Two-way anova example: histamine shock on guinea pig medullar blood vessel surface (mm^2/mm^3)

 # input data

```
replicates <- 10

sex <- 2

controls <- matrix(0, ncol = replicates, nrow = sex)

controls[1,] <- c(6.4, 6.2, 6.9, 6.9, 5.4, 7.5, 6.1, 7.3, 5.9, 6.8)

controls[2,] <- c(4.3, 7.5, 5.2, 4.9, 5.7, 4.3, 6.4, 6.2, 5.0, 5.0)


histamine <- matrix(0, ncol = replicates, nrow = sex)

histamine[1,] <- c(8.4, 10.2, 6.2, 5.4, 5.5, 7.3, 5.2, 5.1, 5.7, 9.8)

histamine[2,] <- c(7.5, 6.7, 5.7, 4.9, 6.8, 6.6, 6.9, 11.8, 6.7, 9.0)
```

 # DOING BY HAND

```
allrecords <- c(controls[1,], histamine[1,],controls[2,], histamine[2,])

xbar <- mean(allrecords)    # overall grand mean for all records
```

 # calculate sums of squares

```
# SSTotal

allxbar <- rep(xbar, length(allrecords))

SST <- sum((allrecords - allxbar) ^2)          # SST = 98.308
```

```
# SSError

xbarijall <- c(rep(mean(controls[1,]), replicates),
rep(mean(histamine[1,]), replicates), rep(mean(controls[2,]),
replicates), rep(mean(histamine[2,]), replicates))

SSE <- sum( (allrecords - xbarijall) ^2 )            # SSE = 80.089


# Treatment: factor A (sex): female vs male

ni <- c(replicates+replicates, replicates+replicates)

xbari <- c(mean(c(controls[1,], histamine[1,])), mean(c(controls[2,],
histamine[2,])))

SSA <- sum((ni*(xbari - xbar) ^2))          # SSA = 1.260


# Treatment: factor B (histamine): histamine vs. control

nj <- c(replicates+replicates, replicates+replicates)

xbarj <- c(mean(c(controls[1,], controls[2,])), mean(c(histamine[1,],
histamine[2,])))

SSB <- sum((nj*(xbarj - xbar) ^ 2))          # SSB = 11.556


# for interactions b/w sex and histamine treatment

# get means in each (i,j) group

xbarij <- c(mean(controls[1,]), mean(histamine[1,]), mean(controls[2,]),
mean(histamine[2,]))

xbari.rep2 <- c(rep(xbari[1],2), rep(xbari[2],2))
```

```
xbarj.rep2 <- rep(c(xbarj[1], xbarj[2]),2)
xbar.rep4 <- rep(xbar, 4)
SSAB <- replicates*sum( (xbarij - xbari.rep2 - xbarj.rep2 + xbar.rep4)^2)
  # SSAB = 5.402

# verify SST = SSA+SSB+SSAB+SSE
SST == SSA+SSB+SSAB+SSE     # TRUE

# use additivity to get SSAB
SSAB = SST - SSA -SSB - SSE
```

 # calculate Mean Square

```
df.A <- 1
MSA <- SSA/df.A                # 1.260
df.B <- 1
MSB <- SSB/df.B                # 11.556
df.AB <- df.A * df.B
MSAB <- SSAB/df.AB             # 5.402
df.error <- 2*2*(replicates-1)
MSE <- SSE/df.error            # 2.225
```

 # calculate F statistic

```
FA <- MSA/MSE                # 0.566
FB <- MSB/MSE                # 5.195
FAB <- MSAB/MSE              # 2.428
```

 # calculate p-value

```
pA <- pf(q=FA, df1 = df.A, df2 = df.error, lower.tail = FALSE)    # 0.456
pB <- pf(q=FB, df1 = df.B, df2 = df.error, lower.tail = FALSE)    # 0.029
pAB <- pf(q=FAB, df1 = df.AB, df2 = df.error, lower.tail = FALSE)   # 0.127
```

 # Use R to do the two-way ANOVA

 # make the input data as a data frame first

```
sexes <- c(rep("Female", 2*replicates), rep("Male", 2*replicates))

treatments <- c(rep("Control", 10), rep("HistamineShock", 10),
                rep("Control", 10), rep("HistamineShock", 10))

bloodVesselSurface <- c(controls[1,], histamine[1,],controls[2,],
histamine[2,])

df <- data.frame(sexes, treatments, bloodVesselSurface)

# data in df look like this:
```

	sexes	treatments	bloodVesselSurface
1	Female	Control	6.4
2	Female	Control	6.2
3	Female	Control	6.9
4	Female	Control	6.9
5	Female	Control	5.4
6	Female	Control	7.5
7	Female	Control	6.1
8	Female	Control	7.3
9	Female	Control	5.9
10	Female	Control	6.8
11	Female	HistamineShock	8.4
12	Female	HistamineShock	10.2
13	Female	HistamineShock	6.2
14	Female	HistamineShock	5.4
15	Female	HistamineShock	5.5
16	Female	HistamineShock	7.3
17	Female	HistamineShock	5.2
18	Female	HistamineShock	5.1
19	Female	HistamineShock	5.7
20	Female	HistamineShock	9.8
21	Male	Control	4.3
22	Male	Control	7.5
23	Male	Control	5.2
24	Male	Control	4.9
25	Male	Control	5.7

26	Male	Control	4.3
27	Male	Control	6.4
28	Male	Control	6.2
29	Male	Control	5.0
30	Male	Control	5.0
31	Male	HistamineShock	7.5
32	Male	HistamineShock	6.7
33	Male	HistamineShock	5.7
34	Male	HistamineShock	4.9
35	Male	HistamineShock	6.8
36	Male	HistamineShock	6.6
37	Male	HistamineShock	6.9
38	Male	HistamineShock	11.8
39	Male	HistamineShock	6.7
40	Male	HistamineShock	9.0

 # running 2-way anova in R

```
twowayanova <- aov(bloodVesselSurface ~ sexes + treatments + sexes:treatments, data = df)

summary(twowayanova)
```

	Df	SumSq	MeanSq	Fvalue	Pr(>F)
sexes	1	1.26	1.260	0.566	0.4566
treatments	1	11.56	11.556	5.195	0.0287 *
sexes:treatments	1	5.40	5.402	2.428	0.1279
Residuals	36	80.09	2.225		

```
---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


interaction.plot(sexes, treatments, bloodVesselSurface)
```

mean of bloodVesselSurface
7.0
6.5
6.0
5.5
treatments
HistamineShock
Control
Female
Male
sexes