A Clinical Study on Middle-Aged, Female Islanders to Determine Potential Anxiety Reduction Therapies

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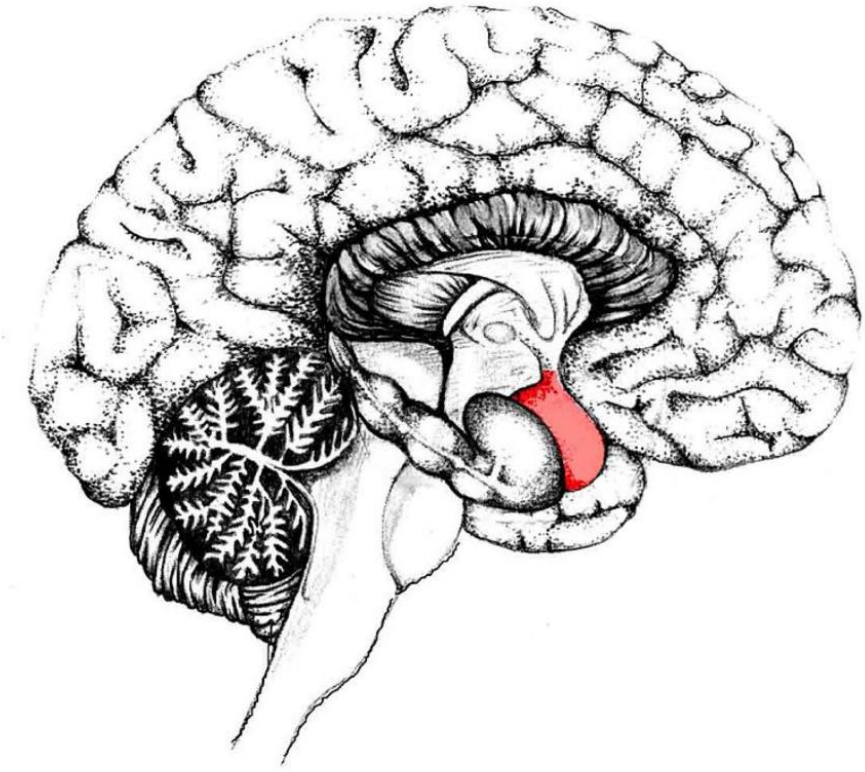

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3. Abstract

Studies have shown that anxiety is the most common mental health concern in the United States, affecting more than three-million Americans yearly [2]. Researchers have linked high cortisol levels to heightened levels of anxiety. If a treatment or therapy could be developed to modulate and lower cortisol levels, the pain of anxiety disorder would be less of a worry or concern. We propose that the following therapies may lead to reduced cortisol levels: sitting with a pet dog, napping, taking psychedelics (e.g. Psilocybin Mushrooms), listening to classical music, taking Alprazolam, and drinking tea. Thus, our study aims to determine which anxiety reduction therapy is most effective, if effective at all, at reducing cortisol levels in saliva in order to help treat anxiety. In order to isolate sources of variation and reduce variability in participants, our study utilizes a repeated measures crossover experimental design. Twenty-four subjects (18-41 year-old women) were sampled from Akkeshi, Maeva, and Hofn to perform our experiment. By nature of the repeated measure experimental design, order effects pose a problem. In order to minimize order effects, a forty-eight hour washout period in which patients receive no treatment was prescribed to each subject in between each of the six treatment applications. For each treatment, we recorded the salivary cortisol levels beforehand, administered the treatment, waited the specified amount of time per treatment, and recorded cortisol levels afterward. Once our data was collected, we created 6 two-sample t-tests to test whether or not there is a significant difference in means between our before and after administration of treatment groups. Additionally, we created box-plots to visualize our data. The results of our t-tests and box-plots show that sitting with a pet dog for 10 minutes, napping for 30 minutes, taking 10 grams of psilocybin mushrooms, and taking 1 milligram of alprazolam were all significant in changing one's cortisol level. This tells us that these treatments and therapies may be effective at providing anxiety relief. Further research on these four treatments show that only three of the four treatments are effective in reducing anxiety. These three treatments are sitting with a pet dog for 10 minutes, taking 10 grams of psilocybin mushrooms, and taking 1 milligram of alprazolam.

