

**The Design of Clinical Trials:- A Blinded Placebo-led Trial to Determine if Caffeine Improves Cognitive Performance.**

This on-line resource contains a data set and lesson plan for conducting a simulated clinical trial using a caffeine supplemented drink to test the hypothesis that caffeine improves cognitive performance. It is ideally run with a large class of undergraduate students (80+) who are in their pre-honours year. The lesson is intended to introduce them to the importance of experimental design and the power of randomisation and placebo controlled trials. The approach used in class is intended to fall short of the ideal experimental and, in practice, this approach encourages students to discuss the shortcomings of the study and the ways in which it might be improved if adequate facilities and time were available. The format is quite robust, can accommodate a number of measured variables and lends itself towards further development as an honours project for students in their final undergraduate year.

**Organisation of the practical:**

* Duration: 3h
* Facilities: 3 workshop rooms suitable for ~50 students (depends on class size). One IT suite
* Equipment required: templates for cognition and reaction time tests (see below)
* Fruit drink labelled A or B. An over-the-counter caffeine supplement added to one of the drink sets by a third party not connected to the lesson.
* A Google Form that is set up to collect the data in real time that the students can access using an on-line link.

**Running of the practical:**

1. Students are introduced to the session with a 15 minute Powerpoint lecture outlining the philosophy behind the design of clinical trials, the importance of the placebo concept and the pharmacology of caffeine. For interest, it can be useful to illustrate the power of the placebo effect by referring students to YouTube clips that discuss homeopathy and the “Memory of Water” controversy published in Nature in 1988 (see E. Dayenas et al (1988) Human basophil degranulation triggered by very dilute antiserum against IgE . Nature 333 (6176): 816–818.).
2. Students are randomised into either group A or B. It is important that they do this themselves so that they become aware that they are part of the experiment. This can be done in a variety of ways but one option is to get them to generate random numbers generated by a programme such as Random.org. Students with even numbers enter group A and odd numbers are group B.
3. A set of data is recorded before the trial starts. This includes gender, smoker, time since last drink, resting pulse (a mobile phone app can be useful for this) as indicators of mood measured using a linear analog scale.
4. Students are provided with a fruit drink labelled A or B according to their randomised grouping. The identity of the drink that contains the caffeine supplement is withheld from both the class leader and the students.
5. Students are provided with a series of cognitive skills tests to complete in each of the workshop rooms. The dataset provided with this lesson plan is based on the following:
	1. “Grid” psychomotor test run in an IT suite which tests the student’s ability to target a computer mouse to a series of highlighted grid intersections. Reaction time an proportion of grid hits, misses and errors are recorded (gridhits, gridmisses, griderror, totalaverage (=reaction time in ms)).
	2. Letter cancellation task (lctcolumn, lctmiss)
	3. Digit substitution task (dsstrows, dssterror)
	4. Memory test (number of pictures recalled under time limit from selection shown)
	5. Stroop test (stroop words - stroopwc; stroop colours stroolscol)
	6. Linear analog measurements (performed before and after consuming drink)
		1. Drowsy-awake
		2. Quick-witted-slow witted
		3. Clear minded - muzzy
		4. Calm - shaky
		5. Dizzy -steady

Students collect the information and enter it in real time using the Google form link.

1. After a de-brief session, students are provided with the complete data set of results for the class. An on-line tutorial is provided to enable them to evaluate the data and perform appropriate statistical analyses.
2. Results can be submitted as a written report however we have found it useful to ask the students to prepare a poster or oral presentation that focusses on one aspect of the data that has been generated. Students are encouraged to discuss the strengths of the placebo led approach as well as the shortcomings of the experiments that they have performed.
3. From their analyses, the students should attempt to identify which drink acted as the placebo and which contained caffeine.
4. The following is the text that the students are provided with to help them analyse their data using “R”:

 ***This tutorial has two aims:***

1. To examine the scientific data which you have gathered in the associated practical class on the effects of caffeine on various aspects of human performance.
2. To introduce and use the statistical programming language **R** to make appropriate and supportable interpretations of the data.

**PLEASE NOTE: R is case sensitive and does not permit spaces in variable names**

You have also been provided with a practice data set in an Excel dataframe called **Caffeine dataset for online tutorial.csv (\*see instructions for downloading this file at the end of this document)**. This is the data which will need to be imported into **R** prior to the analysis.

**PART 1: Terms and Definitions**

The practical investigated how drinking caffeine can affect a number of aspects of your performance. In the first part of this investigation we will focus on the effects of caffeine on your pulse rate – caffeine is a stimulant, and so we might expect drinking caffeine to increase pulse rate, whereas those of you who did not drink caffeine should have a pulse rate which does not change over the measurement interval*.* ***Once you understand this analysis, attempt to analyse some of the other variables that were measured during the practical session.* *Perform your own suite of analyses which you will use as the basis of your optional poster presentation in week 11***

Pulse rate is a **continuous variable** (= a number which has, or could have, some decimal places attached to it)

In addition to measuring your pulse rate after you have drunk whichever drink you were randomly allocated (caffeine or non-caffeine), various other biological variables which might also affect your final pulse rate were measured. These were:

Your initial pulse rate before drinking you allocated drink. This is also a **continuous** variable (see above)

The time since you last knowingly consumed a caffeine-containing drink. Also a **continuous** variable

Whether you consumed a drink containing caffeine or one with no caffeine. This is known as a **categorical variable,** and there are many of these in biology, such as sex, ethnic origin, blood group etc.

