Contents

1.   Introduction

The Time Series Response Analyser (TSRA) aims to reduce the time spent analysing time series data, and the chance of mistakes being made. The principal functionality of the TSRA is the calculation of the area under the curve (AUC) when a response is plotted against time, with a variety of calculation options depending on the context in which the spreadsheet is being used (see Section 3.2). Though AUC calculations are the key feature for which this spreadsheet has been built, a range of outputs which may provide greater transparency in the nature of the collected data are also provided. These include representations of the individual responses, comparisons of the responses at a group level, and further variables including the peak values, the time at which these values occur, and a range of intra-trial variability statistics such as standard deviation (SD) and coefficient of variation (CV). Though these features all exist within the overall output, users are encouraged not to 'look for an effect'. Instead, these features are intended to provide context and extra clarity around any interpretations that are drawn.

Though considerable effort has been put into the design and testing of the TSRA, users take full responsibility for the results generated. As such, users are advised to be rigorous when entering data, and to spot check the calculations before results are published. This spreadsheet will remain completely free to use in the hope that researchers can save considerable time during data analysis, and that results can be published with greater consistency and transparency. A reference in any papers in which results from the TSRA are published would add credibility to the study and, would be greatly appreciated by the authors (see Section 11).

2. User Interface

The user interface in the 'Data Entry' worksheet consists of a range of experimental parameters across the top, and three buttons above a condensed list of instructions down the left-hand side. The area in the middle (initially blank) allows for a theoretically infinite number of trials and timepoints to be entered.

2.1. Start Button

The 'Start' button reveals the 'Experimental Setup' window (Fig. 1), allowing the user to define the parameters and calculation preferences for the time series analysis.

Figure 1. Experimental Setup Window

2.2. Reset Button

The 'Reset' button will clear and hide all subsequent tabs beyond 'Data Entry', clear all raw data if any had been entered in the 'Data Entry' worksheet, and empty the parameter cells across the top of this sheet. Essentially, this button resets the spreadsheet to the condition in which it is initially opened (Fig. 2). All results should be exported before clicking this button as this function cannot be undone.

Figure 2. The user interface upon opening the TSRA or after clicking 'Reset'.

2.3. Analyse Button

The 'Analyse' button performs all functions underlying the output of this spreadsheet. It begins by interpolating any missing values, if this option was chosen in the 'Experimental Setup' window, and subsequently performs all calculations before revealing the various outputs in their respective tabs.

3.   Experimental Setup

The 'Experimental Setup' window (Fig. 1) will appear when the 'Start' button is clicked. This window allows the user to define the parameters of the experiment, and the calculations and analyses they would like the TSRA to perform. The following section provides the restrictions on each parameter. If any of these are violated, an appropriate error message will prompt the user to amend the relevant field.

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3.1. Parameters

Number of Participants: Enter the number of participants for whom there are data. There is no limit to the number of participants that can be entered.

Number of Conditions: Enter the number of conditions to be compared. The TSRA can handle up to 4 conditions. If more are required, analyses should be re-run the required number of times.

Number of Timepoints: Enter the number of timepoints at which the measurement was sampled. This number should **include** the baseline timepoint (time 0). There is no limit to the number of timepoints that can be entered.

Unit of Time: This is an optional field. The dropdown options are 'Minutes' or 'Hours'. If neither of these two options apply, this field can be left blank.

Study Design: This is a mandatory field, which must be either 'Repeated Measures' or 'Independent Groups'. If all participants completed all trials, select 'Repeated Measures' from the dropdown list. Otherwise, if participants each took part in only one trial, this input should be 'Independent Groups'. If a combination of the two are used, select 'Repeated Measures' and use the 'Amend Trials' function after clicking 'Ok'. This field will default to 'Repeated Measures' if only one participant exists.

Area Under the Curve Calculation: The options for this field, as per the dropdown menu, are iAUC, tAUC, iAUCcut, iAUCmin, iAUCmincut, iAUCx and iAUCxcut. Click 'View Options' to see graphical representations of the area that each of these options will calculate (Fig. 3; Section 3.2). If either iAUCx or iAUCxcut is selected, the input field by 'x =' will be unlocked and a numerical value is required to define the absolute threshold.

Interpolate Missing Values: Select 'Yes' or 'No' depending on whether missing values should be interpolated by the TSRA. If 'No' is selected, complete data should be pasted into the spreadsheet with any missing values already handled.