4. Introduction

According to the *National Institute of Mental Health*, 31.1% of U.S. adults have experienced some type of anxiety disorder in their lives [4]. This number increases to 41% in Gen Z and Millennials [18]. By definition, anxiety is a classification of mental health conditions that include but are not limited to General Anxiety Disorder (GAD), Obsessive-Compulsive Disorder (OCD), Panic Disorder, Post-Traumatic Stress Disorder (PTSD), and Social Anxiety Disorder (SAD) [14] . GAD causes major feelings of restlessness and worry, increases irritability, and affects ability to sleep, energy levels as well as concentration. People with severe anxiety also experience headaches, muscle aches, stomachaches, and unexplained pains, affecting their physical health [3]. Due to the frequency of this condition, it is arguably well-studied. Through such research, scientists have found a relationship between Cortisol and Anxiety [13]. Cortisol is the human body's main stress hormone. It is produced and released by the adrenal glands and is triggered by stressful situations and puts the body in "fight or flight" mode to manage stress. In a neuroscience study done at Washington University of St. Louis in 2007, it was found that a positive relationship exists between cortisol levels and anxiety, wherein subjects who experience GAD had higher cortisol levels than healthy controls (no GAD) [8].

Due to cortisol's important role in human's physical and mental wellbeing, we sought to investigate methods in which we can alter its levels, specifically to lower them. Treatments for anxiety can come in many forms, from daily activities to prescription and experimental drugs. The internet often lists many suggestions of activities to reduce stress and relieve anxiety such as playing with a pet, listening to classical music, and consuming tea. Psychiatrists may prescribe medicinal treatments such as Alprazolam (commonly known as Xanax) for those who are diagnosed with anxiety disorders. Recent studies have also shown that Psilocybin, a hallucinogenic commonly found in "Magic Mushrooms" has potential to treat anxiety as well. With this in mind, we wanted to include all three treatment types in our study to analyze which treatment is most effective.

For this study we chose to use six treatments, which included four daily habits/activities, one prescription drug, and one experimental drug. The four daily habit treatments include sitting with a pet dog for 30 minutes, napping for 30 minutes, listening to classical music for 10 minutes, and drinking 250 mL of herbal tea. The prescription drug treatment is a 1 mg dosage of Alprazolam and the experimental drug treatment is a 10 g dosage of Psilocybin Mushrooms. Through these alternative/at-home methods and drugs, we aim to find the best method of decreasing cortisol levels in saliva with the goal of finding which treatment best treats anxiety disorder.

5. Methods

5.1. Participants:

Participants were selected via a virtual human population simulator called *The Islands*. According to a study published by the *American Psychological Association*, Generation Z and Millennials are reported to be the most anxious group of individuals with higher levels of anxiety being seen particularly in women [15]. For the purposes of our experiment we decided to exclude individuals below the age of 18 in order to avoid giving certain treatments to children that are particularly prescribed to adults. Hence, we selected our participants to be within the age range of 18-41 in order to reflect the adults within the Gen Z and Millennial generations. A sample size of 24 was selected in order to achieve a power level of 0.8 for a crossover repeated measures design; more on this is elaborated under the design and procedure sections.

5.2. Design:

The repeated measures crossover experimental design is effective at minimizing variation in participants as the same participants are assigned to all treatments [9]. Within this study, there are two nuisance factors: the order in which the treatment is prescribed (order effect) and variability within participants.

Order effects may lead to progressive error, which are changes in a subject's response that are a direct result of being tested in multiple treatment conditions [12]. In order to minimize order effects, participants were assigned to one of three randomly generated treatment groups where each participant within each group was administered the treatments in a specific, randomly regenerated order (randomly generated in RStudio - see section 5.4.).