Your gender – also a **categorical** variable (see above)

Whether you smoke or not – also a **categorical** variable.

So, there are 5 variables which you have measured which might affect your final pulse rate; 2 of these are continuous and 3 are categorical.

For statistical analysis the variable you are trying to explain (or predict) is known as the **response variable** (RV; in this case final pulse rate), and the variables which might affect the RV are known as **explanatory variables** (EVs; in this case, initial pulse rate, time since last caffeinated drink, gender, smoker/nonsmoker, & drink consumed).

So, our scientific question is a simple and common sense one:

***“Can variation in final pulse rate (RV) be explained by one, or a combination, of the 5 EVs?”***

**How do we address this question?**

First of all we can define three kinds of statistical model to see which is most appropriate to our dataframe:

RV continuous; EVs all categorical – **Analysis of Variance (ANOVA)**

RV continuous; EVs all continuous – **Regression**

RV continuous; EVs a mixture of categorical & continuous – **Analysis of Covariance (ANCOVA)**

Clearly, in this case we have 3 categorical EVs and 2 continuous EVs, so the appropriate technique is **ANCOVA**. This technique is so-called because continuous EVs are known as **covariates**, hence analysis of **covariance**.

**PART 2: Carrying out an ANCOVA in R**

There are 4 stages to this process:

i. Opening the program & creating a project

ii. Importing the dataframe

iii. Carrying out the analysis

iv. Interpreting the final statistical model

**(i)** Opening the program

**R** is opened from a **Graphical User Interface** (GUI) called **RStudio**, which acts as a front end to **R** itself, and which makes many of the processes, such as data import and manipulation easy. Both **R** and **RStudio** are open source freeware, and so can be downloaded onto any machine which has a web connection. To open RStudio follow the following filepath:

**Start > Programs > Statistics > RStudio.** When the program is loaded you will see this screen:

There are 4 panes. **PLEASE NOTE again at this stage that R is *very unforgiving* of typing errors, and it is case-sensitive, so if you get an error message the most likely reason is that you have mistyped a command.**

Having opened the program (which has, in the background, opened the main R program itself) we now need to create a new project. It is good housekeeping practice in R to create a new project for each new piece of work you do. To create a new project execute the file path: **File> New Project> New Directory> Empty Project.** Under “Directory Name” give your project a name of your choice, and tell the program where to create it using “Browse”.

**(ii)** Importing the dataframe

You have been provided with some practice data in the file **Caffeine.csv**. This has been saved as a comma separated values (csv) file (one of the “Save As” options in Excel), which is by far the best way in which to read data into **R**. To read in this dataframe select the “Tools” option from the menu at the top of the screen, and click “Import Dataset”, select “From Text File”, Locate the file and select “Open” . Select Import and you should see a screen like this:



The dataframe is in the top left pane (the Source Editor). To make the data in the dataframe available to **R** we now need to attach the dataframe, which we do by going to the > prompt in the Console (lower left pane), and typing:

**> attach(Caffeine)** [remember that R is case sensitive!]

You will not get any response to this command other than the lack of an error message, which is R’s way of telling you that it has been done. Now the data has been imported and successfully attached and we are ready to begin the analysis.

(iii) Carrying out the analysis

The first stage of any analysis is to visualise the data using graphs. Remember that the hypothesis (H1) is:

***“Drinking caffeine will increase pulse rate”***

Is there any indication that this is true? Well, let’s look at initial pulse rate before any drinks have been consumed. You will find the column headings listed in the order that they appear on the spreadsheet in column AC in the Excel sheet. These are identified by R as Variable columns (in column AB) and are listed as V1(Gender)….. V27(Calmshaky). “pulse1” appears as **Variable 5** (**V5**) in the dataframe). Go to the Console (bottom left) and type:

**> hist(V5)**

A histogram of initial pulse rates will appear in the bottom right pane (Plots). You will see that the peak of the distribution appears between 80 and 90 bpm. So, how does this compare with the situation after drinking caffeine (remember that only half the students drank caffeine). To see the overall pulse rate for the second measurement time type:

**> hist(V6)**

**Q1. How do you interpret the difference between these two graphs? Can a sensible interpretation be made?**

Is there any relationship between the pulse rates at first and second measurement? To look at this type:

**> plot(V5,V6)**

**Q2. What do you think this implies?**

Obviously, the second measurement (“pulse2”) is a mixture of students with different categories of drinks (placebo and caffeinated), so to see any effect we need to separate these, and to do this we first have to do a little housekeeping by telling **R** that “drink” is a categorical variable. Since “drink” is located in column V2, we do this by typing:

**> V2<-factor(V2)**

The “ <-“ symbol is **R**’s version of the “=” sign. We have now told R that “drink” is categorical, so we can now plot them separately. Just type:

**> plot(V2,V6)**

You will now see that the caffeine and decaffeinated treatments are plotted separately. The solid line in the middle of the bars represents the median point.

