The Impact of Nicotine, Caffeine, and Fish Oil on Memory Retention: A Two-Way Randomized Block Design Study

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Abstract

This study examines the effects of nicotine, caffeine, fish oil, and a control on memory retention across varying dosage levels. Utilizing a two-way randomized block design where we block by three age groups (18-30 yrs old, 31-50 yrs old, 60+ yrs old), a total of 252 participants were divided into four treatment groups with each receiving different substances. Each treatment group was further divided into three dosage levels, with each level consisting of seven participants. Our Null Hypotheses are:

Ho: There is no significant difference in memory retention between the different treatment groups (nicotine, caffeine, fish oil, and control group).

Ho: There is no significant difference in memory retention between different dosage levels.

Ho: There is no significant interaction effect between treatment group and dosage level on memory retention.

And our Alternative Hypotheses are:

Ha1: There is a significant difference in memory retention between the different treatment groups (nicotine, caffeine, fish oil, and control group).

Ha2: There is a significant difference in memory retention between different dosage levels.

Ha3: There is a significant interaction effect between treatment group and dosage level on memory retention.

Statistical analyses, includes a two-way ANOVA, conducted to understand the impact of substance type and dosage level on memory retention. The findings of this study have potential implications for understanding the relationship between the intake of these substances and memory performance, with significant potential for applications in the field of cognitive health and neuroscience.

Introduction

In an ever-advancing and rapidly changing world where our performance is measured by tests and strict analysis, our cognitive abilities play a vital role in achieving success. As we face increasing demands to process and retain vast amounts of information, it is no surprise that people are constantly seeking ways to sharpen their minds and enhance learning and memory. It is within this landscape that nootropics have emerged as a beacon of hope, promising to unlock the full potential of our cognitive abilities. Nootropics are substances believed to enhance cognitive functions, including memory, creativity, and focus. These substances encompass a wide spectrum, ranging from natural compounds to synthetic chemicals and even pharmaceutical drugs. Recent research has shed light on the soaring popularity of nootropics, revealing that the global market for these cognitive enhancers reached an astounding value of USD 10.69 billion in 2021 (Polaris, 2022). The prospects for this market are even more captivating, as it is projected to grow at an impressive compound annual growth rate of 14.8% during the forecast period. Among the respected roster of nootropics, one familiar name takes center stage, caffeine. Caffeine, perhaps one of the most widely recognized nootropics, has earned its reputation through countless studies exploring its impact on mental and physical performance. The abundant scientific literature surrounding caffeine testifies to its ability to

improve focus, heighten alertness, and unleash productivity. Acting as a stimulant, caffeine awakens our senses and provides a vital boost to our physical and cognitive performance. Beyond caffeine, other nootropics exhibit remarkable potential in nurturing brain health and safeguarding against neurological disorders such as Alzheimer's disease. For instance, omega-3 fatty acids from fish oil have shown promising effects in bolstering cognitive function and preserving brain health. Additionally, nicotine, when used responsibly, has been found to offer cognitive benefits and holds the potential to enhance memory retention. With an understanding of the profound impact that nootropics can have on our cognitive capacities, this study aims to explore the effects of caffeine, nicotine, and fish oil on short-term memory. Our research goal is to unravel the potential benefits of different substances and dosages on heightened memory retention. By examining the impact of these three substances on short-term memory, we hope to highlight the pathways through which they may serve as cognitive enhancers. We hypothesize that all three substances will have positive effects on short-term memory, with caffeine leading the charge in terms of significance. Through our research, we hope to improve our understanding of the potential cognitive benefits offered by these nootropics and to empower individuals with the knowledge to optimize their own cognitive capabilities and thrive in an era that demands peak mental performance.

Methods

Participants

The participants of this study come from the virtual island simulation. Each participant came from different parts of the island which was randomly selected through a random number generator with each island having an assigned a unique number. The participants were also asked for consent and only those that have given consent were tested. The treatment for each participant was randomly assigned using Google Sheets' native randomize list function.

Design

The study will be set up as a Two-Way with a blocking factor. The parameters for the design are detailed here:

Response Variable	Memory Test Difference Results (in seconds)			
Treatment 1 (Drug)	Control (Sugar Pill)	Nicotine (2mg)	Fish Oil (500 mg)	Caffeine (100mg)
Treatment 2 (Dose)	Single Dose (Tablets)	Double Dose (Tablets)	Triple Dose (Tablets)	
Blocking (Age)	18-30	31-50	60+	

Figure 1: Parameter Diagram

The factor diagram is detailed below:

We chose to block by age (18-30, 31-50, 60+) and focus on nicotine, fish oil, and caffeine as our drugs (in tablet form). Our second factor, dosage, was separated into three categories (single, double, and triple), with a single dosage being the minimum effective dosage of each drug (nicotine: 2mg, fish oil: 500mg, caffeine: 100mg). Our response variable, difference in test score, was the difference in test score (via memory game measured by time finished in seconds) before and after taking the respective drug and dosage.

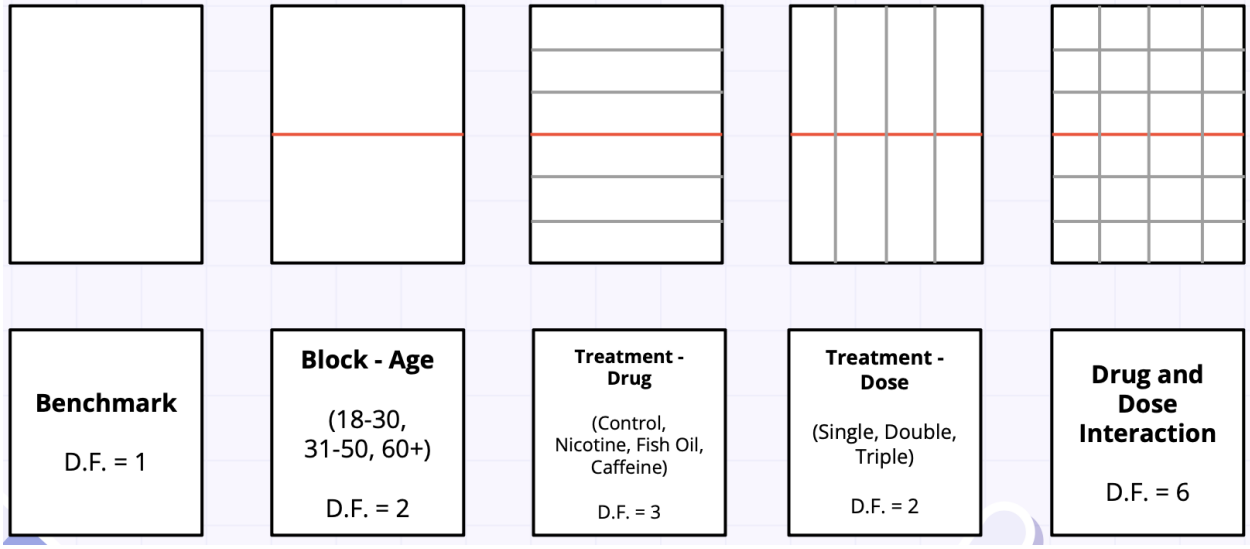


Figure 2: Factor Diagram

Procedure

Step 1: Find subjects from the Island willing to be a part of our experiment. Record each participant in a list in Google Sheets.

