# A guide to robust statistical methods in neuroscience: Figure 8

Rand R. Wilcox & Guillaume A. Rousselet

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## Contents



**Development of Anxiety-Like Behaviors after Mild Traumatic Brain Injury**
Almeida-Suhett et al. (2014)

The experimental groups (5-6 week old male, Sprague–Dawley rats) received a mild controlled cortical impact (CCI). The outcome measure was stereologically estimated total number of GAD-67-positive cells in the basolateral amygdala (BLA). There are three independent groups: sham-treated controls that received a craniotomy, but no CCI injury, measures taken 1 day after CCI, and measures taken 7 days later.

## Dependencies

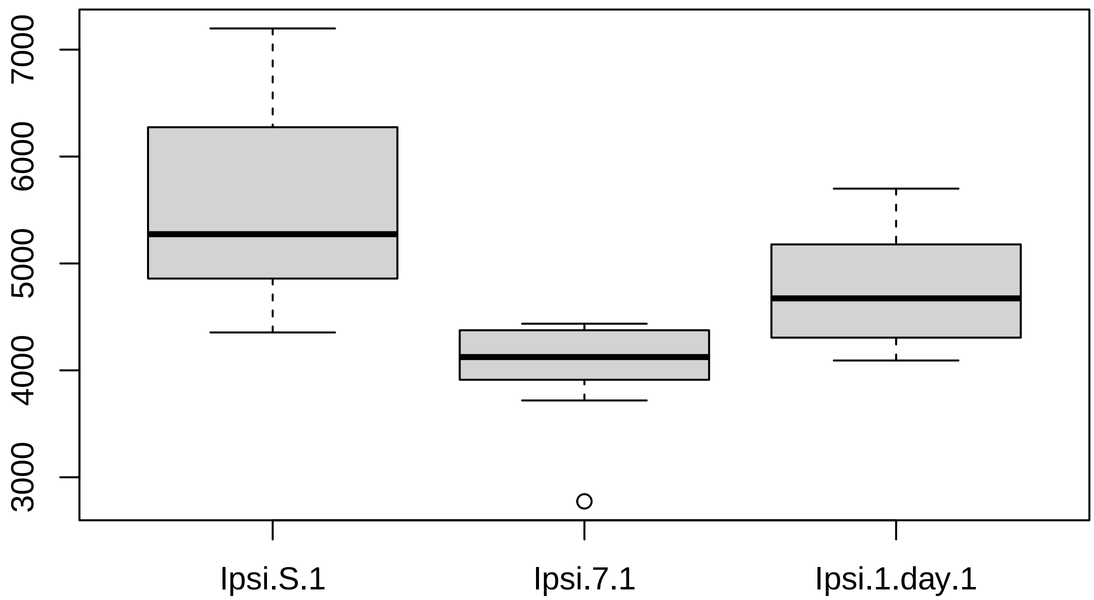
```
library(ggplot2)
library(tibble)
library(tidyr)
source("./code/Rallfun-v40.txt")
source("./code/theme_gar.txt")
```

## Load data

```
# First six columns deal with neurons, final six with GAD-67
prager=read.table('./data/Prager_Behvior_data.tex',skip=1,header=T)
```