**Q3. What can we deduce from this plot?**

It looks as though the picture is not necessarily simple and that we need to consider the effects of all the explanatory variables (EVs) on “pulse2”. The reason for this is because more than one conditions might contribute to the effect on pulse. Indeed, they may act together as an amplified effect. To do this we need to do two things:

1. Carry out the ANCOVA analysis referred to above (remind yourself here why ANCOVA is the most appropriate technique).
2. Selectively exclude explanatory variables which have a non-significant effect. This process simplifies the ANCOVA in a step-wise manner as you will see.

**PART 3: The statistical modelling process**

The object is to arrive at the simplest model which will explain the variation in the RV. This means that, although we have 5 EVs which we can enter into the model, it may well be that most of the variation in the RV is explained by one or two of those EVs, and the rest are statistically redundant. To investigate this we begin as follows (remember to type accurately).

First, we fit the **maximal model** = RV + all 5 EVs. Enter the following information: > model1 <- lm(pulse2 ~ pulse1+drink+gender+smoking+lastdrink) which will be written using the column variable headings as the command:

**>model1<-(V6~V5+V2+V1+V3+V4)** (**STEP 1**)

In words this simply says “fit a linear model (lm), with the RV (“pulse2”) as a function of (~), the EVs “pulse1”,”drink”,”gender”,”smoking” and “lastdrink”. A linear model is a procedure in **R** for fitting an ANCOVA.

If you received no error messages it means that **R** will have fitted the model and you need to ask it to show you the output. To do this type:

**> summary.aov(model1)** (**STEP 2**)

This produces an ANCOVA table where the key columns are the EVs (left hand column) and the statistical significance values (right hand column). 1, 2 or 3 stars in the right hand column indicates statistical significance (to achieve this the Pr(>)F value must be 0.05 or less). If you look at the table you will see that only 2 of the EVs appear to be statistically significant – “pulse1” & “smoking”. To check this we remove the non-significant EVs from the model and fit a second simpler model with only the significant EVs from the first model, as follows:

**> model2 <- lm(V6~V5+V3)**   **(STEP 3)**

To see the output from this new model type:

**> summary.aov(model2)**

We see from the reduced table that both of the included EVs are still significant, as above. However, we need to be sure that in simplifying the model we have not sacrificed accuracy, and to do this we need to compare the two models to show that the simpler model is not a significantly less good predictor of “pulse2” than the maximal model. To check this type:

**> anova(model1,model2) (STEP 4)**

This command uses an analysis of variance to compare the 2 models and you will see that the Pr(>F) value is much greater than 0.05, and therefore the difference between the models is not statistically significant, and the simpler model is preferred.

So far so good, but clearly the table shows that “pulse1” (\*\*\*) has a much more significant effect on “pulse2” than “smoking”(\*). The numbers of asterisks (max.3) indicates the level of significance. So, is it really necessary to retain “smoking” in the model? – we could have an even simpler model than model2, and as we said above, the simplest model is what we should be aiming for. So let’s take “smoking” out of the model and see what effect it has. Type:

**> model3 <- lm(V6~V5) (STEP 5)**

Then type:

**> summary.aov(model3)**

and here it appears that “pulse1” is still a very strong indicator of “pulse2”, BUT have we lost any information by not retaining “smoking” in the model? To determine this we compare the two models as before:

**> anova(model2,model3)**

and here we see that the Pf(>F) value is much less than 0.05, which indicates that not retaining “smoking” in the model has reduced the overall significance of the model, and so it should be reinstated.

So, our simplest statistical model to predict the RV “pulse2” contains only 2 of the 5 EVs –“ smoking” and “pulse1”, with “pulse1” being the more important of the 2 EVs. This simplest model is called the **Minimal Adequate Model** (MAM), and the essence of all statistical modelling efforts is to get from the Maximal Model to the MAM.

Finally, we need to check how much explanatory power our MAM has. To do this type:

**> summary.lm(model2) (STEP 6)**

The output shows that R-squared = 0.2886. This is the key parameter, and is a measure of the model’s explanatory power, because in words it means that 28.86% of the variance in pulse 2 is explained by “pulse1” and “smoking”. This also means, if you think about it, that 71.14% of the variance in “pulse2” must be explained by other things. But, we have already shown that these other things do not include “gender”, ” lastdrink” or “drink”, and so they must be things we have not measured. **You may care to comment on what they could be in your biological interpretation of the outcomes.**

***Further exercises and to generate statistically sound data for your poster or presentation:***

STEPS (1)-(6) above can be repeated for any of the other RVs using the same EVs and the same model simplification process. Include the relevant plots and summary values in your results section (use the Export option in RStudio for plots, table values need copy/paste functions). Please include analysis of at least **ONE** other RV in your poster. To avoid confusion use new model numbers e.g. 4, 5, 6, etc. Please note that the visual analogue scales are listed with the final measures first (i.e. drwsy, qwttd, clearm, dizzy, calm) and those taken at baseline are listed second (drowsyA, quickslow, clearMu, dizzyste, calmshaky).