Step 2: Randomly assign these groups (already divided by block) into different treatment groups for studying. This is done by using the randomize list function in Google Sheets. The different groups are:

1) Single dose of nicotine tablet (2mg), double dose of nicotine (4mg) tablet, triple dose of nicotine tablet (6mg)
2) Single dose of caffeine tablet (100mg), double dose of caffeine tablet (200mg), triple dose of caffeine tablet (300mg)
3) Single dose of fish oil tablet (500mg), double dose of fish oil tablet (1g), triple dose of fish oil tablet (1.5g)

Step 3: For each unit (one islander), give them memory test

Step 4: For each unit, apply the assigned treatments to the islanders by having them take nicotine, caffeine, and fish oil at their respective dosages

Step 5: For each unit, measure their memory again via memory test

Step 6: For each unit, compute the difference in memory score (in seconds) before and after memory test (this will be our response variable)

Data Analysis

Type of Satistical Analysis

This study involves a two-way randomized block design to evaluate the effects of various substances, specifically nicotine, caffeine, and fish oil, on memory retention among 252 participants. The subjects are randomly divided into four groups and each group is assigned to one of the treatments: nicotine, caffeine, fish oil, or a control group (with no active treatment). To add another layer of variation, the study will test different dosage levels within each group, to explore whether dosage has an effect on memory retention. The blocking factor is age, the randomized block design helps to control for variability among participants that might otherwise

confound the results. Statistical analysis for this study will involve a two-way Analysis of Variance (ANOVA). This method is useful for comparing means from three or more groups, while also considering another factor (dosage levels). This test will allow us to determine if there is a significant effect of substance type and dosage level on memory retention. The ANOVA also allows us to test for an interaction effect between substance type and dosage level, which would indicate that the effect of substance type on memory retention depends on the dosage level. If the results of the ANOVA suggest that there are significant differences among the groups, post-hoc tests (such as Tukey's HSD) can be conducted to make pairwise comparisons between the groups to determine which specific groups differ from each other. Lastly, assumptions of the ANOVA (independence, normality, and homogeneity of variances) should be checked and, if violated, suitable transformations or non-parametric alternatives should be considered.

Sample Size Determination

In our research, we set a statistical power at 0.8. This ensures an 80% chance of correctly identifying a significant effect if one truly exists. We also established a significance level (alpha) at 0.05, reflecting a 5% risk of incorrectly rejecting the null hypothesis. Furthermore, we have chosen a relatively small effect size of 0.25 to represent the meaningful difference between groups. Our study employs a two-way complete block design. After employing G*Power for sample size calculation, we found that we need 252 participants based on the factor with the highest degree of freedom (6, in the case of interaction). Each of the treatment groups will include 21 individuals, which are further split into three dosage levels, resulting in 7 participants per dosage level for each treatment.

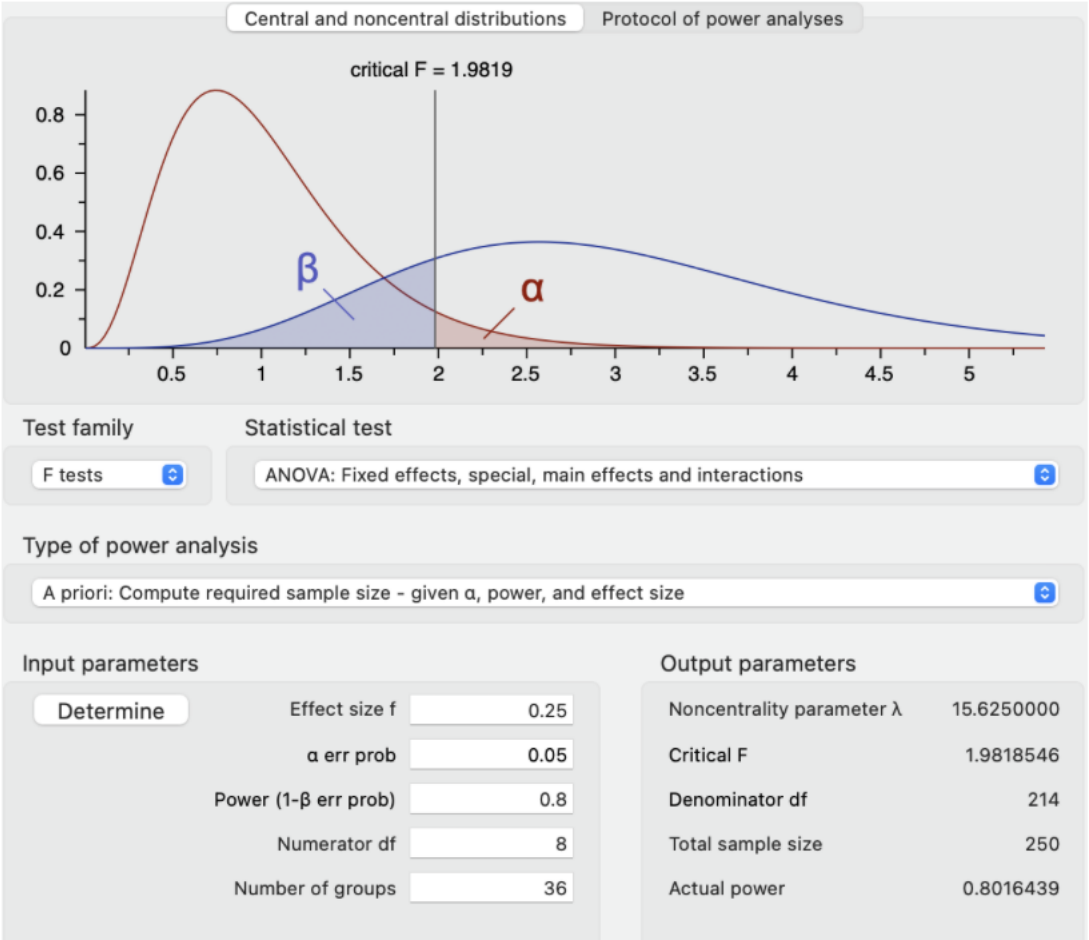


Figure 3: Sample Size

Results

Anova Analysis

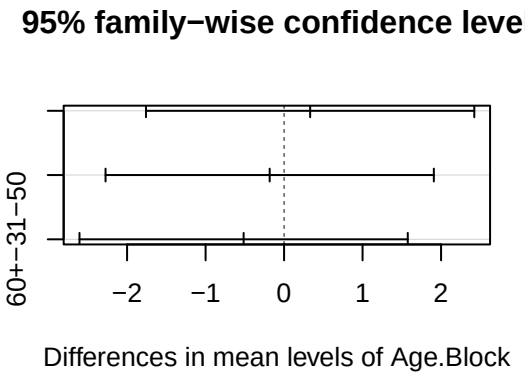
```
library(ggplot2)

data <- read.csv("101B_Final_Project.csv")

data$Age.Block <- as.factor(data$Age.Block)
data$Drug <- as.factor(data$Drug)
data$Dose <- as.factor(data$Dose)
model1 <- aov(Difference ~ Age.Block + Drug*Dose, data = data)
summary(model1)
```