3.2. Amending Trials

If certain trials do not align with the existing data (for example if certain participants completed some, but not all, trials), rows can be added or removed from the 'Data Entry' tab. Click 'Amend Trials' and the window shown in Figure 3 will appear.
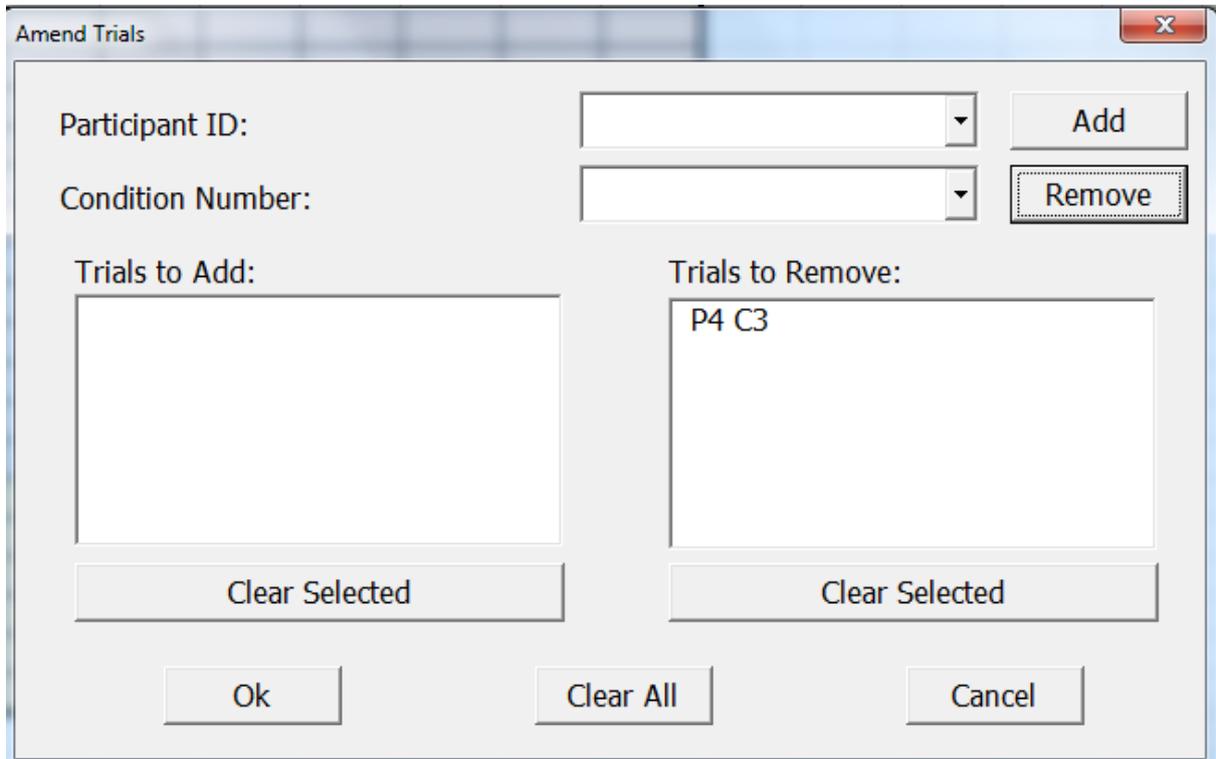

Figure 3. Amend trials window

To amend the list of trials, select the Participant ID and Condition Number from the dropdown menu, and choose whether you would like to add or remove the relevant trial. In the example (Fig. 3), the trial corresponding to Participant 4 Condition 3 will be removed when 'Ok' is clicked. Multiple trials can be added and removed at the same time. Selecting trials from the 'Trials to Add' and 'Trials to Remove' boxes will allow them to be cleared, and clicking 'Clear All' will empty both boxes.
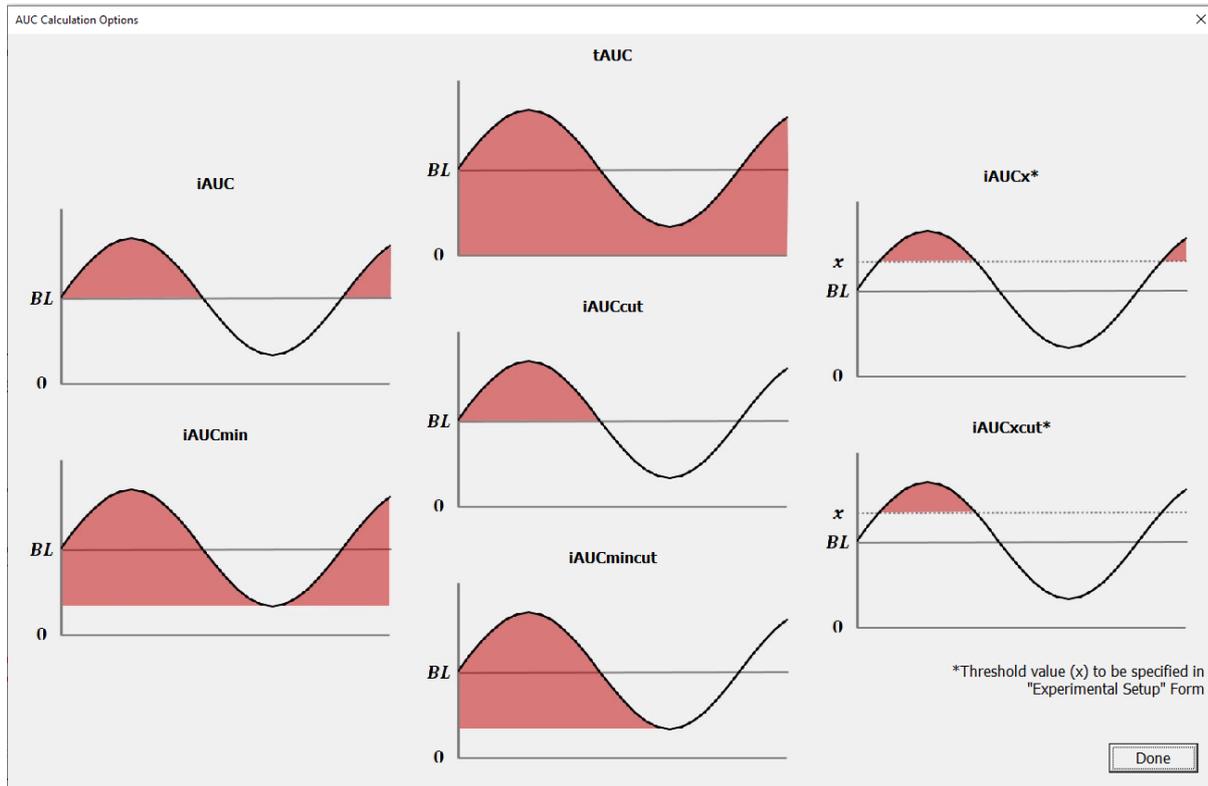
Figure 4. Area under the curve calculation options.