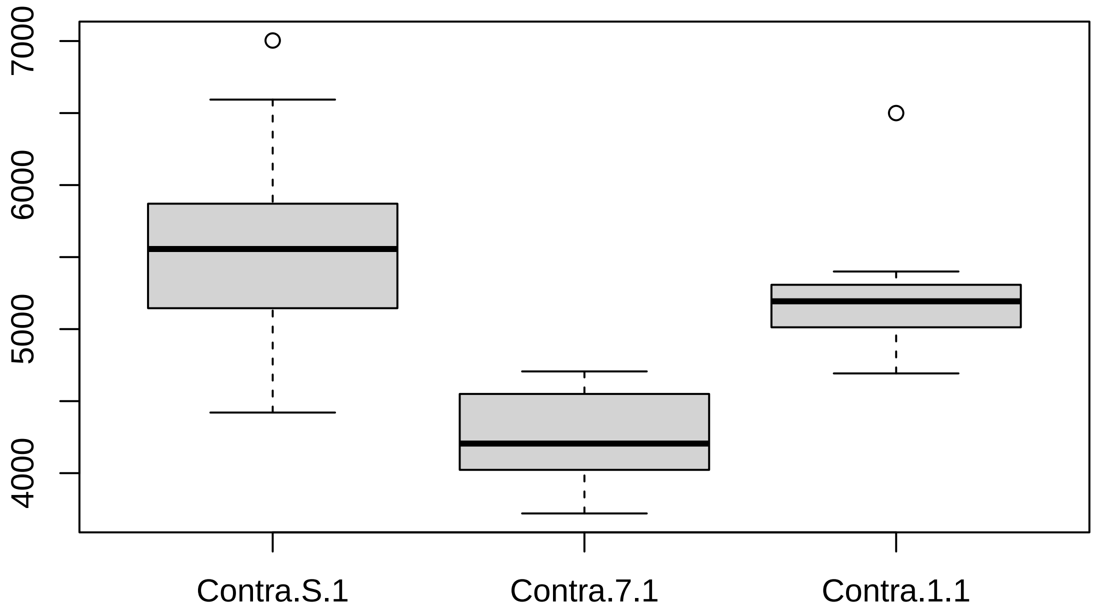
## base R ipsi boxplots

```
boxplot(prager[,c(7,9,11)])
```




## base R contra boxplots

```
boxplot(prager[,c(8,10,12)])
```




\# Make ggplot2 figure

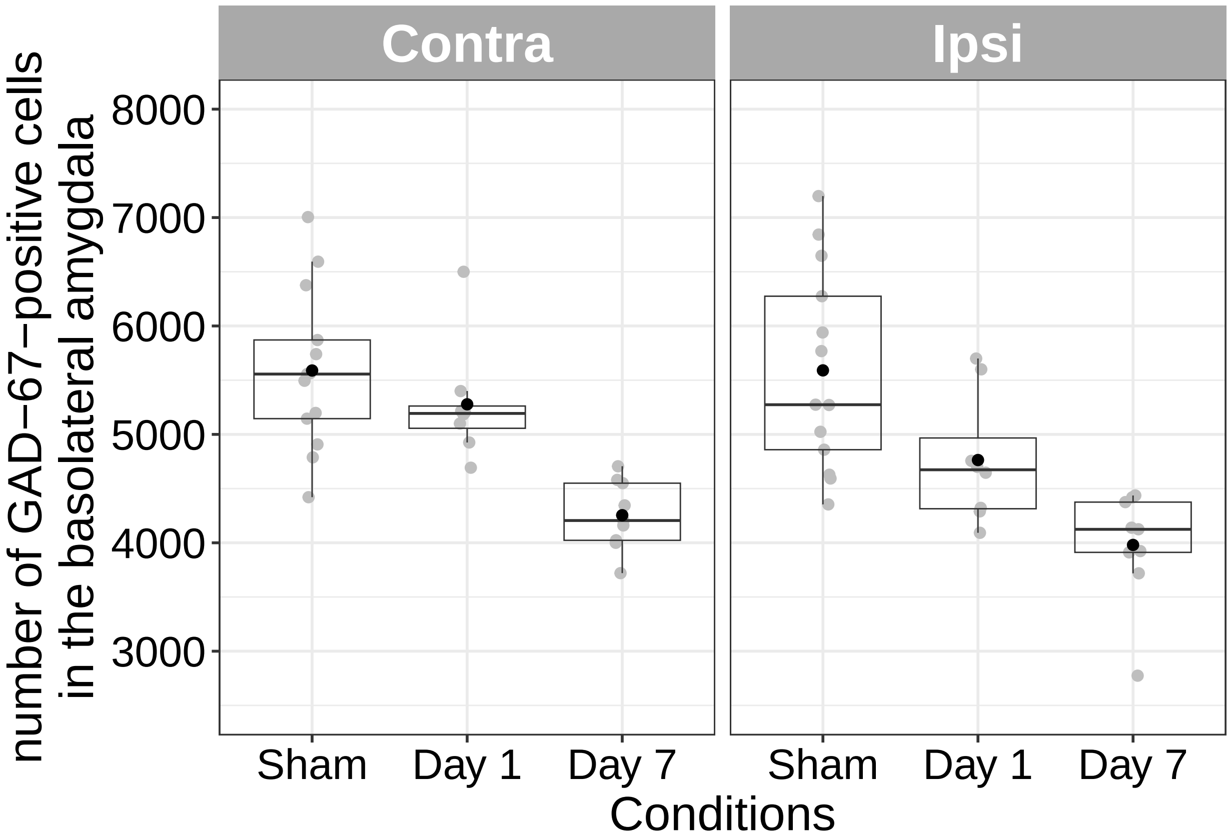
### Load data and reformat

```
# ipsi <- prager[,c(7,9,11)]
# contra <- prager[,c(8,10,12)]
df <- as_tibble(prager[,7:12])
df <- gather(df, group, BLA, na.rm=TRUE)
df <- separate(df, group, c("location", "condition"), sep = "\\.")
df$BLA <- as.numeric(df$BLA)
df$location <- as.factor(df$location)
df$condition <- factor(df$condition, levels = c("S", "1", "7"))
```

