To reproduce figures for Tan et al., Munc13 paper:

[Text typed after ">>" is to be entered in the MATLAB command window.]

**NOTE**: The hierarchical bootstrap code was written with clarity in mind and not optimized for execution speed. Sampling in this way is rather slow, so it takes about 45 minutes to generate 100k bootstrap replicates on a typical laptop computer (as compared to about 2 minutes for the non-hierarchical version). This could be sped up tremendously using parallel 'for' loops ('parfor' in MATLAB) on a multip-processor machine.

1. Tan et al., Figure 4 - figure supplement 2, Panel B:

>> **plot\_ANOVA\_Munc13**('dataset4- AP evoked EPSC.xlsx',0.25,0);

This plots all 4 experimental groups. Panel B corresponds to Group A (top left subplot)

2. Tan et al., Figure 4 - figure supplement 2, Panel C:

Mocked up in PowerPoint: **hierarchical\_bootstrap\_figures.pptx**

3. Tan et al., Figure 4 - figure supplement 2, Panel D:

>> [Tr,Tb,allMu,SE,CI,pH0] = **hBS\_Munc13\_function**('dataset4- AP evoked EPSC.xlsx', 1, 100000,0.05, 1);

% NOTE: This will take about 45 minutes to run on a typical laptop computer.

4. All 100k bootstrap iterations for all data files were run overnight (requires several hours for 100k replicates of all data files). This is just a wrapper script that calls the hBS\_Munc13\_function above. Note that it processes each data file twice: once performing a hierarchical bootstrap and once performing a non-hierarchical bootstrap.

>> **run\_all\_Munc13**

needs file containing all data file names: **all\_data\_files\_names.mat**

This script saves all of the bootstrap replicates of T\* for each file in its own .mat file. So any given histogram can be reproduced (without having to re-run the entire bootstrap) by loading the corresponding .mat file and using 'plot\_Tboot\_Histogram'.

The script also saves summary statistics for each bootstrap in an Excel spreadsheet:

**results\_bootstrap\_100k\_replicates-14Oct2022.xlsx**

So, for example, the histogram in panel B above could also be reproduced with:

>> load 'dataset4- AP evoked EPSC\_hFlag\_1\_nBoot\_100000.mat'

>> Treal = 1.5172; % experimental value for T (from above Excel file)

>> alpha = 0.05; % for 95% CI

>> h = **plot\_Tboot\_Histogram**(Tb,Treal, alpha);

5. Two-factor ANOVAs were performed with:

>> **run\_ANOVA\_Munc13**

Results written to Excel file: **results\_ANOVA.xlsx**

6. Tan et al., Figure 4, panel N:

>> load 'dataset4- AP evoked EPSC\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,1.5172, 0.05);

7. Tan et al., Figure 4, panel O:

>> load 'dataset 3- AP evoked IPSC\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,2.393, 0.05);

Note: Could also accomplish this with:

>> [Tr,Tb,allMu,SE,CI,pH0] = **hBS\_Munc13\_function**('dataset 3- AP evoked IPSC.xlsx', 1, 100000,0.05, 1);

% This would take about 45 minutes to run on a typical laptop computer. Similar results can be obtained

% with fewer bootstrap replicates: generating 10,000 replicates takes about 4.5 minutes.

8. Tan et al., Figure 5, panel K:

>> load 'dataset 2-sucrose E-v1\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,3.1595, 0.05);

9. Tan et al., Figure 5, panel L:

>> load 'dataset 1-sucrose IPSC-v4\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,1.6399, 0.05);

10. Tan et al., Figure 6, panel K:

>> load 'dataset 5-EPSC-PPR(50ms)\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,1.061, 0.05);

11. Tan et al., Figure 6, panel L:

>> load 'dataset 6-IPSC-PPR(20ms)\_hFlag\_1\_nBoot\_100000.mat'

>> h = **plot\_Tboot\_Histogram**(Tb,1.035, 0.05);