Technical Note on Data Processing

This note documents the processing procedures for dorsal root ganglion discharge data collected from AA rat models before and after acupuncture stimulation. The corresponding article is titled "Acupuncture-Induced Dorsal Root Potentials and Mast Cell Degranulation in AA Rats."

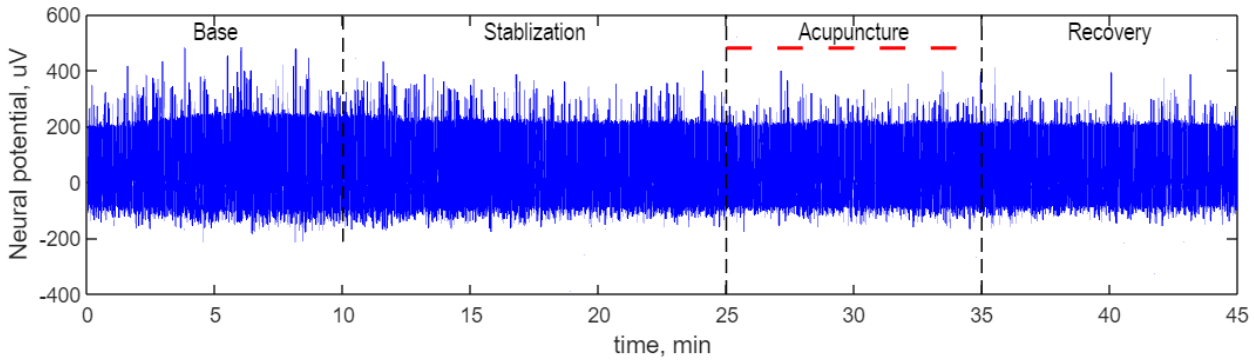
1. Original data

The experimental methods and data acquisition procedures are detailed in the original article. Here, we provide pre-processed and cleaned raw data (note: pre-processing primarily involved removing irrelevant redundant data from the experimental process and performing temporal alignment). The data is located in the "source_data" folder, which contains three subfolders: "acu", "saline", and "dscg". Each subfolder contains neural electrical signal data stored in files named "animal_{xx}.mat".

Each data recording spans 45 minutes, comprising baseline (10 min), stabilization (15 min), acupuncture (10 min), and recovery (10 min) phases. The sampling rate is 20 kHz.

The following code plots a time-domain data.

```
close all; clearvars; clc;
datafile = './source_data/ACU1.mat'; % AUTHORS: please alter here to plot different
results
%
load(datafile); % the result is stored as variable 'data'
fs = 20000;
x = 1e6*data; t = [0:length(x)-1]/fs/60; % time in minutes, voltage in uV
figure(1); set(gcf,'position',[0,0,1200,300]);
plot(t(1:20:end),x(1:20:end),'b-','linewidth',1); hold on; % to reduce ploting
efforts, plot every 20 points (0.01s)
set(gca,'fontsize',12);
for(ii = [10,25,35]) plot([ii,ii],get(gca,'ylim'),'k--','linewidth',1); hold on; end
loc = get(gca,'ylim'); loc2 = loc(2);
for(ii = 1:2:10) plot([25+ii-1,25+ii],0.8*loc2+[0,0],'r-','linewidth',2); end
xloc = [5,17.5,30,40]; anno = {'Base','Stablization','Acupuncture','Recovery'};
for ii = 1:length(xloc)
    text(xloc(ii),0.9*loc2,anno{ii},'fontname','arial narrow','fontsize',14,
'HorizontalAlignment','center');
end
xlabel('time, min', 'FontSize',14); ylabel('Neural potential, uV','FontSize',14);
```



2. Calculate the firing rate

2.1 Detecting neural spikes

To calculate neuronal firing rates, it is first necessary to detect neural impulses (spikes). Although more advanced and sophisticated detection methods exist, we employ a simple and rapid approach here, based on the following facts:

- Neuronal discharges form a relatively high positive potential.
- Neurons have a refractory period after firing.

Accordingly, the `fun_detect_spikes_original` function detects neuronal discharges based on time-domain data. This function has two parameters: first, the threshold (voltages above this value are considered neuronal discharges, set at 50μV in this study); second, the refractory period (only one neuronal discharge can occur within the same refractory period, set at 4ms in this study). These two parameters may have minor quantitative effects on the results of this study, but they essentially do not affect the qualitative outcomes.

2.2 Calculation of Firing Rate

Firing rate is defined as the number of neural impulses per unit time. We processed the entire 45-minute dataset using a 10-second sliding window (with no overlap between consecutive windows), resulting in 270 firing rate values. This calculation is implemented in the `fun_calc_fr` function.

2.3 Batch Processing

The following code performs batch analysis on the data from each group of animals and stores the firing rate results for each group in a corresponding `FR_original.mat` file within their respective subfolders.

```
% AUTHORS: this code section may take a while, please be patient. ;p
close all; clearvars; clc;
folders = {'ACU','SALINE','DSCG'}; fs = 20000;
for i = 1:length(folders)
    folder = folders{i};
    SpikeLoc = {}; FR = [];
    for animal = 1:7
        filename = sprintf('./source_data/%s%d.mat',folder,animal);
        load(filename); % to get data
        [spikeloc, spikevol] = fun_detect_spikes(data,50e-6,5e-3*fs);
        data = data*0; data(spikeloc) = 1;
        fr = fun_calc_fr(data,10,fs);
        SpikeLoc{animal} = spikeloc;
        FR(animal,:) = fr;
    end
    savefile = sprintf('./source_data/%s_FR_original.mat',folder);
    save(savefile,'SpikeLoc','FR');
end
```