### Create figure

```
ggplot(df, aes(condition, BLA)) + theme_gar +
  geom_jitter(colour = "grey", width = 0.05) +
  geom_boxplot(outlier.colour = "grey70", outlier.shape = 16,
               outlier.size = 3, outlier.alpha = 0, size = 0.25, alpha = 0) +
  stat_summary(fun = "mean",  geom = "point", size = 1.5) +
  theme(axis.text.x = element_text(size = 16),
        axis.text.y = element_text(size = 16),
        axis.title.x = element_text(size = 18),
        axis.title.y = element_text(size = 18),
        plot.title = element_text(size = 20),
        legend.position="none") +
  scale_x_discrete(breaks = c("S", "1", "7"),
                   labels = c("Sham", "Day 1", "Day 7")) +
  labs(x = "Conditions",
       y = "number of GAD-67-positive cells\nin the basolateral amygdala") +
  scale_y_continuous(limits = c(2500, 8000), breaks = seq(3000, 8000, 1000)) +
  facet_grid(. ~ location,
```

```
             labeller = labeller(group = c(Ipsi = "Ipsilateral",
                                           Contra = "Contralateral"))) +
  theme(strip.text.x = element_text(size = 20, colour = "white"),
        strip.background = element_rect(colour="darkgrey", fill="darkgrey"))
```




```
# save figure
ggsave(filename='./figures/figure8.pdf',width=6,height=6)
```

## Comparisons: ipsilateral conditions

### Standard t-test

#### Day 7 vs control (sham)

```
t.test(prager[,7],prager[,9],var.equal =T)
```

```
##
##  Two Sample t-test
##
## data:  prager[, 7] and prager[, 9]
## t = 4.6903, df = 20, p-value = 0.0001406
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##   894.0488 2326.2425
## sample estimates:
```

```
## mean of x mean of y
##  5590.382  3980.237
```

### Day 1 vs control (sham)

```
t.test(prager[,7],prager[,11],var.equal =T)
```

```
##
##  Two Sample t-test
##
## data:  prager[, 7] and prager[, 11]
## t = 2.2366, df = 19, p-value = 0.0375
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##    53.10298 1601.01163
## sample estimates:
## mean of x mean of y
##  5590.382  4763.325
```

So by Bonferroni, fail to reject sham vs. Day 1. But reject sham vs Day 7. Note, however, that for Day 1, the improvement on the Bonferroni method derived by Hochberg (1988) rejects.

```
p.adjust(c(0.0375, 0.0001406), method = "hochberg")
```

```
## [1] 0.0375000 0.0002812
```

Now repeat this using a 20% trimmed mean using a non-bootstrap method followed by a bootstrap method, then compare medians, then Cliff's improvement of WMW, next compare medians using the Harrell–Davis estimator, followed by the symmetry test, and finally use the median of D=X-Y.

Again focus on Ipsi and day 1 vs. sham.