**Overleaf:**

\*Practice Data Set: Copy and paste to Excel and save as either text (.txt) or comma separated file (.csv) as filename “Caffeine dataset for online tutorial.csv”

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Male | A | Yes | 12 | 95 | 91 | 19 | 10 | 3 | 563 | 17 | 5 | 4 | 1 | 8 | 9 | 24 | 5.7 | 4.2 | 6.1 | 3.4 | 4.5 | 3.3 | 9.1 | 6.1 | 3.4 | 4.5 | V1 | Gender | Column A |
| Male | B | No | 12 | 75 | 68 | 24 | 5 | 2 | 612 | 25 | 7 | 7 | 0 | 12 | 9.8 | 19.2 | 7.6 | 2.4 | 2.8 | 5.8 | 6.4 | 2.4 | 6 | 6.8 | 5.4 | 5.2 | V2 | drink | Column B |
| Female | B | No | 24 | 78 | 74 | 15 | 14 | 3 | 529 | 12 | 4 | 6 | 0 | 12 | 14.09 | 17.46 | 7.75 | 3.5 | 4 | 6.5 | 4.5 | 3.5 | 6 | 5.75 | 9.25 | 1 | V3 | smoking | Column C |
| Male | B | No | 18 | 72 | 66 | 10 | 19 | 4 | 547 | 18 | 3 | 6 | 0 | 11 | 13 | 15 | 8 | 3.2 | 3.5 | 7.1 | 2.8 | 7.4 | 2 | 2.9 | 7.9 | 1.4 | V4 | lastdrink | Column D |
| Male | A | No | 12 | 65 | 56 | 16 | 13 | 11 | 638 | 12 | 7 | 4 | 1 | 8 | 8.1 | 26.6 | 1.4 | 6.5 | 3.9 | 5.1 | 1.5 | 2.5 | 6.1 | 3.9 | 5.9 | 2 | V5 | pulse1 | Column E |
| Female | B | Yes | 24 | 78 | 65 | 12 | 17 | 7 | 437 | 25 | 18 | 6 | 0 | 8 | 9 | 15.5 | 6.5 | 1.1 | 3.5 | 7.3 | 7 | 0.9 | 8 | 7 | 7.5 | 5 | V6 | pulse2 | Column F |
| Female | A | No | 12 | 95 | 82 | 9 | 20 | 74 | 364 | 18 | 25 | 5 | 0 | 9 | 10.17 | 17.73 | 7.9 | 2.1 | 1.5 | 1.8 | 6.8 | 9.9 | 2.3 | 0.2 | 10.2 | 0 | V7 | gridhits | Column G |
| Male | B | Yes | 24 | 57 | 56 | 20 | 9 | 5 | 646 | 15 | 3 | 5 | 0 | 11 | 7 | 13 | 8 | 2 | 2 | 9.5 | 1 | 8 | 2 | 2 | 9.5 | 1 | V8 | gridmisses | Column H |
| Male | A | No | 15 | 75 | 72 | 18 | 11 | 4 | 570 | 17 | 4 | 6 | 0 | 11 | 7.98 | 13.43 | 7.7 | 3 | 3.1 | 7.5 | 2.6 | 7.9 | 3.6 | 4.1 | 7.3 | 4.9 | V9 | griderror | Column I |
| Male | B | Yes | 24 | 94 | 83 | 16 | 13 | 24 | 577 | 24 | 8 | 4 | 0 | 8 | 8.3 | 10.1 | 6 | 7 | 7 | 4 | 6 | 5 | 8 | 5 | 6 | 3 | V10 | totalaverage | Column J |
| Male | A | No | 15 | 66 | 64 | 12 | 17 | 1 | 476 | 14 | 8 | 4 | 0 | 8 | 9.51 | 19.8 | 8.8 | 0.6 | 0.5 | 8.35 | 4.3 | 7.25 | 2.3 | 0.9 | 7.3 | 3.7 | V11 | lctcolumn | Column K |
| Male | B | No | 12 | 65 | 58 | 22 | 7 | 6 | 447 | 12 | 12 | 6 | 0 | 10 | 8.86 | 18.71 | 6.8 | 3.5 | 5.3 | 6.4 | 6.6 | 4 | 5.4 | 6.3 | 5.2 | 4 | V12 | lctmiss | Column L |
| Female | A | No | 24 | 83 | 84 | 14 | 15 | 3 | 509 | 17 | 5 | 6 | 0 | 8 | 8.05 | 14.03 | 4 | 6 | 6 | 3 | 7 | 3 | 7 | 8 | 2 | 8 | V13 | dsstrows | Column M |
| Male | A | No | 168 | 84 | 84 | 17 | 12 | 1 | 727 | 16 | 12 | 4 | 0 | 8 | 9.3 | 22.3 | 6 | 5 | 6 | 3 | 7 | 5 | 6 | 6 | 4 | 4 | V14 | dssterror | Column N |
