FITC-combined-perm-assay

CTB

2023-03-25

```r
csv_list <- list.files(path="D:/Microscopy/IHC images/FITC-perf-analysis/FITC/processing/MAXproj/CSVs",
csv_cols <- c("Label", "Area", "Mean", "Min", "Max", "%Area")
fitc_csvs <- as.data.frame(matrix(,0,length(csv_cols)))
names(fitc_csvs) <- csv_cols
fitc_csvs
```

```
## [1] Label Area  Mean  Min   Max   %Area
## <0 rows> (or 0-length row.names)
```

```r
for (i in csv_list) {
  test <- read_csv(i, show_col_types=FALSE)
  test <- test %>% select(Label:"%Area")
  fitc_csvs <- rbind(fitc_csvs, test)
}
```

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## * `` -> `...1`
```

```r
fitc_csvs <- fitc_csvs %>% mutate(
  mouseID = as.factor(str_extract(Label, "^[:digit:]+")),
  tissue = as.factor(str_extract(Label, "cap|A[V]?|PVN"))
)


fitc_csvs <- fitc_csvs %>% mutate(
  tissue = as.factor(case_when(tissue %in% c("A", "AV", "V", "cap") ~ "retina",
                      tissue == "PVN" ~ "brain")),
  objLabel = str_extract(Label, "(?<=:).*"),
  vesselType = as.factor(str_extract(objLabel, "^[:alpha:]")),
  vesselNum = as.numeric(str_extract(objLabel, "[:digit:]+")),
  location = str_extract(objLabel, "(?<=-).*"),
  img = str_extract(Label, ".*(?=_f)")
) %>% na.omit() %>% select(!Label)

fitc_csvs <- fitc_csvs %>% mutate(
  vesselType = case_when(vesselType %in% c("A", "AV", "V") ~ "AV",
                      vesselType == "C" ~ "C"))

#fixing problems when there's, e.g., A1 and V1 both being changed to AV1
fitc_csvs <- fitc_csvs %>% ungroup() %>%
  group_by(mouseID, img,vesselType, location) %>%
  mutate(vesselNum = ifelse(duplicated(vesselNum), vesselNum + 1, vesselNum))

fitc_csvs <- fitc_csvs %>% ungroup() %>%
  pivot_wider(id_cols = c(img, mouseID, tissue, vesselType, vesselNum),
              names_from = location,
              values_from = c(Area, Mean, Min, Max, "%Area"))

fitc_csvs
```

```
## # A tibble: 736 x 15
##     img     mouseID tissue vesse~1 vesse~2 Area_~3 Area_~4 Mean_~5 Mean_~6 Min_i~7
##    <chr>   <fct>   <fct>  <chr>     <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 29-pe~ 29      retina C             1    69.4    154.    29.6    7.13       9
## 2 29-pe~ 29      retina C             2    63.6    147.    36.4    6.9       12
## 3 29-pe~ 29      retina C             3    67.7    151.    39.0    6.89       9
## 4 29-pe~ 29      retina C             5    52.9    164.    20.2    7.37       9
## 5 29-pe~ 29      retina C             7    60.7    151.    35.5    7.25       8
## 6 29-pe~ 29      retina C             8    79.2    208.    36.1    6.78       8
## 7 29-pe~ 29      retina C             9    84.2    220.    27.2    7.39       7
## 8 29-pe~ 29      retina C            10    74.4    208.    33.8    6.94      10
## 9 29-pe~ 29      retina C            11    54.6    149     34.8    6.53       8
## 10 29-pe~ 29      retina C            12    52.8    136.    19.5    7.24       9
## # ... with 726 more rows, 5 more variables: Min_outside <dbl>,
## #   Max_inside <dbl>, Max_outside <dbl>, `%Area_inside` <dbl>,
## #   `%Area_outside` <dbl>, and abbreviated variable names 1: vesselType,
## #   2: vesselNum, 3: Area_inside, 4: Area_outside, 5: Mean_inside,
## #   6: Mean_outside, 7: Min_inside
```

```r
fitc_csvs <- fitc_csvs %>% unnest() %>%
  mutate(treatment = as.factor(case_when(mouseID %in% c(29, 62) ~ "untreated",
                            mouseID %in% c(85, 86) ~ "saline",
                            mouseID %in% c(83, 84) ~ "morphine")))
```

```
## Warning: `cols` is now required when using unnest().
## Please use `cols = c()`
```

```r
levels(fitc_csvs$vesselType)
```

```
## NULL
```

```r
fitc_diffs <- fitc_csvs %>%
  group_by(img,mouseID, treatment, tissue, vesselType, vesselNum) %>%
  mutate(Mean_OI = Mean_outside/Mean_inside,
         Max_OI = Max_outside/Max_inside) %>%
  select(img:Area_inside, treatment:Max_OI)
fitc_diffs
```

```
## # A tibble: 736 x 9
## # Groups:   img, mouseID, treatment, tissue, vesselType, vesselNum [736]
##     img           mouseID tissue vesse~1 vesse~2 Area_~3 treat~4 Mean_OI Max_OI
##    <chr>           <fct>   <fct>  <chr>     <dbl>   <dbl> <fct>     <dbl>  <dbl>
## 1 29-periph-cap-~ 29      retina C             1    69.4 untrea~   0.241  0.314
## 2 29-periph-cap-~ 29      retina C             2    63.6 untrea~   0.189  0.343
## 3 29-periph-cap-~ 29      retina C             3    67.7 untrea~   0.177  0.471
## 4 29-periph-cap-~ 29      retina C             5    52.9 untrea~   0.365  0.452
## 5 29-periph-cap-~ 29      retina C             7    60.7 untrea~   0.204  0.269
## 6 29-periph-cap-~ 29      retina C             8    79.2 untrea~   0.188  0.208
## 7 29-periph-cap-~ 29      retina C             9    84.2 untrea~   0.272  0.703
## 8 29-periph-cap-~ 29      retina C            10    74.4 untrea~   0.205  0.288
## 9 29-periph-cap-~ 29      retina C            11    54.6 untrea~   0.188  0.205
## 10 29-periph-cap-~ 29      retina C            12    52.8 untrea~   0.372  0.545
```

```
## # ... with 726 more rows, and abbreviated variable names 1: vesselType,
## #   2: vesselNum, 3: Area_inside, 4: treatment
```

```
fitc_diffs_norm <- fitc_diffs %>% ungroup() %>%
  group_by(img, mouseID, treatment, tissue, vesselType) %>%
  summarize(n= n(),
            totArea = sum(Area_inside),
            meanOI = mean(Mean_OI),
            maxOI = mean(Max_OI),
            meanOI_norm = meanOI/totArea*100000,
            maxOI_norm = maxOI/totArea*100000)
```

```
## 'summarise()' has grouped output by 'img', 'mouseID', 'treatment', 'tissue'.
## You can override using the '.groups' argument.
```

```
fitc_diffs_norm
```

```
## # A tibble: 55 x 11
## # Groups:   img, mouseID, treatment, tissue [46]
##    img          mouseID treat~1 tissue vesse~2     n totArea meanOI maxOI meanO~3
##    <chr>        <fct>   <fct>   <fct>  <chr>   <int>   <dbl>  <dbl> <dbl>   <dbl>
##  1 29-periph-~  29      untrea~ retina C          14   5515.  0.251 0.319    4.55
##  2 29-periph-~  29      untrea~ retina C          25   2735.  0.221 0.310    8.08
##  3 29-periph-~  29      untrea~ retina C          19   2069.  0.257 0.230   12.4
##  4 29-periph-~  29      untrea~ retina C          21   5423.  0.207 0.271    3.81
##  5 29-periph-~  29      untrea~ retina C          15   6519.  0.237 0.396    3.64
##  6 29-periph-~  29      untrea~ retina C          24   7224.  0.257 0.337    3.56
##  7 29-PVN-ish~  29      untrea~ brain  C          23  12265.  0.278 0.271    2.27
##  8 29-PVN-ish~  29      untrea~ brain  C          27   9249   0.254 0.442    2.75
##  9 62-central~  62      untrea~ retina AV          2  12599.  0.428 0.645    3.40
## 10 62-central~  62      untrea~ retina C           8   3461.  0.287 0.654    8.29
## # ... with 45 more rows, 1 more variable: maxOI_norm <dbl>, and abbreviated
## #   variable names 1: treatment, 2: vesselType, 3: meanOI_norm
```

```
levels(fitc_diffs_norm$vesselType)
```

```
## NULL
```

```
fitc_diffs_norm_trt <- fitc_diffs_norm %>% group_by(treatment, vesselType) %>%
  summarize(n= n(),
            Mean_OI = mean(meanOI),
            Max_OI = mean(maxOI),
            Mean_OI_norm = mean(meanOI_norm),
            Max_OI_norm = mean(maxOI_norm))
```

```
## 'summarise()' has grouped output by 'treatment'. You can override using the
## '.groups' argument.
```