3. Statistics and Visualization

3.1 Statistics on Firing Rate

First, calculate the average firing rate (f) for each animal across different stages (baseline, stabilization, acupuncture, recovery). Then, compute the mean and standard error of these firing rates. Finally, use repeated measures ANOVA (rmANOVA) to assess differences between the stages. The result will be stored in `FR_statistics.mat`, the following code deals with the statistics.

```
format compact
format short
folders = {'ACU','SALINE','DSCG'};
for kk = 1:length(folders)
    folder = folders{kk};
    fprintf(1,'%s \n',repmat('#',1,100));
    fprintf(1,' ANALYSISING %s\n',folder);
    fprintf(1,'%s \n',repmat('#',1,100));
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile);
    window_in_seconds = 10; % FR data is calculated every 10 seconds
    timepoints = [10,15,10,10]; % duration of the stages, in min
    timepoints = [cumsum(timepoints)];
    timepoints = timepoints*60/10;
    FRacuperiod = FR(:,timepoints(2)+1:timepoints(3));
    FRtwist = FRacuperiod(:,[ 1:6,13:18,25:30,37:42,49:54]);
    FRretain= FRacuperiod(:,[7:12,19:24,31:36,43:48,55:60]);
    FRbase = mean( FR(:,1:timepoints(1)), 2 );
    FRstab = mean( FR(:,timepoints(1)+1:timepoints(2)), 2 );
    FRtwis = mean( FRtwist, 2 );
    FRreta = mean( FRretain,2 );
    FRreco = mean( FR(:,timepoints(3)+1:end), 2 );
    FRtable = array2table( [FRbase,FRstab,FRtwis,FRreta,FRreco],'VariableNames',
{'f_base','f_stab','f_twist','f_retain','f_reco'} );
    fprintf(1,'Table. Neural discharge frequency for each animal, divided by time
stages\n')
    disp(FRtable);
    fprintf(1,'%s \n',repmat('-',1,30));
    % statistics
    means = mean(FRtable{:,:});      % average
    stds = std(FRtable{:,:});        % standard deviation
    stderrs = stds / sqrt(height(FRtable));  % standard error
    statsTable = table(means', stds', stderrs', ...
        'VariableNames', {'mean', 's.d.', 's.e.'}, ...
        'RowNames', FRtable.Properties.VariableNames);
    fprintf(1,'Table. mean, std and se results\n')
    disp(statsTable); fprintf(1,'%s \n',repmat('-',1,30));
    % rm-ANOVA and post-hoc comparisons
    withinDesign = table([1 2 3 4 5]', 'VariableNames', {'Stage'});
    withinDesign.Properties.Description = 'Within-subject factor: Stage';
    rm = fitrm(FRtable, 'f_base-f_reco ~ 1', 'WithinDesign', withinDesign);
    ranovaResults = ranova(rm);
```

```
    fprintf(1,'Result. RM-ANOVA report\n')
    disp(ranovaResults);
    fprintf(1,'%s \n',repmat('-',1,30));
    % Post-hoc comparisons
    multcompareResults = multcompare(rm, 'Stage');
    fprintf(1,'Result. Post-hoc comp. report\n');
    disp(multcompareResults);
end
```

```
#################################################################################################
 ANALYSISING ACU
#################################################################################################
Table. Neural discharge frequency for each animal, divided by time stages
    f_base    f_stab    f_twist    f_retain    f_reco
    ______    ______    _______    ________    ______
    75.028    73.139     76.15       72.34     73.108
    29.912    32.433     43.26      39.417     41.922
    42.838    42.171    56.623       42.97     44.643
     11.99    14.063    24.107      19.657     19.613
    17.507    17.002    27.397      18.677     18.593
    23.865    21.998    25.937      22.547      21.12
    35.393    33.933    42.133      37.173     38.883
------------------------------
Table. mean, std and se results
                mean      s.d.      s.e.
               ______    ______    ______
    f_base      33.79    20.972    7.9267
    f_stab     33.534    20.125    7.6064
    f_twist     42.23     19.03    7.1925
    f_retain   36.111    18.843     7.122
    f_reco      36.84    19.507    7.3729
------------------------------
Result. RM-ANOVA report
                        SumSq     DF    MeanSq      F         pValue       pValueGG      pValueHF      pValueLB
                        ______    __    ______    ______    __________    _________    __________    _________
    (Intercept):Stage    344.62    4     86.155    11.763    1.9732e-05    0.0019813    0.00033339    0.013975
    Error(Stage)         175.78    24     7.324
------------------------------
Result. Post-hoc comp. report
    Stage_1    Stage_2    Difference    StdErr      pValue       Lower      Upper
    _______    _______    __________    _______    _________    _______    _______
       1          2          0.25619    0.69154     0.99488    -2.3379     2.8503
       1          3            -8.439     1.9838    0.029152     -15.88    -0.99767
       1          4            -2.321     1.7247      0.6776    -8.7906     4.1487
       1          5             -3.05     1.9825      0.5771     -10.487     4.3867
       2          1         -0.25619    0.69154     0.99488    -2.8503     2.3379
       2          3          -8.6952     1.5242   0.0072145     -14.413    -2.9779
       2          4          -2.5771     1.0739     0.23417    -6.6056     1.4513
       2          5          -3.3062     1.3648     0.22816    -8.4257     1.8133
       3          1            8.439     1.9838    0.029152    0.99767      15.88
       3          2           8.6952     1.5242   0.0072145     2.9779      14.413
       3          4           6.1181     1.4278    0.028223    0.76236      11.474
       3          5            5.389     1.4034    0.045365    0.12467      10.653
       4          1            2.321     1.7247      0.6776    -4.1487     8.7906
       4          2           2.5771     1.0739     0.23417    -1.4513     6.6056
       4          3          -6.1181     1.4278    0.028223     -11.474    -0.76236
       4          5         -0.72905    0.50974     0.63329    -2.6412     1.1831
       5          1             3.05     1.9825      0.5771    -4.3867      10.487
       5          2           3.3062     1.3648     0.22816    -1.8133     8.4257
       5          3          -5.389     1.4034    0.045365     -10.653    -0.12467
       5          4          0.72905    0.50974     0.63329    -1.1831     2.6412
#################################################################################################
```

ANALYSISING SALINE

Table. Neural discharge frequency for each animal, divided by time stages

f_base	f_stab	f_twist	f_retain	f_reco
16.435	18.789	23.297	19.46	18.442
24.085	22.557	28.233	25.713	23.267
11.473	11.466	18.95	12.763	11.972
16.998	17.887	33.663	19.743	19.897
21.787	21.296	24.613	21.003	20.04
52.608	52.258	55.227	50.293	49.343
43.2	44.516	50.35	44.453	43.93

Table. mean, std and se results

	mean	s.d.	s.e.
f_base	26.655	15.307	5.7855
f_stab	26.967	15.214	5.7504
f_twist	33.476	14.015	5.2972
f_retain	27.633	14.108	5.3323
f_reco	26.699	14.125	5.3389

Result. RM-ANOVA report

	SumSq	DF	MeanSq	F	pValue	pValueGG	pValueHF	pValueLB
(Intercept):Stage	239.99	4	59.998	14.539	3.6672e-06	0.0034524	0.0017699	0.0088325
Error(Stage)	99.041	24	4.1267					

Result. Post-hoc comp. report

Stage_1	Stage_2	Difference	StdErr	pValue	Lower	Upper
1	2	-0.31143	0.48994	0.96334	-2.1492	1.5264
1	3	-6.821	1.814	0.049505	-13.625	-0.016429
1	4	-0.97762	0.72135	0.6726	-3.6835	1.7283
1	5	-0.043333	0.81029	1	-3.0828	2.9962
2	1	0.31143	0.48994	0.96334	-1.5264	2.1492
2	3	-6.5095	1.6526	0.040748	-12.709	-0.3103
2	4	-0.66619	0.62465	0.81728	-3.0093	1.677
2	5	0.2681	0.59424	0.98927	-1.961	2.4972
3	1	6.821	1.814	0.049505	0.016429	13.625
3	2	6.5095	1.6526	0.040748	0.3103	12.709
3	4	5.8433	1.4329	0.035107	0.46816	11.219
3	5	6.7776	1.2115	0.0079607	2.233	11.322
4	1	0.97762	0.72135	0.6726	-1.7283	3.6835
4	2	0.66619	0.62465	0.81728	-1.677	3.0093
4	3	-5.8433	1.4329	0.035107	-11.219	-0.46816
4	5	0.93429	0.29551	0.097112	-0.17419	2.0428
5	1	0.043333	0.81029	1	-2.9962	3.0828
5	2	-0.2681	0.59424	0.98927	-2.4972	1.961
5	3	-6.7776	1.2115	0.0079607	-11.322	-2.233
5	4	-0.93429	0.29551	0.097112	-2.0428	0.17419