5.3. Instruments:

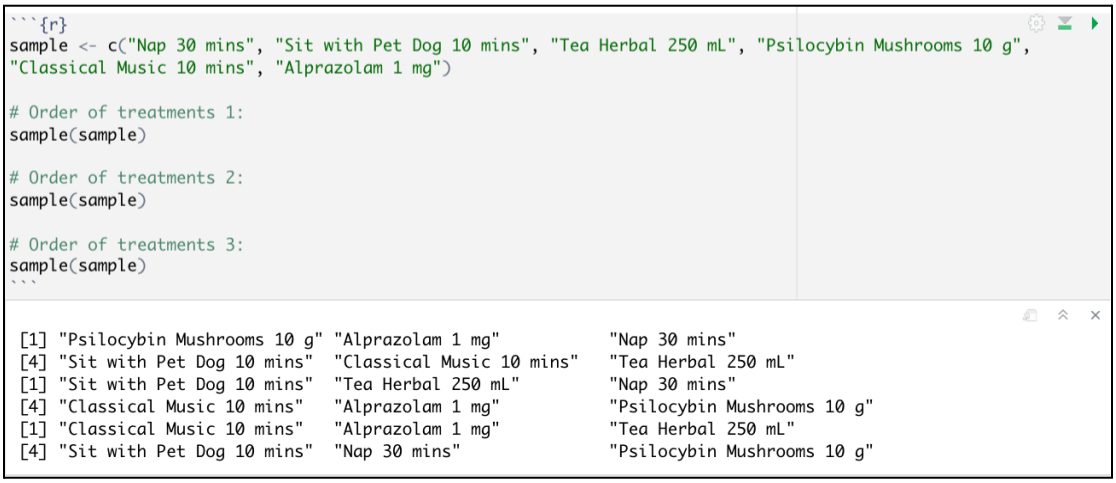
Our study was conducted by prescribing virtual tasks to people on the Island (refer to section 5.1.) in which we specifically sampled twenty-four women ages eighteen to forty-one. Cortisol levels were measured using a salivary cortisol test and recorded; we opted to use a salivary cortisol test over a blood cortisol test as it "may be more accurate in that less stress on the system during the production of the specimen means less interference with the factors being tested" [17]. Needles and syringes might cause panic and anxiety in participants prior to

application of the treatment. Since we are testing cortisol levels, it is especially important that we ensure that participants experience changes in cortisol levels due to only the treatment and not extraneous variables. The six virtual tests used to administer our treatments were “Sit with Pet Dog 10 mins”, “Nap 30 mins”, “Psilocybin Mushrooms 10 g”, “Classical Music 10 mins”, “Alprazolam 1 mg”, and “Tea Herbal 250 mL”. To record our data, we used Google Sheets and created csv files for the collected information (https://www.google.com/sheets/about/). The analysis of our data was conducted using base R and select packages in R Studio (https://www.rstudio.com/products/rstudio/download/).

5.4. Procedure:

Step 1: Find subjects from the Island that consent to being a part of our experiment. The subjects must be females aged eighteen to forty-one. For our experiment, we sampled one hundred subjects and used R Studio to extract twenty-four random subjects from our sample as participants in our study.

Step 2: In order to minimize order effects in our experiment, we randomly divided our participants into three different groups where each group received a specific order of the same six treatments. The three different groups are as follows (in order from left to right):



```r
sample <- c("Nap 30 mins", "Sit with Pet Dog 10 mins", "Tea Herbal 250 mL", "Psilocybin Mushrooms 10 g",
"Classical Music 10 mins", "Alprazolam 1 mg")

# Order of treatments 1:
sample(sample)

# Order of treatments 2:
sample(sample)

# Order of treatments 3:
sample(sample)
```

```
[1] "Psilocybin Mushrooms 10 g" "Alprazolam 1 mg"           "Nap 30 mins"
[4] "Sit with Pet Dog 10 mins"  "Classical Music 10 mins"   "Tea Herbal 250 mL"
[1] "Sit with Pet Dog 10 mins"  "Tea Herbal 250 mL"         "Nap 30 mins"
[4] "Classical Music 10 mins"   "Alprazolam 1 mg"           "Psilocybin Mushrooms 10 g"
[1] "Classical Music 10 mins"   "Alprazolam 1 mg"           "Tea Herbal 250 mL"
[4] "Sit with Pet Dog 10 mins"  "Nap 30 mins"               "Psilocybin Mushrooms 10 g"
```

Figure 1

Note 1: The above three groups were randomly generated in RStudio using the sample() function in base R.

