

Using R to Evaluate the Effect of Rotenone and Paraquat Exposure on the Zebrafish (*Danio rerio*) Model

By: Hope Tucker

How do Rotenone and Paraquat Relate to Parkinson's Disease?

- Parkinson's Disease: neurodegenerative disease characterized by:
 - α -synuclein aggregates, reduction in glutathione, increase in oxidative stress, loss of dopaminergic neurons

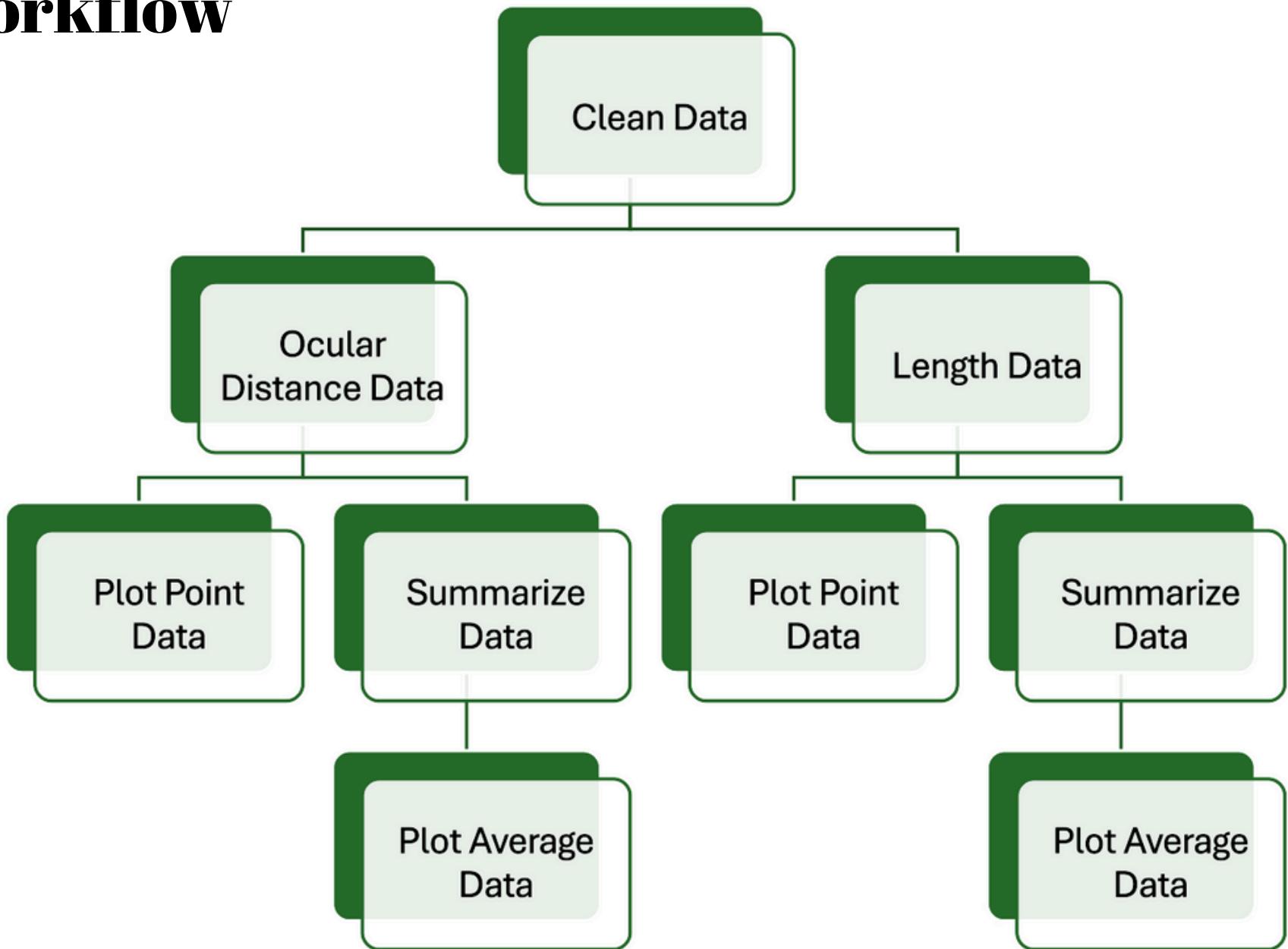
How do Rotenone and Paraquat Relate to Parkinson's Disease?