3.3. Area Under the Curve Calculation Options (Fig. 4)

iAUC: the whole AUC above the baseline value

tAUC: the whole AUC above 0.

iAUCcut: the AUC above the baseline value until the curve cuts below the baseline value for the first time.

iAUCmin: the whole AUC above the minimum value.

iAUCmincut: the AUC above the minimum value until the minimum value is reached for the first time.

iAUCx:* the whole AUC above the user-defined threshold (x).

iAUCxcut:* the AUC above the user-defined threshold (x), until the curve cuts below the user-defined threshold (x) for the first time.

*User-defined threshold (x) to be specified in the 'Experimental Setup' form.

4. Inserting Raw Data

When pasting data into the TSRA 'Data Entry' tab, it is vital the user right clicks in the destination cell and selects 'Paste Values' from the 'Special Paste' options (Fig. 5). This is to prevent the cells being influenced by the raw data source formatting. If data are pasted using an inappropriate method, and the formatting is affected, click 'Reset' to begin the process again.
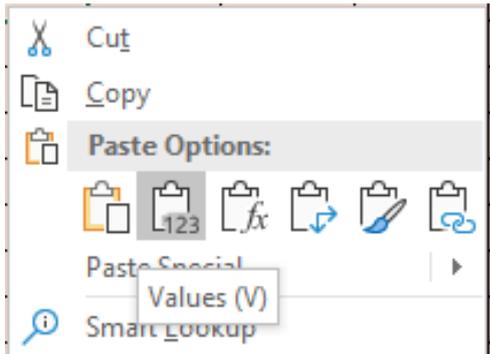

Figure 5. When pasting data, right click in the destination cell and select "Paste Values".

If the missing value interpolation option has not been selected, ensure any missing values have already been dealt with. Under these circumstances, all coloured cells in the 'Data Entry' tab should contain data once they have been pasted into the appropriate fields (Fig. 6).

*Disclaimer: Users take full responsibility for results generated using this spreadsheet

Reset		Number of Participants:	8		Number of Timepoints:	7	Study Design:	Repeated Measures	Interpolate:	No