Note 2: Order effects "refer to the order of the conditions having an effect on the participants' behavior" [11]. It is important to reduce order effects in order to minimize bias responses, thus increasing the significance of our experimental results and the confidence in which we can draw conclusions on our study.

Important Note: Repeat steps 3-7 for each of the six treatments (in the specified order depending on which of the the three groups they were assigned to) for each unit (one islander)
* Skip step 7 for the last treatment

Step 3: Measure salivary cortisol
Step 4: Apply the assigned treatment
Step 5: Wait the allotted amount of time for the treatment
Step 6: Measure salivary cortisol
Step 7: Wait 48 hours (washout period) prior to gathering data for the next treatment

6. Data Analysis

6.1. Type of Statistical Analysis

Using R, we will conduct two-sample t-tests for each of our six treatments to test whether or not the administration of each treatment causes a statistically significant change in mean cortisol level for before and after treatment groups. By comparing the value of the difference in means, we can determine which treatment decreases cortisol level the most. We utilized the Fisher LSD Test to see if any treatment group significantly distinguishes themselves amongst other treatments. Through these two statistical analysis methods we will be able to identify which treatment is the best for anxiety disorder.

6.2. Sample Size Determination:

In this study, we decided to use a power of 0.8, which is the probability that we will correctly reject the false null hypothesis. In other words, it is the probability that ourtest will "find a statistically significant difference when such a difference actually exists" [16]. Generally, the power should be 0.8 or greater [16]. An alpha level of 0.05 was used, which is the probability of rejecting the null hypothesis when the null hypothesis is true. Based on Cohen's suggestions, the G*Power software provides a conventional effect size value of 0.25 for small effect sizes [7]. We determined the value of the rest of the parameters in G*Power as follows:

- -α err prob - We chose a Type I error rate (0.05).
- Number of groups - Our value for this parameter is 6 since we have 6 total treatments in this study
- Number of measurements - We determined that the number of measurements is 2 since cortisol levels are recorded twice (before and after administration of treatment)
- Corr. among rep measures - We decided that the approximate correlation among columns of repeated measures is 0.7 (this value should be somewhat high)
- Nonsphericity correlation - Since the sphericity assumption is met, the nonsphericity correlation is set to a default value of 1

Using G*Power, our required sample size is 24, which is relatively low compared to other experimental designs but makes sense in our repeated measures design. In repeated measures crossover experimental designs, a lower sample size can be used since each participant in the study is administered all six treatments. The greater statistical power that results from this design allows us to use fewer subjects to detect a desired effect size [10].

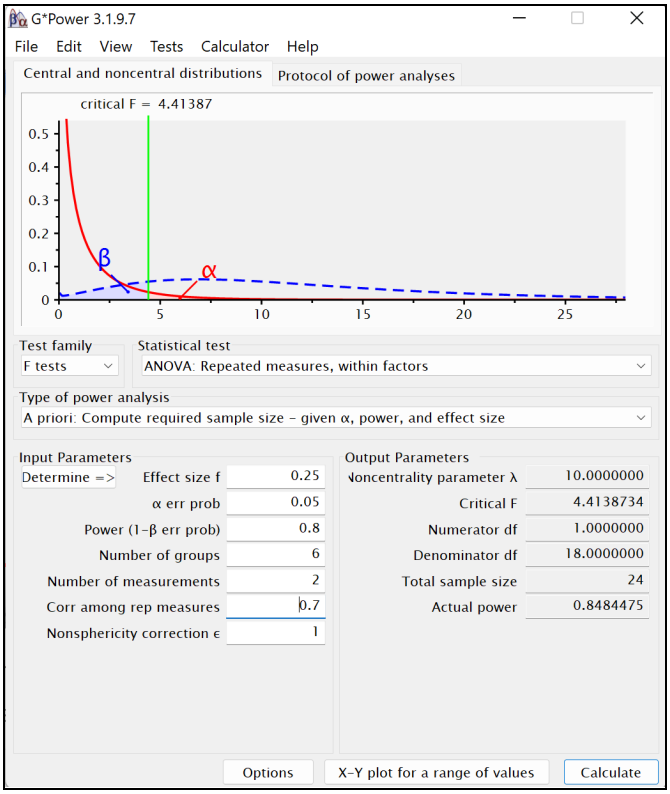


Figure 2

7. Results

7.1. Model Validity:

In our study, we used a two-sample t-test. In order to check the validity of our model, we need to check that our data values are independent, normally distributed, continuous, have equal variances, and are randomly sampled.

