2 WAY ANOVA

FACTOR AND LEVEL

- **Factors**: are the variables (treatments) in the study that we believe will influence the results.
- **Levels**: are the "values" of that factor in an experiment.

Example: antihypertensive drug

Group	
Experimental	Drug: 1 unit/d
	Drug: 3 unit/d
Control	Placebo

FACTOR DESIGN

- A Factorial Design is an experimental setup that consists of multiple factors and their separate and conjoined influence on the subject of interest in the experiment.
- A factor is an independent variable in the experiment and a level is a subdivision of a factor.

CONTENTS

- Introduction of Factorial design
- Graphic method
- GLM method
- Interpretation

1. INTRODUCTION OF FACTORIAL DESIGN

- Design 1: Does haptic feedback improve performance?
- A group of technology students wanted to see if haptic feedback (forces and vibrations applied through a joystick) is helpful in navigating a simulated game environment. To do this, they plan to randomly assign each of 60 students to one of the three jo [...] es and record the time it takes to co [...] navigation mission.

Joystick	n
1	20
2	20
3	20
Total	60

Took one week

- Design 1: Does haptic feedback improve performance?
- It turns out that their simulated game has several different difficulty levels. Suppose that a second experiment is planned to compare these levels. A similar experimental design will be used, with the four difficulty levels randomly assigned to 60 s. All students will use the standard j

Difficulty	n
1	15
2	15
3	15
4	15
Total	60

Took one week

- **Design 2: Does haptic feedback improve performance regardless of difficulty level?**

Here is a picture of the two-way design with the sample sizes:

	Difficulty				
Joystick	1	2	3	4	Total
1	5	5	5	5	20
2	5	5	5	5	20
3	5	5	5	5	20
Total	15	15	15	15	60

cell

2. GRAPHIC METHOD

- Example 1: In order to evaluate the protective effect of two drugs on renal system, 25 mice were selected and randomly allocated to 4 groups, and finally the serum BUN levels were examined.

2✖2 factorial design

Table 1. serum BUN levels after being treated by drug A and/or B.

	Use drug A (a1)		Not use Drug A (a2)	
	Use Drug B (b1)	Not use Drug B (b2)	Use Drug B (b1)	Not use Drug B (b2)
	12.01	13.90	13.96	15.78
	13.78	14.56	14.01	15.01
	12.87	13.78	13.46	14.89
	13.86	13.67	12.98	14.22
	12.76	14.46	14.02	15.41
	13.24	14.52	14.23	
	13.90	14.23		
$\overline{xi}$	13.20	14.16	13.78	15.06

Table 2. Averaged value of 2×2 factorial design.

Drug A	Drug B	
	b1	b2
a1	13.20	14.16
a2	13.78	15.06

SIMPLE EFFECT

- Simple effects (sometimes called simple main effects) are differences among particular cell means within the design. More precisely, <u>a simple effect is the effect of one independent variable within one level of</u>

Drug A	Drug B		Average	b2-b1
	b1	b2		
a1	13.20	14.16	13.68	0.96
a2	13.78	15.06	14.42	1.28
Average	13.49	14.61	-	1.12
a2-a1	0.58	0.90	0.74	-

The simple effect of A is 0.58, when B is fixed to level 1.

0.58=13.78-13.20

MAIN EFFECT

- A main effect is an outcome that is a consistent difference between levels of a factor.
- The average of simple effects.

Drug A	Drug B		Average	b2-b1
	b1	b2		
a1	13.20	14.16	13.68	0.96
a2	13.78	15.06	14.42	1.28
Average	13.49	14.61	-	1.12
a2-a1	0.58	0.90	0.74	-

Main effect of A$=\frac{(0.58+0.90)}{2}$=0.74 Main effect of B$=\frac{(0.96+1.28)}{2}$=1.28

INTERACTION EFFECT

- An *interaction effect* exists (or just "interaction") when the effect of one independent variable depends on the level of another