ANALYSISING DSCG

Table. Neural discharge frequency for each animal, divided by time stages

f_base	f_stab	f_twist	f_retain	f_reco
17.732	17.45	19.197	18.767	16.997
23.667	25.538	24.627	24.297	24.34
28.408	28.237	32.607	34.387	38.883
37.102	32.66	32.35	30.123	30.397
35.698	37.724	38.813	36.253	35.487
20.543	17.132	23.777	16.867	16.445
18.42	19.458	22.443	18.82	17.535

```
------------------------------
Table. mean, std and se results
                mean      s.d.      s.e.
              _______    ______    ______
    f_base      25.939    8.0012    3.0242
    f_stab      25.457    7.9531     3.006
    f_twist     27.688    6.9998    2.6457
    f_retain    25.645    7.9768     3.015
    f_reco      25.726    9.3234    3.5239
------------------------------
Result. RM-ANOVA report
                         SumSq     DF    MeanSq       F        pValue     pValueGG    pValueHF    pValueLB
                        _______    __    ______    _______    _______    ________    ________    ________
    (Intercept):Stage    23.146     4    5.7866    0.89858    0.48024    0.42627     0.45092     0.37976
    Error(Stage)         154.55    24    6.4397
------------------------------
Result. Post-hoc comp. report
    Stage_1    Stage_2    Difference    StdErr     pValue      Lower     Upper
    _______    _______    __________    _______    _______    _______    ______
       1          2          0.48159    0.95783    0.98403    -3.1114    4.0745
       1          3           -1.749     1.1772     0.6043    -6.1648    2.6667
       1          4          0.29381     1.5375    0.99961    -5.4736    6.0613
       1          5          0.21238     2.0295    0.99996    -7.4004    7.8251
       2          1         -0.48159    0.95783    0.98403    -4.0745    3.1114
       2          3          -2.2306     1.0058     0.2877    -6.0037    1.5424
       2          4         -0.18778     1.0903    0.99974    -4.2776     3.902
       2          5         -0.26921     1.7511    0.99983    -6.8377    6.2993
       3          1            1.749     1.1772     0.6043    -2.6667    6.1648
       3          2           2.2306     1.0058     0.2877    -1.5424    6.0037
       3          4           2.0429     1.0538     0.3899    -1.9099    5.9957
       3          5           1.9614     1.6192      0.747    -4.1124    8.0352
       4          1         -0.29381     1.5375    0.99961    -6.0613    5.4736
       4          2          0.18778     1.0903    0.99974     -3.902    4.2776
       4          3          -2.0429     1.0538     0.3899    -5.9957    1.9099
       4          5        -0.081429    0.78376    0.99997    -3.0214    2.8586
       5          1         -0.21238     2.0295    0.99996    -7.8251    7.4004
       5          2          0.26921     1.7511    0.99983    -6.2993    6.8377
       5          3          -1.9614     1.6192      0.747    -8.0352    4.1124
       5          4         0.081429    0.78376    0.99997    -2.8586    3.0214
```

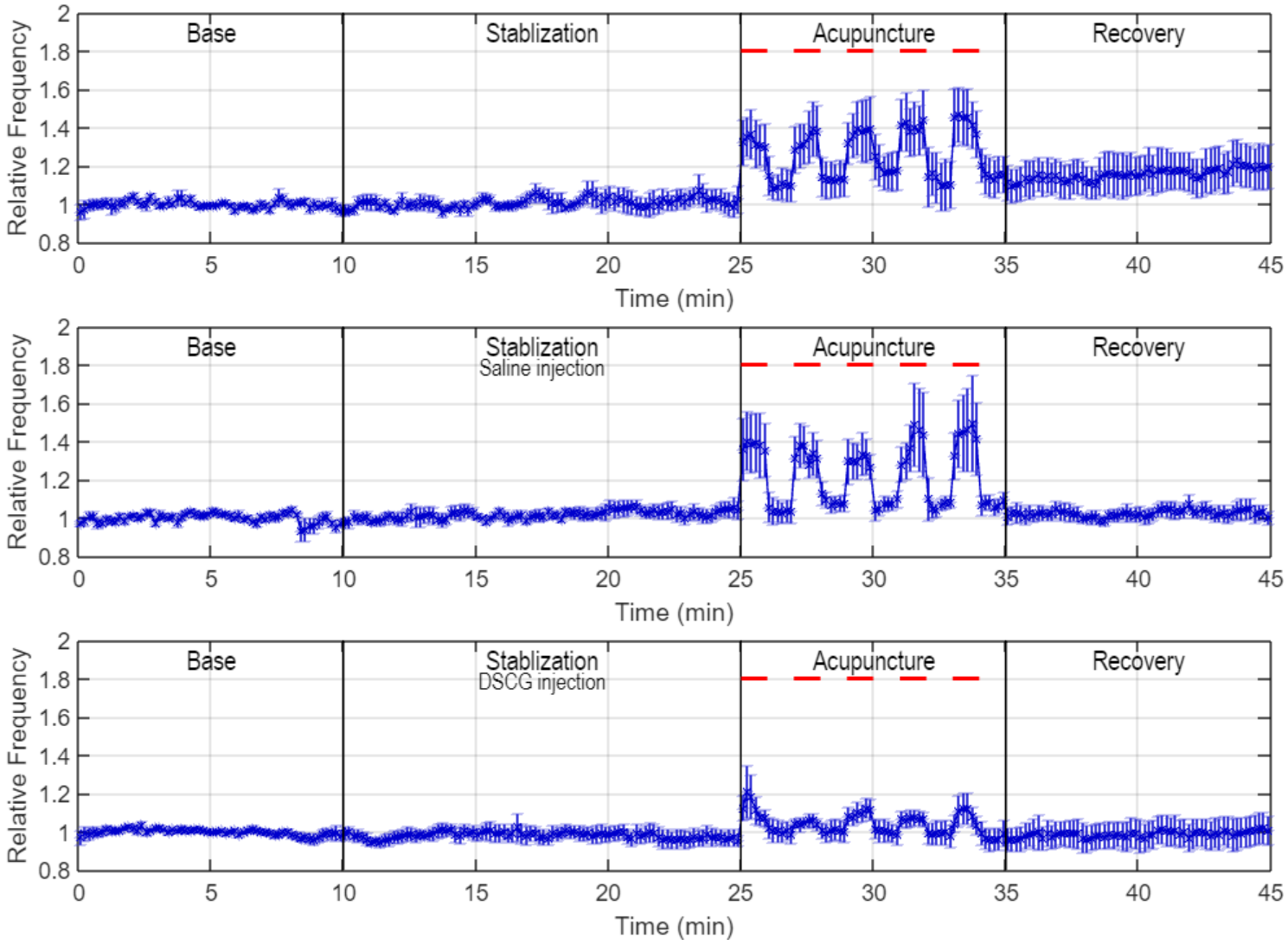
3.2 Plot time-frequency curve

To eliminate individual differences in f_base, we used the normalized frequency f/f_base as the plotting metric to illustrate the changes in neural firing rates throughout the experimental process for each group of animals. This was achieved through the following code.

```
folders = {'acu','saline','dscg'}; % change the folder name for analysis of
different groups, i.e. acu, saline, or dscg
fig = figure(1); clf; set(gcf,'position',[0,0,900,220*length(folders)]);
tiledlayout(length(folders),1,"TileSpacing","tight","Padding","tight");
for k = 1:length(folders)
    nexttile(k);
    folder = folders{k};
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile); [n,nT] = size(FR); FRnorm = FR;
    FRbase = mean( FR(:,1:60),2 );
    for i = 1:n
        FRnorm(i,:) = FRnorm(i,:)/FRbase(i);
```

```
    end
    mean_FRnorm = mean(FRnorm);
    std_FRnorm = std(FRnorm); se_FRnorm = std_FRnorm/sqrt(n);
    t = ( 5+(0:length(mean_FRnorm)-1)*10 )/60; % in minutes

    errorbar(t,mean_FRnorm,se_FRnorm,'*-','color',[0,0,0.8]);
    set(gca,'xlim',[0,45],'ylim',[0.8,2.0],'fontsize',12);
    xlabel('Time (min)'); ylabel('Relative Frequency'); grid on; hold on;
    for ii = [10,25,35]
        plot([ii,ii],get(gca,'ylim'),'k-','linewidth',1);
    end
    xloc = [5,17.5,30,40]; yloc = 1.9; anno =
{'Base','Stablization','Acupuncture','Recovery'};
    anno2 = {'','Saline injection','DSCG injection'};
    for ii = 1:2:10
        plot([25+ii-1,25+ii],1.8+[0,0],'r-','linewidth',2);
    end
    for ii = 1:length(xloc)
        text(xloc(ii),yloc,anno{ii},'fontname','arial narrow','fontsize',14,...
            'HorizontalAlignment','center','color','k');
        if ii == 2
            text(xloc(ii),yloc-0.11,anno2{k},'fontname','arial
narrow','fontsize',12,...
            'HorizontalAlignment','center','color','k');
        end
    end
    set(gca,'fontsize',12,'ytick',[0.8:0.2:2]);
end
```