In section 5.4, we mention that our data is a subset of individuals randomly sampled from a sample of 100 subjects that consented to be a part of this experiment; RStudio was used to generate this random sample. Since our data values are cortisol levels, our measurements are continuous, and since the cortisol level of one participant does not depend on the cortisol level of another participant, our data values are independent.

To test for the assumption of normality, we will conduct multiple Shapiro-Wilk tests. Our results are as follows:

```
        Shapiro-Wilk normality test

data:  dog$difference
W = 0.9675, p-value = 0.6059
```

```
        Shapiro-Wilk normality test

data:  tea$difference
W = 0.97186, p-value = 0.7129
```

```
        Shapiro-Wilk normality test

data:  alp$difference
W = 0.9674, p-value = 0.6033
```

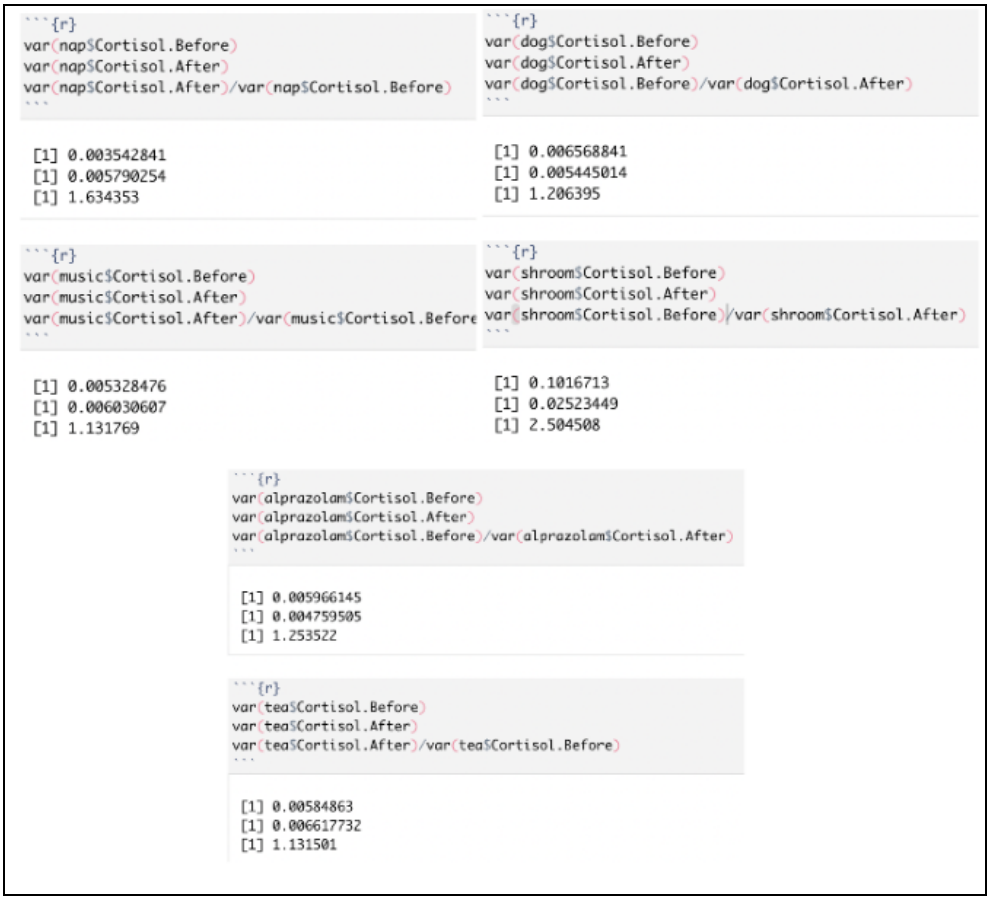
```
        Shapiro-Wilk normality test

data:  music$difference
W = 0.97097, p-value = 0.691
```

```
        Shapiro-Wilk normality test

data:  nap$difference
W = 0.97894, p-value = 0.8755
```

```
        Shapiro-Wilk normality test

data:  shrooms$difference
W = 0.97206, p-value = 0.718
```