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- Rotenone ($C_{23}H_{22}O_6$):
 - common piscicide
 - damages dopaminergic (DA) neurons
 - in zebrafish, exposure to rotenone exposure induced a decrease in DA neurons with a correlating impairment of motor activity
 - induces formation of α -synuclein aggregates and increased ROS

How do Rotenone and Paraquat Relate to Parkinson's Disease?

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 - in zebrafish, exposure to rotenone exposure induced a decrease in DA neurons with a correlating impairment of motor activity
 - induces formation of α-synuclein aggregates and increased ROS
- Paraquat ($C_{12}H_{14}Cl_2N_2$):
 - common herbicide
 - induces ROS production, lipid peroxidation, accumulation of α-synuclein, mitochondrial dysfunction, and low levels of glutathione in zebrafish and rodent models

Workflow



Research Questions

- Does exposure to rotenone, paraquat, and/or a combination of rotenone and paraquat affect the ocular distance of zebrafish?
- Does exposure to rotenone, paraquat, and/or a combination of rotenone and paraquat affect the length of zebrafish?
- Does treating zebrafish with a combination of rotenone and paraquat result in a greater effect on zebrafish morphology than compared to treating zebrafish with individual rotenone or paraquat?

Ocular Distance

```
2 all_data_clean <- na.omit(all_data_at)
3 view(all_data_clean)
4
5 #Ocular Distance Data Point Plot
6 library(tidyverse)
7 library(ggplot2)
8 library(ggthemes)
9 "Ocular Distance Point Data" <- ggplot(all_data_clean, aes(x = beaker, y = ocular_distance_mm)) +
10   geom_point(aes(color = treatment)) +
11   labs(color = "Treatment",
12       title = "The Effect of Rotenone and Paraquat
13           on Ocular Distance",
14       x = "Beaker Treatment", y = "Ocular Distance (mm)") +
15   scale_color_manual(
16     labels = c("paraquat"= "Paraquat",
17               "rotenone" = "Rotenone",
18               "rotenone_paraquat" = "Rotenone + Paraquat"),
19     values = c("paraquat" = "#deepskyblue",
20               "rotenone" = "#darkgoldenrod1",
21               "rotenone_paraquat" = "#chartreuse4")) +
22   theme(axis.title = element_text(size = 17),
23         axis.text = element_text(size = 14),
24         plot.title = element_text(size = 20),
25         legend.title = element_text(size = 17),
26         legend.text = element_text(size = 14)) +
27   theme_bw()
28 #Saving Ocular Distance Data Point Plot
29 ggsave(filename = "OcularDistancePointData.png",