```
fitc_diffs_norm_trt
```

```
## # A tibble: 6 x 7
## # Groups:   treatment [3]
##   treatment vesselType     n Mean_OI Max_OI Mean_OI_norm Max_OI_norm
##   <fct>     <chr>      <int>   <dbl>  <dbl>        <dbl>       <dbl>
## 1 morphine  AV             4   0.331  0.592         2.85        5.20
## 2 morphine  C             16   0.235  0.455         5.60       11.6
## 3 saline    AV             4   0.301  0.715         3.63        8.85
## 4 saline    C             16   0.277  0.456         4.81        9.25
## 5 untreated AV             1   0.428  0.645         3.40        5.12
## 6 untreated C             14   0.272  0.401         4.88        7.05
```

```
levels(fitc_diffs_norm_trt$vesselType)
```

```
## NULL
```

```
fitc_diffs_norm %>% group_by(img,mouseID,vesselType) %>% ggplot(aes(x=treatment, y=meanOI)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID))+
  facet_wrap(tissue~vesselType)
```
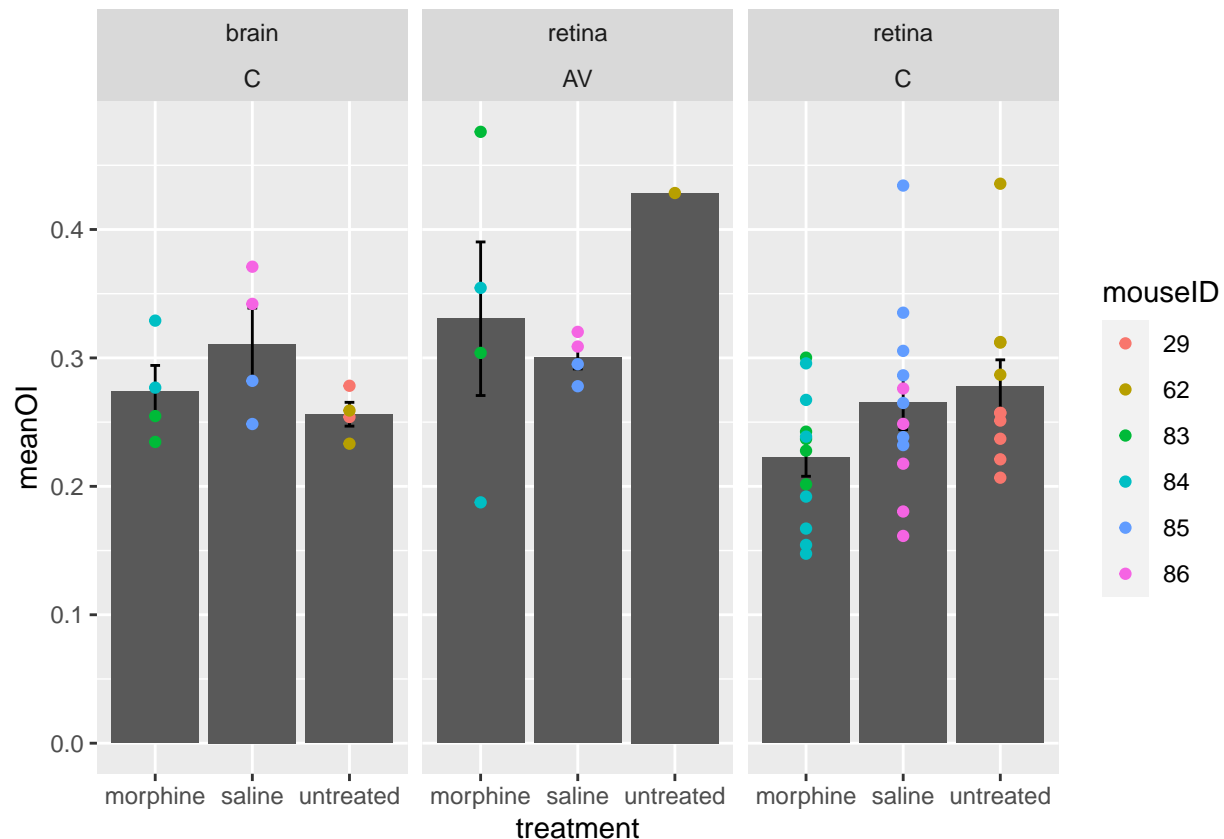