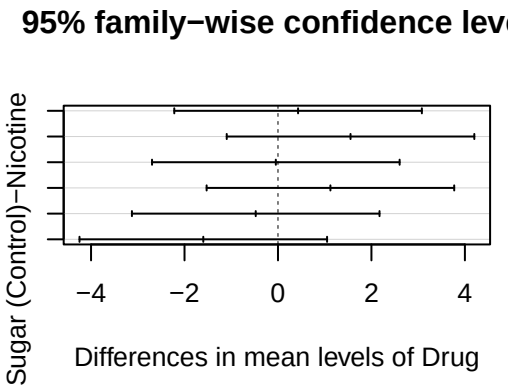
```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Age.Block     2     11    5.74   0.174  0.841
## Drug          3    105   34.85   1.055  0.369
## Dose          2     12    5.93   0.180  0.836
## Drug:Dose     6    208   34.70   1.051  0.393
## Residuals   238   7859   33.02
```

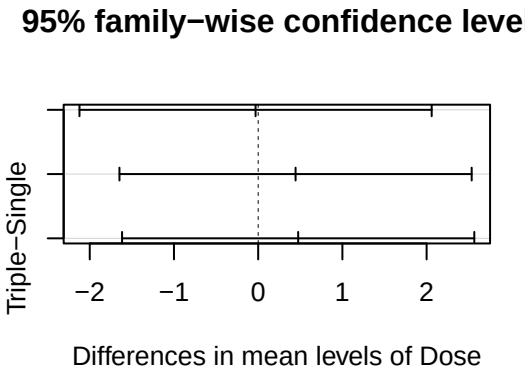
Given the anova table results above, we can see that none of the factors that we presented seemed to be significant. Using an alpha level of 0.05, we can not reject any of the null hypotheses that we had stated. This means that the anova table suggests that none of our factors were effective in enhancing memory retention.

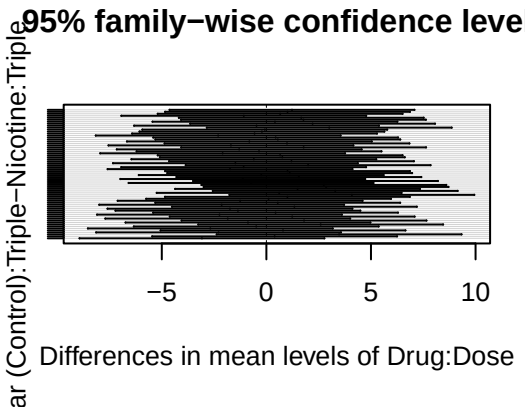
TukeyHSD

```
# TukeyHSD(model1)
par(mfrow=c(2,2))
plot(TukeyHSD(model1))
```







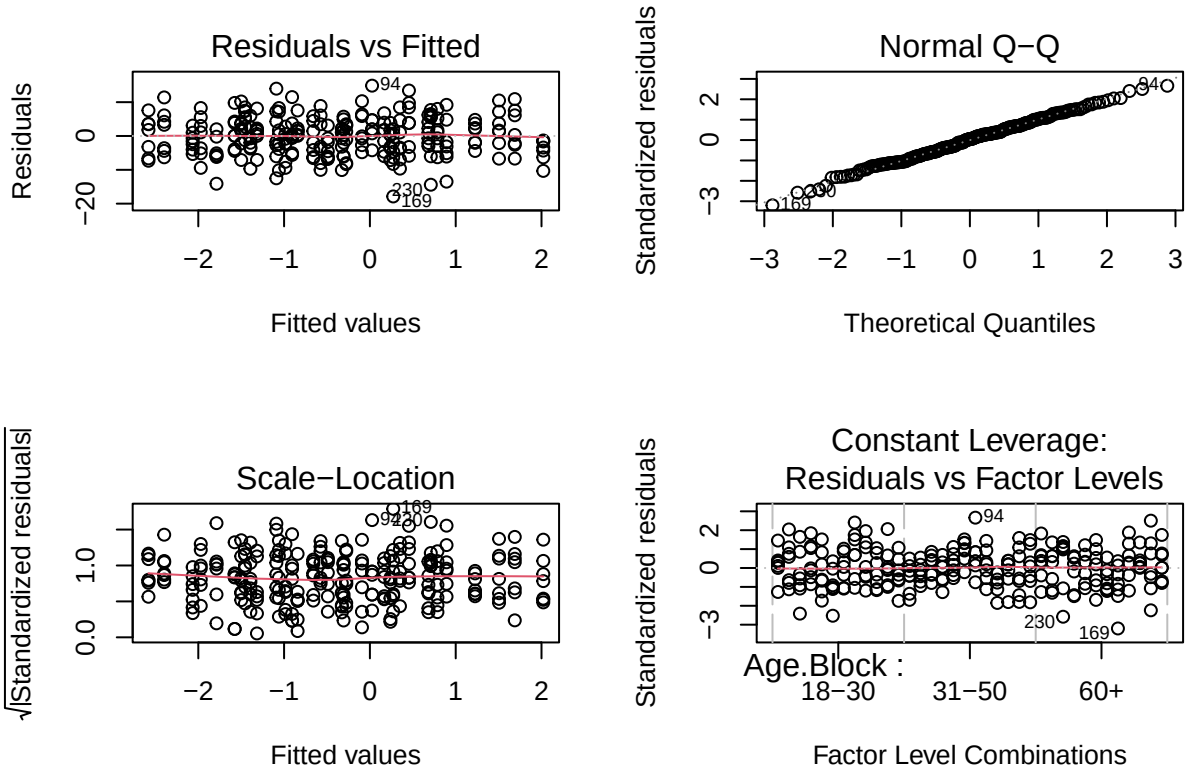



```
par(mfrow=c(1,1))
```

Given that none of the factors were significant, there is no use in performing a Post Hoc analysis as we know that none of the pairwise confidence intervals will be significant. However for the sake of thoroughness, it is provided above.