30        plot = last_plot(),
31        width = 4,
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```

Ocular Distance

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Ocular Distance

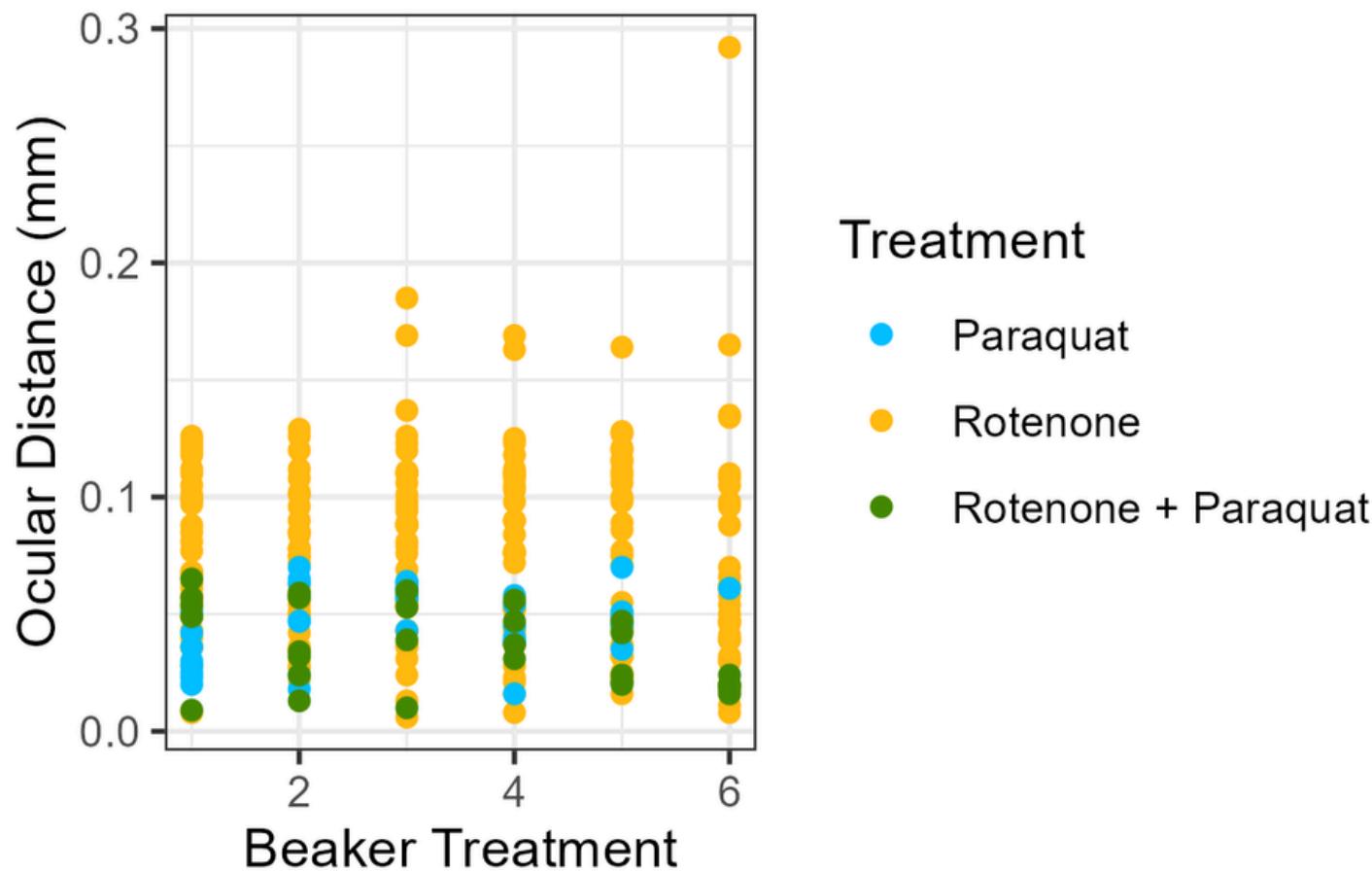
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Ocular Distance

The Effect of Rotenone and Paraquat on Ocular Distance



Length

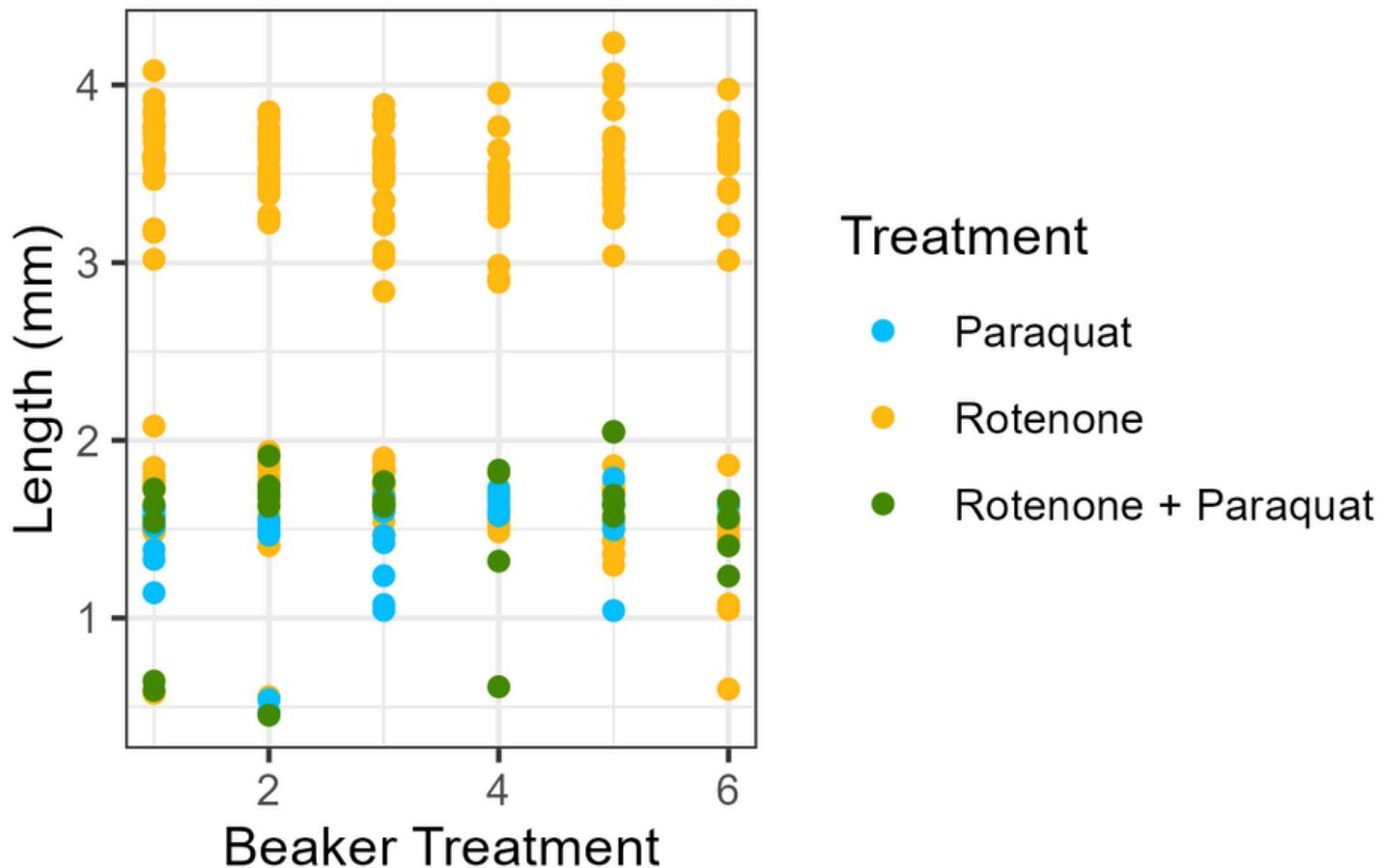
```
35 #Length Data Point Plot|
36 "Length Point Data" <- ggplot(all_data_clean, aes(x = beaker, y = length_mm)) +
37   geom_point(aes(color = treatment), na.rm = TRUE) +
38   labs(color = "Treatment",
39         title = "The Effect of Rotenone and Paraquat
40         on Length",
41         x = "Beaker Treatment", y = "Length (mm)") +
42   scale_color_manual(
43     labels = c("paraquat"= "Paraquat",
44               "rotenone" = "Rotenone",
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55 #Saving Length Data Point Plot
56 ggsave(filename = "LengthPointData.png",
57         plot = last_plot(),
58         width = 4,
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59         height = 3,
60         units = "in")
```

Length

The Effect of Rotenone and Paraquat on Length



Mean Ocular Distance