| Male | B | Yes | 8 | 86 | 98 | 18 | 11 | 4 | 444 | 28 | 19 | 6 | 0 | 11 | 8.3 | 13.8 | 6.9 | 2.7 | 4.7 | 4.8 | 5 | 1 | 5.8 | 8.9 | 6.6 | 4.8 | V15 | pictures | Column O |
| Male | A | No | 24 | 70 | 63 | 17 | 12 | 4 | 486 | 19 | 17 | 5 | 0 | 4 | 8.5 | 14.2 | 6 | 5 | 5.1 | 4.3 | 6.3 | 4.9 | 4.2 | 7.3 | 3.5 | 5.2 | V16 | stroopwc | Column P |
| Male | B | No | 48 | 61 | 63 | 15 | 14 | 16 | 531 | 18 | 17 | 5 | 1 | 8 | 8 | 19 | 4.7 | 4.3 | 4.9 | 7.1 | 0.7 | 5.3 | 4.7 | 4 | 5.6 | 0.6 | V17 | stroopscol | Column Q |
| Male | A | No | 24 | 76 | 86 | 18 | 11 | 2 | 446 | 17 | 3 | 5 | 0 | 9 | 9.6 | 21.3 | 7.4 | 2.1 | 2 | 7.5 | 7 | 5.9 | 3 | 4.9 | 7.5 | 6.8 | V18 | drwsy | Column R |
| Female | B | No | 24 | 62 | 82 | 13 | 16 | 26 | 611 | 13 | 11 | 6 | 1 | 11 | 11.19 | 20.34 | 2 | 9.7 | 9.4 | 6.4 | 4.7 | 1 | 8.7 | 8.7 | 5.2 | 5 | V19 | qwttd | Column S |
| Female | B | No | 100 | 83 | 73 | 18 | 11 | 5 | 477 | 19 | 5 | 3 | 0 | 9 | 9.3 | 16.4 | 4 | 6 | 7 | 10 | 1 | 4 | 6 | 7 | 10 | 1 | V20 | clearm | Column T |
| Female | A | No | 4 | 73 | 74 | 11 | 18 | 1 | 399 | 18 | 7 | 6 | 0 | 9 | 8.4 | 13.4 | 6.8 | 3.8 | 5.9 | 4.5 | 6.4 | 4.1 | 4.8 | 4.5 | 7.4 | 6.5 | V21 | dizzy | Column U |
| Female | B | No | 20 | 87 | 88 | 24 | 5 | 10 | 370 | 30 | 60 | 4 | 0 | 9 | 9.9 | 11.7 | 8 | 2 | 1.5 | 7 | 5 | 8 | 1.5 | 1.5 | 7 | 3 | V22 | calm | ColumnV |
| Male | B | Yes | 27 | 55 | 120 | 17 | 12 | 14 | 506 | 9 | 1 | 2 | 0 | 10 | 8.05 | 22 | 5 | 3 | 2 | 4 | 1 | 5 | 5 | 3 | 7 | 1 | V23 | drowsyA | Column W |
| Male | A | No | 16 | 74 | 79 | 23 | 6 | 8 | 538 | 16 | 6 | 6 | 0 | 12 | 9 | 14 | 7.7 | 4 | 4.1 | 8.7 | 1.5 | 7.5 | 2.2 | 0.9 | 10 | 0.7 | V24 | quickslow | Column X |
| Male | B | No | 24 | 79 | 77 | 17 | 12 | 1 | 517 | 18 | 31 | 4 | 0 | 10 | 9.37 | 19.5 | 7.2 | 2 | 1 | 9 | 1 | 7.5 | 3 | 4 | 8 | 2 | V25 | clearMu | Column Y |
| Female | B | No | 4 | 52 | 68 | 17 | 12 | 1 | 576 | 21 | 8 | 7 | 0 | 9 | 9 | 15 | 7.5 | 4.5 | 2.5 | 8.2 | 7.5 | 3.2 | 7 | 7.5 | 7 | 1.8 | V26 | dizzyste | Column Z |
| Male | A | No | 48 | 80 | 86 | 8 | 21 | 3 | 513 | 27 | 20 | 3 | 0 | 11 | 10.3 | 21.4 | 8.6 | 2 | 7.6 | 4 | 9.9 | 7.5 | 3.2 | 5.2 | 7 | 3.6 | V27 | calmshaky | Column AA |
| Male | A | No | 30 | 68 | 69 | 16 | 13 | 4 | 501 | 15 | 6 | 5 | 0 | 9 | 8.2 | 14.6 | 6.7 | 2.2 | 4.4 | 4.6 | 5.7 | 5 | 4.7 | 5.2 | 4.1 | 5.6 |  |  |  |
| Male | A | Yes | 15 | 70 | 70 | 9 | 20 | 60 | 400 | 23 | 25 | 5 | 0 | 9 | 9.98 | 15.55 | 4 | 4 | 2 | 6 | 3 | 3 | 5 | 2 | 6 | 1 |  |  |  |
| Male | B | No | 24 | 50 | 51 | 9 | 20 | 11 | 513 | 21 | 11 | 2 | 0 | 8 | 17 | 21 | 2.2 | 6 | 6.3 | 2.7 | 6.8 | 2.7 | 7.2 | 8.2 | 2.2 | 8.5 |  |  |  |