To test the assumption of normality, we chose to use the Shapiro-Wilk Test and tested the difference of cortisol levels before and after treatment. In order to pass the Shapiro-Wilk Test, data must have a p-value larger than 0.05, which signifies that it does not significantly deviate from a normal distribution. In the case of our data, all treatments showed p-values larger than 0.05, which indicates that they are close to being normally distributed and valid.

To test for the assumption of equal variances for our two sample t-tests, we must ensure that “the ratio of the larger variance to the smaller variance is less than 4” [20]. If this ratio is less than 4, it is safe to assume that the variances are approximately equal.



Since the ratio of variances for our before and after administration of treatment groups for all six treatments are less than 4, our variances for all six two sample t-tests are approximately equal; the assumption of equal variances is met.

7.2. T-tests:

In our two-sample t-test, we tested the following hypotheses:

H0: The mean salivary cortisol level before and after the treatment is the same.

Ha: The mean salivary cortisol level after treatment is less than before treatment.

```
        Welch Two Sample t-test

data:  tea$Cortisol.Before and tea$Cortisol.After
t = -0.073128, df = 45.826, p-value = 0.529
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 -0.03992809         Inf
sample estimates:
mean of x mean of y
0.2257500 0.2274167
```

Figure 3

```
        Welch Two Sample t-test

data:  shroom$Cortisol.Before and shroom$Cortisol.After
t = 2.9939, df = 33.755, p-value = 0.002562
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 0.09472461        Inf
sample estimates:
mean of x mean of y
0.4780417 0.2603333
```

Figure 5

```
        Welch Two Sample t-test

data:  dog$Cortisol.Before and dog$Cortisol.After
t = 4.8867, df = 45.601, p-value = 6.53e-06
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 0.07176893        Inf
sample estimates:
mean of x mean of y
0.2306667 0.1213333
```

Figure 4

```
        Welch Two Sample t-test

data:  nap$Cortisol.Before and nap$Cortisol.After
t = -5.3119, df = 43.479, p-value = 1
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 -0.1378927        Inf
sample estimates:
mean of x mean of y
0.1171667 0.2219167
```

Figure 6

```
        Welch Two Sample t-test

data:  music$Cortisol.Before and music$Cortisol.After
t = -0.17237, df = 45.825, p-value = 0.568
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 -0.04027271         Inf
sample estimates:
mean of x mean of y
0.2210417 0.2247917
```

Figure 7

```
        Welch Two Sample t-test

data:  alprazolam$Cortisol.Before and alprazolam$Cortisol.After
t = 6.5969, df = 45.425, p-value = 1.941e-08
alternative hypothesis: true difference in means is greater than 0
95 percent confidence interval:
 0.1039621       Inf
sample estimates:
mean of x mean of y
0.2273333 0.0878750
```

Figure 8

Based on this, we conducted two-sample t-tests in R for every treatment to check the significance in the difference of means. We found that 1mg dose of alprazolam, sitting with pet dog for 30 minutes, and 10g dose of Psilocybin were statistically significant, shown by P-value less than alpha = 0.05. This indicates that these three treatments were able to reduce salivary cortisol levels.