```
62 #Average Ocular Distance Data Bar Plot
63 #Summarize Data
64 mean_sd_ocular <- all_data_clean |>
65   group_by(beaker, treatment) |>
66   summarise(
67     mean_ocular_distance = mean(ocular_distance_mm),
68     sd_ocular_distance = sd(ocular_distance_mm),
69     .groups = "drop"
70   )
71 view(mean_sd_ocular)
72 #Plot Data
73 "Average Ocular Distance Data" <- ggplot(mean_sd_ocular, aes(x = beaker, y = mean_ocular_distance, fill = treatment)) +
74   geom_bar(stat = "identity", position = position_dodge(width = 0.9)) +
75   geom_errorbar(aes(ymin = mean_ocular_distance - sd_ocular_distance,
76                      ymax = mean_ocular_distance + sd_ocular_distance),
77                 width = 0.2,
78                 position = position_dodge(width = 0.9)) +
79   labs(
80     title = "Mean Ocular Distance across Rotenone and Paraquat Treatments",
81     subtitle = "With Standard Deviation Error Bars",
82     x = "Beaker", y = "Ocular Distance (mm)") +
83   scale_fill_manual(
84     name = "Treatment",
85     labels = c("paraquat" = "Paraquat",
86               "rotenone" = "Rotenone",
87               "rotenone_paraquat" = "Rotenone + Paraquat"),
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91   theme_bw() +
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96     plot.subtitle = element_text(size = 14),
97     legend.title = element_text(size = 14),
98     legend.text = element_text(size = 12)
99   )
100 #Saving Average Ocular Distance
101 ggsave(filename = "AverageOcularDistanceData.png",
102         plot = last_plot(),
103         width = 9,
104         height = 7,
105         units = "in")
```

Mean Ocular Distance

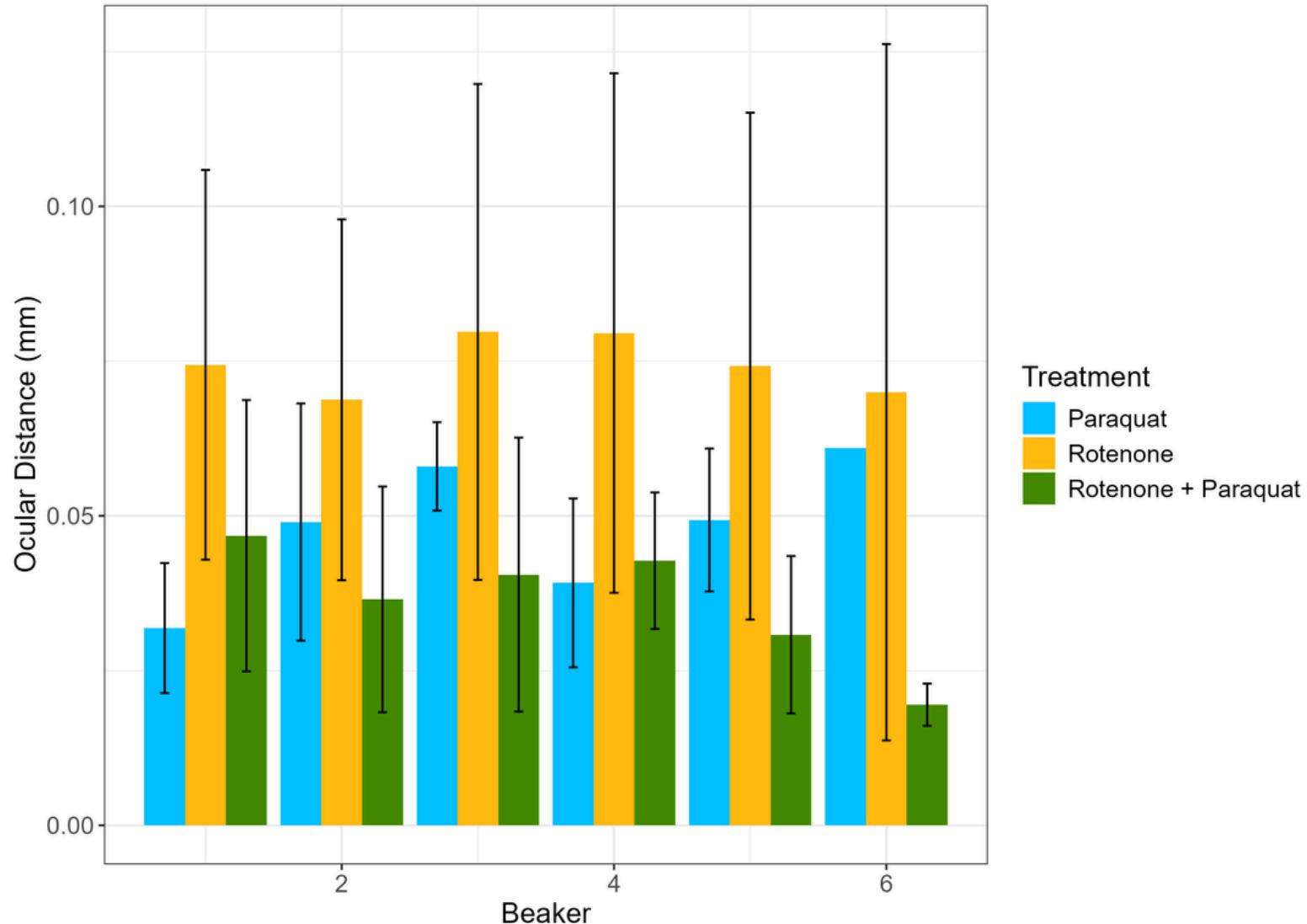
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71 view(mean_sd_ocular)
72 #Plot Data
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75   geom_errorbar(aes(ymin = mean_ocular_distance - sd_ocular_distance,
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80     title = "Mean Ocular Distance across Rotenone and Paraquat Treatments",
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67     mean_ocular_distance = mean(ocular_distance_mm),
68     sd_ocular_distance = sd(ocular_distance_mm),
69     .groups = "drop"
70   )
71 view(mean_sd_ocular)
72 #Plot Data
73 "Average Ocular Distance Data" <- ggplot(mean_sd_ocular, aes(x = beaker, y = mean_ocular_distance, fill = treatment)) +
74   geom_bar(stat = "identity", position = position_dodge(width = 0.9)) +
75   geom_errorbar(aes(ymin = mean_ocular_distance - sd_ocular_distance,
76                      ymax = mean_ocular_distance + sd_ocular_distance),
77                 width = 0.2,
78                 position = position_dodge(width = 0.9)) +
79   labs(
80     title = "Mean Ocular Distance across Rotenone and Paraquat Treatments",
81     subtitle = "With Standard Deviation Error Bars",
82     x = "Beaker", y = "Ocular Distance (mm)") +
83   scale_fill_manual(
84     name = "Treatment",
85     labels = c("paraquat" = "Paraquat",
86               "rotenone" = "Rotenone",
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```

Mean Ocular Distance

Mean Ocular Distance across Rotenone and Paraquat Treatments
With Standard Deviation Error Bars



Mean Length