Residual Diagnostics

```
par(mfrow=c(2,2))
plot(model1)
```

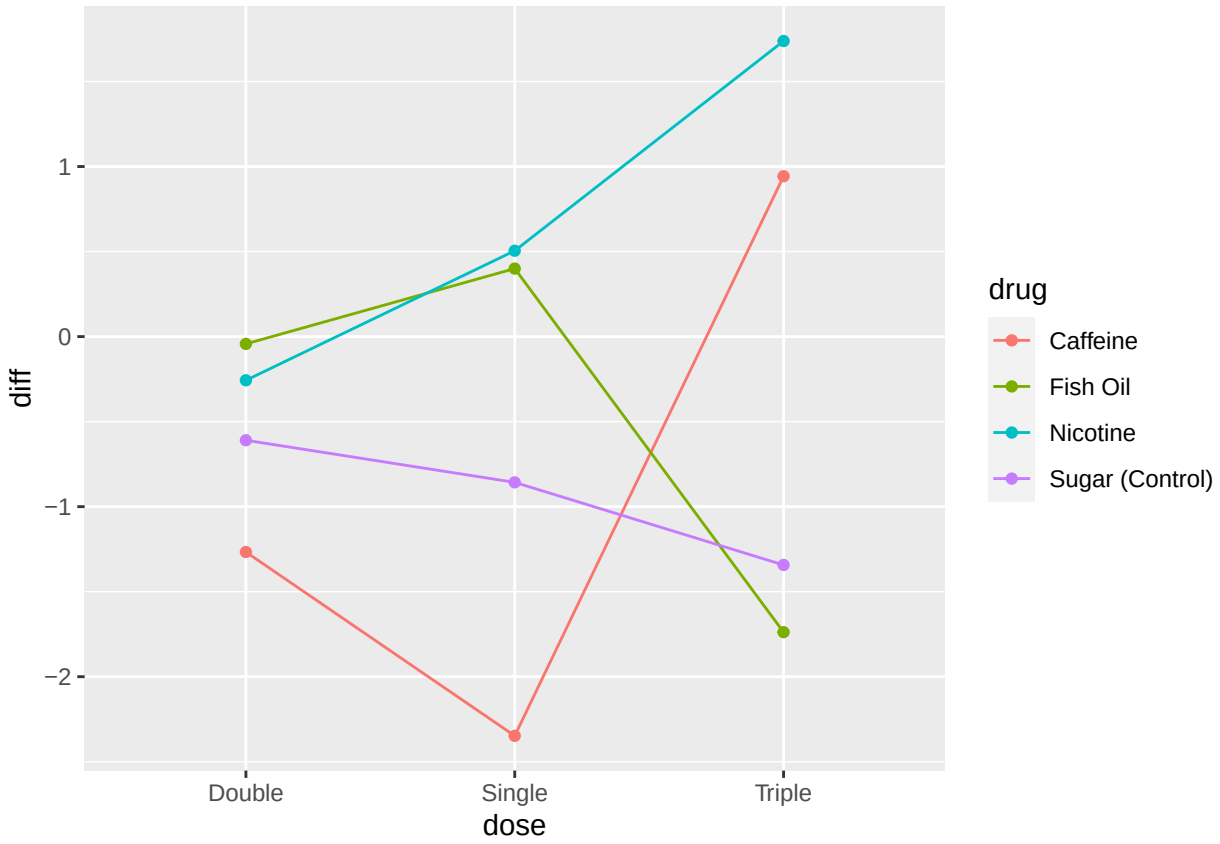



```
par(mfrow=c(1,1))
```

The diagnostics plots above show that we have met our model assumptions. The Normal QQ plot shows that the residuals are normally distributed with some deviation along the tail ends but not enough to consider it a violation of normality. The other plots also show that there is no apparent pattern in the residuals and therefore constant variance is maintained.