```
fitc_diffs_norm %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI_norm)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
```

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```
geom_point(aes(col=mouseID))+
facet_wrap(tissue~vesselType, scales="free_y")
```
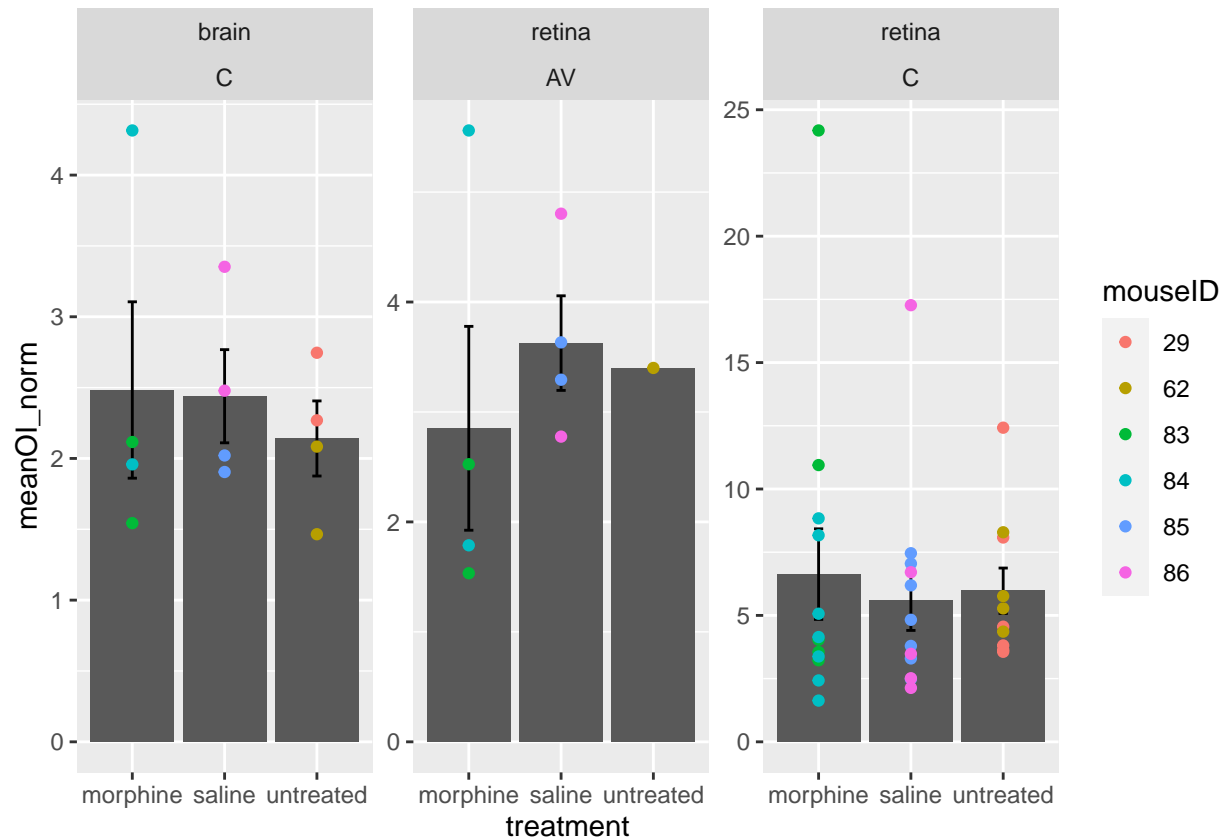


```
#across vessel types
fitc_diffs_norm2 <- fitc_diffs %>% group_by(treatment, mouseID, tissue) %>%
  summarize(n= n(),
            totArea = sum(Area_inside),
            meanOI = mean(Mean_OI),
            maxOI = mean(Max_OI),
            meanOI_norm = meanOI/totArea*100000,
            maxOI_norm = maxOI/totArea*100000)
```

```
## 'summarise()' has grouped output by 'treatment', 'mouseID'. You can override
## using the '.groups' argument.
```

```
fitc_diffs_norm2
```

```
## # A tibble: 12 x 9
## # Groups:   treatment, mouseID [6]
##    treatment mouseID tissue     n totArea meanOI maxOI meanOI_norm maxOI_norm
##    <fct>     <fct>   <fct>  <int>   <dbl>  <dbl> <dbl>       <dbl>      <dbl>
## 1 morphine  83      brain     70  27236.  0.243 0.463       0.893       1.70
## 2 morphine  83      retina    49  62998.  0.272 0.463       0.431      0.736
## 3 morphine  84      brain     55  21763.  0.299 0.467       1.37        2.15
```

```
##  4 morphine   84         retina   54  55293.  0.222 0.424       0.402       0.766
##  5 saline     85         brain    34  27011.  0.265 0.390       0.982       1.44
##  6 saline     85         retina   95  65370.  0.305 0.437       0.466       0.668
##  7 saline     86         brain    68  24868.  0.357 0.488       1.44        1.96
##  8 saline     86         retina   44  44847.  0.219 0.494       0.487       1.10
##  9 untreated 29          brain    50  21514.  0.265 0.363       1.23        1.69
## 10 untreated 29          retina  118  29486.  0.237 0.307       0.805       1.04
## 11 untreated 62          brain    51  28348.  0.247 0.413       0.873       1.46
## 12 untreated 62          retina   48  36894.  0.354 0.545       0.959       1.48
```
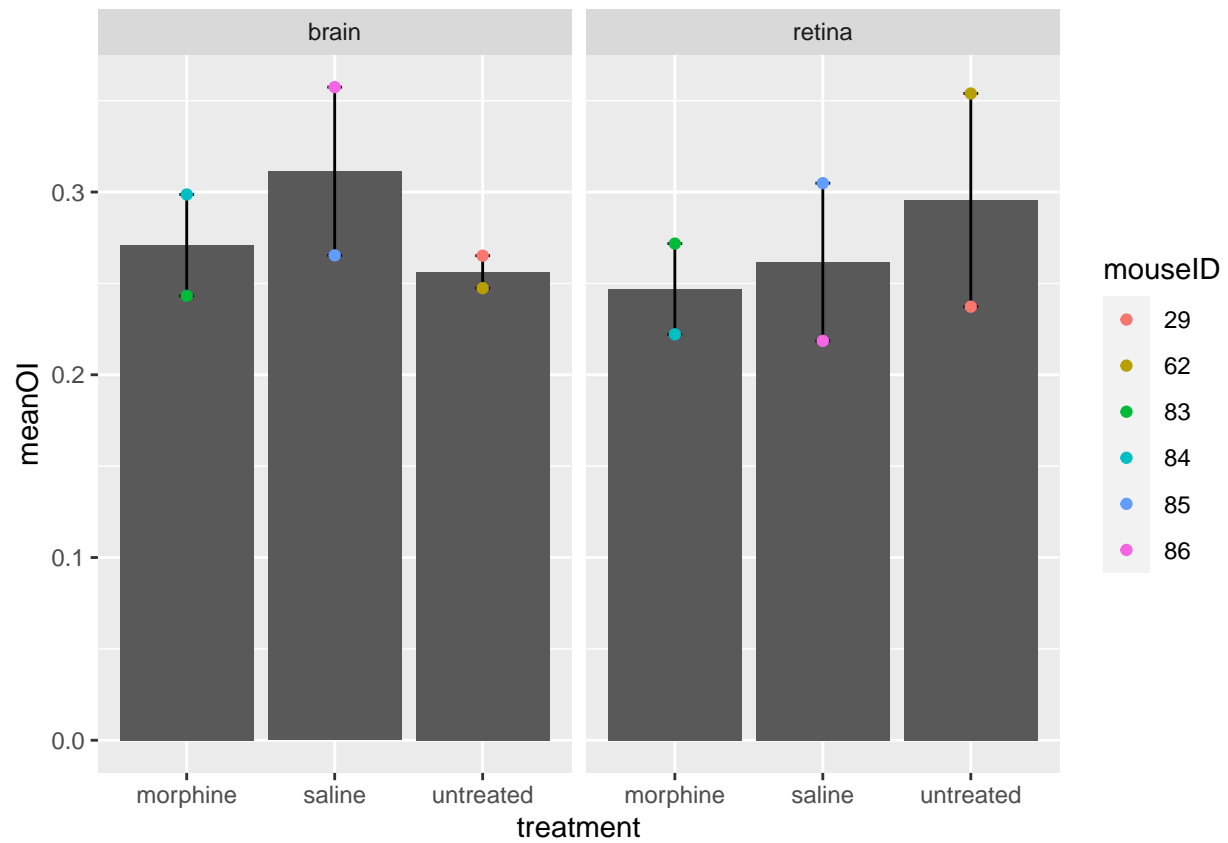
```r
fitc_diffs_norm_trt2 <- fitc_diffs_norm2 %>% group_by(treatment, tissue) %>%
  summarize(n= n(),
          Mean_OI = mean(meanOI),
          Max_OI = mean(maxOI),
          Mean_OI_norm = mean(meanOI_norm),
          Max_OI_norm = mean(maxOI_norm))
```

```
## 'summarise()' has grouped output by 'treatment'. You can override using the
## '.groups' argument.
```

```r
fitc_diffs_norm_trt2
```
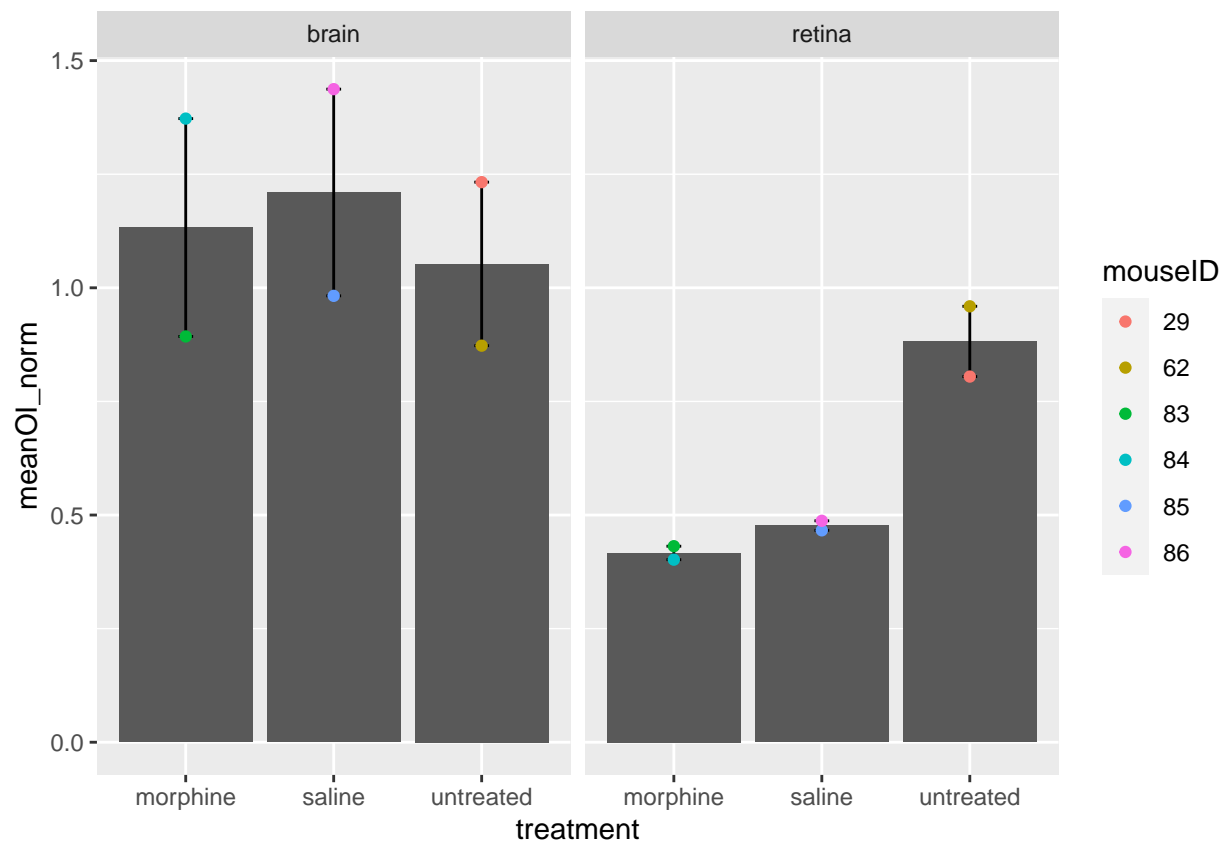
```
## # A tibble: 6 x 7
## # Groups:   treatment [3]
##    treatment tissue     n Mean_OI Max_OI Mean_OI_norm Max_OI_norm
##    <fct>     <fct> <int>   <dbl>  <dbl>        <dbl>       <dbl>
## 1 morphine  brain      2   0.271  0.465        1.13        1.92
## 2 morphine  retina     2   0.247  0.443        0.417       0.751
## 3 saline    brain      2   0.311  0.439        1.21        1.70
## 4 saline    retina     2   0.262  0.466        0.477       0.885
## 5 untreated brain      2   0.256  0.388        1.05        1.57
## 6 untreated retina     2   0.296  0.426        0.882       1.26
```

```r
fitc_diffs_norm2 %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID)) +
  facet_wrap(~tissue)
```

```
fitc_diffs_norm2 %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI_norm)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID)) +
  facet_wrap(~tissue)
```

```r
csv_list_dex <- list.files(path="D:/Microscopy/IHC images/FITC-perf-analysis/FITC-dextran/processing/MA
csv_cols_dex <- c("Label", "Area", "Mean", "Min", "Max", "%Area")
fitc_csvs_temp_dex <- as.data.frame(matrix(,0,length(csv_cols_dex)))
names(fitc_csvs_temp_dex) <- csv_cols_dex
fitc_csvs_temp_dex
```

```
## [1] Label Area  Mean  Min   Max   %Area
## <0 rows> (or 0-length row.names)
```

```r
for (i in csv_list_dex) {
  test <- read_csv(i, show_col_types=FALSE)
  test <- test %>% select(Label:"%Area")
  fitc_csvs_temp_dex <- rbind(fitc_csvs_temp_dex, test)
}
```

```
## New names:
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```