```
107 #Mean Length Data Bar Plot
108 #Summarize Data
109 mean_sd_length <- all_data_clean |>
110   group_by(beaker, treatment) |>
111   summarise(
112     mean_length = mean(length_mm),
113     sd_length = sd(length_mm),
114     .groups = "drop"
115   )
116 view(mean_sd_length)
117 #Plot Data
118 "Average Length Data" <- ggplot(mean_sd_length, aes(x = beaker, y = mean_length, fill = treatment)) +
119   geom_bar(stat = "identity", position = position_dodge(width = 0.9)) +
120   geom_errorbar(aes(ymax = mean_length + sd_length,
121                     ymin = mean_length - sd_length),
122                 width = 0.2,
123                 position = position_dodge(width = 0.9)) +
124   labs(
125     title = "Mean Length across Rotenone and Paraquat Treatments",
126     subtitle = "With Standard Deviation Error Bars",
127     x = "Beaker", y = "Length (mm)") +
128   scale_fill_manual(
129     name = "Treatment",
130     labels = c("paraquat" = "Paraquat",
131               "rotenone" = "Rotenone",
132               "rotenone_paraquat" = "Rotenone + Paraquat"),
133     values = c("paraquat" = "#deepskyblue",
134               "rotenone" = "#darkgoldenrod1",
135               "rotenone_paraquat" = "#chartreuse4")) +
136   theme_bw() +
137   theme(
138     axis.title = element_text(size = 14),
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147         plot = last_plot(),
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Mean Length

