AARGII

Previously, AARGI carried out all the necessary time-consuming and computationally intensive data processing that did not require ongoing user input. Thus, AARGI was designed such that the user could command large amounts of data to be analysed at a time and then attend to other tasks. In AARGII, more of the user's ongoing attention is required at each step and so there is no need (indeed, it could even be unhelpful) to enable multiple experiments to be selected at one time.

User interface sections

6. Keyword(s)

Keyword(s): Same guidelines apply as for AARGI - see AARGI help pdf.

7. Experiment

Experiment: Same guidelines apply as for AARGI, except the user can only select one experiment at a time.

8. Cell tracing

The objective at this stage of the analysis is to build a representation of the cell, which is then used to measure distances between each ROI and its connected start point. The user must manually specify start points and white lines - white lines represent dendrites. Connections between white lines and ROIs (representing the spine neck) are coloured green. These green lines are generated once the user has set all white lines and start points. Green lines cannot be created manually. They can only be deleted manually.

Experiment: This text box will be updated once the user selects an experiment for analysis.

Show ROIs from condition: This text box will be updated with the last condition given in the keywords section. The selected condition will show not only ROIs established in this condition, but also ROIs produced in any previous condition.

Resolution: This is the microscope resolution, which the user should calculate independently.

Max. distance to ROI: Maximum distance to ROI from the closest point on a dendrite (or more precisely, a white line representing a dendrite). It is useful to specify this value, otherwise the algorithm will connect all ROIs, including those that are associated with dendrites the user wishes to exclude from analysis, to a dendrite the user wishes to include. Such undesired connections can be deleted, but this could be a tedious task if there are many of them.

New build checkbox: Checked by default. If pre-existing data is detected, the user will be asked whether or not it should be overwritten once s/he clicks the 'Launch' button. The user will build a skeletal outline of each cell. This process involves four steps of user intervention:

1. Build start points: This will be the default 0 distance point. Every white line will be connected directly or indirectly to a start point.
2. Build white lines: Carried out manually by the user. These are segmented lines, which should be used to precisely follow the shape of the dendrites. Once this is complete and the user presses the 'a' key, AARGII will automatically try to establish connections between each ROI and its nearest white line (provided it is within the range determined by the 'Max. distance to ROI' value).
3. Deleting unwanted or invalid connections between ROIs and dendrites followed by distance measurement between each ROI and the start points they are connected to along a path of green and white lines.
4. Final confirmation: Left clicking on a ROI will make its centre appear blue as well as the centre of any ROI along the same line which is also closer to the connected start point. Any ROI with a black centre will be excluded from further analysis. When the

user presses the ‘a’ key, a final image of the cell will be presented with only the ROIs selected for inclusion being displayed in the image.

Modify old build checkbox: When checked, the ‘Modify’ subregion of the AARGII user-interface is enabled. The Modify subregion allows the user to choose which part of the analysis should be modified. There are three options each represented by a radio button:

Add start point(s) and/or white line(s): when selected, the algorithm will load any start points. Note that is not possible (with the first release of AARG) to selectively delete start points or white lines. If the user wishes to exclude ROIs from analysis for a specific dendrite the best current option would be to not select these ROIs in stage 4 (see ‘New build checkbox’). If the user wishes to exclude the line in the image (e.g. for presenting the image in a figure or presentation slide), then this can be done with slight modifications to the relevant matlab scripts, if the user is familiar enough with matlab. Otherwise, it is necessary to re-do the analysis.

Remove ROI connection(s): When this radio button is selected, AARGII will load all data up to and including green lines connecting the ROIs to their white lines. The user may then delete more green line connections before proceeding.

Skip to ROI selection: This option will take the user straight to stage 4 (see ‘New build checkbox’). No deletion of green lines is possible with this option selected.

9. Review data

Enter frame rate (Hz): the frame rate is the number of frames per second the data was acquired at. This information is needed to calculate the decay time constant in seconds.

Launch review: AARGII will prepare the data for review by the user if this has not previously been done. This preparation involves taking each trace for each ROI and cutting it into segments, with each segment containing a single event - or what AARG has detected as an event up to this point. With each button press the user will be presented with at least five new figure windows: 1) The first window is a trace of the

most active ROI for the first condition. Green dots indicate the point of baseline measurement for the nearest event further along the time axis. The red dots are the peaks of each event, which were found in AARGI. 2) Histogram of the data presented in the first figure window. Subsequent pairs of figure windows show the same type of data, but for subsequent conditions. The final figure window shows individual events that have been cut out of the traces for each ROI. The raw data trace is overlaid with a fitted curve, which can be used to measure the decay time constant.