```
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## New names:
## * `` -> `...1`
```

```r
fitc_csvs_dex <- fitc_csvs_temp_dex %>% mutate(
  mouseID = as.factor(str_extract(Label, "^[:digit:]+")),
  tissue = as.factor(str_extract(Label, "cap|A[V]?|PVN"))
)

fitc_csvs_dex <- fitc_csvs_dex %>% mutate(
  tissue = as.factor(case_when(tissue %in% c("A", "AV", "V", "cap") ~ "retina",
                      tissue == "PVN" ~ "brain")),
  objLabel = str_extract(Label, "(?<=:).*"),
  vesselType = as.factor(str_extract(objLabel, "^[:alpha:]")),
  vesselNum = as.numeric(str_extract(objLabel, "[:digit:]+")),
  location = str_extract(objLabel, "(?<=-).*"),
  img = str_extract(Label, ".*(?=_f)")
) %>% na.omit() %>% select(!Label)

fitc_csvs_dex <- fitc_csvs_dex %>% mutate(
  vesselType = case_when(vesselType %in% c("A", "AV", "V") ~ "AV",
                      vesselType == "C" ~ "C"))

#fixing problems when there's, e.g., A1 and V1 both being changed to AV1
fitc_csvs_dex <- fitc_csvs_dex %>% ungroup() %>%
  group_by(mouseID, img,vesselType, location) %>%
  mutate(vesselNum = ifelse(duplicated(vesselNum), vesselNum + 1, vesselNum))

fitc_csvs_dex <- fitc_csvs_dex %>% ungroup() %>%
  pivot_wider(id_cols = c(img, mouseID, tissue, vesselType, vesselNum),
              names_from = location,
              values_from = c(Area, Mean, Min, Max, "%Area"))
```