Drug A	Drug B		Average	b2-b1
	b1	b2		
a1	13.20	14.16	13.68	0.96
a2	13.78	15.06	14.42	1.28
Average	13.49	14.61	-	1.12
a2-a1	0.58	0.90	0.74	-

$$AB = BA = \frac{(a_2b_2 - a_1b_2) - (a_2b_1 - a_1b_1)}{2}$$

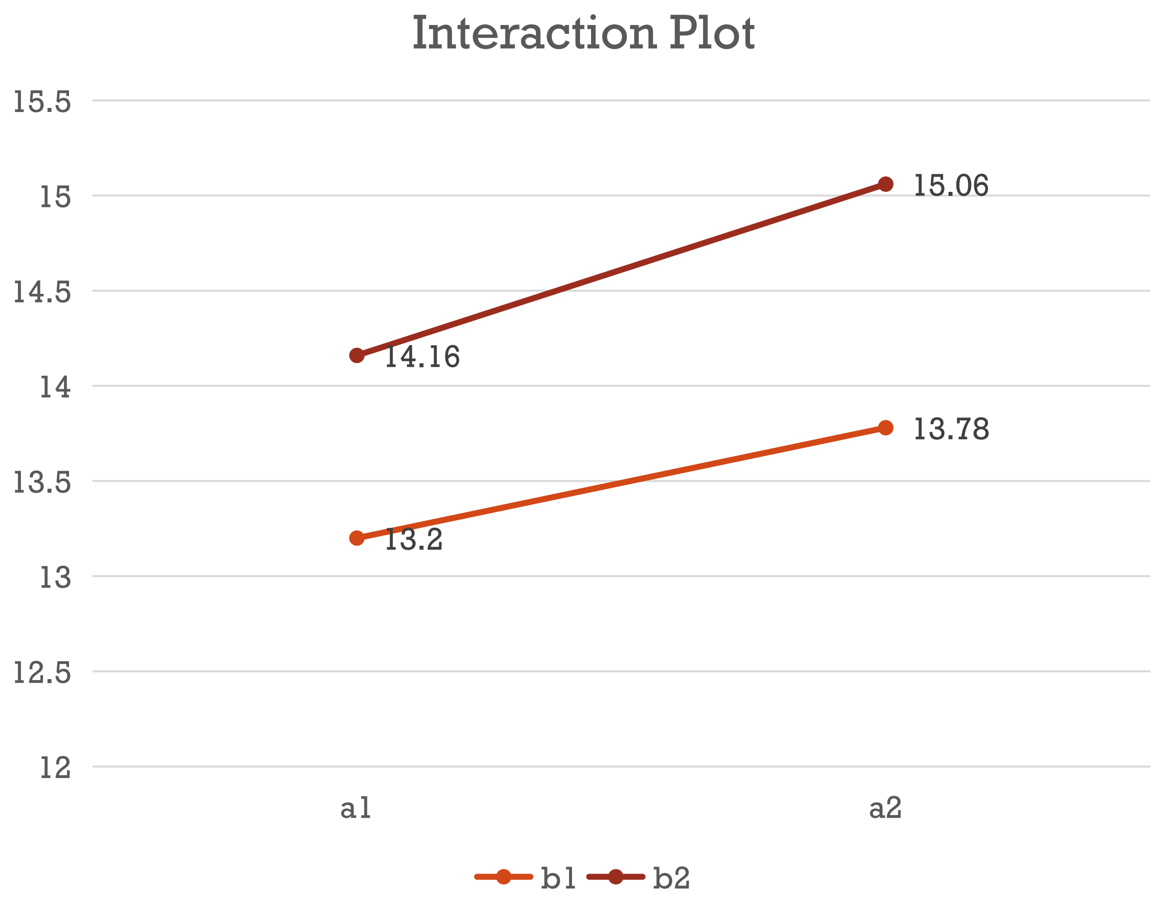
$$= \frac{(b_2a_2 - b_1a_2) - (b_2a_1 - b_1a_1)}{2} = \frac{0.90 - 0.58}{2} = 0.16$$