## Ipsi: Sham vs. Day 1

### Non-bootstrap 20% trimmed mean

```
yuen(prager[,7],prager[,11])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $est.1
## [1] 5520.637
##
## $est.2
## [1] 4719.068
##
## $ci
## [1] -107.2539 1710.3905
##
## $p.value
```

```
## [1] 0.07908206
##
## $dif
## [1] 801.5683
##
## $se
## [1] 420.6771
##
## $teststat
## [1] 1.905424
##
## $crit
## [1] 2.160379
##
## $df
## [1] 12.99937
```

### 20% trimmed mean + bootstrap

```
set.seed(44)
nboot <- 2000
trimpb2(prager[,7],prager[,11], nboot=nboot)
```

```
## $Est1
## [1] 5520.637
##
## $Est2
## [1] 4719.068
##
## $p.value
## [1] 0.031
##
## $ci
## [1]   67.46667 1591.94278
##
## $est.dif
## [1] 801.5683
```

In this situation, with relatively small sample sizes, the expectation is that trimpb2 (bootstrap method) is better than yuen. As illustrated here, the choice between these two methods can make a practical difference.

### medians + bootstrap

```
set.seed(44)
medpb2(prager[,7],prager[,11], nboot=nboot)
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $p.value
```

```
## [1] 0.079
##
## $ci
## [1] -153.965 1919.925
##
## $est1
## [1] 5273.74
##
## $est2
## [1] 4673.385
##
## $est.dif
## [1] 600.355
```

### Improved Wilcoxon-Mann-Whitney

```
cidv2(prager[,7],prager[,11])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $d.hat
## [1] 0.5576923
##
## $d.ci
## [1] 0.04469286 0.83794455
##
## $p.value
## [1] 0.04
##
## $p.hat
## [1] 0.2211538
##
## $p.ci
## [1] 0.08102772 0.47765357
##
## $summary.dvals
##         P(X<Y) P(X=Y)    P(X>Y)
## [1,] 0.2211538      0 0.7788462
```

### Harrell-Davis estimator + bootstrap

```
set.seed(44)
pb2genMC(prager[,7],prager[,11],est=hd, nboot=nboot)
```

```
## $est.1
## [1] 5464.464
##
## $est.2
```

```
## [1] 4659.006
##
## $ci
## [1]   -2.811687 1679.964766
##
## $p.value
## [1] 0.052
##
## $sq.se
## [1] 182482.2
##
## $n1
## [1] 13
##
## $n2
## [1] 8
```

### Symmetry test based on D=X-Y

```
set.seed(44)
cbmhd(prager[,7],prager[,11], nboot=nboot)
```

```
## $q
## [1] 0.25
##
## $Est1
## [1] 90.77639
##
## $Est2
## [1] 1611.567
##
## $sum
## [1] 1702.343
##
## $ci
## [1]  302.0517 2978.2497
##
## $p.value
## [1] 0.014
```

If the two distributions are identical, the distribution of $D = X - Y$ should be symmetric about zero, so the sum of the lower and upper quartiles should be zero. The symmetry test performed by `cbmhd` indicates that this is not the case. Even the lower quartile is estimated to be greater than zero.

### Median of the typical difference between randomly sampled observations from each group

That is,focus on D=X-Y rather than the median of X and Y.

```
set.seed(44)
wmwpb(prager[,7],prager[,11], nboot=nboot)
```

```
## $estimate
## [1] 688.155
##
```

