*Sig (2-tailed)*	*Mean Difference*	*Lower*	*Upper*
Classical	-2.1099	47	0.04	-0.7437	-1.453	-0.036
Heavy Metal	2.1396	47	0.03761	0.8604	0.05141	1.670

Table 4: *Output for individual t-tests for the levels of music*

Since the ANOVA analysis indicated that music is a significant factor, we performed a t-test to determine the difference in the effect of Classical and Heavy Metal on melatonin levels. The results indicated that listening to classical music led to a significant decrease in melatonin levels, while heavy metal had the opposite effect (i.e. led to a significant increase in melatonin levels).

6. Discussion

Our study demonstrated some results that were unexpected from our initial hypotheses based off of existing literature so it is important to discuss some of the limiting factors of our study. Our primary limitations lied in data collection as the window of observation and treatment couldn't be performed at peak melatonin. Ideally we would like to observe individuals at their peak melatonin levels over a long period of time as there are a lot of nuisance factors that can present themselves when individuals are treated during the day such as noise levels, substance ingestion and more and testing at night when an individual is sleeping would produce more reliable and more inferential data. Unfortunately, the parameters of our experiment could not accommodate entirely for this limitation as we could not observe islanders while they were asleep and during the times of 3 to 4 AM where melatonin levels are highest. Furthermore, the length exposure of music as our treatment could have affected melatonin levels and is a limitation to our study as we can only interpret our results based on the treatment time suggested by previous academic papers. One last limitation of our experiment is that the scope of our experiment studies only two psychoactive which diminishes the power of test to be able to generalize our findings to all CNS substances. As a result, in future research we would like to increase the scope of our treatments with CNS substances to encompass more varieties of these substances in order to be able to better generalize our findings. Moreso, it would be important to understand how CNS substances affect sleep if it does not influence melatonin levels and how the different aspects that differentiate the treatments of music directly affect melatonin levels.

7. Conclusion

Upon analyzing our results from the ANOVA tables, the Tukey post-hoc test and the interaction plots shown above, we were able to arrive at some conclusions for our study. Our study showed that there was a sign of a significant difference in the mean melatonin level across the 6 conditions which allowed us to reject the null hypothesis for ANOVA where the mean melatonin levels would be the same. By looking further into the individual conditions, we found that alcohol and Valerian have no significant effect on blood melatonin levels and that heavy metal lead to an increase in melatonin while classical music lead to a significant decrease in melatonin.

From the post-hoc test, we found that the combinations of treatments of no drugs with heavy metal and alcohol with classical music as well as the combination of no drugs with heavy metal and no drugs with classical music were the two significant groupings of conditions. This post-hoc test reaffirms the results in the ANOVA that demonstrated that music does have a statistically significant effect on blood melatonin levels. Further insights we gained from our study specifically was that age was not a significant blocking factor and by look at the vertical scatterplot for interaction, we saw that condition six with no drugs and heavy metal had a higher mean than all the other conditions and helps us conclude that heavy metal music has a significant effect on the rise of blood melatonin levels.

8. References

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