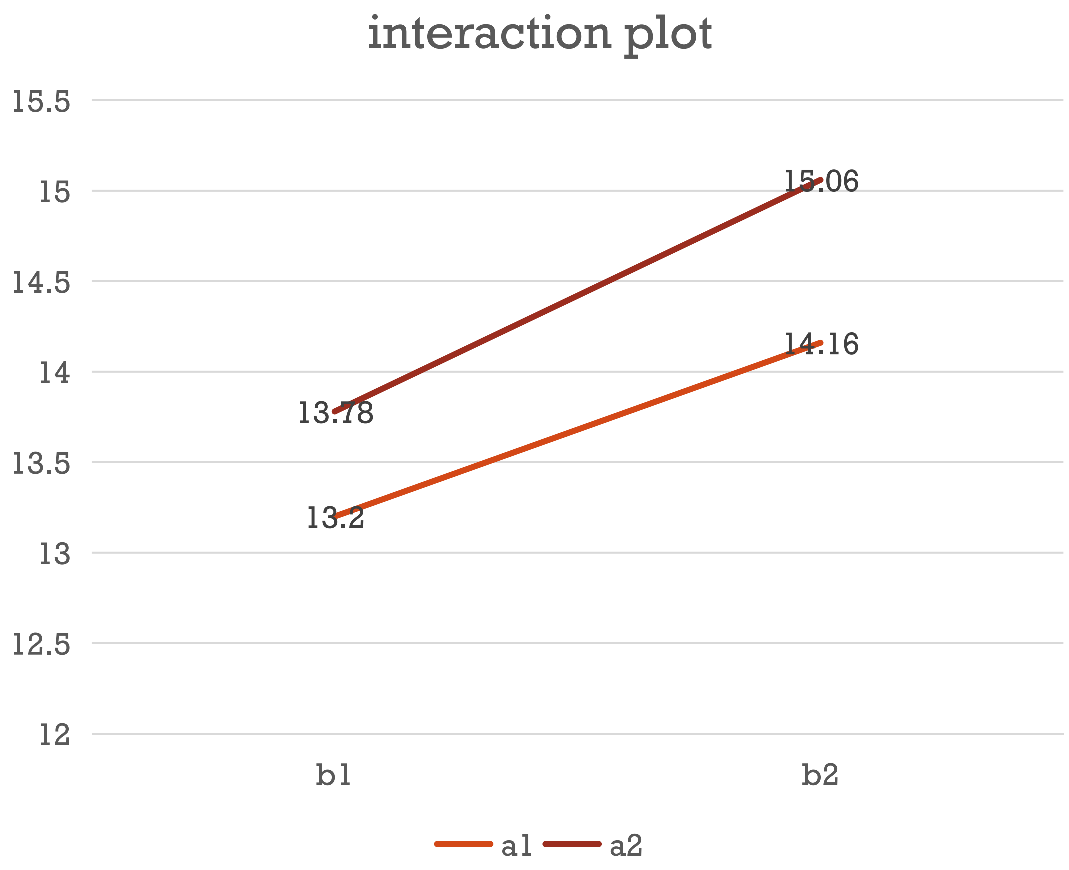
$$\frac{b_2a_2 - b_1a_2 - b_2a_1 + b_1a_1}{2} = \frac{b_2(a_2-a_1) + b_1(a_1-a_2)}{2}$$