Interaction Plots

```
dose <- data$Dose
drug <- data$Drug
diff <- data$Difference
ggplot() + aes(x = dose, color = drug, group = drug, y = diff) + stat_summary(fun = mean, geom = "point
stat_summary(fun = mean, geom = "line")
```




```
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```
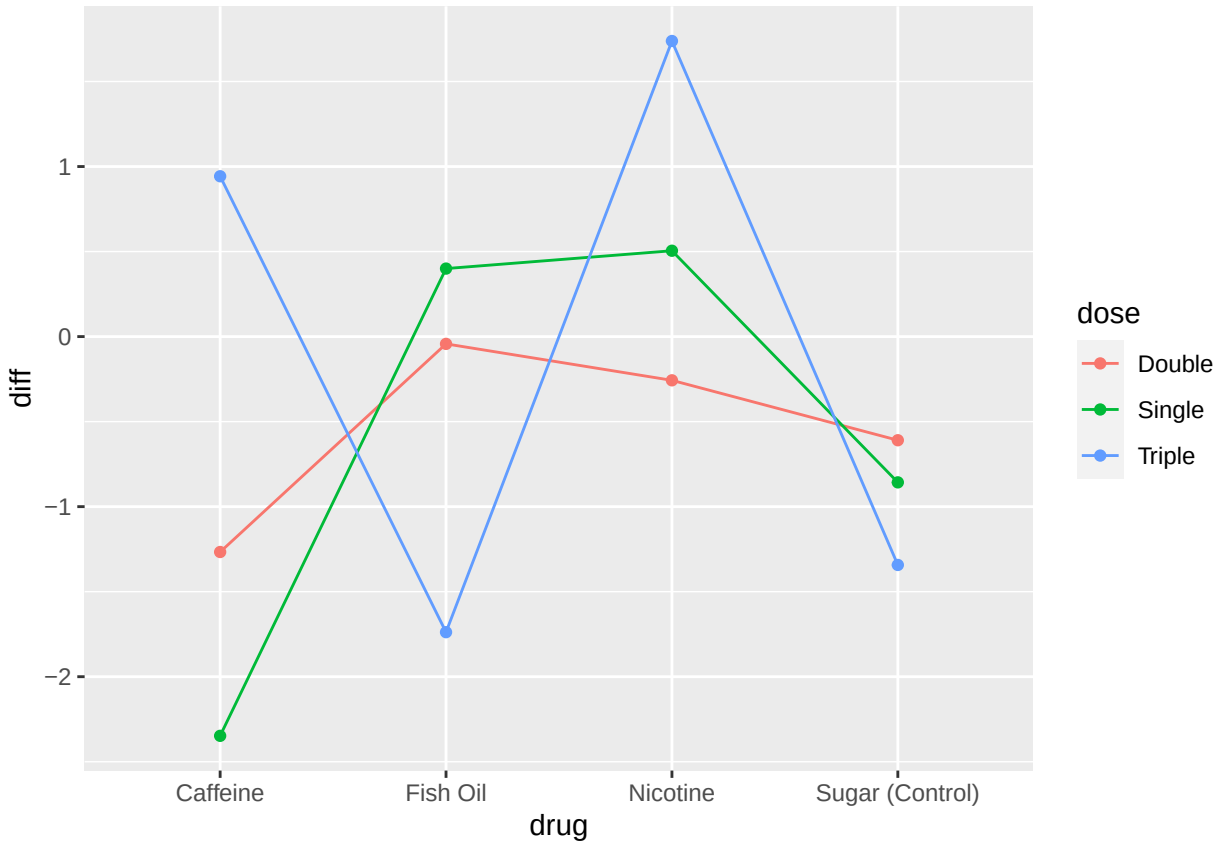
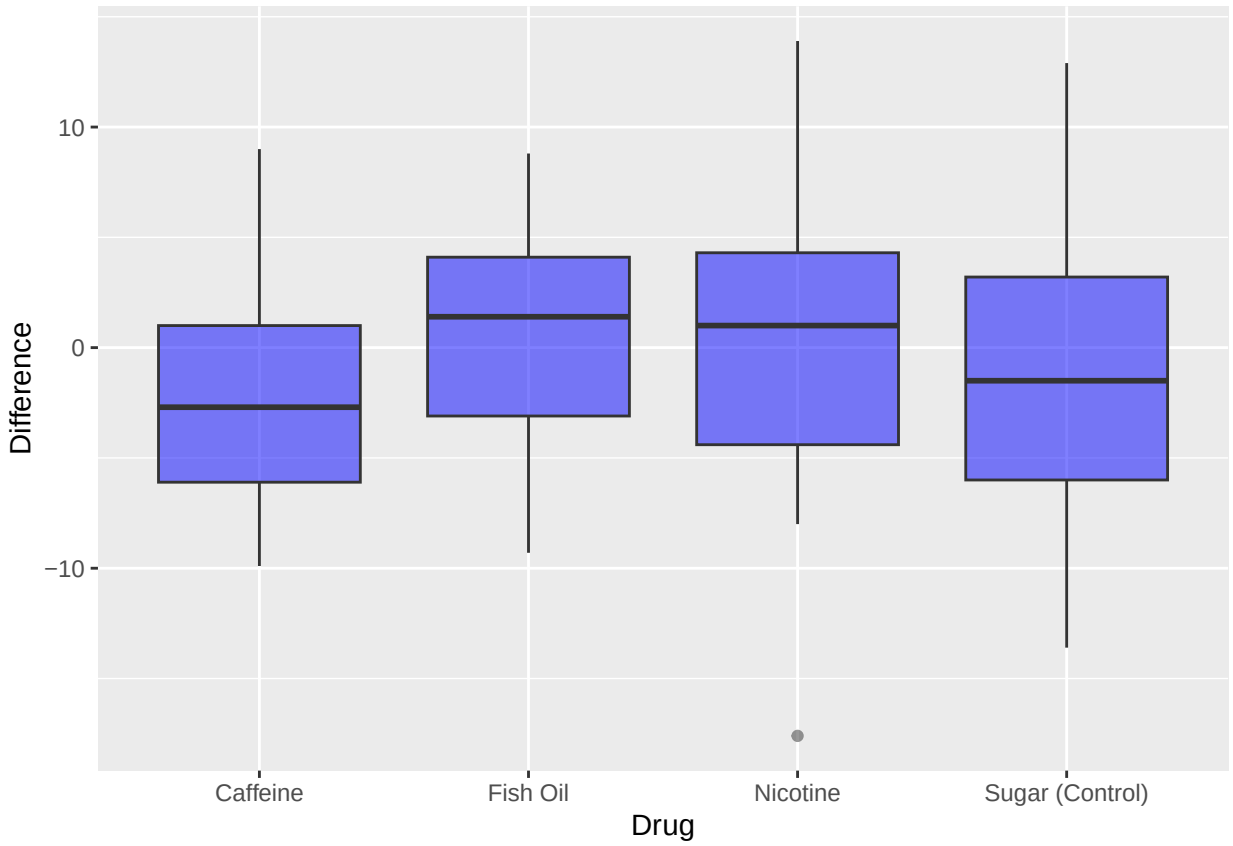



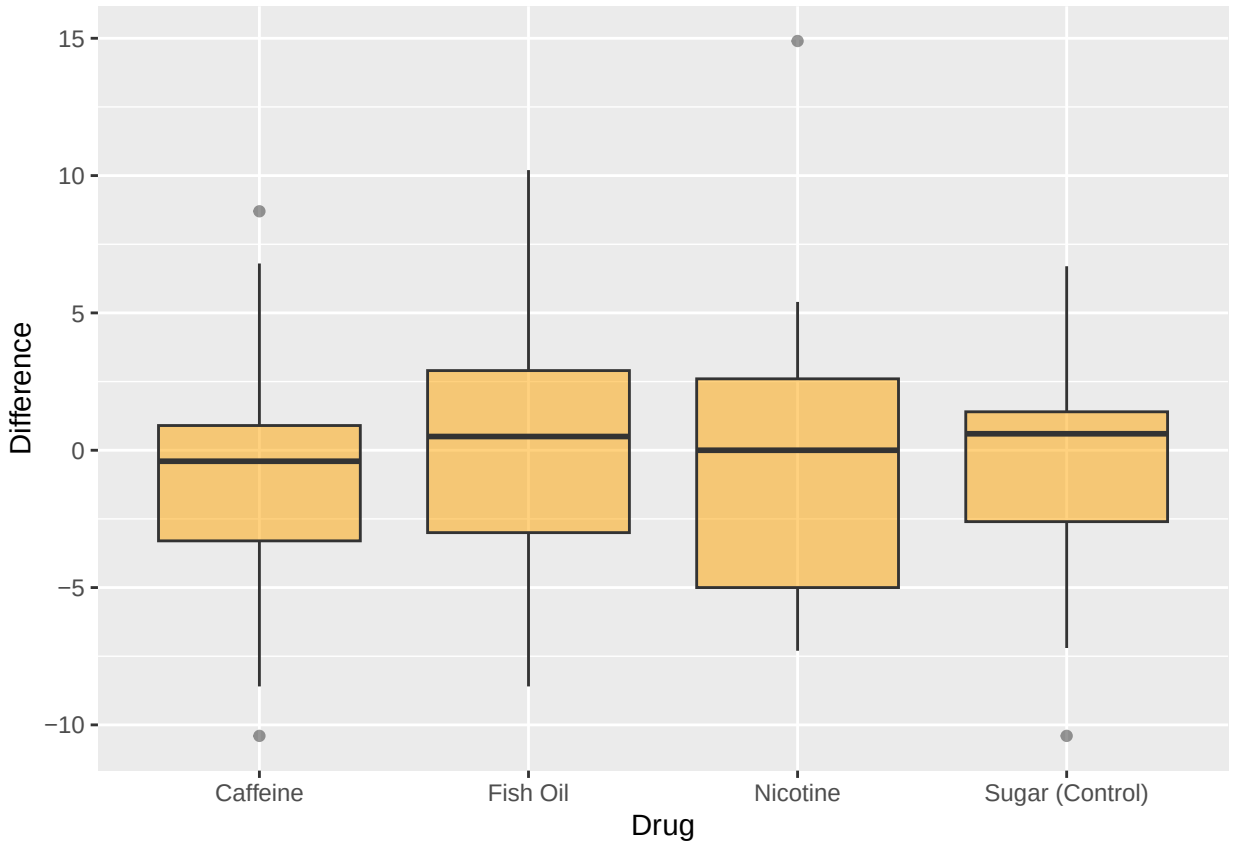
The interaction plots demonstrate that although it may seem as if there is significant interaction between the two variables, there is in fact no significant interaction for drug and dosage. The lines do not generally move in the same direction or at the same steepness but our anova table already told us that it would not be significant

Box Plots

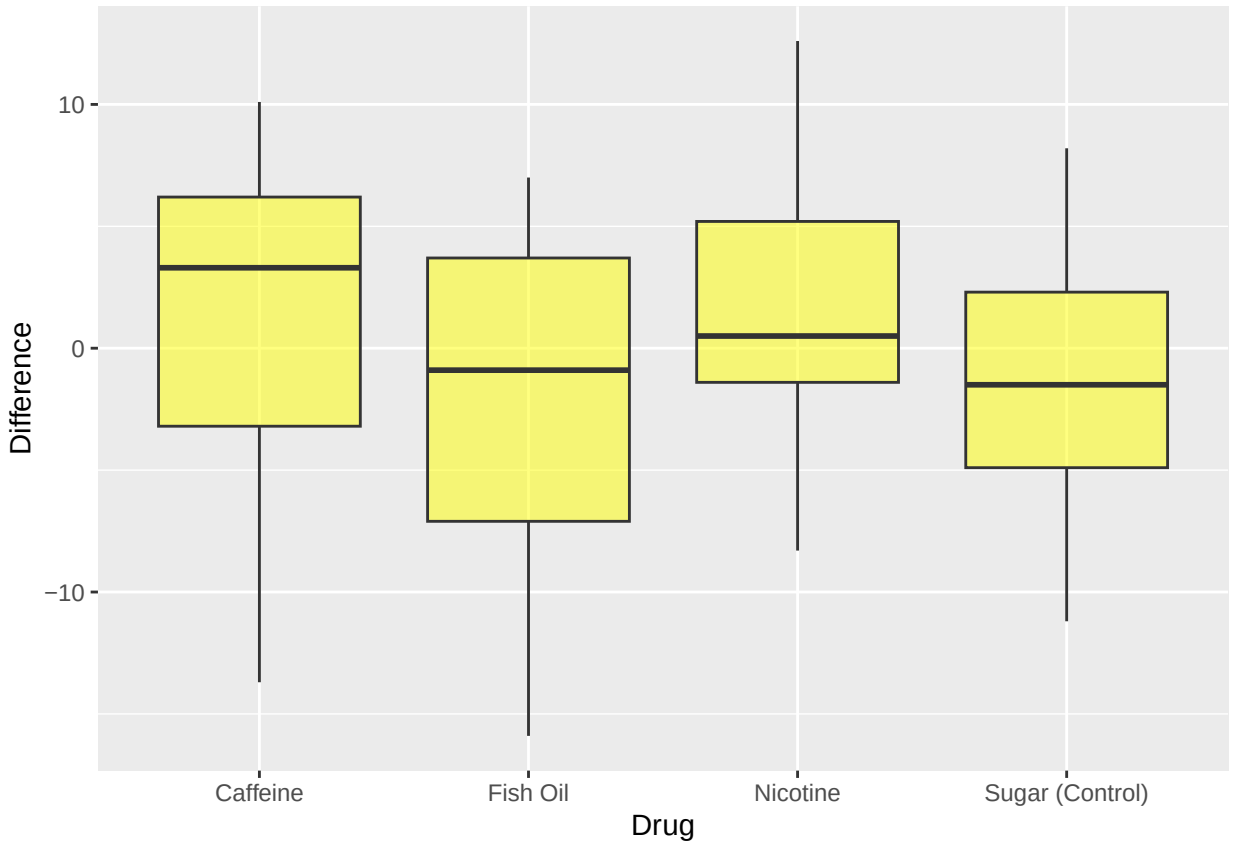
```
ggplot(data[data$Dose == "Single",], aes(Drug, Difference)) + geom_boxplot(fill = "Blue", alpha = 0.5)
```



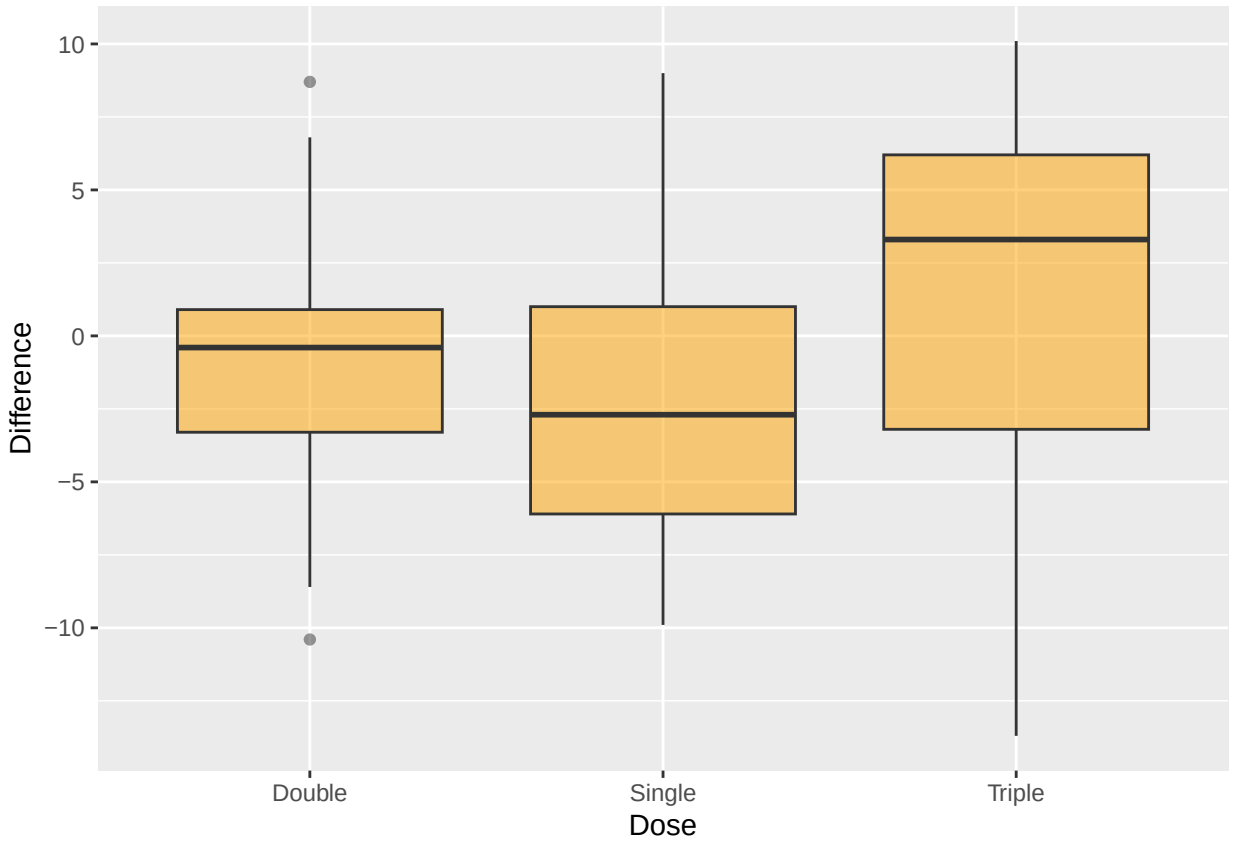