| Male | A | No | 17 | 65 | 70 | 14 | 15 | 9 | 729 | 18 | 21 | 6 | 0 | 10 | 11.7 | 17.5 | 3.1 | 6.8 | 6.9 | 4.9 | 5.2 | 4.3 | 3.1 | 5.2 | 8 | 3 |  |  |  |
| Female | B | No | 24 | 68 | 66 | 15 | 14 | 8 | 661 | 14 | 9 | 8 | 0 | 6 | 9 | 17.9 | 8 | 4.7 | 4.3 | 4.8 | 3 | 7.3 | 3 | 3 | 10 | 2 |  |  |  |
| Female | A | No | 7 | 90 | 71 | 9 | 20 | 6 | 714 | 4 | 9 | 4 | 0 | 12 | 8.56 | 16.47 | 8 | 5.9 | 0.4 | 9.6 | 0.4 | 9.3 | 8.5 | 9.8 | 0.5 | 0.3 |  |  |  |
| Female | A | No | 25 | 84 | 75 | 16 | 13 | 2 | 600 | 16 | 6 | 6 | 0 | 8 | 9.45 | 13.59 | 6.4 | 4.6 | 4.5 | 3.4 | 5.9 | 2.8 | 6.3 | 6.1 | 5.1 | 4.4 |  |  |  |
| Male | A | No | 48 | 79 | 67 | 13 | 16 | 24 | 616 | 16 | 26 | 5 | 0 | 9 | 10 | 15 | 1.8 | 8 | 9 | 1.9 | 2.2 | 0.8 | 9.3 | 9.3 | 1.8 | 4.3 |  |  |  |
| Female | B | Yes | 24 | 72 | 77 | 23 | 6 | 5 | 509 | 15 | 5 | 5 | 0 | 9 | 13.2 | 14.9 | 7 | 2.5 | 4 | 4.5 | 7.5 | 3 | 7 | 7 | 8 | 5 |  |  |  |
| Male | A | No | 100 | 84 | 80 | 8 | 21 | 18 | 761 | 17 | 10 | 4 | 3 | 9 | 17 | 24 | 3.7 | 6 | 3.7 | 5.7 | 2 | 7 | 5.5 | 6.7 | 7 | 6.5 |  |  |  |
| Female | A | No | 22 | 70 | 72 | 14 | 15 | 2 | 544 | 25 | 17 | 5 | 0 | 10 | 8 | 20 | 7.6 | 2.8 | 6.7 | 7.3 | 1.3 | 7.3 | 2.8 | 5.8 | 6.9 | 1.8 |  |  |  |
| Female | A | No | 5 | 67 | 61 | 13 | 16 | 2 | 537 | 18 | 11 | 6 | 0 | 7 | 8.4 | 23.4 | 6.4 | 0.5 | 0.4 | 5.5 | 5.4 | 5.5 | 2.5 | 1.5 | 6 | 0.5 |  |  |  |
| Male | B | No | 14 | 80 | 71 | 17 | 12 | 4 | 446 | 17 | 4 | 4 | 0 | 11 | 11.1 | 18.3 | 8.4 | 2 | 6.4 | 3.2 | 6.4 | 6.8 | 2.6 | 3.6 | 5.4 | 5.1 |  |  |  |
| Female | A | No | 25 | 63 | 65 | 18 | 11 | 4 | 670 | 24 | 41 | 6 | 0 | 9 | 7.6 | 14.1 | 6.8 | 1.8 | 3.2 | 7.2 | 1.2 | 7.5 | 2 | 2.8 | 7.7 | 0.8 |  |  |  |
| Female | B | No | 24 | 85 | 75 | 10 | 19 | 4 | 536 | 18 | 20 | 6 | 0 | 8 | 7.5 | 19.4 | 6.4 | 2.3 | 3.2 | 8.8 | 1 | 3.4 | 3.9 | 6.1 | 9.2 | 0.6 |  |  |  |
| Female | B | No | 96 | 73 | 60 | 16 | 13 | 52 | 594 | 21 | 13 | 5 | 0 | 7 | 10.85 | 19.45 | 7.4 | 3.6 | 6.7 | 7.4 | 3.8 | 6.2 | 3 | 5.7 | 9.1 | 4.6 |  |  |  |
| Female | B | No | 24 | 74 | 75 | 10 | 19 | 21 | 807 | 37 | 83 | 5 | 0 | 11 | 10.25 | 18.15 | 9.3 | 1.7 | 1.2 | 8 | 3.5 | 7.4 | 2.5 | 1.8 | 7.8 | 1.5 |  |  |  |
| Female | A | No | 20 | 79 | 83 | 14 | 15 | 0 | 600 | 11 | 3 | 4 | 0 | 11 | 13.5 | 14.9 | 6.8 | 2 | 2.4 | 7.1 | 2.4 | 7.7 | 3 | 3.3 | 6.3 | 3.3 |  |  |  |
| Female | A | No | 2 | 90 | 77 | 6 | 23 | 6 | 666 | 14 | 25 | 3 | 0 | 5 | 9.31 | 21.51 | 1.3 | 8.9 | 9.1 | 3.6 | 7.8 | 1.7 | 6.3 | 8.2 | 2.3 | 8 |  |  |  |
| Female | A | No | 24 | 60 | 57 | 13 | 19 | 6 | 555 | 21 | 32 | 4 | 0 | 7 | 11 | 24 | 5 | 3.5 | 3.5 | 5.5 | 2.2 | 6.5 | 2.5 | 2.7 | 7 | 3 |  |  |  |