```
## Warning: Values from `%Area`, `Area`, `Max`, `Mean` and `Min` are not uniquely identified; output wil
## * Use `values_fn = list` to suppress this warning.
## * Use `values_fn = {summary_fun}` to summarise duplicates.
## * Use the following dplyr code to identify duplicates.
##   {data} %>%
##     dplyr::group_by(img, mouseID, tissue, vesselType, vesselNum, location) %>%
```

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```
##       dplyr::summarise(n = dplyr::n(), .groups = "drop") %>%
##       dplyr::filter(n > 1L)
```

```
fitc_csvs_dex
```

```
## # A tibble: 406 x 15
##     img      mouseID tissue vesse~1 vesse~2 Area_~3 Area_~4 Mean_~5 Mean_~6 Min_i~7
##     <chr>    <fct>   <fct>  <chr>     <dbl> <list>  <list>  <list>  <list>  <list>
##  1 2-cen~ 2         retina AV            1 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  2 2-cen~ 2         retina AV            2 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  3 2-cen~ 2         retina C             1 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  4 2-cen~ 2         retina C             2 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  5 2-cen~ 2         retina C             3 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  6 2-cen~ 2         retina C             4 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  7 2-cen~ 2         retina C             5 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  8 2-cen~ 2         retina C             6 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  9 2-cen~ 2         retina C             7 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 10 2-cen~ 2         retina C             8 <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## # ... with 396 more rows, 5 more variables: Min_outside <list>,
## #   Max_inside <list>, Max_outside <list>, '%Area_inside' <list>,
## #   '%Area_outside' <list>, and abbreviated variable names 1: vesselType,
## #   2: vesselNum, 3: Area_inside, 4: Area_outside, 5: Mean_inside,
## #   6: Mean_outside, 7: Min_inside
```

```
fitc_csvs_dex <- fitc_csvs_dex %>% unnest() %>%
  mutate(treatment = as.factor(case_when(mouseID %in% c(99) ~ "untreated",
                            mouseID %in% c(2,3) ~ "saline",
                            mouseID %in% c(5,6) ~ "morphine")))
```

```
## Warning: 'cols' is now required when using unnest().
## Please use 'cols = c(Area_inside, Area_outside, Mean_inside, Mean_outside, Min_inside,
##     Min_outside, Max_inside, Max_outside, '%Area_inside', '%Area_outside')'
```

```
fitc_csvs_dex
```

```
## # A tibble: 407 x 16
##     img      mouseID tissue vesse~1 vesse~2 Area_~3 Area_~4 Mean_~5 Mean_~6 Min_i~7
##     <chr>    <fct>   <fct>  <chr>     <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
##  1 2-cen~ 2         retina AV            1   2377.   1266.    88.8    11.2       3
##  2 2-cen~ 2         retina AV            2   7931.   2219.    50.9    13.9       0
##  3 2-cen~ 2         retina C             1    53.6    171.    47.0    15.9      28
##  4 2-cen~ 2         retina C             2    55.4    204.    46.8    13.2      26
##  5 2-cen~ 2         retina C             3    61.3    163.    45.5    15.0      27
##  6 2-cen~ 2         retina C             4   110.     299.    47.2    15.4      26
##  7 2-cen~ 2         retina C             5    56.4    171.    77.6    14.2      29
##  8 2-cen~ 2         retina C             6   3464.   3501.   109.     13.4       4
##  9 2-cen~ 2         retina C             7   1194.   1017.   113.     15.5      23
## 10 2-cen~ 2         retina C             8    55.1    194.    43.6    15.9      23
## # ... with 397 more rows, 6 more variables: Min_outside <dbl>,
## #   Max_inside <dbl>, Max_outside <dbl>, '%Area_inside' <dbl>,
## #   '%Area_outside' <dbl>, treatment <fct>, and abbreviated variable names
## #   1: vesselType, 2: vesselNum, 3: Area_inside, 4: Area_outside,
## #   5: Mean_inside, 6: Mean_outside, 7: Min_inside
```