		Number of Conditions:	3	Unit of Time:	Minutes	AUC Calculation:	iAUC

	Key	Time (mins)	0	15	30	45	60	90	120
Start	Condition 1	Participant ID	(BL)						
	Condition 2	1	4.69	6.14	7.61	8.45	6.51	4.12	5.48
	Condition 3	2	5.37	6.91	6.53	6.18	7.16	6.80	5.69
Amend Trials		3	5.62	7.69	5.99	5.51	5.91	4.49	4.67
		4	5.44	7.20	8.35	6.84	5.25	4.62	5.17
		5	4.38	7.56	8.00	5.70	5.50	6.01	5.12
Analyse		6	4.96	6.59	8.03	6.39	5.59	5.60	3.81
		7	5.71	9.10	9.00	7.60	6.56	5.14	2.83
		8	5.18	7.61	7.69	7.97	6.63	6.46	6.03
Instructions		1	4.97	6.55	7.88	7.53	6.20	4.37	3.84
1. Click "Start"		2	5.06	7.17	5.56	3.70	4.10	5.77	4.77
		3	5.61	7.39	10.70	8.59	6.02	5.70	4.56
2. Enter experimental setup variables and click "Ok"		4	5.45	6.68	7.25	6.36	5.18	5.80	5.09
Max number of conditions = 4		5	4.84	6.44	9.35	7.31	7.51	5.03	4.38
		6	5.79	8.18	8.30	5.25	2.63	3.03	4.05
3. Check variables at the top of the sheet, the coloured cells		7	5.67	7.50	7.89	4.68	5.18	2.54	2.34
and the Participant ID values all match requirements		8	5.46	6.69	7.18	6.45	6.51	5.74	5.38
Click "Amend Trials" to manipulate rows as required		1	5.05	7.10	9.71	10.21	8.73	6.61	6.06
		2	5.18	6.68	7.47	6.67	6.14	5.75	6.69
4. Populate coloured cells with relevant data		3	5.82	7.67	9.67	8.89	9.54	8.20	7.09
To paste data, right-click and **select special "Paste Values"**		4	5.09	7.51	8.40	8.20	7.37	7.60	6.45
		5	4.75	7.92	6.94	5.48	5.00	5.09	3.89
5. Click "Analyse"		6	5.64	6.87	7.71	6.64	5.24	4.28	4.36
		7	5.50	8.98	8.41	5.89	5.27	4.27	4.28
6. Click "Export" on the last worksheet to open (Mac) or save		8	5.58	7.98	9.27	9.52	8.41	6.01	6.82
(Windows) tabulated data in separate excel workbook									
Data **must be exported** as this file resets upon opening (i.e.									
saving this file **will not save** any inputs or outputs)									

Figure 6. If the interpolate missing values option has not been chosen, all coloured cells should contain data before 'Analyse' is clicked.

5. Missing Value Interpolation

The interpolation of missing values is an optional feature. For the missing values to be handled by the TSRA, the 'Yes' option should be selected in the 'Experimental Design' form that is shown when 'Start' is clicked. If 'No' is selected at this stage, any missing values should be handled before raw data are pasted into the sheet.

Missing values are handled using linear interpolation (Fig. 7). A straight line is drawn between the two closest existing values either side of any that are missing, and the values are then calculated as a function of the equation of that line at each respective timepoint. This means any number of single or consecutive values can be interpolated, assuming the baseline values and values under the last timepoint are known. If any of these mandatory values are not know for a given trial, these should be imputed using an appropriate method of the user's choice before being pasted into the 'Data Entry' tab.
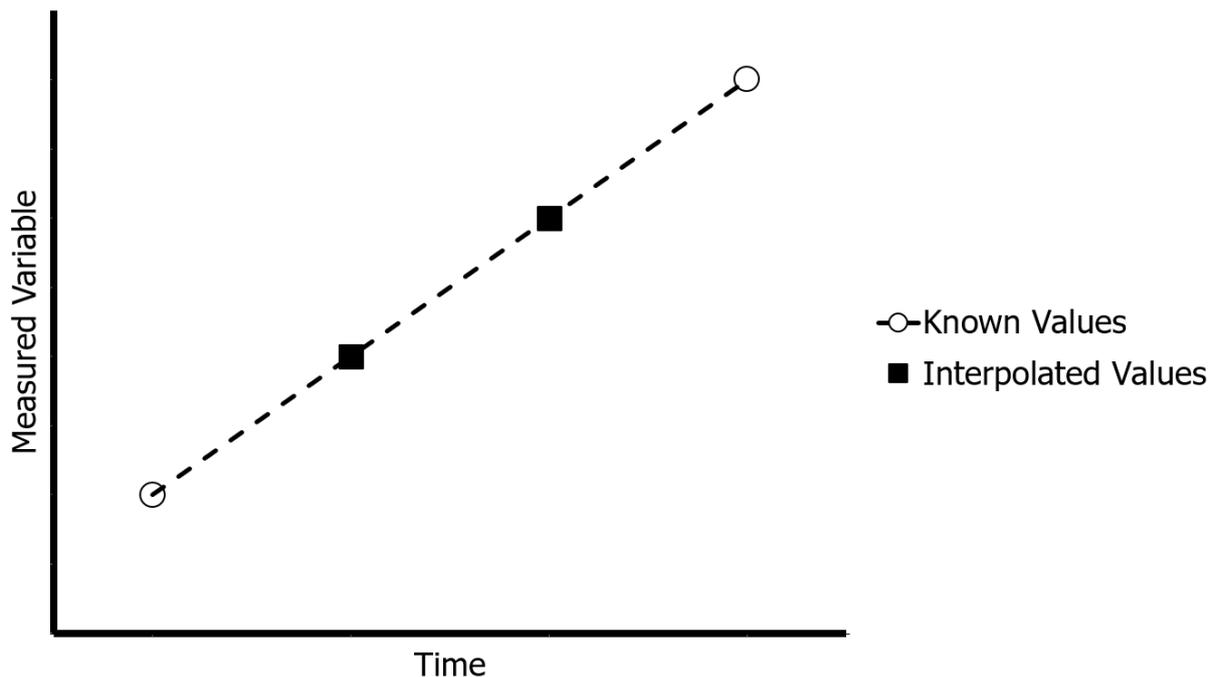
Figure 7. Process by which missing values are interpolated from known values.