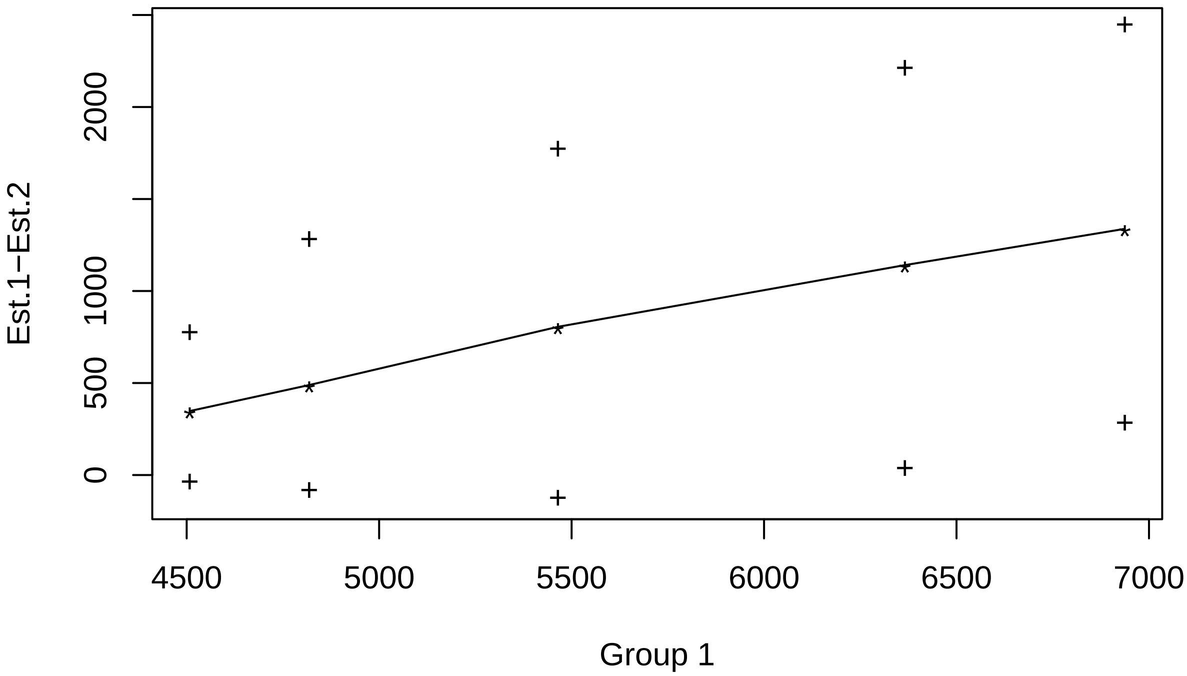
```
## $ci
## [1]  158.72 1574.49
##
## $p.value
## [1] 0.02
```

Did not reject with medians, $p = 0.086$, but based on the median of typical difference, $p = 0.021$, merely illustrating that the point of view can matter.

### Shift function (compare deciles)

Comparing quantiles via qcomhd, the 0.75 and 0.9 quantiles differ ($p = 0.40$ and $p < 0.001$), otherwise no significant differences.

```
set.seed(44)
qcomhd(prager[,7],prager[,11], nboot=nboot)
```




```
##          q n1 n2    est.1    est.2 est.1_minus_est.2     ci.low     ci.up
## [1,] 0.10 13  8 4507.802 4160.134          347.6679  -49.35483  762.4518
## [2,] 0.25 13  8 4818.187 4329.077          489.1101  -95.29215 1268.4374
## [3,] 0.50 13  8 5464.464 4659.006          805.4579 -137.23238 1759.5477
## [4,] 0.75 13  8 6365.796 5225.087         1140.7096   24.19753 2198.9816
## [5,] 0.90 13  8 6937.136 5599.383         1337.7528  270.63915 2434.3647
##      p-value adj.p.value
## [1,]   0.072       0.072
## [2,]   0.051       0.072
## [3,]   0.062       0.072
## [4,]   0.010       0.040
## [5,]   0.000       0.000
```

## Ipsi: Sham vs. Day 7

### Non-bootstrap 20% trimmed mean

```
yuen(prager[,7],prager[,9])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 9
##
## $est.1
## [1] 5520.637
##
## $est.2
## [1] 4087.336
##
## $ci
## [1]  651.7428 2214.8591
##
## $p.value
## [1] 0.002158218
##
## $dif
## [1] 1433.301
##
## $se
## [1] 351.3947
##
## $teststat
## [1] 4.078892
##
## $crit
## [1] 2.224161
##
## $df
## [1] 10.1337
```

### 20% trimmed mean + bootstrap

```
set.seed(44)
nboot <- 2000
trimpb2(prager[,7],prager[,9], nboot=nboot)
```

```
## $Est1
## [1] 5520.637
##
## $Est2
## [1] 4087.336
##
## $p.value
## [1] 0
```

```
##
## $ci
## [1]  873.060 2222.917
##
## $est.dif
## [1] 1433.301
```

### medians + bootstrap

```
set.seed(44)
medpb2(prager[,7],prager[,9], nboot=nboot)
```

```
## $n1
## [1] 13
##
## $n2
## [1] 9
##
## $p.value
## [1] 0
##
## $ci
## [1]  648.66 2362.85
##
## $est1
## [1] 5273.74
##
## $est2
## [1] 4124.29
##
## $est.dif
## [1] 1149.45
```