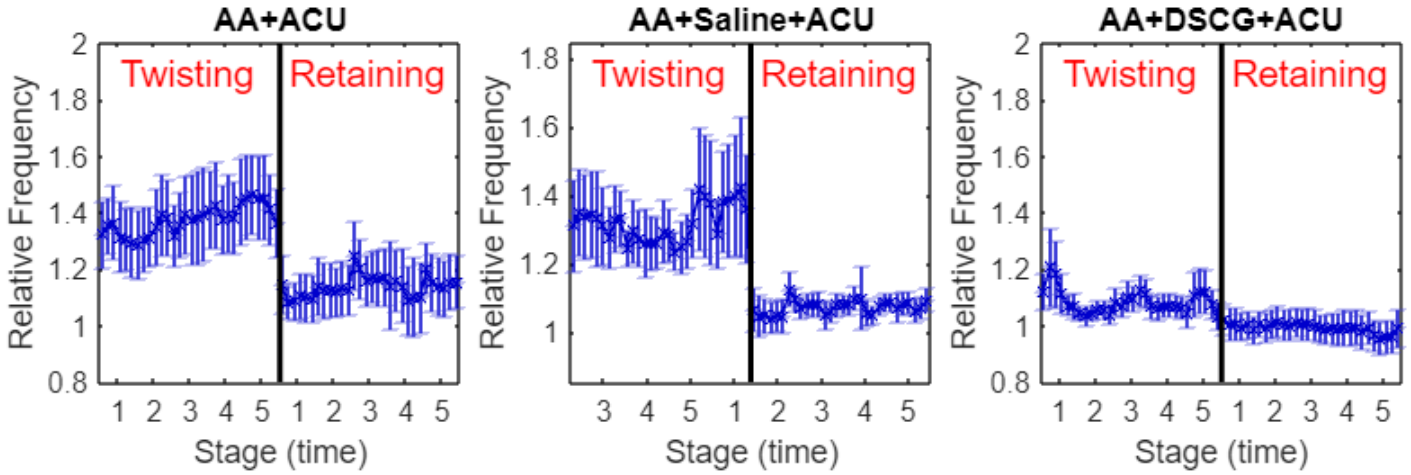
4. Twisting vs. Retaining

Here, we compare the differences in neural discharge rates between the twisting phase and the retaining phase (five each) during the acupuncture stage and plot a comparison chart.

```
folders = {'ACU','SALINE','DSCG'};
titles = {'AA+ACU','AA+Saline+ACU','AA+DSCG+ACU'};
fig = figure(1); clf; set(gcf,'position',[0,0,250*length(folders),250]);
tiledlayout(1,length(folders),"TileSpacing","compact","Padding","compact");
for k = 1:length(folders)
    nexttile(k);
    folder = folders{k};
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile); FRnorm = FR; FRbase = mean( FR(:,1:60),2 );
    [n,nT] = size(FR);
    for i = 1:n
        FRnorm(i,:) = FRnorm(i,:)/FRbase(i);
    end
    %
```

```
    idx = 150+[1:60]; idx = reshape(idx,6,10);
    idx_twist = idx(:,1:2:end); idx_retain= idx(:,2:2:end);
    FRtwist = FR(:,idx_twist(:)); FRretain= FR(:,idx_retain(:));
    FRtwist_norm = FRnorm(:,idx_twist(:)); FRretain_norm = FRnorm(:,idx_retain(:));
    t = 1:60;
    y = [FRtwist_norm, FRretain_norm];
    mean_y = mean(y);
    std_y = std(y); se_y = std_y/sqrt(n);
    errorbar(t,mean_y,se_y,'*-','color',[0,0,0.8]); hold on;
    set(gca,'xlim',[0.5,60.5],'ylim',[0.8,2],'fontsize',10);
    xlabel('Stage (time)', 'FontSize',11); ylabel('Relative
Frequency','fontsize',11);
    title(titles{k});
    plot([30.5,30.5],[0.8,2],'k-','linewidth',2);
    text( 15,1.9,'Twisting',
'FontSize',14,'HorizontalAlignment','center','color','r' );

text( 45,1.9,'Retaining','FontSize',14,'HorizontalAlignment','center','color','r' );
    set(gca,'xtick',[3.5:6:60],'xticklabel',[1 2 3 4 5 1 2 3 4 5],'ytick',
[0.8:0.2:2.0]);
    set(gca,'fontsize',11);
end
```



For statistical analysis, we will employ the following averaging procedures on either the firing rate (FR) or normalized firing rate (FRnorm) data. Average data across the 6 timepoints for each animal to obtain a single value per subject, then average these values across the 7 animals in the group.

```
folders = {'ACU','SALINE','DSCG'};
for kk = 1:length(folders)
    folder = folders{kk};
    fprintf(1,'%s \n',repmat('#',1,100));
    fprintf(1,' ANALYSISING %s\n',folder);
    fprintf(1,'%s \n',repmat('#',1,100));
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile); FRnorm = FR; FRbase = mean( FR(:,1:60),2 );
```

```
    [n,nT] = size(FR);
    for i = 1:n
        FRnorm(i,:) = FRnorm(i,:)/FRbase(i);
    end
    %
    idx = 150+[1:60]; idx = reshape(idx,6,10);
    idx_twist = idx(:,1:2:end); idx_retain= idx(:,2:2:end);
    FRtwist = FR(:,idx_twist(:)); FRretain= FR(:,idx_retain(:));
    FRtwist_norm = FRnorm(:,idx_twist(:)); FRretain_norm = FRnorm(:,idx_retain(:));
    % statistics
    [FRtwist_mean, FRtwist_se, FRretain_mean, FRretain_se] = deal(zeros(5,1));
    [FRtwist_n_mean, FRtwist_n_se, FRretain_n_mean, FRretain_n_se] =
deal(zeros(5,1));
    for i = 1:5
        twist_i = 6*(i-1)+[1:6]; retain_i = 6*(i-1)+[1:6];
        FRtwist_i = FRtwist(:,twist_i); FRtwist_norm_i = FRtwist_norm(:,twist_i);
        FRretain_i = FRretain(:,retain_i); FRretain_norm_i =
FRretain_norm(:,retain_i);
        [m,sd,se] = fun_mean_se(FRtwist_i,2);  FRtwist_mean(i)=m;  FRtwist_se(i) =
se;
        [m,sd,se] = fun_mean_se(FRretain_i,2); FRretain_mean(i)=m; FRretain_se(i) =
se;
        [m,sd,se] = fun_mean_se(FRtwist_norm_i,2);  FRtwist_n_mean(i)=m;
FRtwist_n_se(i) = se;
        [m,sd,se] = fun_mean_se(FRretain_norm_i,2); FRretain_n_mean(i)=m;
FRretain_n_se(i) = se;
    end
    stats = [FRtwist_mean,FRtwist_se,FRretain_mean,FRretain_se];
    statsTable = array2table(stats,'VariableNames',
{'Twist(Avg)','Twist(SE)','Retain(Avg)','Retain(SE)'});
    fprintf(1,'Table. The discharge frequency (average and se) for each of the 5
epochs\n');
    disp(statsTable);
    fprintf(1,'%s\n', repmat('-',1,30));
    % alternatively, using the rmANOVA
    X1 = FRtwist; X2 = FRretain;
    X = zeros(7,10);
    for i = 1:5
        idx = 6*(i-1)+[1:6];
        X1i = X1(:,idx); X2i = X2(:,idx);
        X1ii = mean(X1i,2); X2ii = mean(X2i,2);
        X(:,2*i-1) = X1ii;
        X(:,2*i  ) = X2ii;
    end
    t = array2table(X,'VariableNames',
{'X1','X2','X3','X4','X5','X6','X7','X8','X9','X10'});
    rm_model = fitrm(t, 'X1-X10 ~ 1', 'WithinDesign', (1:10)');
    ranova_result = ranova(rm_model);
    fprintf(1,'Results: rm-ANOVA report \n');
    disp(ranova_result);
```

```
    fprintf(1,'%s\n', repmat('-',1,30));
    posthoc_result = multcompare(rm_model, 'Time', 'ComparisonType', 'lsd');
    posthoc_result = posthoc_result{:,:};
    fprintf(1,'Results: Post-hoc comparison \n');
    for i = 1:2:10
        j = i+1;
        idx = find( posthoc_result(:,1)==i & posthoc_result(:,2)==j );
        pvalue = posthoc_result(idx,5);
        fprintf('Epoch %d of 5 (Twisting vs. Retaining): p = %6.5f\n', (i+1)/2,
pvalue);
    end
end
```

```
################################################################################################################
 ANALYSISING ACU
################################################################################################################
Table. The discharge frequency (average and se) for each of the 5 epochs
    Twist(Avg)    Twist(SE)    Retain(Avg)    Retain(SE)
    __________    _________    ___________    __________
      41.376       7.4754         35.19         6.813
      41.219       7.1096         35.85        7.0754
      41.893       7.0327        37.983        7.6423
      42.867       7.0844        34.979        7.2991
      43.793       7.3402        36.555        7.1699
------------------------------
Results: rm-ANOVA report
                        SumSq     DF    MeanSq      F         pValue       pValueGG     pValueHF     pValueLB
                        ______    __    ______    ______    _________    _________    _________    _________
    (Intercept):Time    729.33     9    81.037    8.4404    9.758e-08    0.014119     0.0087977    0.027148
    Error(Time)         518.46    54    9.6011
------------------------------
Results: Post-hoc comparison
Epoch 1 of 5 (Twisting vs. Retaining): p = 0.00231
Epoch 2 of 5 (Twisting vs. Retaining): p = 0.00378
Epoch 3 of 5 (Twisting vs. Retaining): p = 0.01426
Epoch 4 of 5 (Twisting vs. Retaining): p = 0.03314
Epoch 5 of 5 (Twisting vs. Retaining): p = 0.00133
################################################################################################################
 ANALYSISING SALINE
################################################################################################################
Table. The discharge frequency (average and se) for each of the 5 epochs
    Twist(Avg)    Twist(SE)    Retain(Avg)    Retain(SE)
    __________    _________    ___________    __________
      33.633       5.3054         26.667         5.329
      32.931       5.2317          27.91        5.1584
      32.443       5.5934          27.75        5.3394
       33.65       5.2043         27.971        5.5211
      34.724       5.6533         27.867        5.4427
------------------------------
Results: rm-ANOVA report
                        SumSq     DF    MeanSq      F         pValue       pValueGG     pValueHF     pValueLB
                        ______    __    ______    ______    _________    _________    _________    _________
    (Intercept):Time    626.72     9    69.635    7.3765    6.3684e-07    0.017647      0.0106      0.034828
    Error(Time)         509.77    54    9.4401
------------------------------
Results: Post-hoc comparison
Epoch 1 of 5 (Twisting vs. Retaining): p = 0.01237
Epoch 2 of 5 (Twisting vs. Retaining): p = 0.00062
Epoch 3 of 5 (Twisting vs. Retaining): p = 0.00217
Epoch 4 of 5 (Twisting vs. Retaining): p = 0.02326
Epoch 5 of 5 (Twisting vs. Retaining): p = 0.05794
```