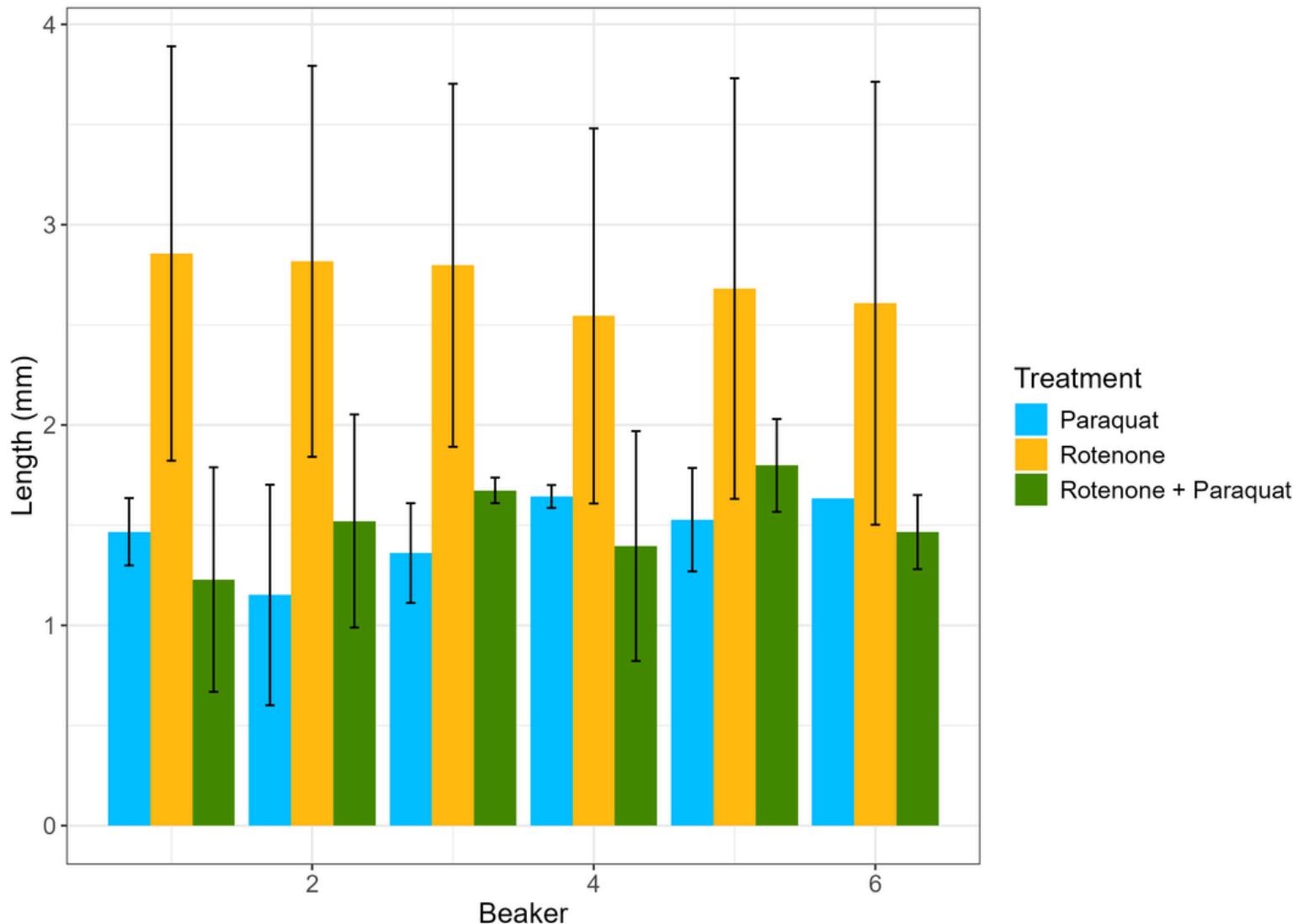
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109 mean_sd_length <- all_data_clean |>
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111   summarise(
112     mean_length = mean(length_mm),
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115   )
116 view(mean_sd_length)
117 #Plot Data
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124   labs(
125     title = "Mean Length across Rotenone and Paraquat Treatments",
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Mean Length

Mean Length across Rotenone and Paraquat Treatments
With Standard Deviation Error Bars



Sources

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<https://forum.posit.co/t/error-in-check-aesthetics/135885>
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