$$= \frac{(b_2-b_1)(a_2-a_1)}{2}$$

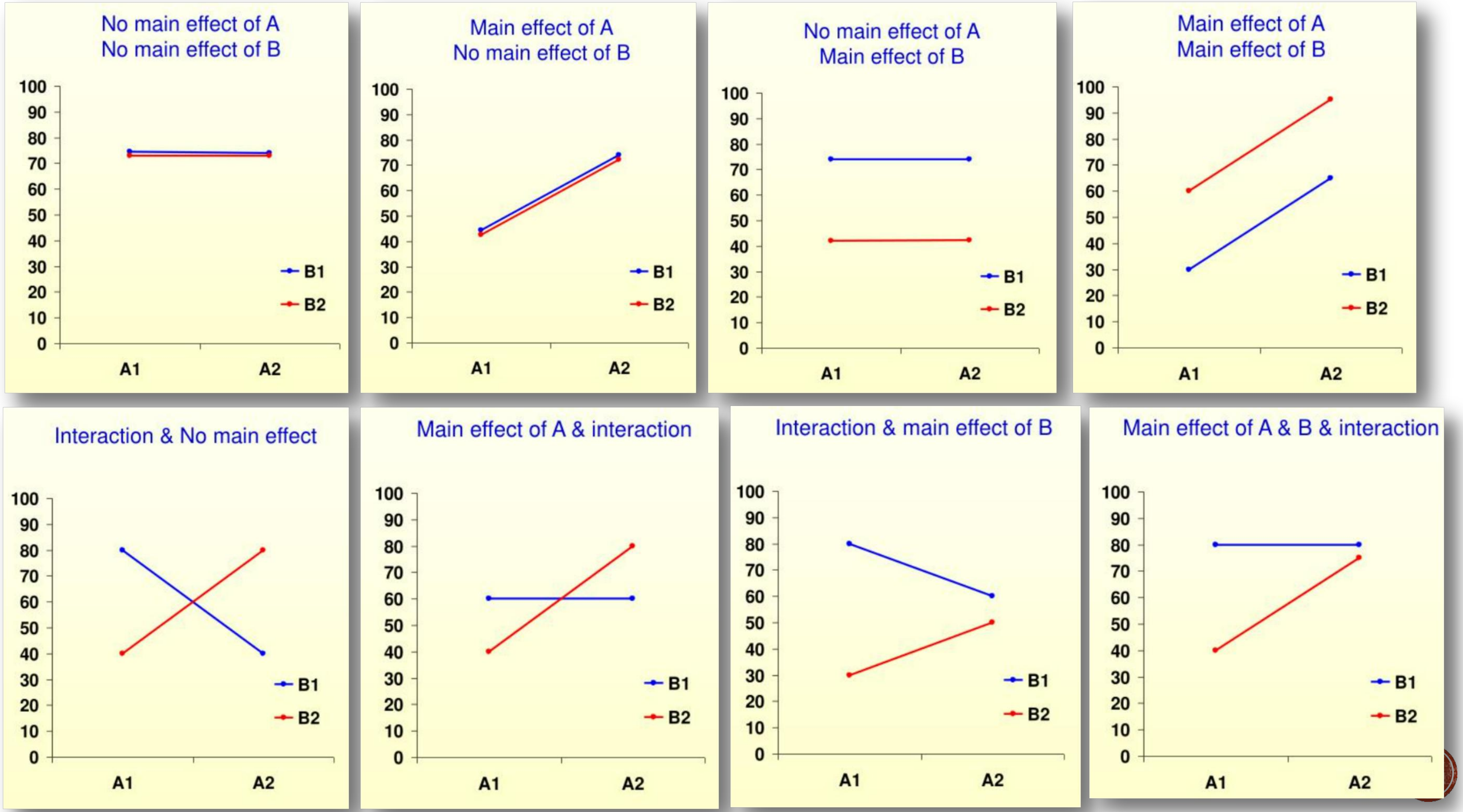
MATHEMATICALLY

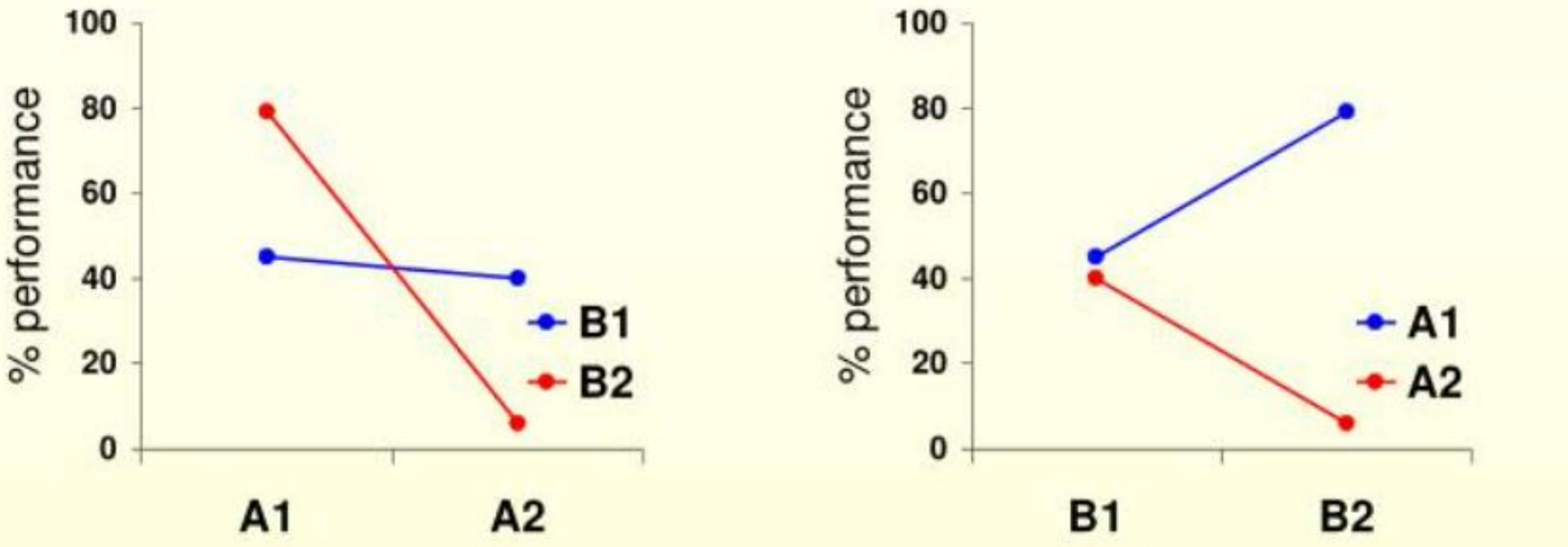
- Interaction is present when the differences between means representing the effect of a factor A at one level of B do not equal the corresponding differences at another level of factor B.
- An interaction is present when one of the independent variables does not have a constant effect at all levels of the other independent variable.