To further this, we also chose to run a Fisher LSD test to confirm our results. The result is shown below:

```
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    5  1.613  0.3226   42.17 <2e-16 ***
Residuals  138  1.056  0.0076
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$statistics
      MSerror  Df       Mean       CV  t.value        LSD
  0.007648862 138 0.05772917 151.4967 1.977304 0.04992075

$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   6  0.05

$means
         Difference        std  r         LCL         UCL    Min    Max      Q25     Q50      Q75
dog     0.107416667 0.02399985 24  0.07211736  0.14271597  0.052  0.148  0.09800  0.1060  0.12850
music  -0.003750000 0.01995266 24 -0.03904930  0.03154930 -0.032  0.030 -0.01750 -0.0115  0.01400
nap    -0.104750000 0.02274480 24 -0.14004930 -0.06945070 -0.157 -0.057 -0.11525 -0.1020 -0.09350
shroom  0.217708333 0.20847927 24  0.18240903  0.25300764  0.021  0.783  0.07575  0.1410  0.26325
tea    -0.001666667 0.01949954 24 -0.03696597  0.03363264 -0.044  0.034 -0.00925 -0.0030  0.01100
xanax   0.131416667 0.02362003 24  0.09611736  0.16671597  0.060  0.178  0.12025  0.1345  0.14400

$comparison
NULL

$groups
         Difference groups
shroom  0.217708333      a
xanax   0.131416667      b
dog     0.107416667      b
tea    -0.001666667      c
music  -0.003750000      c
nap    -0.104750000      d
```

Figure 9

Based on the LSD test result, we were able to see that 10g dose of Psilocybin was most effective, shown by the largest difference in mean salivary cortisol levels before and after treatment. The LSD test result also shows that sitting with a pet dog for 30 minutes and 1mg dose of Alprazolam are equally effective in lowering salivary cortisol levels. The other three treatments are shown to be counter effective or not effective. This is seen in that napping for 30 minutes statistically showed an increase in salivary cortisol.

7.3. Graphs and Visualizations:

The following graphs are box-plots that help visualize the difference in means before and after administration of a treatment for our six treatments. As seen below, sitting with a pet dog for 10 minutes, napping for 30 minutes, taking 10 grams of psilocybin mushrooms, and taking 1 milligram of alprazolam were all significant in changing one’s cortisol level. In other words, the difference in means between the before and after groups for these treatments appear to be visually significant.