| Female | B | No | 4 | 86 | 76 | 11 | 18 | 13 | 789 | 20 | 28 | 6 | 0 | 9 | 9.52 | 16.99 | 9.9 | 0.7 | 3.5 | 8.8 | 7 | 9.7 | 1.2 | 1.7 | 9.4 | 7.3 |  |  |  |
| Male | A | Yes | 18 | 74 | 64 | 16 | 13 | 2 | 513 | 15 | 8 | 4 | 0 | 11 | 11 | 24 | 0 | 2 | 10 | 9 | 0 | 6 | 0 | 9 | 10 | 8 |  |  |  |
| Male | B | No | 25 | 98 | 86 | 17 | 12 | 1 | 423 | 6 | 14 | 6 | 0 | 7 | 12.6 | 26.3 | 7.3 | 2.9 | 1.6 | 9.4 | 3.6 | 5.6 | 4.8 | 5 | 7.8 | 6 |  |  |  |
| Male | B | No | 25 | 70 | 68 | 12 | 17 | 3 | 544 | 15 | 10 | 4 | 0 | 9 | 7.81 | 14.38 | 6.1 | 4.3 | 4.8 | 7.9 | 6.5 | 3.5 | 7.6 | 5.3 | 7.5 | 3.4 |  |  |  |
| Female | A | No | 24 | 86 | 76 | 23 | 6 | 5 | 593 | 26 | 21 | 4 | 0 | 10 | 8 | 12 | 6 | 4 | 6 | 6 | 2 | 2 | 6 | 6 | 4 | 7 |  |  |  |
| Male | B | No | 12 | 90 | 99 | 9 | 20 | 10 | 609 | 18 | 18 | 6 | 0 | 7 | 11.9 | 17.15 | 8 | 3 | 3 | 8 | 4 | 4 | 7 | 5 | 8 | 3 |  |  |  |
| Female | A | No | 18 | 89 | 83 | 18 | 11 | 6 | 644 | 21 | 29 | 7 | 0 | 10 | 11.14 | 17.57 | 6 | 4 | 5 | 4.7 | 5 | 2.7 | 6 | 6.5 | 5 | 5 |  |  |  |
| Male | B | No | 24 | 84 | 76 | 20 | 9 | 3 | 479 | 5 | 10 | 4 | 0 | 8 | 20 | 30 | 5.2 | 2 | 3 | 4 | 2 | 4 | 1 | 2 | 3 | 1.5 |  |  |  |
| Female | A | No | 24 | 84 | 78 | 17 | 12 | 1 | 494 | 14 | 12 | 6 | 0 | 10 | 9.4 | 18.6 | 6.2 | 4 | 3.9 | 6.9 | 5.1 | 3.2 | 7.4 | 7.4 | 7.2 | 5.2 |  |  |  |
| Female | A | No | 24 | 66 | 51 | 20 | 9 | 8 | 715 | 13 | 9 | 5 | 0 | 12 | 9 | 17 | 4.91 | 2.26 | 3.33 | 4.17 | 2.9 | 2.1 | 3.17 | 5.59 | 0.25 | 6.49 |  |  |  |
| Male | A | No | 24 | 79 | 81 | 19 | 10 | 5 | 555 | 30 | 17 | 6 | 0 | 12 | 10 | 17 | 6.5 | 1.5 | 1.5 | 9 | 2.5 | 7.5 | 2 | 2 | 8 | 2 |  |  |  |
| Male | A | No | 24 | 84 | 75 | 19 | 10 | 5 | 553 | 14 | 5 | 4 | 0 | 5 | 9.98 | 18.66 | 5.5 | 4 | 6 | 5 | 5 | 8.7 | 6 | 5.5 | 4.5 | 6 |  |  |  |
| Female | B | No | 24 | 73 | 59 | 8 | 21 | 11 | 646 | 20 | 29 | 4 | 0 | 7 | 14 | 27 | 4 | 7.5 | 6 | 5.5 | 7 | 8.5 | 1.8 | 0.5 | 10 | 0 |  |  |  |
| Female | B | Yes | 72 | 85 | 77 | 16 | 13 | 11 | 593 | 14 | 12 | 6 | 0 | 6 | 13.3 | 22 | 2 | 8 | 7 | 3 | 3 | 1 | 8 | 8 | 3 | 1.5 |  |  |  |
| Female | B | No | 24 | 90 | 70 | 12 | 17 | 2 | 648 | 18 | 31 | 6 | 2 | 7 | 14.89 | 24.27 | 8.8 | 4 | 2.5 | 7.9 | 2 | 3.5 | 6 | 5.5 | 7.2 | 3.7 |  |  |  |
| Female | B | No | 3 | 70 | 59 | 11 | 18 | 26 | 609 | 24 | 68 | 6 | 0 | 6 | 12.6 | 29.59 | 10.4 | 2.2 | 9.6 | 6.7 | 8.4 | 10 | 1 | 1 | 8.2 | 7 |  |  |  |
| Female | B | No | 6 | 82 | 78 | 16 | 13 | 2 | 618 | 20 | 9 | 5 | 0 | 6 | 10.45 | 16.04 | 9.3 | 1.7 | 1.2 | 7.6 | 6.8 | 7.4 | 2.5 | 1.8 | 7.8 | 5.5 |  |  |  |
| Female | A | No | 24 | 78 | 62 | 14 | 15 | 3 | 635 | 20 | 20 | 4 | 3 | 8 | 10 | 23 | 6 | 3 | 4 | 7 | 0.5 | 4.2 | 3.8 | 2 | 7.5 | 1.5 |  |  |  |