Parallel, no interaction


No main effect of A
No main effect of B
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Main effect of A
No main effect of B
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
No main effect of A
Main effect of B
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Main effect of A
Main effect of B
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Interaction & No main effect
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Main effect of A & interaction
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Interaction & main effect of B
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2
Main effect of A & B & interaction
100 90 80 70 60 50 40 30 20 10 0
A1 A2
B1
B2



Main effect of A, interaction

Same data; changed factor illustrated on X-axis.

Plotting the data in different ways can help interpretation

3. GLM METHOD

Two-Way ANOVA Data Layout

j

i

Factor A	Factor B			
	1	2	...	b
1	X_{111} X_{11n}	X_{121} X_{12n}	... ...	X_{1b1} X_{1bn}
2	X_{211} X_{21n}	X_{221} X_{22n}	... ...	X_{2b1} X_{2bn}
:	:	:	:	:
a	X_{a11} X_{a1n}	X_{a21} X_{a2n}	... ...	X_{ab1} X_{abn}

Observation k in each cell

X_{ijk}

Level i Factor A

Level j Factor B

A cell/condition

$i = 1, \ldots, a$

$j = 1, \ldots, b$

$k = 1, \ldots, n$

There are a X b treatment combinations

TWO-WAY ANOVA INTERACTION MODEL

- The population (cell) means μ_{ij} may differ, but all populations have the same standard deviation σ. The μij and σ are unknown parameters.

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk}$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2)$$

$$\mu_{ij} \to \bar{X}_{ij} = \frac{1}{n_{ij}} \sum_{i=1}^{k} X_{ijj}$$

$$\sigma^2 \to S_p^2 = \frac{\sum (n_{ij} - 1) S_{ij}^2}{\sum (n_{ij} - 1)}$$

Let x_{ijk} represent the *k*th observation from the population having Factor A at level *i* and Factor B at level *j*.

TWO-WAY ANOVA INTERACTION MODEL

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \underbrace{\boxed{\alpha}}_{\text{Baseline group mean}} + \underbrace{\underbrace{\boxed{\tau_i}}_{\text{Main effect of A}} + \underbrace{\boxed{\gamma_j}}_{\text{Main effect of B}} + \underbrace{\boxed{\omega_{ij}}}_{\text{interaction}}}_{\text{treatment}} + \varepsilon_{ijk}$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2)$$

Let x_{ijk} represent the kth observation from the population having Factor A at level i and Factor B at level j.