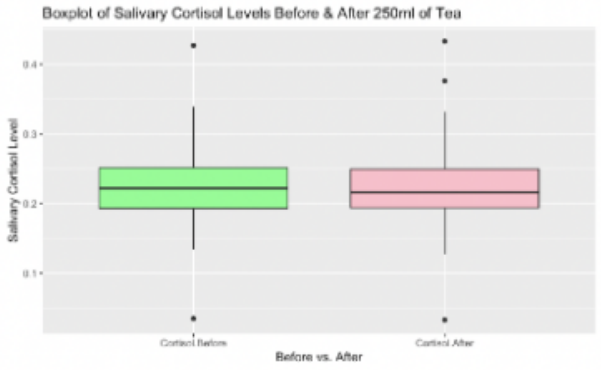


Figure 10

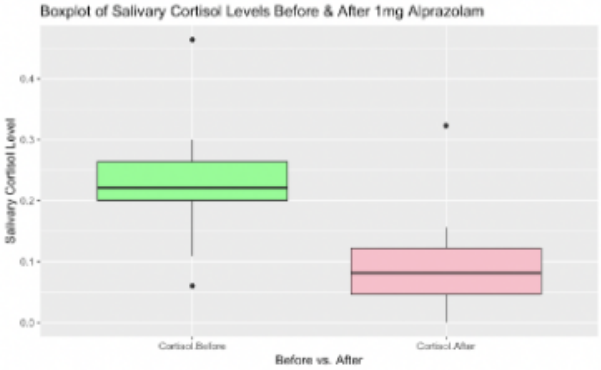


Figure 11

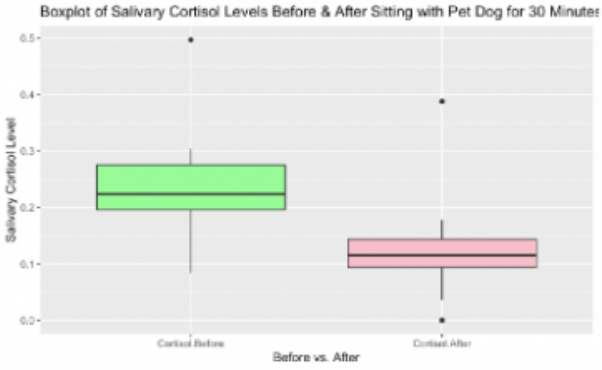


Figure 12

Figure 13

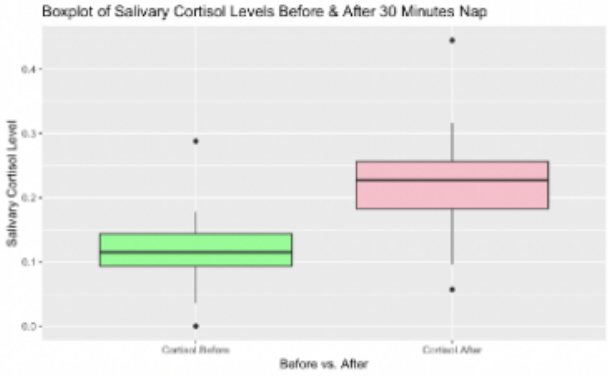


Figure 14

Figure 15

8. Discussion

8.1. Strengths:

By utilizing a crossover design we are able to ensure that all participants are in both the control group and in all of the treatment groups. This allowed us to achieve a high statistical power while being able to detect an effect size with fewer participants. The higher statistical power also allowed us to control for the variability between the participants. Through this method we were able to efficiently compare the treatments and gather results.

8.2. Weaknesses:

The suggested washout period for each treatment varied and it was not entirely clear as to what the correct wait times should be for each treatment within the Island simulator. Although we waited a total of 48 hours between administering each treatment, there was still the possibility of the effect of one treatment trickling down to the other treatments. Another disadvantage of this design is that subjects may choose to withdraw from the study before going through all the treatments. Due to this, we had to obtain the consent of a few new participants in order to complete the experiment with a large enough sample size and power level.

8.3. Suggestions for Further Improvements:

Although a power level of 0.8 is generally accepted as good enough in order to have a good chance of finding a statistically significant difference, in the future we would increase our sample size in order to achieve a higher power level. This would also help in the event that some participants withdraw from the experiment. Another possible improvement would be to explore different designs such as a between subjects, or independent measures design. This type of design is set up so that each participant is assigned to only one treatment group. Through this process we could test all treatments at the same time as well as avoid the effects of one treatment trickling down to another [5].

9. Conclusion

Over 41% of Gen Z and Millennials struggle with anxiety on a daily basis and this percentage is even worse amongst college students in particular [18]. Hence why it is important to study different methods of treating anxiety. All treatments that were selected for this experiment were chosen based on some notion of being an alleviator for anxiety. By implementing a crossover repeated measures experimental design we were able to conclude which of the selected treatments were actually significant. By measuring the before and after levels of salivary cortisol tests, we were able to analyze which treatments were effective in reducing one's cortisol level, which correlates to reducing anxiety.

From our results we were able to determine that sitting with a pet dog for 10 minutes, napping for 30 minutes, taking 10 grams of psilocybin mushrooms, and taking 1 milligram of alprazolam were all significant in changing one's cortisol level. However, this does not mean that all of these treatments help reduce anxiety. In fact, napping for 30 minutes causes one's cortisol levels to increase, thus increasing one's anxiety. Although it may sound strange, research from the *National Library of Medicine* indicates that cortisol levels increase when being awoken from a nap [19]. This research is consistent with our findings and thus explains the significance of the 30 minute nap treatment. Sitting with a pet dog for 30 minutes, taking 10 grams of psilocybin mushrooms, and taking 1 milligram of alprazolam were treatments that all resulted in a p-value less than a significance level of 0.05. This indicates that these treatments are effective in reducing cortisol levels and subsequently anxiety. By implementing the Fisher LSD method, we can see that psilocybin mushrooms in particular showed the greatest difference in means, indicating that it is the most effective treatment for reducing anxiety. Although taking naps, listening to calming classical music, and drinking herbal tea seem like effective ways to reduce anxiety, our findings did not indicate that these treatments could significantly reduce one's cortisol levels. These methods all resulted in p-values higher than a significance level of 0.05 and did show any indication of being good treatments for decreasing anxiety. Thus, we conclude that the medicinal treatments of alprazolam and psilocybin mushrooms are the most effective drugs for reducing anxiety, with dogs being the best non-drug treatment for decreasing cortisol levels.

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