Please note that excluding participants with a certain amount of missing data may be more appropriate than using linear interpolation. Indeed, linear interpolation may not always be the most appropriate method to handle missing data, so the user is advised to use this feature only if they feel it is justified within their context. The 'Peak' and 'Time to Peak' features within the TSRA may also be skewed through

linear interpolation depending on which values are missing within certain trials (Fig. 8). Values imputed by the TSRA cannot be differentiated from the raw data, so it is recommended that users make a note of these cells before clicking 'Analyse'. Imputed values can then be screened in the output.
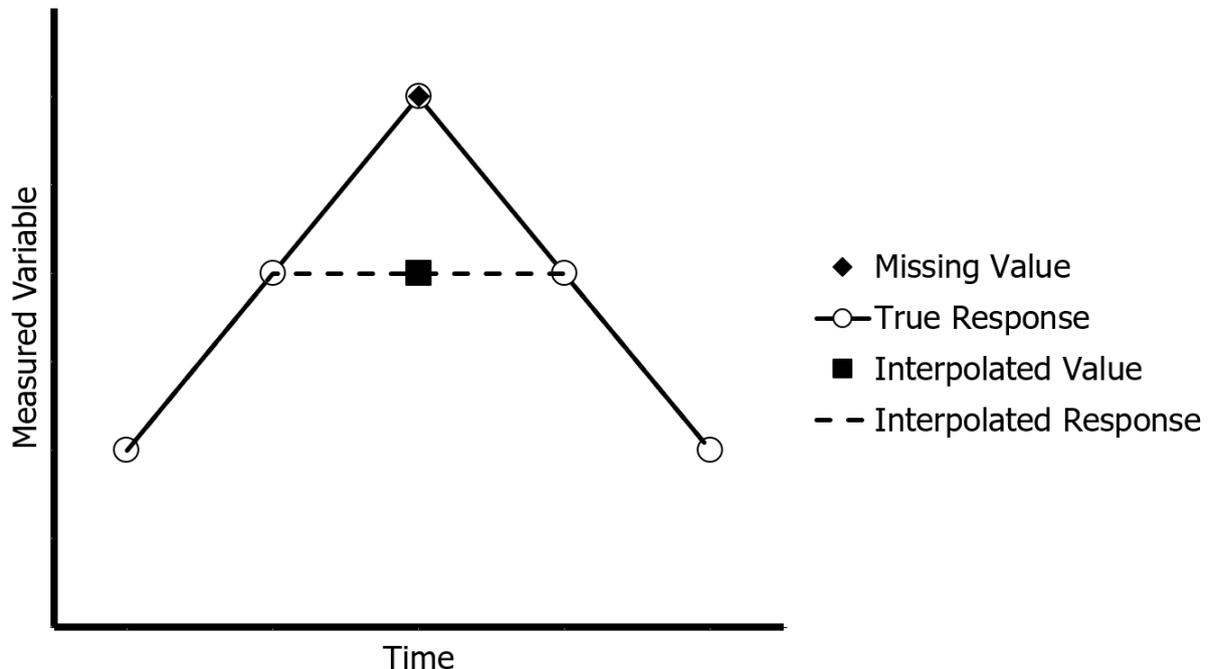

Figure 8. Scenario in which linear interpolation might mask a true response.

6.   Data Entry Checks

A few simple checks before clicking 'Analyse' will mitigate the chance of an error message appearing. All obvious errors are covered by specific error messages that correspond to the user making appropriate changes. There are however certain errors which the TSRA may not be able to identify.

All values relating to the experimental design at the top of the sheet should reflect the study design and required calculations. If iAUCx or iAUCxcut have been selected, an absolute threshold value (x) must exist.

If missing value interpolation is off, ensure every coloured cell on the sheet contains a value. This includes the blue row containing the timepoint increments, the Participant ID column and all raw data fields (Fig. 6). If missing value interpolation is on, the same checks apply except that certain raw data points may be missing. Note that a greater number of interpolated values is likely to result in an estimation that is

less reflective of the true response (see Section 4). Though an unlimited number of raw data points can be interpolated, all baseline values and all values at the final timepoint are required for this function to operate.

Finally, check that the values have been pasted into the correct rows, so that each set of responses corresponds to the appropriate participant number and condition. The TSRA will not identify errors of this type, meaning they may propagate and/or go unnoticed when analysing results at a group level.

Note: The Participant ID numbers are not restricted. These can be manually changed to reflect the unique identifiers used by the researcher. Extra care should be taken in ensuring consistency and accuracy if these are manipulated.