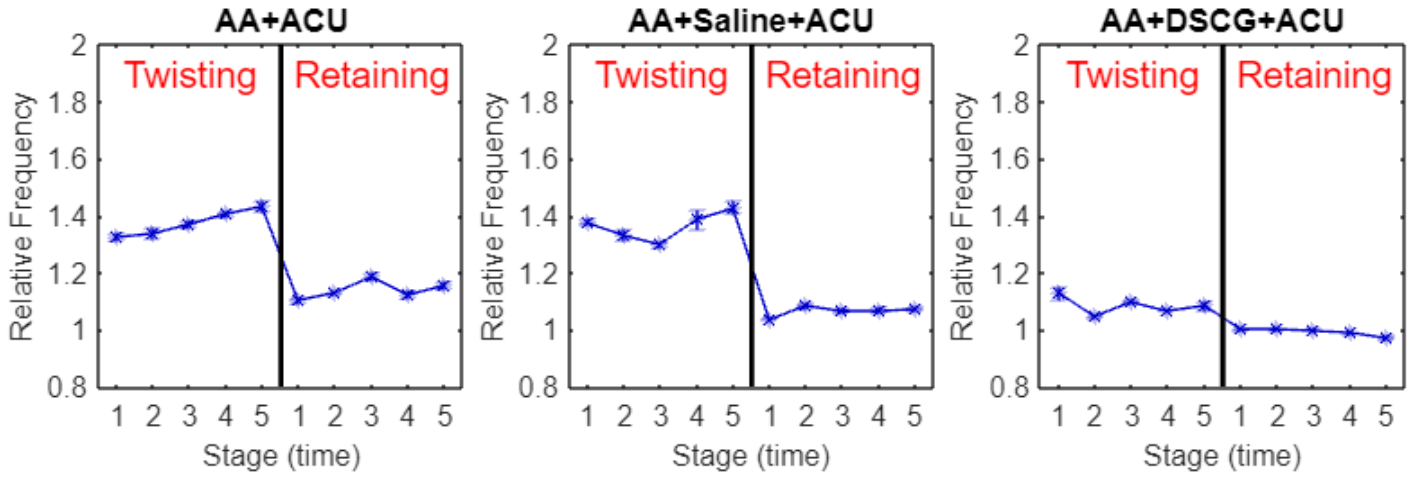
```
#################################################################################################
 ANALYSISING DSCG
#################################################################################################
Table. The discharge frequency (average and se) for each of the 5 epochs
    Twist(Avg)    Twist(SE)    Retain(Avg)    Retain(SE)
    __________    _________    ___________    __________
        28.5       2.5525        25.779         2.8626
      26.893       2.7923         25.86         2.9293
      28.179       2.9352         25.81         3.0133
      27.231       2.5478        25.588          3.139
      27.636       2.7753        25.188         3.1802
------------------------------
Results: rm-ANOVA report
                         SumSq     DF    MeanSq      F       pValue    pValueGG    pValueHF    pValueLB
                         ______    __    ______    ______    ______    ________    ________    ________
    (Intercept):Time      87.36     9    9.7066    2.3671    0.0246    0.12379     0.081528    0.17484
    Error(Time)          221.44    54    4.1007
------------------------------
Results: Post-hoc comparison
Epoch 1 of 5 (Twisting vs. Retaining): p = 0.06773
Epoch 2 of 5 (Twisting vs. Retaining): p = 0.32742
Epoch 3 of 5 (Twisting vs. Retaining): p = 0.07229
Epoch 4 of 5 (Twisting vs. Retaining): p = 0.20432
Epoch 5 of 5 (Twisting vs. Retaining): p = 0.15218
```

```
folders = {'acu','saline','dscg'};
titles = {'AA+ACU','AA+Saline+ACU','AA+DSCG+ACU'};
fig = figure(1); clf; set(gcf,'position',[0,0,250*length(folders),250]);
tiledlayout(1,length(folders),"TileSpacing","compact","Padding","compact");
for k = 1:length(folders)
    nexttile(k);
    folder = folders{k};
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile); FRnorm = FR; FRbase = mean( FR(:,1:60),2 );
    [n,nT] = size(FR);
    for i = 1:n
        FRnorm(i,:) = FRnorm(i,:)/FRbase(i);
    end
    %
    idx = 150+[1:60]; idx = reshape(idx,6,10);
    idx_twist = idx(:,1:2:end); idx_retain= idx(:,2:2:end);
    FRtwist = FR(:,idx_twist(:)); FRretain= FR(:,idx_retain(:));
    FRtwist_norm = FRnorm(:,idx_twist(:)); FRretain_norm = FRnorm(:,idx_retain(:));

    [FRtwist_n_mean, FRtwist_n_se, FRretain_n_mean, FRretain_n_se] =
deal(zeros(5,1));
    method = 3;
    for i = 1:5
        twist_i = 6*(i-1)+[1:6]; retain_i = 6*(i-1)+[1:6];
        FRtwist_norm_i = FRtwist_norm(:,twist_i);
        FRretain_norm_i = FRretain_norm(:,retain_i);
        [m,sd,se] = fun_mean_se(FRtwist_norm_i,method);  FRtwist_n_mean(i)=m;
FRtwist_n_se(i) = se;
```

```
        [m,sd,se] = fun_mean_se(FRretain_norm_i,method); FRretain_n_mean(i)=m;
FRretain_n_se(i) = se;
    end
    mean_y = [FRtwist_n_mean; FRretain_n_mean];
    se_y = [FRtwist_n_se; FRretain_n_se];
    errorbar(1:length(mean_y),mean_y,se_y,'*-','color',[0,0,0.8]); hold on;
    set(gca,'xlim',[0.5,10.5],'ylim',[0.8,2]); set(gca,'fontsize',11);
    xlabel('Stage (time)', 'FontSize',11); ylabel('Relative
Frequency','fontsize',11);
    title(titles{k});
    plot([5.5,5.5],[0.5,2],'k-','linewidth',2);
    text( 3,1.9,'Twisting',
'FontSize',14,'HorizontalAlignment','center','color','r' );

text( 8,1.9,'Retaining','FontSize',14,'HorizontalAlignment','center','color','r' );
    set(gca,'xtick',[1:1:10],'xticklabel',[1 2 3 4 5 1 2 3 4 5],'ytick',
[0.8:0.2:2.0]);
end
```