### Improved Wilcoxon-Mann-Whitney

```
cidv2(prager[,7],prager[,9])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 9
##
## $d.hat
## [1] 0.9487179
##
## $d.ci
## [1] 0.6620536 0.9932113
##
## $p.value
## [1] 1e-04
##
```

```
## $p.hat
## [1] 0.02564103
##
## $p.ci
## [1] 0.003394325 0.168973180
##
## $summary.dvals
##          P(X<Y) P(X=Y)   P(X>Y)
## [1,] 0.02564103      0 0.974359
```

### Harrell-Davis estimator + bootstrap

```
set.seed(44)
pb2genMC(prager[,7],prager[,9],est=hd, nboot=nboot)
```

```
## $est.1
## [1] 5464.464
##
## $est.2
## [1] 4082.849
##
## $ci
## [1]  784.4126 2263.0703
##
## $p.value
## [1] 0
##
## $sq.se
## [1] 149403
##
## $n1
## [1] 13
##
## $n2
## [1] 9
```

### Symmetry test based on D=X-Y

```
set.seed(44)
cbmhd(prager[,7],prager[,9], nboot=nboot)
```

```
## $q
## [1] 0.25
##
## $Est1
## [1] 761.1658
##
## $Est2
## [1] 2398.585
##
## $sum
## [1] 3159.751
```

```
##
## $ci
## [1] 2014.913 4375.765
##
## $p.value
## [1] 0
```

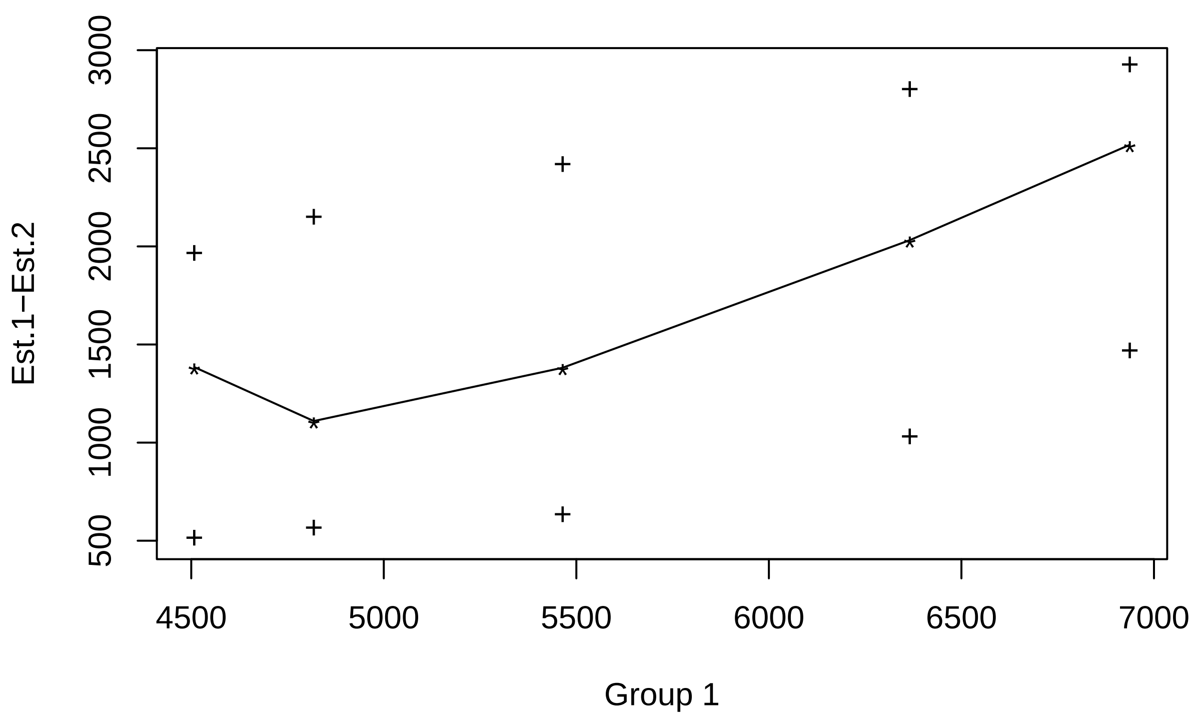
**Median of the typical difference between randomly sampled observations from each group**

```
set.seed(44)
wmwpb(prager[,7],prager[,9], nboot=nboot)
```

```
## $estimate
## [1] 1520.63
##
## $ci
## [1]  851.22 2362.85
##
## $p.value
## [1] 0
```