```
ggplot(data[data$Dose == "Double",], aes(Drug, Difference)) + geom_boxplot(fill = "Orange", alpha = 0.5)
```



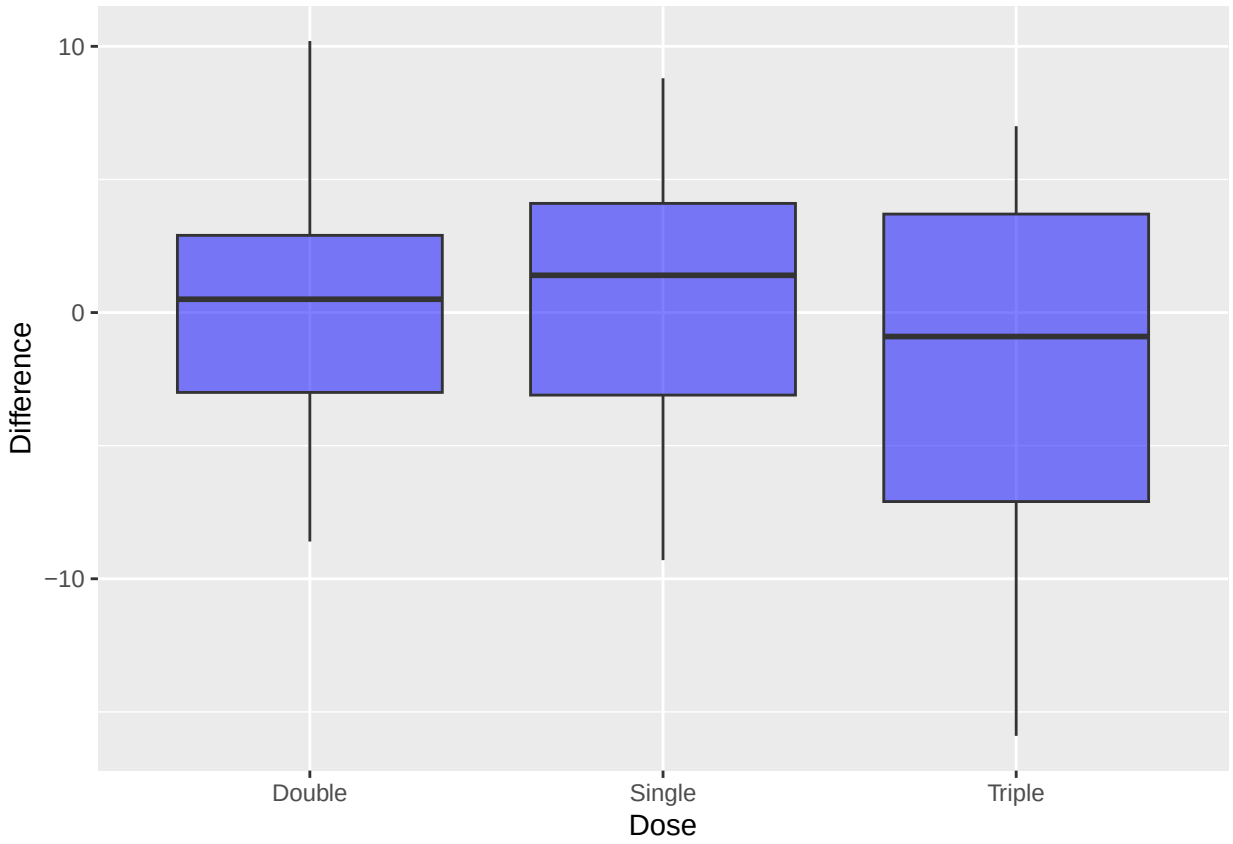