7. Output Tabs

Upon clicking 'Analyse', a range of tabs will appear after 'Data Entry' containing the TSRA outputs. If only one participant is analysed, only the 'Tabulated Data' and 'Individual Responses' tabs will appear. In all other cases, the following four tabs are made visible:

7.1. Tabulated Data

The 'Tabulated Data' tab contains all raw data and calculation outputs in tabular form (Fig. 9). Each row corresponds to an individual trial, which can be identified by the Participant ID (first) and Condition (second) columns. In this format, each condition is denoted by an integer. The next set of columns are the raw data as they were entered on the 'Data Entry' tab, including any values that were interpolated by the TSRA. Subsequent columns include the AUC calculation for each segment (shaded grey), the method of which is determined by the initial user input, with the AUC for each trial calculated as the sum of each segment (shaded green). The header of each of the shaded grey and green columns confirm the method of AUC calculation. The following column, called 'Peak', is the maximum value for each trial (shaded pale blue), with the time at which that value first occurred in the next 'Time to Peak' column (shaded orange). The final two columns display the standard deviation (shaded dark blue) and coefficient of variation (shaded yellow) for each trial.

Figure 9. An example of the "Tabulated Data" tab from the TSRA output.

7.2. Individual Responses

The 'Individual Responses' tab allows the user to scroll through each participant's response to the condition(s) on a case-by-case basis (Fig. 10). Use the slicer to the right of the graph to scroll though each Participant ID. If a repeated measures research design has been selected, the colour of each condition will correspond to the cell fill colour in the 'Data Entry' tab. If an independent groups research design has been used, all plots will be in black. The legend at the top right of the graph identifies the condition number that the selected individual participated in.
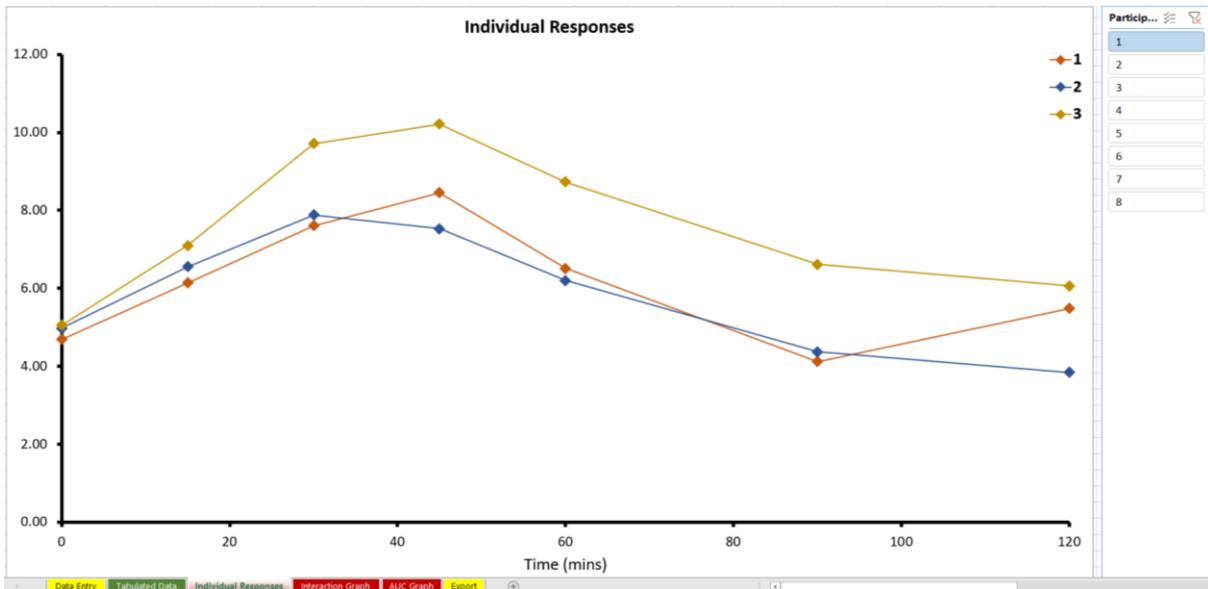

Figure 10. An example of the "Individual Responses" tab from the TSRA output.