**Shift function (compare deciles)**

```
set.seed(44)
qcomhd(prager[,7],prager[,9], nboot=nboot)
```




```
##         q n1 n2    est.1    est.2 est.1_minus_est.2    ci.low    ci.up p-value
## [1,] 0.10 13  9 4507.802 3122.971          1384.831  502.3565 1953.559       0
## [2,] 0.25 13  9 4818.187 3708.413          1109.774  553.7445 2138.005       0
## [3,] 0.50 13  9 5464.464 4082.849          1381.614  621.8554 2406.406       0
```

```
## [4,] 0.75 13  9 6365.796 4334.162          2031.635 1018.5626 2788.815       0
## [5,] 0.90 13  9 6937.136 4419.506          2517.629 1456.5692 2914.107       0
##      adj.p.value
## [1,]           0
## [2,]           0
## [3,]           0
## [4,]           0
## [5,]           0
```

## Comparisons: contralateral conditions

Repeat the above using contra.

First do T-tests:

```
t.test(prager[,8],prager[,10],var.equal =T)  # Day 7 vs control (sham)
```

```
##
##  Two Sample t-test
##
## data:  prager[, 8] and prager[, 10]
## t = 5.0662, df = 20, p-value = 5.904e-05
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##   785.1479 1884.2401
## sample estimates:
## mean of x mean of y
##  5589.168  4254.474
```

```
t.test(prager[,8],prager[,12],var.equal =T)
```

```
##
##  Two Sample t-test
##
## data:  prager[, 8] and prager[, 12]
## t = 1.032, df = 19, p-value = 0.315
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -320.5262  944.0407
## sample estimates:
## mean of x mean of y
##  5589.168  5277.411
```

So based on Student's T, sham vs. Day 1, not significant, sham vs. Day 7 is.

Now look at sham vs. Day 7 using robust methods.

### Contra: Sham vs. Day 7

#### Non-bootstrap 20% trimmed mean

```
yuen(prager[,8],prager[,10])
```

```
## $n1
```

```
## [1] 13
##
## $n2
## [1] 9
##
## $est.1
## [1] 5539.229
##
## $est.2
## [1] 4266.234
##
## $ci
## [1]  720.8929 1825.0963
##
## $p.value
## [1] 0.0003365192
##
## $dif
## [1] 1272.995
##
## $se
## [1] 251.7097
##
## $teststat
## [1] 5.057392
##
## $crit
## [1] 2.193406
##
## $df
## [1] 11.3205
```

#### Bootstrap + trimmed means

```
set.seed(44)
trimpb2(prager[,8],prager[,10], nboot = nboot)
```

```
## $Est1
## [1] 5539.229
##
## $Est2
## [1] 4266.234
##
## $p.value
## [1] 0
##
## $ci
## [1]  840.9737 1783.4533
##
## $est.dif
## [1] 1272.995
```

### Medians

```
set.seed(44)
medpb2(prager[,8],prager[,10], nboot = nboot)
```

```
## $n1
## [1] 13
##
## $n2
## [1] 9
##
## $p.value
## [1] 0
##
## $ci
## [1]  647.71 1740.07
##
## $est1
## [1] 5556.19
##
## $est2
## [1] 4205.32
##
## $est.dif
## [1] 1350.87
```

### Cliff

```
cidv2(prager[,8],prager[,10])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 9
##
## $d.hat
## [1] 0.9487179
##
## $d.ci
## [1] 0.6620536 0.9932113
##
## $p.value
## [1] 1e-04
##
## $p.hat
## [1] 0.02564103
##
## $p.ci
## [1] 0.003394325 0.168973180
##
## $summary.dvals
##          P(X<Y) P(X=Y)   P(X>Y)
```

```
## [1,] 0.02564103       0 0.974359
```