```
ggplot(data[data$Dose == "Triple",], aes(Drug, Difference)) + geom_boxplot(fill = "Yellow", alpha = 0.5)
```



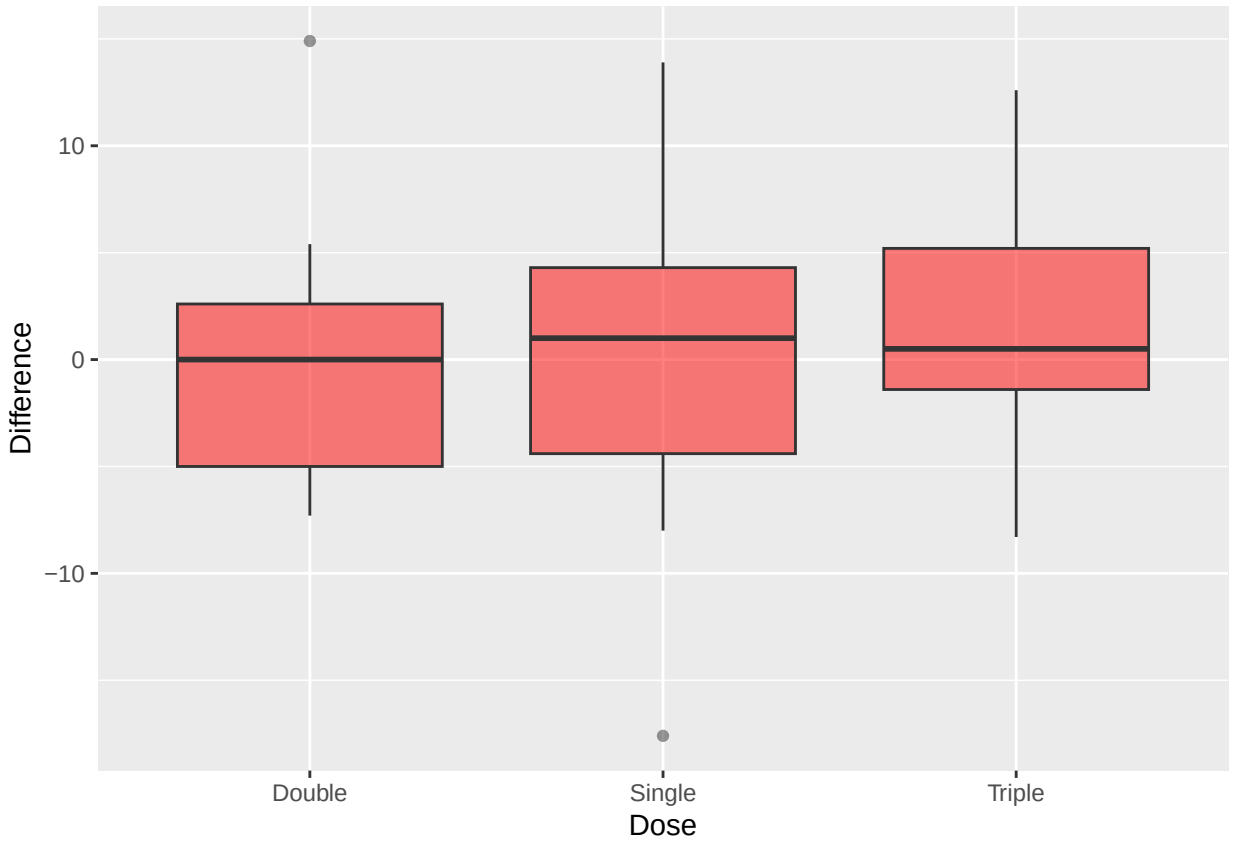