| Female | A | No | 24 | 63 | 58 | 12 | 17 | 1 | 599 | 7 | 9 | 6 | 0 | 2 | 9.6 | 18.7 | 4.1 | 6.9 | 5.3 | 7.8 | 3.6 | 5.8 | 3.2 | 2.9 | 7.7 | 1.6 |  |  |  |
| Male | B | No | 17 | 92 | 81 | 23 | 6 | 1 | 530 | 15 | 3 | 5 | 0 | 11 | 11 | 19 | 7.5 | 2.75 | 3.75 | 3.75 | 8.75 | 1.75 | 7 | 6.75 | 1 | 6.5 |  |  |  |
| Female | B | No | 25 | 68 | 59 | 8 | 21 | 9 | 492 | 16 | 17 | 4 | 1 | 7 | 13.6 | 29.1 | 6.9 | 4.6 | 3.7 | 6.1 | 3.2 | 3 | 6.6 | 6.8 | 8.7 | 5.9 |  |  |  |
| Male | B | No | 24 | 71 | 62 | 21 | 8 | 1 | 622 | 23 | 11 | 4 | 0 | 9 | 10 | 17 | 7.5 | 2 | 2 | 8.5 | 6 | 7.5 | 3.5 | 2.5 | 7 | 3.5 |  |  |  |
| Male | A | No | 27 | 78 | 69 | 5 | 24 | 36 | 603 | 17 | 32 | 7 | 7 | 7 | 9.4 | 17 | 6.7 | 5 | 5.2 | 6 | 4.3 | 6.5 | 4.7 | 4.8 | 6 | 4 |  |  |  |
| Female | B | No | 48 | 63 | 97 | 16 | 13 | 167 | 447 | 20 | 1 | 5 | 0 | 4 | 9.4 | 20.1 | 8.7 | 1.9 | 0.8 | 0.6 | 9.2 | 3.7 | 5.3 | 4.4 | 5 | 2.5 |  |  |  |
| Male | B | No | 48 | 77 | 60 | 17 | 12 | 2 | 481 | 20 | 23 | 4 | 0 | 3 | 10.7 | 23.8 | 7.4 | 2.6 | 2.2 | 8.7 | 2.7 | 3.6 | 7 | 7 | 6 | 0.5 |  |  |  |
| Female | B | No | 48 | 80 | 70 | 13 | 16 | 0 | 522 | 33 | 53 | 8 | 0 | 8 | 8.49 | 15.47 | 6.5 | 4.5 | 2.5 | 8 | 4.1 | 5.1 | 5.1 | 5.2 | 8.3 | 3.5 |  |  |  |
| Male | B | No | 19 | 61 | 65 | 22 | 7 | 1 | 586 | 14 | 0 | 4 | 2 | 7 | 6 | 19 | 6.3 | 5.7 | 6.4 | 7.7 | 6.1 | 3.1 | 6.5 | 5.3 | 5.4 | 2.3 |  |  |  |
| Female | A | No | 19 | 69 | 66 | 12 | 17 | 1 | 599 | 20 | 6 | 5 | 0 | 7 | 11 | 17 | 7 | 3 | 5 | 6 | 2.5 | 5 | 5 | 3 | 7.5 | 1 |  |  |  |
| Female | A | No | 48 | 79 | 80 | 5 | 24 | 3 | 872 | 18 | 5 | 4 | 0 | 10 | 13 | 22 | 5.5 | 8 | 6.1 | 7.2 | 1.9 | 6.4 | 6.4 | 4.2 | 7.7 | 1.5 |  |  |  |
| Male | B | No | 24 | 87 | 81 | 14 | 15 | 0 | 517 | 25 | 36 | 6 | 1 | 10 | 15 | 17 | 9.9 | 0.4 | 0.5 | 9.4 | 8.3 | 8.2 | 1.8 | 0.7 | 9.6 | 4.4 |  |  |  |
| Male | A | No | 24 | 110 | 101 | 14 | 15 | 5 | 743 | 13 | 12 | 3 | 2 | 8 | 15 | 35 | 6 | 2.5 | 5 | 10 | 2 | 6 | 5 | 7.5 | 10 | 4.5 |  |  |  |
| Female | B | No | 28 | 84 | 71 | 15 | 14 | 3 | 677 | 14 | 12 | 4 | 0 | 7 | 12.87 | 16.27 | 9 | 2 | 0.5 | 8 | 5.5 | 7.5 | 4 | 0.5 | 9.5 | 0.5 |  |  |  |
| Male | A | No | 24 | 82 | 56 | 18 | 11 | 5 | 699 | 20 | 19 | 6 | 0 | 10 | 11 | 20 | 9.6 | 0.8 | 9.9 | 4.9 | 6.9 | 3.9 | 7.3 | 2.2 | 7.4 | 0.9 |  |  |  |
| Female | A | No | 18 | 83 | 78 | 20 | 9 | 6 | 509 | 16 | 11 | 6 | 0 | 7 | 12 | 23 | 6 | 4.5 | 4.5 | 7.3 | 6.7 | 5 | 4.5 | 5.2 | 6.7 | 3.6 |  |  |  |
| Female | A | No | 24 | 91 | 87 | 17 | 12 | 2 | 752 | 12 | 5 | 4 | 0 | 8 | 9 | 29 | 4.3 | 7.3 | 3.1 | 7.1 | 4.3 | 3.2 | 6.4 | 3 | 7.5 | 3.2 |  |  |  |