7.3. Interaction Graph

The default output in the 'Interaction Graph' tab is a line graph showing the group responses across time for each condition (Fig. 11). Each point on the graph is the mean value for all participants in that condition at that timepoint, with the error bars representing the standard error of the mean. The boxes to the right of the graph under 'Conditions Shown' can be unchecked to remove certain conditions from the graph, and the measures of central tendency under 'Error Bars' can be changed to the user's preference. The 'Update Graph' button must be clicked for any of these changes to be reflected in the graph.
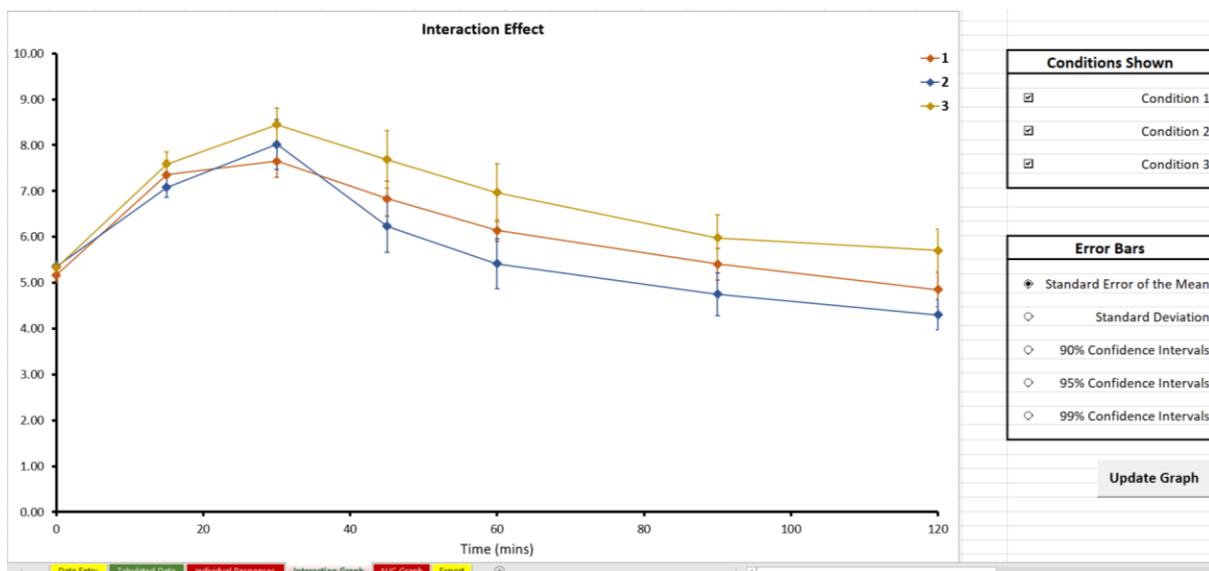

Figure 11. An example of the "Interaction Graph" tab from the TSRA output.

7.4. AUC Graph

The default output in the 'AUC Graph' tab is a bar chart comparing the results of the chosen AUC calculation between conditions (Fig. 12). Individual responses are superimposed as straight lines between conditions if a repeated measures research design was selected, or as diamond markers for an individual groups research design. The error bars above each condition default to the standard error of the mean, though these can be changed using the option buttons to the right. This graph can be manipulated to represent the chosen AUC calculation, 'Peak' or 'Time to Peak' by choosing the relevant option button, and the individual plots can be removed by unchecking the appropriate box. The 'Update Graph' button must be clicked for any of these changes to be reflected in the graph.
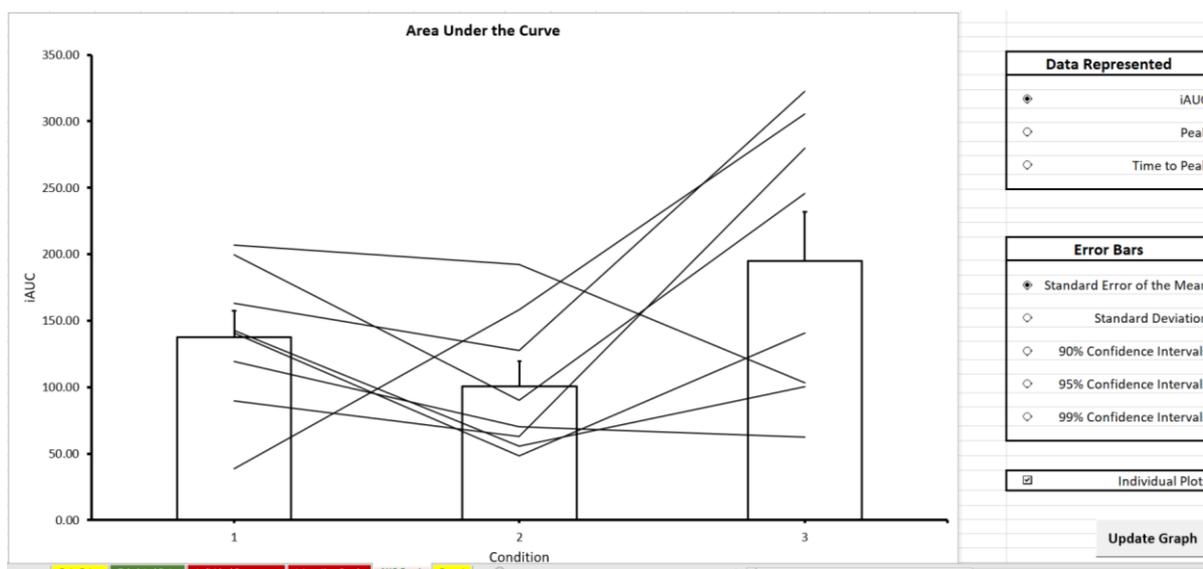
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Figure 12. An example of the "AUC Graph" tab from the TSRA output.