```
ggplot(data[data$Drug == "Caffeine",], aes(Dose, Difference)) + geom_boxplot(fill = "Orange", alpha = 0
```



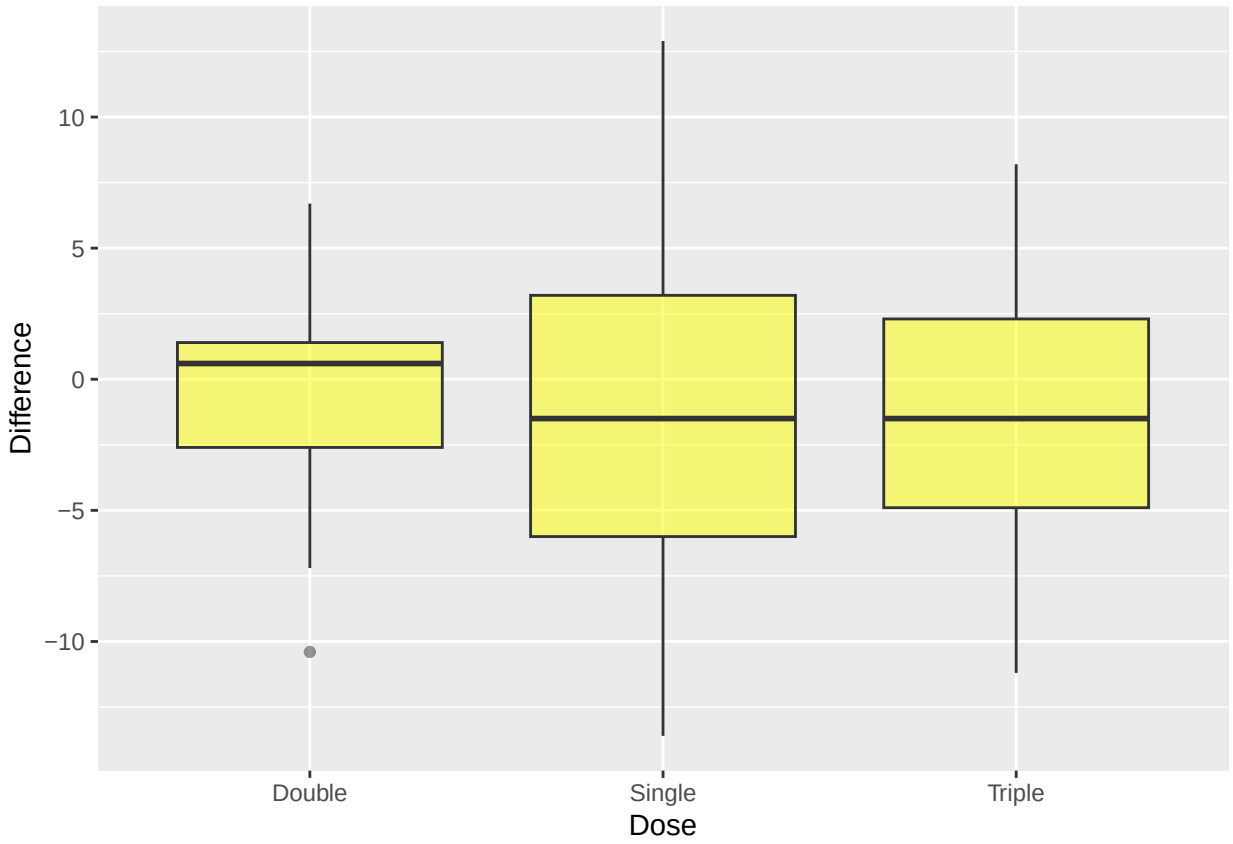

```
ggplot(data[data$Drug == "Fish Oil",], aes(Dose, Difference)) + geom_boxplot(fill = "Blue", alpha = 0.5)
```




```
ggplot(data[data$Drug == "Nicotine",], aes(Dose, Difference)) + geom_boxplot(fill = "Red", alpha = 0.5)
```




```
ggplot(data[data$Drug == "Sugar (Control)",], aes(Dose, Difference)) + geom_boxplot(fill = "Yellow", al
```



When interpreting the boxplots for this model, there seems to be a greater variation within each level of factor than the variation between the levels, which is in line with our initial anova analysis. Most of the boxplots seem to be similar in spread, median value, and shape meaning that there is not an observable difference in their mean values.

Discussion and Conclusions

Through this study, we explored the effects of different drugs of nicotine, fish oil, and caffeine and their dosage level on memory retention.

From the very beginning, we were shown that the effects of the drugs and their dosage level were not significant in the means for the difference in memory score. When using the two-factor anova test with blocking, none of our terms ended up being significant. The Age Block and Dosage level were far from different in their means and their p-values were 0.841 and 0.836 respectively. The Drug factor and interaction term between Drug and Dosage seemed to be slightly more different from each other with p-values of 0.369 and 0.393 respectively.

Looking at our interaction plots, one could initially conclude that the interaction term might be significant but in fact they are not. They seem to be "intersecting" each other but that is simply not enough to conclude that interaction is significant and again we confirmed through the anova table results that this is the case.

The boxplots that were created definitely helped put into perspective what was really happening in this experiment. As stated earlier, there was a lot more variation within each level of the factors than variation between the levels of the factors. This presents two points to address. The first is that our factors are not significant and there doesn't seem to be a change in means by factor. The second is that our subjects may be highly variable and therefore using a Latin Squares design might be more beneficial to see significant results. Additionally, we observed some outliers that we could have possibly removed but there were only a few and most likely would have not changed the results of the model.

There are many limitations and future work we would like to address. As noted in the previous paragraph, we

could have used a latin squares design to have possibly seen some better results. There was a lot of variability coming from the participants and a Latin Squares would be able to reduce that variability and possibly give us better results. Secondly, we had discussed the use of a different control group treatment. The sugar pill was what we thought the best control group would have been but after running the experiment, we would like to try the experiment again with simply testing the subjects again after waiting for a certain period of time. Since sugar is known to increase energy levels, there could be a correlation between energy and memory retention and therefore might have not really been a baseline treatment. Another limitation that was brought onto this experiment was the wait time. After administering the drug, we tested the memory score immediately after which might have altered results since we didn't give the participants time to have the drugs take full effect. Therefore if we had the time and resources to repeat this experiment, we would wait longer in between taking the drug and testing their memory. We would take it even further to test different wait times of the drugs and what would be the most effective waiting time for each drug to take effect in increasing memory retention.

References

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