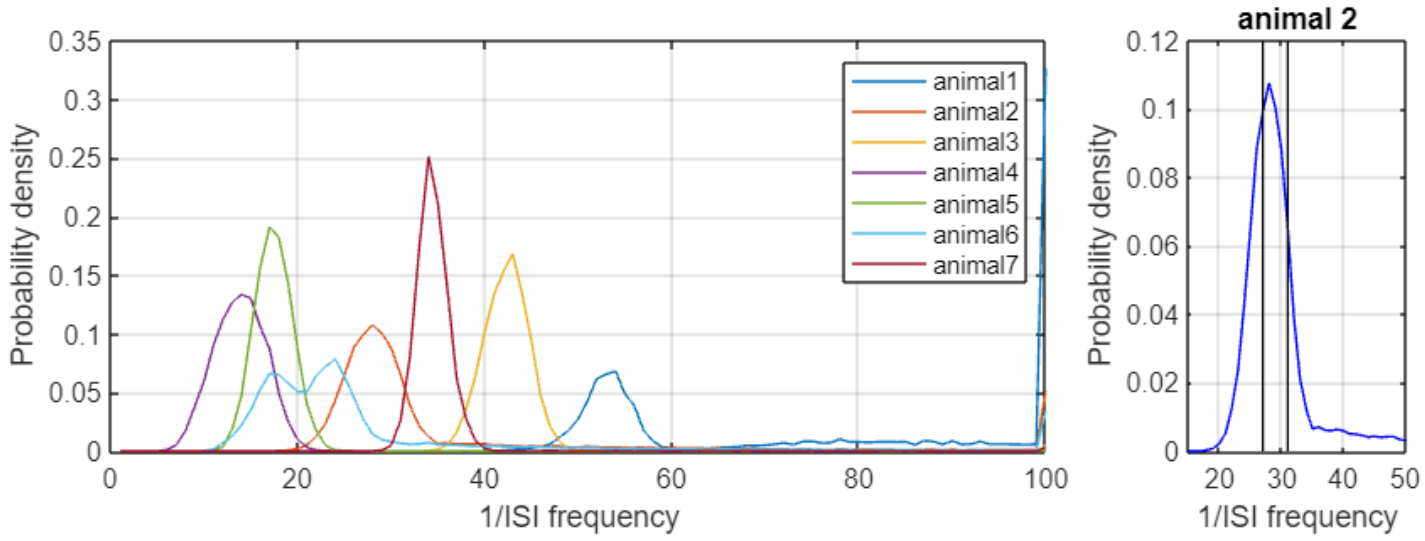


5. Analysis of ISI

We classified the 1/ISI (inter-spike interval) frequencies into low, medium, and high bands based on the probability density function derived from histograms. For each nerve, firing events were divided into three equal segments according to the distribution, with two threshold frequencies established to delineate the respective bands. This methodology was specifically designed to enhance the detection of experimental phase-dependent effects on 1/ISI frequencies while minimizing confounding influences from inter-individual variability. The approach was applied to both baseline and stabilization phases, as illustrated in the following example.

```
FRfile = './source_data/ACU_FR_original.mat';
load(FRfile);
figure(1); set(gcf,'position',[0,0,800,300]);
tiledlayout(1,4,"TileSpacing","compact","Padding","compact");
nexttile(1,[1,3]);
for animal = 1:7
    spikeloc = SpikeLoc{animal}; fs = 20000;
```

```
    % calculate the ISIf
    ISIf = fs./diff(spikeloc);
    ISIft = spikeloc(1:end-1)/fs/60; % in minutes
    ISIf_ = ISIf(ISIft<25); % baseline and stabilization stages
    bins = [1:100];
    dist = hist(ISIf_,bins);
    plot(bins, dist/sum(dist),'-'); hold on; grid on;
    if animal==2
        dist2 = dist;
    end
end
xlabel('1/ISI frequency'); ylabel('Probability density'); set(gca,'fontsize',11);
legend({'animal1','animal2','animal3','animal4','animal5','animal6','animal7'});
nexttile(4); y = dist2/sum(dist2);
plot(bins,y,'b-'); xlabel('1/ISI frequency'); ylabel('Probability density');
title('animal 2');
set(gca,'xlim',[15,50]); grid on; hold on; set(gca,'fontsize',11);
cy = cumsum(y); tmp1 = find(cy>=1/3); tmp2 = find(cy>=2/3);
f1 = bins(tmp1(1)); f2 = bins(tmp2(1));
plot([f1,f1],get(gca,'ylim'),'k-'); plot([f2,f2],get(gca,'ylim'),'k-');
```