```
fitc_diffs_dex <- fitc_csvs_dex %>%
  group_by(img,mouseID, treatment, tissue, vesselType, vesselNum) %>%
  mutate(Mean_OI = Mean_outside/Mean_inside,
         Max_OI = Max_outside/Max_inside) %>%
  select(img:Area_inside, treatment:Max_OI)
fitc_diffs_dex
```

```
## # A tibble: 407 x 9
## # Groups:   img, mouseID, treatment, tissue, vesselType, vesselNum [406]
##    img          mouseID tissue vesse~1 vesse~2 Area_~3 treat~4 Mean_OI Max_OI
##    <chr>        <fct>   <fct>  <chr>     <dbl>   <dbl> <fct>     <dbl>  <dbl>
##  1 2-central-AV-4~ 2     retina AV          1   2377.  saline   0.126  0.578
##  2 2-central-AV-4~ 2     retina AV          2   7931.  saline   0.272  0.842
##  3 2-central-AV-4~ 2     retina C           1    53.6 saline   0.339  0.474
##  4 2-central-AV-4~ 2     retina C           2    55.4 saline   0.283  0.492
##  5 2-central-AV-4~ 2     retina C           3    61.3 saline   0.329  0.585
##  6 2-central-AV-4~ 2     retina C           4   110.  saline   0.326  0.390
##  7 2-central-AV-4~ 2     retina C           5    56.4 saline   0.183  0.196
##  8 2-central-AV-4~ 2     retina C           6  3464.  saline   0.123  0.624
##  9 2-central-AV-4~ 2     retina C           7  1194.  saline   0.137  0.965
## 10 2-central-AV-4~ 2     retina C           8    55.1 saline   0.364  0.591
## # ... with 397 more rows, and abbreviated variable names 1: vesselType,
## #   2: vesselNum, 3: Area_inside, 4: treatment
```

```
fitc_diffs_norm_dex <- fitc_diffs_dex %>% ungroup() %>%
  group_by(img, mouseID, treatment, tissue, vesselType) %>%
  summarize(n= n(),
            totArea = sum(Area_inside),
            meanOI = mean(Mean_OI),
            maxOI = mean(Max_OI),
            meanOI_norm = meanOI/totArea*100000,
            maxOI_norm = maxOI/totArea*100000)
```

```
## `summarise()` has grouped output by 'img', 'mouseID', 'treatment', 'tissue'.
## You can override using the `.groups` argument.
```

```
fitc_diffs_norm_dex
```

```
## # A tibble: 34 x 11
## # Groups:   img, mouseID, treatment, tissue [27]
##    img          mouseID treat~1 tissue vesse~2     n totArea meanOI maxOI meanO~3
##    <chr>        <fct>   <fct>   <fct>  <chr>   <int>   <dbl>  <dbl> <dbl>   <dbl>
##  1 2-central-~  2       saline  retina AV         2  10307.  0.199 0.710    1.93
##  2 2-central-~  2       saline  retina C          8   5051.  0.260 0.540    5.16
##  3 2-periph-c~  2       saline  retina C         11   9785.  0.219 0.289    2.24
##  4 2-periph-c~  2       saline  retina C          7  10749.  0.340 0.462    3.17
##  5 2-PVN-4avg~  2       saline  brain  C         41   7679.  0.276 0.504    3.60
##  6 2-PVN-4avg~  2       saline  brain  C         18   9331.  0.263 0.358    2.82
##  7 3-central-~  3       saline  retina AV         2  13449.  0.287 0.551    2.14
##  8 3-central-~  3       saline  retina C          4   2370.  0.208 0.528    8.78
##  9 3-periph-c~  3       saline  retina C          1  10161.  0.248 0.514    2.44
## 10 3-periph-c~  3       saline  retina C          7   6661.  0.425 0.709    6.38
```

```
## # ... with 24 more rows, 1 more variable: maxOI_norm <dbl>, and abbreviated
## #   variable names 1: treatment, 2: vesselType, 3: meanOI_norm
```
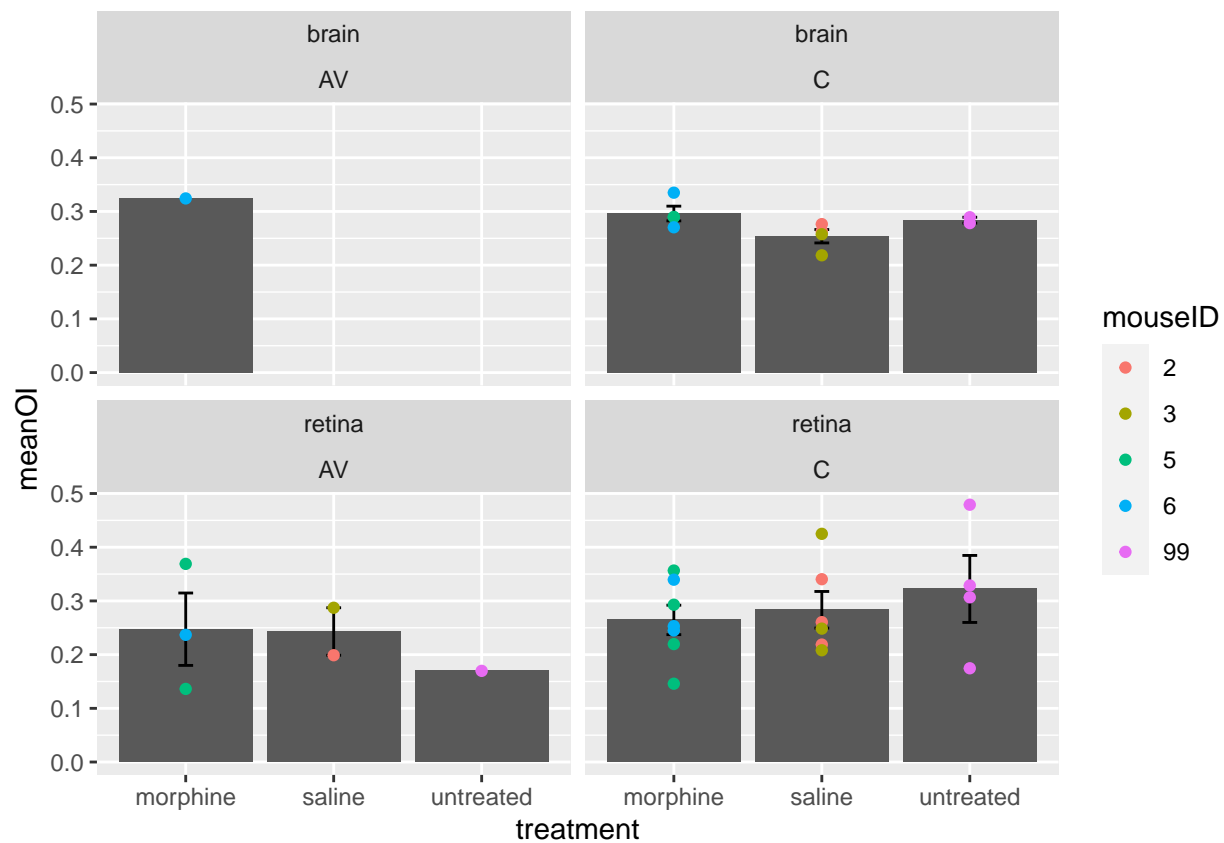
```
fitc_diffs_norm_trt_dex <- fitc_diffs_norm_dex %>% group_by(treatment, vesselType) %>%
  summarize(n= n(),
            Mean_OI = mean(meanOI),
            Max_OI = mean(maxOI),
            Mean_OI_norm = mean(meanOI_norm),
            Max_OI_norm = mean(maxOI_norm))
```

```