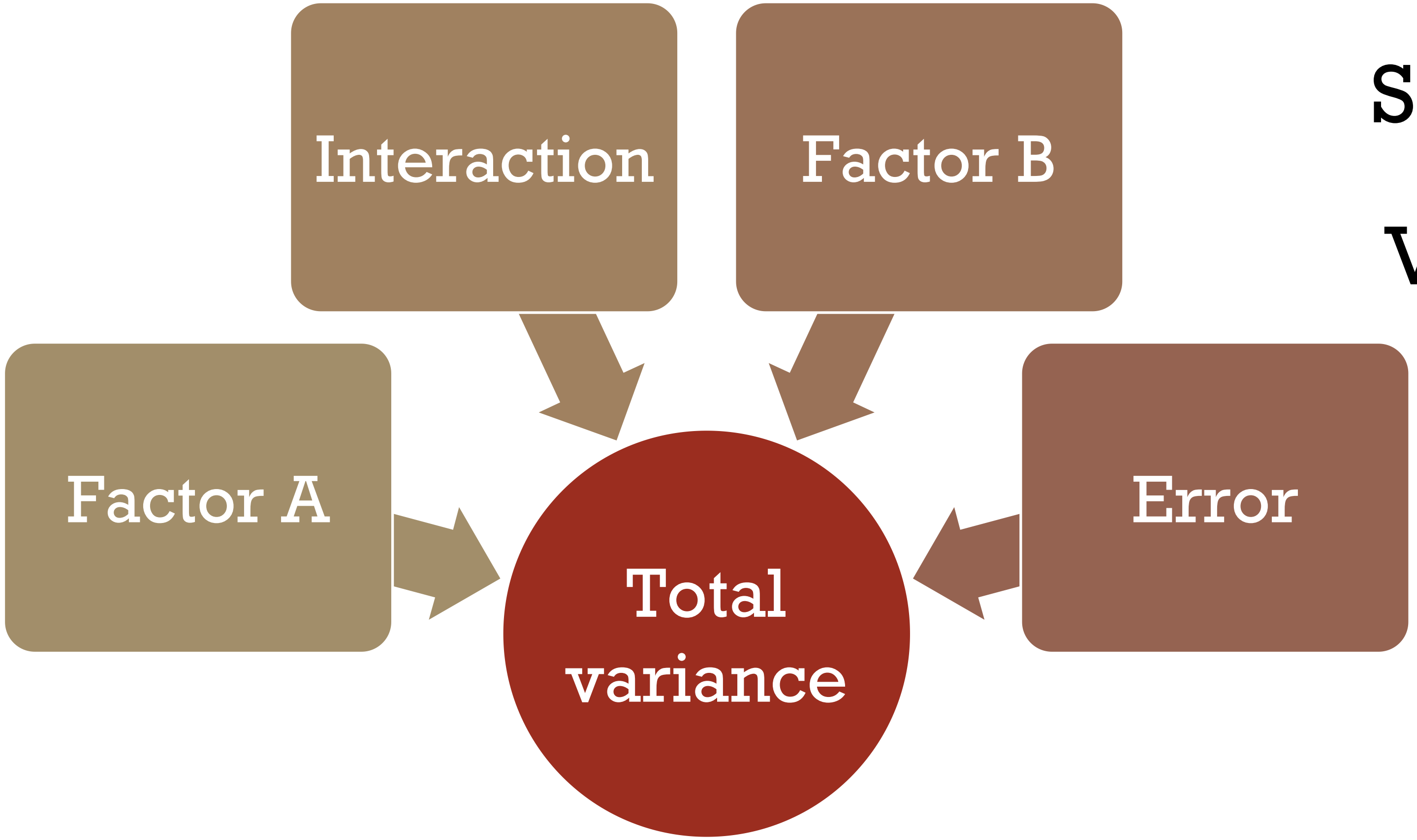
If the interaction term is not important

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \underbrace{\boxed{\alpha}}_{\text{Baseline group mean}} + \underbrace{\boxed{\tau_i}}_{\text{Main effect of A}} + \underbrace{\boxed{\gamma_j}}_{\text{Main effect of B}} + \underbrace{\boxed{\phantom{(\tau\gamma)_{ij}}}}_{\text{interaction}} + \varepsilon_{ijk}$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2)$$

Let x_{ijk} represent the kth observation from the population having Factor A at level i and Factor B at level j.

Variance Decomposition Using 2-way ANOVA



$$SS_T = SS_A + SS_B + SS_{A:B} + SS_E$$

$$V_T = V_A + V_B + V_{AB} + V_E$$

Equations

$$SS_{Total} = \sum_{i=1}^{i}\sum_{i=1}^{j}\sum_{i=1}^{k}(X_{ijk} - \bar{X})^2 = \sum x^2 - \frac{(\sum x)^2}{N}, \bar{X} \text{ is the overall mean}$$

$$SS_{treatment} = \sum_{i=1}^{a}\sum_{j=1}^{b} n_{ij}(\bar{X}_{ij} - \bar{X})^2$$

Cell mean

mean of all observations in the ith row of the data matrix averaged across all columns.

$$SSA = \sum_{i=1}^{a} \mathrm{n}_i(\bar{X}_{i.} - \bar{X})^2$$

$$SSB = \sum_{j=1}^{b} \mathrm{n}_j(\bar{X}_{.j} - \bar{X})^2$$

mean of all observations in the jth column of the data matrix averaged across all rows.