8.   Export (Windows Version)

The 'Export' tab contains a single button that allows the user to save the results of the TSRA as a separate .xlsx file in a user-defined location. Simply click 'Export', fill in the required fields, select the data to be included and click "Ok" (Fig. 13).
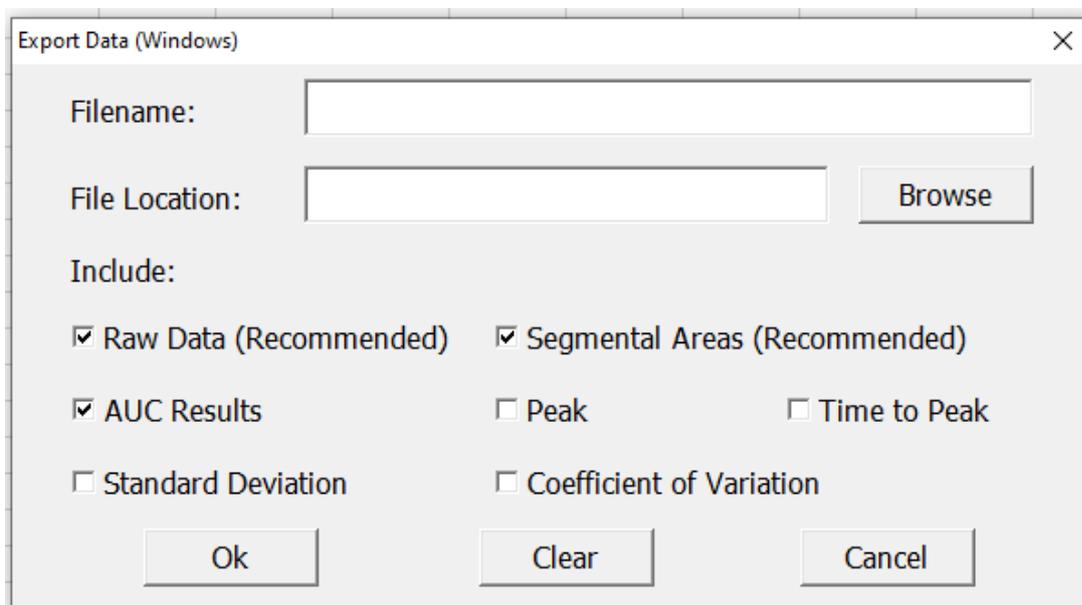

Figure 13. Export Data Window.

8.1. Export Options

The export functionality uses the 'Tabulated Data' tab as a template and removes any columns that are not selected to be included. The following options are available when exporting data from the TSRA:

Raw Data: The exported table will include the raw data values as they were initially entered by the user (including any interpolated values). These are recommended in the output to provide transparency in the calculations.

Segmental Areas: The exported table will include the segmental areas, the sum of which result in the chosen AUC calculation. If the AUC calculation forms part of the export, these segmental areas are recommended to provide transparency in the calculations.

AUC Results: The exported table will include the column containing the AUC results.

Peak: The exported table will include the column containing the maximum raw data value for each trial.

Time to Peak: The exported table will include the column containing the time at which the maximum raw data value occurred for the first time during each trial.

Standard Deviation: The exported table will include the column containing the standard deviation of the raw data for each trial.

Coefficient of Variation: The exported table will include the column containing the coefficient of variation of the raw data for each trial.

8.2. Error Handling

Although the export function is designed to provide appropriate error messages if fields are completed incorrectly, the user is advised to mitigate the chance of these occurring with the following checks:

Filename: The filename field cannot contain **\ / : * ? " < > |** or be left blank.

File Location: The chosen file location must exist, and the user account must have permission to save a file in the desired location. The chance of an error in this field is likely to be reduced by using the 'Browse' button.

Other: If a file of the given name already exists in the chosen directory, an error message will be displayed asking if the user would like it to be overwritten. As this cannot be undone, ensure any data that may be lost is saved. In addition, if a file of

the same name is currently open, **it will be automatically saved and closed**, even if the open file exists in a different directory.

9. Feedback and Final Comments

If as a user of the TSRA you have any feedback, identify any problems with the spreadsheet or have suggested improvements, please go to *URL* to contact the author. As mentioned in Section 1 of this document, a reference to the TSRA in any publications containing results obtained using this spreadsheet would be greatly appreciated (See Section 12). The export functionality of this spreadsheet is also recommended to be used when publishing datasets alongside research papers, allowing full data transparency and reducing the chance of human error compared to manual calculations.

10. Acknowledgements

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11. Citation

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