## 'summarise()' has grouped output by 'treatment'. You can override using the
## '.groups' argument.
```

```
fitc_diffs_norm_trt_dex
```

```
## # A tibble: 6 x 7
## # Groups:   treatment [3]
##   treatment vesselType     n Mean_OI Max_OI Mean_OI_norm Max_OI_norm
##   <fct>     <chr>      <int>   <dbl>  <dbl>        <dbl>       <dbl>
## 1 morphine  AV             4   0.267  0.599        14.5        19.1
## 2 morphine  C             11   0.276  0.439         4.56        8.64
## 3 saline    AV             2   0.243  0.630         2.03        5.49
## 4 saline    C             10   0.272  0.486         3.70        7.12
## 5 untreated AV             1   0.170  0.972         2.43       13.9
## 6 untreated C              6   0.309  0.453         2.71        4.45
```

```
fitc_diffs_norm_dex %>% group_by(img,mouseID,vesselType) %>% ggplot(aes(x=treatment, y=meanOI)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID))+
  facet_wrap(tissue~vesselType)
```

```
fitc_diffs_norm_dex %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI_norm)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID))+
  facet_wrap(tissue~vesselType, scales="free_y")
```
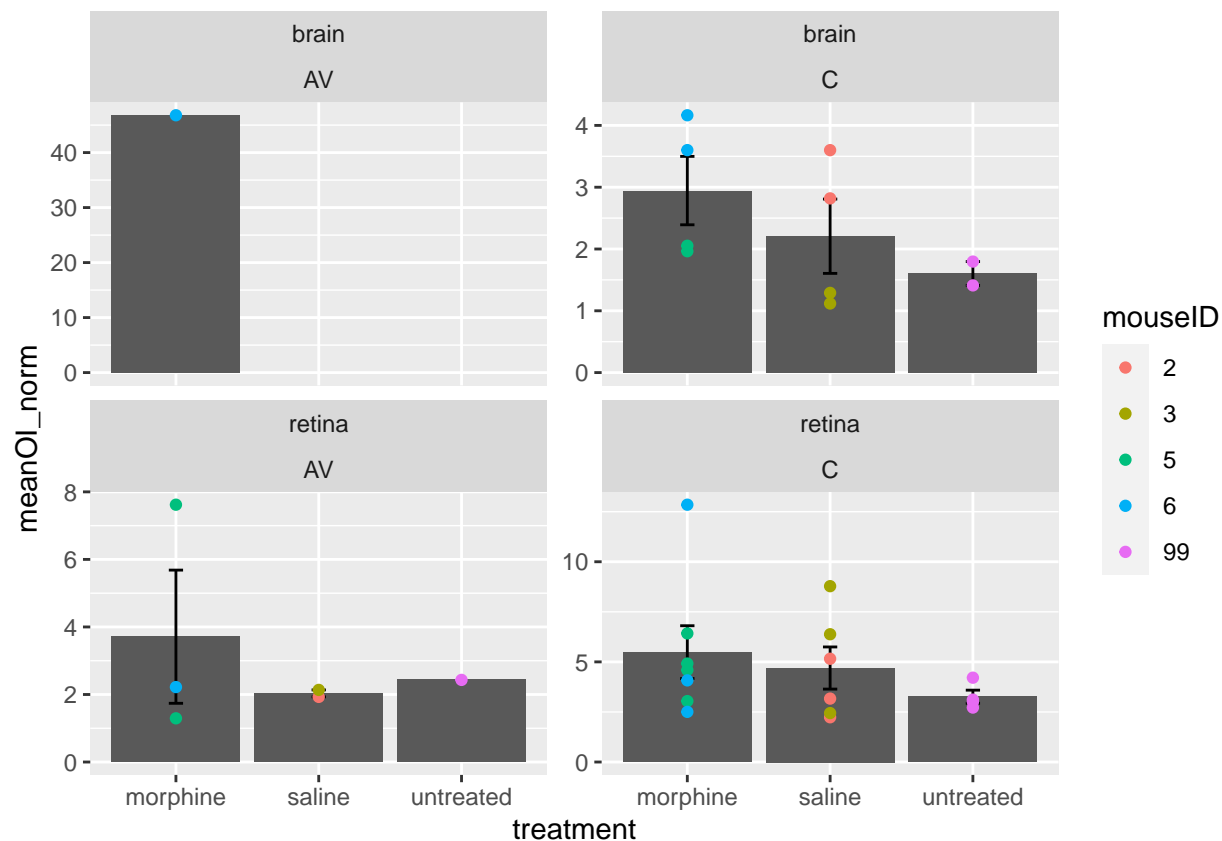
```
#across vessel types
fitc_diffs_norm2_dex <- fitc_diffs_dex %>% group_by(treatment, mouseID, tissue) %>%
  summarize(n= n(),
            totArea = sum(Area_inside),
            meanOI = mean(Mean_OI),
            maxOI = mean(Max_OI),
            meanOI_norm = meanOI/totArea*100000,
            maxOI_norm = maxOI/totArea*100000)
```

```
## 'summarise()' has grouped output by 'treatment', 'mouseID'. You can override
## using the '.groups' argument.
```

```
fitc_diffs_norm2_dex
```

```
## # A tibble: 10 x 9
## # Groups:   treatment, mouseID [5]
##    treatment mouseID tissue     n totArea meanOI maxOI meanOI_norm maxOI_norm
##    <fct>     <fct>   <fct>  <int>   <dbl>  <dbl> <dbl>       <dbl>      <dbl>
## 1 morphine  5       brain     48  28856.  0.290 0.393        1.00       1.36
## 2 morphine  5       retina    55  39139.  0.309 0.429        0.790      1.10
## 3 morphine  6       brain     61  16494.  0.308 0.532        1.87       3.22
## 4 morphine  6       retina    24  30743.  0.277 0.488        0.903      1.59
## 5 saline    2       brain     59  17010.  0.272 0.459        1.60       2.70
## 6 saline    2       retina    28  35892.  0.260 0.434        0.723      1.21
## 7 saline    3       brain     20  39976.  0.238 0.476        0.595      1.19
```

```
##  8 saline    3         retina   14  32641.  0.331 0.621         1.01          1.90
##  9 untreated 99        brain    44  35830.  0.286 0.376         0.797         1.05
## 10 untreated 99        retina   54  45545.  0.350 0.468         0.768         1.03
```
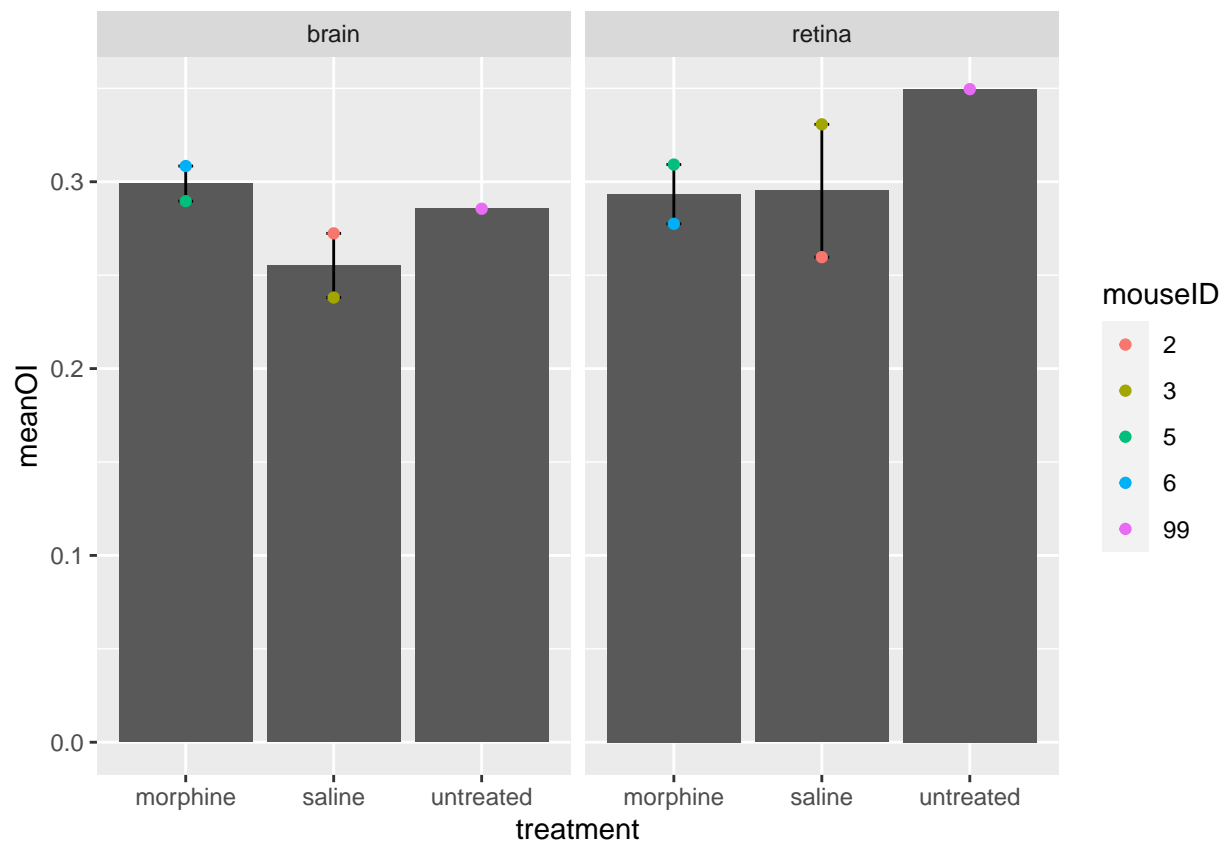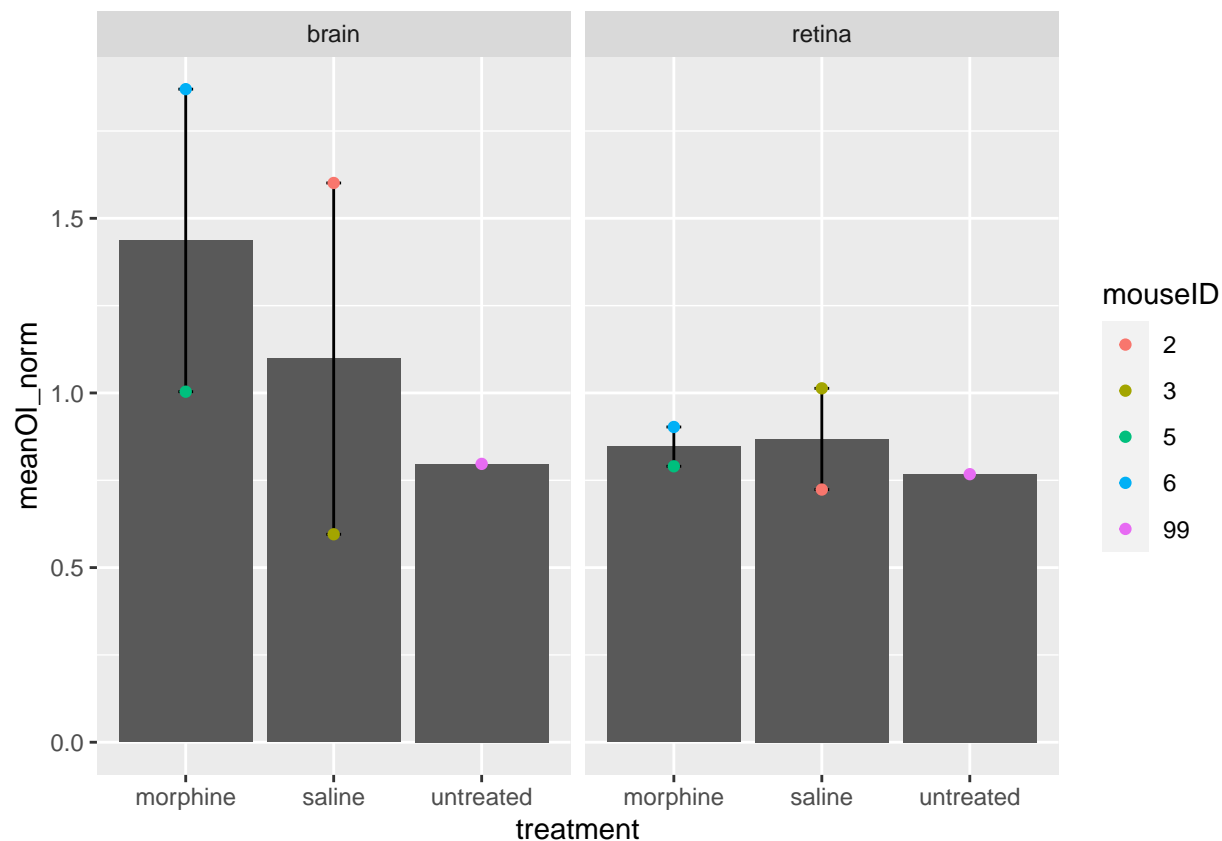
```r
fitc_diffs_norm_trt2_dex <- fitc_diffs_norm2 %>% group_by(treatment, tissue) %>%
  summarize(n= n(),
            Mean_OI = mean(meanOI),
            Max_OI = mean(maxOI),
            Mean_OI_norm = mean(meanOI_norm),
            Max_OI_norm = mean(maxOI_norm))
```

```
## `summarise()` has grouped output by 'treatment'. You can override using the
## `.groups` argument.
```

```r
fitc_diffs_norm_trt2_dex
```

```
## # A tibble: 6 x 7
## # Groups:   treatment [3]
##   treatment tissue     n Mean_OI Max_OI Mean_OI_norm Max_OI_norm
##   <fct>     <fct> <int>   <dbl>  <dbl>        <dbl>       <dbl>
## 1 morphine  brain     2   0.271  0.465         1.13        1.92
## 2 morphine  retina    2   0.247  0.443         0.417       0.751
## 3 saline    brain     2   0.311  0.439         1.21        1.70
## 4 saline    retina    2   0.262  0.466         0.477       0.885
## 5 untreated brain     2   0.256  0.388         1.05        1.57
## 6 untreated retina    2   0.296  0.426         0.882       1.26
```