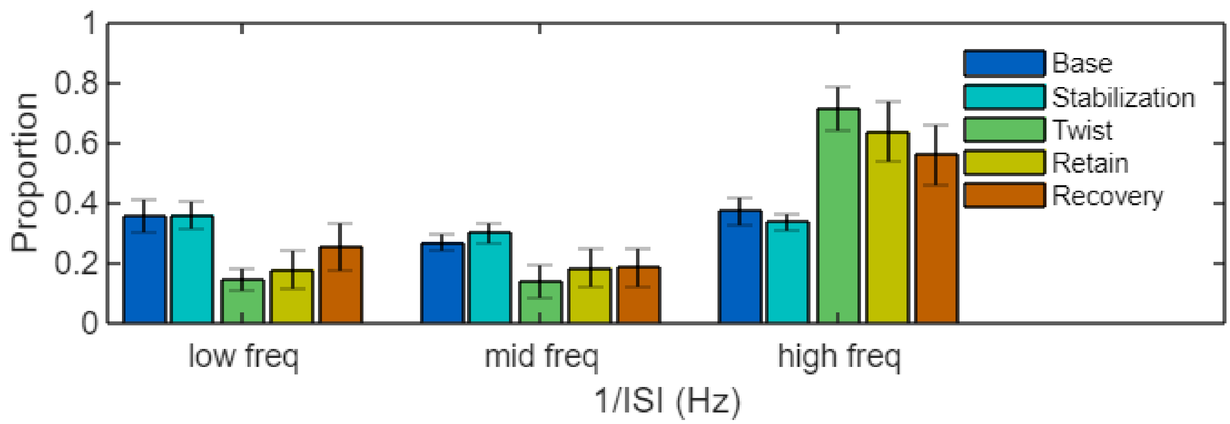


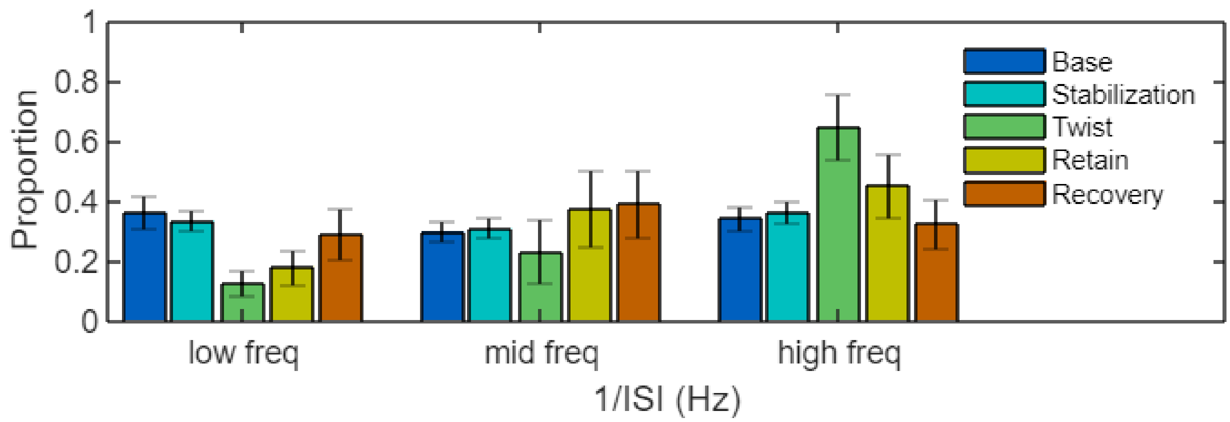

```
folders = {'ACU','SALINE','DSCG'};
figure(1); set(gcf,'position',[0,0,650,220*length(folders)]);
tiledlayout(length(folders),1,"TileSpacing","Compact","Padding","Compact");
for kk = 1:length(folders)
    folder = folders{kk};
    nexttile(kk);
    FRfile = sprintf('./source_data/%s_FR_original.mat',folder);
    load(FRfile); fs = 20000;
    ISInumber = zeros(7,5,3); % 7 animals, 5 stages, 3 bands
    for animal = 1:7
        spikeloc = SpikeLoc{animal};
        ISI = diff(spikeloc)/fs;
```

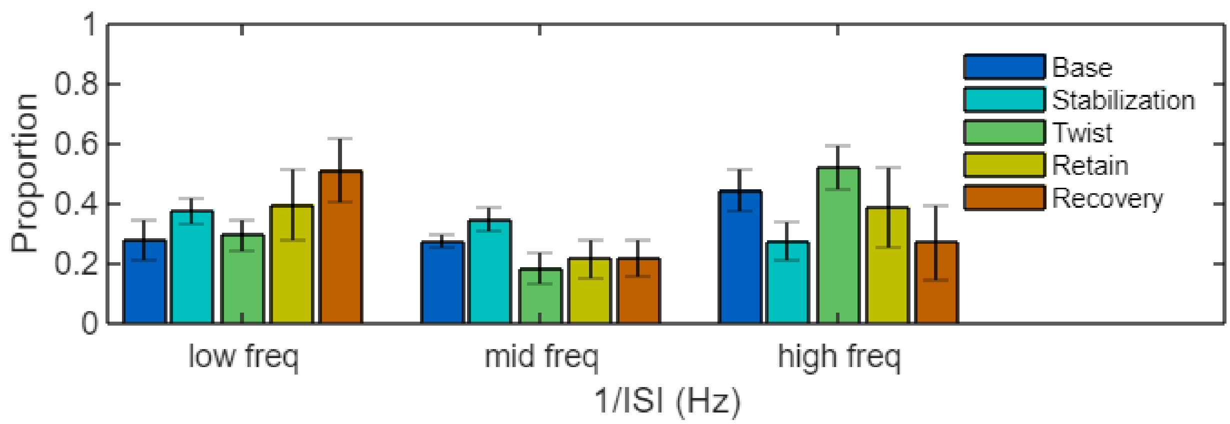
```
        ISIf = 1./ISI;
        ISIft = spikeloc(1:end-1)/fs/60; % in minutes
        stage_base  = (ISIft<=10);
        stage_stab  = (ISIft>10)&(ISIft<=25);
        stage_twist = (ISIft>25)&(ISIft<=35)&(mod(floor(ISIft-25),2)==0);
        stage_retain= (ISIft>25)&(ISIft<=35)&(mod(floor(ISIft-25),2)~=0);
        stage_reco  = (ISIft>35);
        stage_filter = {stage_base,stage_stab,stage_twist,stage_retain,stage_reco};

        % determine the frequency threshold
        ISIf_ = ISIf(ISIft<25); % baseline and stabilization stages
        bins = [1:100]; dist = hist(ISIf_,bins); y = dist/sum(dist);
        cy = cumsum(y); tmp1 = find(cy>=1/3); tmp2 = find(cy>=2/3);
        f1 = bins(tmp1(1)); f2 = bins(tmp2(1));
        %
        fre = [f1,f2];
        band_low = (ISIf<=fre(1));
        band_mid = (ISIf>fre(1))&(ISIf<fre(2));
        band_high= (ISIf>=fre(2));
        band_filter = {band_low, band_mid, band_high};
        for ii = 1:length(stage_filter)
            for jj = 1:length(band_filter)
                ISInumber(animal,ii,jj) = sum( stage_filter{ii} & band_filter{jj} );
            end
        end
    end
    ISIprop = 0*ISInumber;
    for animal = 1:7
        ISIn = reshape(ISInumber(animal,:,:),5,3);
        ISIp = ISIn*0;
        sum_ISIn = sum(ISIn,2);
        for stage = 1:5
            ISIp(stage,:) = ISIn(stage,:)/sum_ISIn(stage);
        end
        ISIprop(animal,:,:) = ISIp;
    end
    colors = jet(5)*0.75;
    ISIp_mean = reshape( mean(ISIprop,1), 5, 3 );
    ISIp_se = reshape( std(ISIprop,[],1),5,3) / sqrt(7);
    % plot the results
    ISIp_mean = ISIp_mean(:);
    ISIp_se = ISIp_se(:);
    x = [0.67, 0.83, 1, 1.167, 1.33, 1.67, 1.83, 2.0, 2.167, 2.33, 2.67, 2.83, 3.0,
3.167, 3.33];
    for k = 1:5
        b(k) =
bar(x(k:5:end),ISIp_mean(k:5:end),'barwidth',0.14,'facecolor',colors(k,:)); hold on;
    end
    errorbar(x,ISIp_mean,ISIp_se,'k.','capsize',10); set(gca,'xlim',
[0.55,4.3],'ylim',[0,1]);
```

```
    % title(folder);
    xlabel('1/ISI (Hz)'); ylabel('Proportion');
    set(gca,'xtick',[1,2,3],'xticklabel',{'low freq','mid freq','high
freq'},'fontsize',12);
    set(gca,'ytick',[0:0.2:1.0]);
    legend(b,
{'Base','Stabilization','Twist','Retain','Recovery'},'location','northeast',...
        'FontSize',10,'box','off');
    %
    file = sprintf('source_data/%s_ISIp.mat',folder);
    save(file,'ISIprop');
end
```







```
% the table
folders = {'ACU','SALINE','DSCG'};
ISIp = zeros(3,7,5,3); % group, animal, stage, frequency_band
for fidx = 1:length(folders)
    load(['source_data\',folders{fidx},'_ISIp.mat']);
    ISIp(fidx,:,:,:) = ISIprop;
end
%
output = '';
fband_text = {'low-frequency','mid-frequency','high-frequency'};
group_text = {'AA+ACU','AA+Saline+ACU','AA+DSCG+ACU'};
output = [output, sprintf('%15s |%13s|%13s|%13s|%13s|%13s|\n', ...
    'Stage','Base','Stabilization','Twisting','Retaining','Recovery')];
sep = repmat('-',1,35);
for fband = 1:3
    output = [output, sprintf('%s %15s %s\n', sep,fband_text{fband},sep)];
    for group = 1:3
        output = [output, sprintf('%15s |', group_text{group})];
        for stage = 1:5
            tmp = ISIp(group,:,stage,fband);
            output = [output, sprintf(' %3.2f ± %3.2f |',mean(tmp),std(tmp)/
sqrt(length(tmp)))];
        end
        output = [output,newline];
    end
end
fprintf(1,'1/ISI proportions report\n\n'); disp(output); % the result for table 2
```

```
1/ISI proportions report

          Stage |         Base|Stabilization|     Twisting|    Retaining|     Recovery|
----------------------------------   low-frequency ----------------------------------
         AA+ACU | 0.36 ± 0.05 | 0.36 ± 0.05 | 0.15 ± 0.03 | 0.18 ± 0.07 | 0.25 ± 0.08 |
  AA+Saline+ACU | 0.36 ± 0.05 | 0.33 ± 0.03 | 0.13 ± 0.04 | 0.18 ± 0.06 | 0.29 ± 0.09 |
    AA+DSCG+ACU | 0.28 ± 0.07 | 0.38 ± 0.04 | 0.30 ± 0.05 | 0.40 ± 0.12 | 0.51 ± 0.11 |
----------------------------------   mid-frequency ----------------------------------
         AA+ACU | 0.27 ± 0.03 | 0.30 ± 0.03 | 0.14 ± 0.05 | 0.18 ± 0.06 | 0.19 ± 0.06 |
  AA+Saline+ACU | 0.30 ± 0.03 | 0.31 ± 0.03 | 0.23 ± 0.11 | 0.37 ± 0.13 | 0.39 ± 0.11 |
    AA+DSCG+ACU | 0.27 ± 0.02 | 0.35 ± 0.04 | 0.18 ± 0.05 | 0.22 ± 0.06 | 0.22 ± 0.06 |
----------------------------------  high-frequency ----------------------------------
         AA+ACU | 0.37 ± 0.05 | 0.34 ± 0.03 | 0.72 ± 0.07 | 0.64 ± 0.10 | 0.56 ± 0.10 |
  AA+Saline+ACU | 0.34 ± 0.04 | 0.36 ± 0.04 | 0.64 ± 0.11 | 0.45 ± 0.11 | 0.32 ± 0.08 |
    AA+DSCG+ACU | 0.44 ± 0.07 | 0.28 ± 0.06 | 0.52 ± 0.07 | 0.39 ± 0.13 | 0.27 ± 0.12 |
```