In most cases, the user will need to use only five keys on the keyboard to complete the review process: ‘a’ to accept both the peak amplitude measurement and the curve fit, ’v’ to accept the amplitude measurement and reject the curve fit as well as ‘r’ to reject both the amplitude measurement and the curve fit. ‘Shift + u’ takes the user backwards and undoes the previous choice. All data is saved with each key stroke made by the user. So, if the user quits or Matlab crashes, all progress will be saved. Furthermore, any time the ‘Launch review’ button is clicked, the user will be taken back to their last point of progress. Although this stage is heavily reliant on user intervention, the time and effort required from the user is minimised by AARG’s cutting of the ROI trace into event segments.

10. Graph data

Graph setup: This button will open another user interface (graphAARG), where the user can plot experiments from the same data set after applying any customizations that may be necessary.

10c. Properties

Customize start point distances: checking this checkbox will enable the ‘Get Branch IDs’ button and the three text boxes beneath this button. These items should be used to change the distance values of selected start points from the default value of 0.

Get Branch IDs: after clicking this button, the user will be prompted to select a number of experiments for which branch IDs are required. Any experiments selected at this point should also appear in the Experiment list of the graph AARG interface. Images of the selected experiments will be generated showing the white lines built by the AARGII cell tracing algorithm and each of the white lines will be labelled with its branch ID number. Note these values down.

Experiment directory index: each experiment containing a start point requiring a customized start point value should be listed here in the form of its order in the Experiment list. For example, if there are six experiments: A, B, C, D, E and F where B has a single start point (AND with a single branch emanating from it) requiring a customized distance value, B will be represented as ‘2’ in the ‘Experiment directory index’ text box (because B is the second experiment in the list). If D also has two start points requiring customized values, ‘2,4,4’ should be entered in this text box. Note that commas should be entered between each value.

Branch IDs: experiment B has only a single start point with a single branch extending from it. In such cases, the ID value can only be ‘1’, but it is recommended that the user always confirm this using the ‘Get Branch IDs’ button. For experiment D, there are two branches - one emanating from each of the two start points. One of these branches will have a branch ID of ‘1’ and other will be some other integer value - imagine that it is ‘7’ in this example. So, the following should be entered in the ‘Branch IDs’ text box: ‘1,1,7’.

Custom start-point distances: custom distance values for each start point in each experiment that requires them should be derived using Fiji/Image J or equivalent

software. Imagine that the distance values for each of the start points in our experiments are as follows (start points are identified according to the branches that emanate from them): ‘B,1’: 83.2μm; ‘D,1’: 138μm; ‘D,7’: 146.1μm. In this case, the ‘Custom start point distances’ should be filled out as: ’83.2,138,146.1’.

Note that the values in ‘Experiment directory index’, ‘Branch IDs’ and ‘Custom start point distances’ have to exist as a triplet. If the order is changed in one text box, the order of values in the other text boxes has to change appropriately as well. For example, if instead of ‘2,4,4’, we have ‘4,4,2’, then IDs should be: ‘1,7,1’ (or ‘7,1,1’), and distances should be: ‘138,146.1,83.2’ (or ‘146.1,138,83.2’).

Configure graphs: this brings up the configureGraphs interface. This allows the user to change the default settings for graph plot (which will be necessary in almost all cases). There are two types of graph that will be plotted: Histograms and median plots. Some controls apply to one graph type independently of the second, while other controls apply to all graphs:

All graphs: By default, AARG will plot the amplitude data across 20μm segments of dendrite. An upper limit can be applied to the distances plotted in the ‘maximum distance’ text box. The ‘hide unnecessary tick labels’ will remove tick labels (and axis labels) from graphs that are not presented at the left or bottom edge of the graph group. This feature can help save time later if the user is well aware of how s/he wants the data to be displayed.

Histograms: By default, each graph will have a maximum x- and y- axes value determined by the contents of the graph. If plotting data for the first time, it is recommended not to set any maximum value for the x- or y- axes and leave the question marks in place. This will allow the user to judge what the most suitable maximum values will be. The default bin width for each histogram is 200 for the amplitude graphs and the default bin width in the case of plotting decay constants is 1.

Median plots: synaptic event amplitudes are positively skewed. In such cases, taking the mean average would not be the most accurate measure of the average event amplitude. The median is more appropriate. The median average from each experiment is calculated for each distance and plotted accordingly. T-test results and their p-values can be plotted for each graph.