### Harrell-Davis estimator

```
set.seed(44)
pb2genMC(prager[,8],prager[,10],est=hd, nboot = nboot)
```

```
## $est.1
## [1] 5521.839
##
## $est.2
## [1] 4249.646
##
## $ci
## [1]  803.2068 1746.5590
##
## $p.value
## [1] 0
##
## $sq.se
## [1] 60471.64
##
## $n1
## [1] 13
##
## $n2
## [1] 9
```

### Symmetry test based on D=X-Y

```
cbmhd(prager[,8],prager[,10], nboot = nboot)
```

```
## $q
## [1] 0.25
##
## $Est1
## [1] 785.1764
##
## $Est2
## [1] 1843.747
##
## $sum
## [1] 2628.923
##
## $ci
## [1] 1733.151 3557.159
##
## $p.value
## [1] 0
```

### Median of the typical difference

```
set.seed(44)
wmwpb(prager[,8],prager[,10], nboot = nboot)
```

```
## $estimate
## [1] 1220.35
##
## $ci
## [1]  851.385 1774.280
##
## $p.value
## [1] 0
```

So even setting FWE (Familywise error, the probability of one or more Type I errors) equal to 0.001, all of the robust methods reject.

## Contra: sham versus Day 1

### Non-bootstrap 20% trimmed mean

```
yuen(prager[,8],prager[,12])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $est.1
## [1] 5539.229
##
## $est.2
## [1] 5171.145
##
## $ci
## [1] -172.6276  908.7954
##
## $p.value
## [1] 0.1607523
##
## $dif
## [1] 368.0839
##
## $se
## [1] 243.418
##
## $teststat
## [1] 1.512148
##
## $crit
## [1] 2.22133
##
```

```
## $df
## [1] 10.23131
```

### Bootstrap + trimmed means

```
set.seed(44)
trimpb2(prager[,8],prager[,12], nboot = nboot)
```

```
## $Est1
## [1] 5539.229
##
## $Est2
## [1] 5171.145
##
## $p.value
## [1] 0.232
##
## $ci
## [1] -233.6828  851.5967
##
## $est.dif
## [1] 368.0839
```

### Medians

```
set.seed(44)
medpb2(prager[,8],prager[,12], nboot = nboot)
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $p.value
## [1] 0.234
##
## $ci
## [1] -242.74  770.85
##
## $est1
## [1] 5556.19
##
## $est2
## [1] 5193.155
##
## $est.dif
## [1] 363.035
```

### Cliff

```
cidv2(prager[,8],prager[,12])
```

```
## $n1
## [1] 13
##
## $n2
## [1] 8
##
## $d.hat
## [1] 0.2884615
##
## $d.ci
## [1] -0.2329653  0.6810648
##
## $p.value
## [1] 0.3
##
## $p.hat
## [1] 0.3557692
##
## $p.ci
## [1] 0.1594676 0.6164826
##
## $summary.dvals
##         P(X<Y) P(X=Y)    P(X>Y)
## [1,] 0.3557692      0 0.6442308
```

### Harrell-Davis estimator

```
set.seed(44)
pb2genMC(prager[,8],prager[,12],est=hd, nboot = nboot)
```

```
## $est.1
## [1] 5521.839
##
## $est.2
## [1] 5180.874
##
## $ci
## [1] -210.2182  829.9328
##
## $p.value
## [1] 0.18
##
## $sq.se
## [1] 63691.75
##
## $n1
## [1] 13
##
## $n2
```

```
## [1] 8
```

### Symmetry test based on D=X-Y

```r
set.seed(44)
cbmhd(prager[,8],prager[,12], nboot = nboot)
```

```
## $q
## [1] 0.25
##
## $Est1
## [1] -240.373
##
## $Est2
## [1] 861.5555
##
## $sum
## [1] 621.1825
##
## $ci
## [1] -464.5627 1691.4453
##
## $p.value
## [1] 0.276
```

### Median of the typical difference

```r
set.seed(44)
wmwpb(prager[,8],prager[,12], nboot = nboot)
```

```
## $estimate
## [1] 340.475
##
## $ci
## [1] -254.83  802.18
##
## $p.value
## [1] 0.263
```

So for contra, sham versus day 1, no significant differences.