```r
fitc_diffs_norm2_dex %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID)) +
  facet_wrap(~tissue)
```
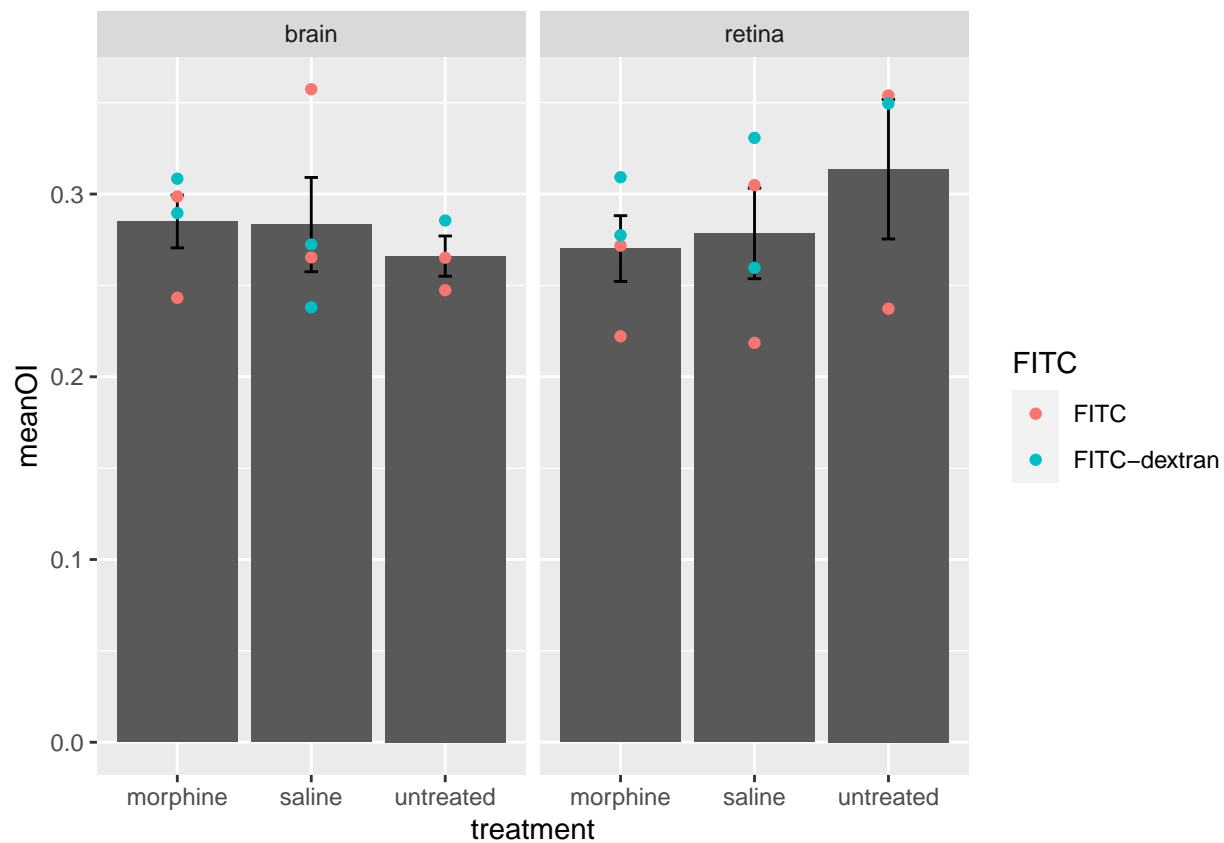
```
fitc_diffs_norm2_dex %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI_norm)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=mouseID)) +
  facet_wrap(~tissue)
```

```
fitc_diffs_norm2 <- fitc_diffs_norm2 %>% mutate(FITC = as.factor("FITC"))
fitc_diffs_norm2_dex <- fitc_diffs_norm2_dex %>% mutate(FITC = as.factor("FITC-dextran"))

fitc_diffs_comb <- rbind(fitc_diffs_norm2, fitc_diffs_norm2_dex) %>% ungroup()


fitc_diffs_comb %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
  geom_point(aes(col=FITC)) +
  facet_wrap(~tissue)
```

```
fitc_diffs_comb %>% group_by(mouseID) %>% ggplot(aes(x=treatment, y=meanOI_norm)) +
    stat_summary(fun = 'mean', geom="bar") +
    stat_summary(fun.data = mean_se, geom = "errorbar", width=.1) +
    geom_point(aes(col=FITC)) +
    facet_wrap(~tissue)
```
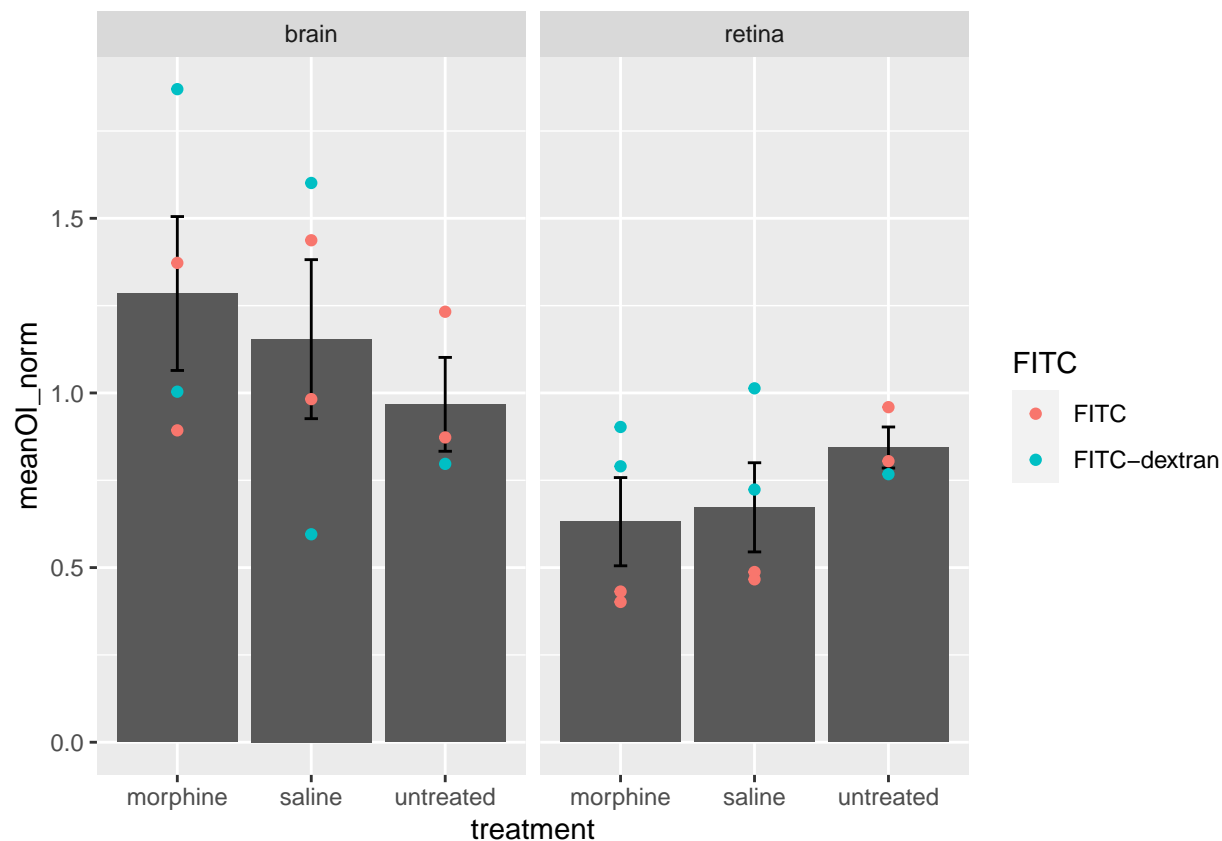
```
fitc_anova <- lmer(meanOI ~ tissue * treatment * FITC + (1|mouseID), data=fitc_diffs_comb)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
comb_anova <- lmer(meanOI ~ tissue * treatment + (1|mouseID), data=fitc_diffs_comb)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
anova(fitc_anova, comb_anova)
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: fitc_diffs_comb
## Models:
## comb_anova: meanOI ~ tissue * treatment + (1 | mouseID)
## fitc_anova: meanOI ~ tissue * treatment * FITC + (1 | mouseID)
##            npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## comb_anova    8 -65.837 -57.108 40.918  -81.837
## fitc_anova   14 -62.051 -46.777 45.026  -90.051 8.2147  6     0.2228
```
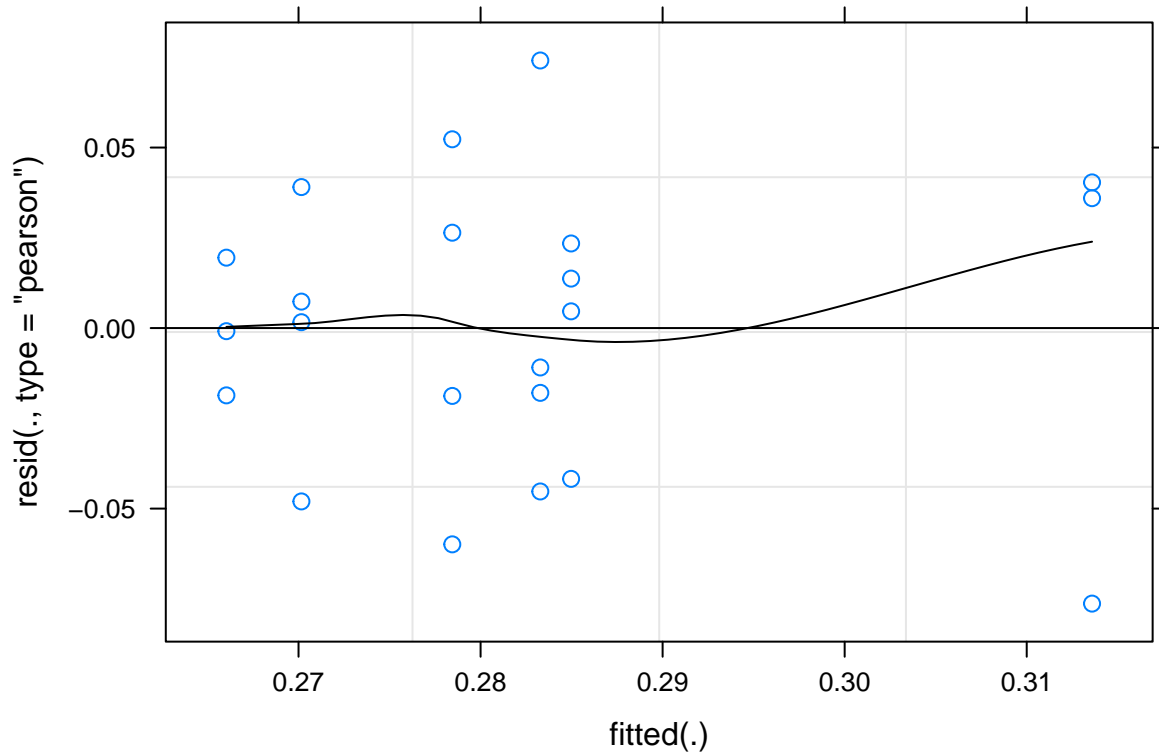
```
shapiro.test(fitc_diffs_comb$meanOI)
```

```
## 
##  Shapiro-Wilk normality test
## 
## data:  fitc_diffs_comb$meanOI
## W = 0.95603, p-value = 0.4133
```
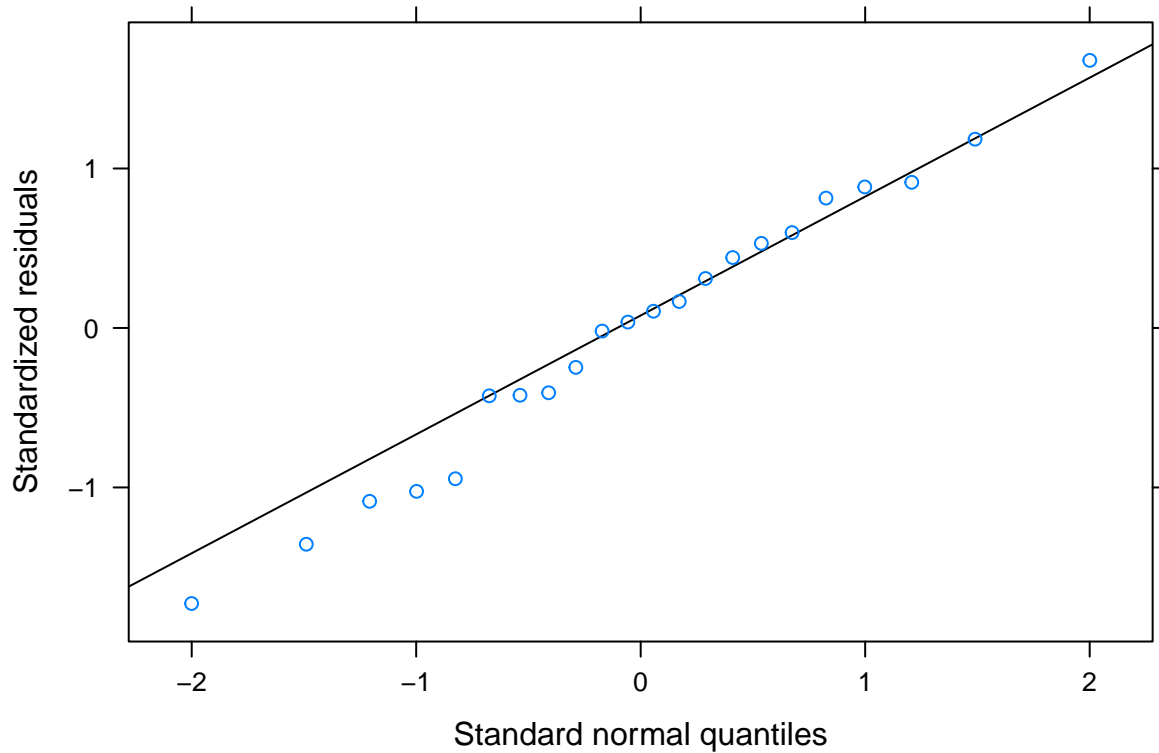
```
car::leveneTest(meanOI ~ tissue * treatment, data=fitc_diffs_comb)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  5  0.3743 0.8589
##       16
```

```
plot(comb_anova, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(comb_anova)
```

```
anova(comb_anova)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                     Sum Sq    Mean Sq NumDF DenDF F value Pr(>F)
## tissue           0.0004671 0.00046706     1    16  0.2394 0.6313
## treatment        0.0005336 0.00026682     2    16  0.1367 0.8732
## tissue:treatment 0.0036910 0.00184551     2    16  0.9458 0.4090
```

```
emmeans::emmeans(comb_anova, pairwise ~ treatment | tissue)$contrasts
```

```
## tissue = brain:
##  contrast              estimate     SE df t.ratio p.value
##  morphine - saline      0.00169 0.0312 16   0.054  0.9984
##  morphine - untreated   0.01894 0.0337 16   0.561  0.8422
##  saline - untreated     0.01725 0.0337 16   0.511  0.8670
##
## tissue = retina:
##  contrast              estimate     SE df t.ratio p.value
##  morphine - saline     -0.00829 0.0312 16  -0.265  0.9620
##  morphine - untreated  -0.04343 0.0337 16  -1.287  0.4222
##  saline - untreated    -0.03514 0.0337 16  -1.042  0.5624
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
emmeans::emmeans(comb_anova, pairwise ~ tissue | treatment)$contrasts
```

```
## treatment = morphine:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina  0.01481 0.0312  8   0.474  0.6480
##
## treatment = saline:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina  0.00484 0.0312  8   0.155  0.8808
##
## treatment = untreated:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina -0.04755 0.0361  8  -1.318  0.2239
##
## Degrees-of-freedom method: kenward-roger
```