$$SSAB = SS_{treatment} - SSA - SSB$$

$$SSerror = SST - SS_{treatment}$$

Table 3: Interaction Model ANOVA Table.				
Source	**DF**	**SS**	**MS**	**F-statistics**
A	$I-1$	SS_A	$MS_A=SS_A/df_A$	MS_A/MS_E
B	$J-1$	SS_B	$MS_B=SS_B/df_B$	MS_B/MS_E
A:B (interaction)	$(I-1)(J-1)$	SS_{AB}	$MS_{AB}=SS_{AB}/df_{AB}$	MS_{AB}/MS_E
Error	$N-IJ$	SS_E	$MS_E=SS_E/df_E$	
Total	$N-1$	SS_{Total}		

ASSUMPTIONS

- Independence
- Normality
- Homogeneity of variance

STEP 1. STATE HYPOTHESIS

1. H0: There is no interaction between factors
   H1: There is a significant interaction between factors
2. H0: There is no effect of Factor A on the response variable
   H1: There is an effect of Factor A on the response variable
3. H0: There is no effect of Factor B on the response variable
   H1: There is an effect of Factor B on the response variable

$\alpha = 0.05$

Step 2. Calculation test statistic for example 1

$$\bar{X} = \frac{\sum_{i=1}^{i}\sum_{i=1}^{j}\sum_{i=1}^{k} X_{ijk}}{N} = 13.9804$$

$$SS_{Total} = \sum_{i=1}^{i}\sum_{i=1}^{j}\sum_{i=1}^{k}(X_{ijk} - \bar{X})^2 = \sum_{i=1}^{i}\sum_{i=1}^{j}\sum_{i=1}^{k}(X_{ijk} - 13.9804)^2 = \boxed{16.8405}$$

$$SS_{treatment} = \sum_{i=1}^{a}\sum_{j=1}^{b} n_{ij}(\bar{X}_{ij} - \bar{X})^2$$

$$= 7\times(13.2-13.98)^2 + 7\times(14.16-13.98)^2 + 6\times(13.78-13.98)^2 + 5\times(15.06-13.98)^2$$

$$= \boxed{10.5576}$$

Step 2. Calculation test statistic for example 1

$$\bar{X}_{1.} = 13.681,\ \bar{X}_{2.} = 14.3609,$$

$$SSA = \sum_{i=1}^{a} \mathrm{n_i}(\bar{X}_{i}. - \bar{X})^2$$

$$= 14 \times (13.681 - 13.98)^2 + 11 \times (14.3609 - 13.98)^2$$

$$= \boxed{2.8440}$$

$$\bar{X}_{.1} = 13.468,\ \bar{X}_{.2} = 14.536,$$

$$SSB = \sum_{j=1}^{b} \mathrm{n_j}(\bar{X}_{.j} - \bar{X})^2$$

$$= 13 \times (13.468 - 13.98)^2 + 12 \times (14.536 - 13.98)^2$$

$$= \boxed{7.1194}$$

Step 2. Calculation test statistic for example 1

$$SSAB = SS_{treatment} - SSA - SSB$$

$$= 10.5576 - 2.8440 - 7.1194 = \boxed{0.5927}$$

$$SSerror = SST - SS_{treatment}$$

$$= 16.8405 - 10.5576 = \boxed{6.2844} \; (according \text{ original data})$$

Table 3-a: Interaction Model ANOVA Table.					
Source	**DF**	**SS**	**MS**	**F-statistics**	**P**
A	1	2.8440	2.8440	9.5035	<0.05
B	1	7.1194	7.1194	23.790	<0.05
A:B (interaction)	1	0.5927	0.5927	1.9806	>0.05
Error	21	6.2844	0.2992		
Total	24	16.8405			

$$F_{0.05,(1,21)} = 4.32$$

- Step 3. Conclusion

- *Drug A and B has renal protective effect, but there is no interaction effect between A and B.*

ADVANTAGES OF 2-WAY ANOVA

- EFFICIENCY
- Control over a second variable by including it in the design as an independent variable
- Greater generalizability
- Evaluate for Interaction

4. INTERPRETATION

4 CONDITIONS

results	Main× and interaction×
	Main√ and interaction×
	interaction√ but is dominated by the main
	interaction√ and it dominates the main

Disulfiram-Like Reaction

1. 1st-generation sulfonylurea
2. Procarbazine
3. Cephalosporins
4. Griseofulvin
5. Metronidazole

Medicine Keys