The low-, medium-, and high-frequency 1/ISI values for each phase were calculated as changes relative to the baseline firing rate, followed by repeated-measures ANOVA (rm-ANOVA).

```
group_names = {'ACU', 'SALINE', 'DSCG'};
band_names = {'Low', 'Mid', 'High'};
stage_names = {'Base', 'Stabilization', 'Twisting', 'Retaining', 'Recovery'};
output = sprintf('%15s |%13s|%13s|%13s|%13s|\n', ...
    'Groups','Stabilization','Twisting','Retaining','Recovery');
Pvalues = zeros(3,3,4); % group,band,stage
```

```
for fband = 1:3
    output = [output, sprintf('%s %15s %s\n', sep,fband_text{fband},sep)];
    for group = 1:3
        output = [output, sprintf('%15s |', group_text{group})];
        group_band_data = squeeze(ISIp(group, :, :, fband)); % [7 animals × 5
stages]
        data_matrix = group_band_data;
        rm_table = array2table(data_matrix, 'VariableNames', stage_names);
        rm_table.Animal = (1:size(data_matrix, 1))';
        within_factor = table([1 2 3 4 5]', 'VariableNames', {'Stage'});
        rm = fitrm(rm_table, 'Base-Recovery ~ 1', 'WithinDesign', within_factor);
        mc = multcompare(rm, 'Stage', 'comparisonType','lsd');
        mc = mc{:,:};
        for stage = 2:5
            pp = find( (mc(:,1)==1) & (mc(:,2)==stage) );
            pvalue = mc(pp,5);
            output = [output, sprintf(' %11.5f |',pvalue)];
            Pvalues(group,fband,stage-1) = pvalue;
        end
        output = [output,newline];
    end
end
fprintf(1,'P-values of 1/ISI proportion (Other stages vs Base) \n\n'); disp(output);
```

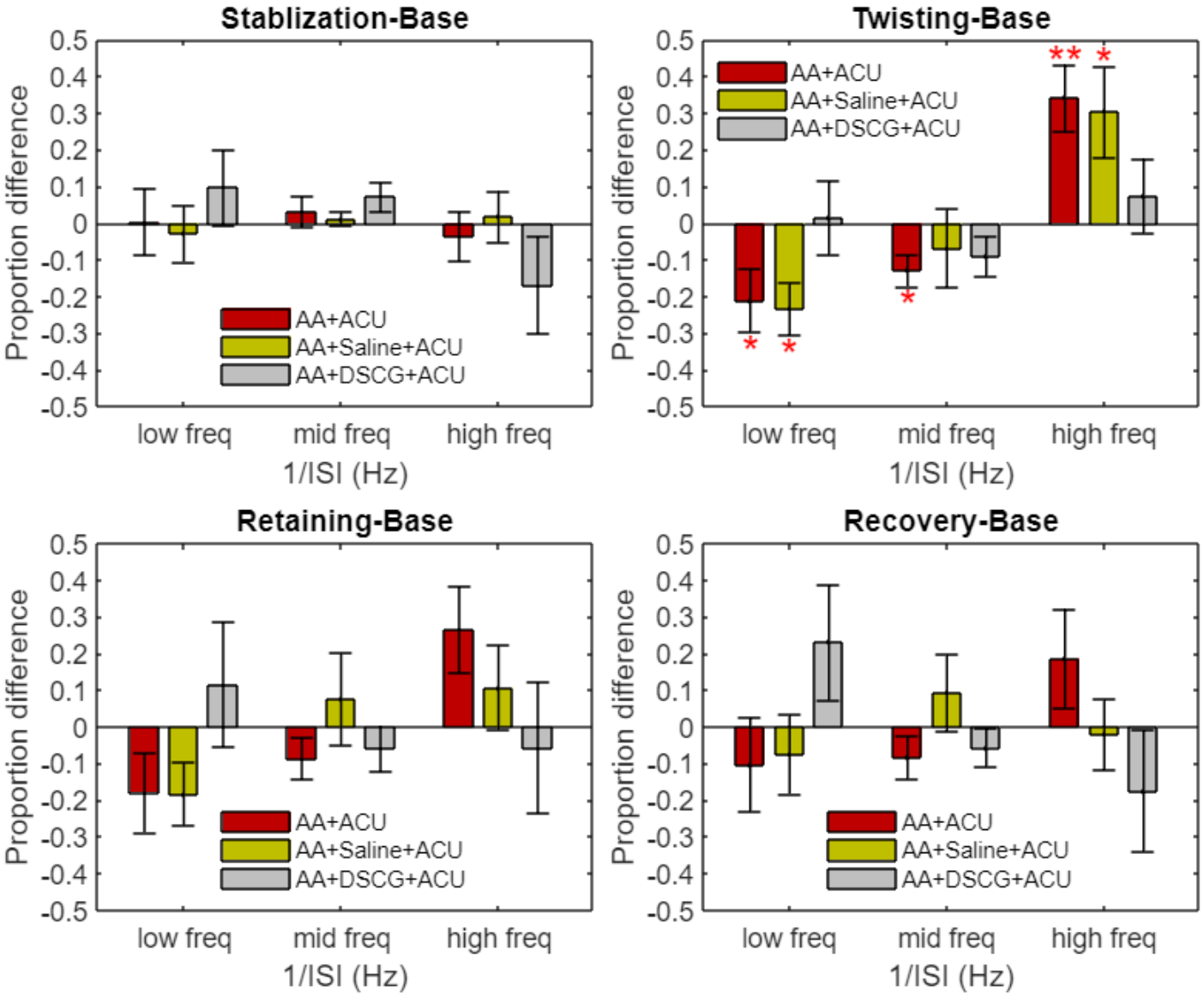
```
P-values of 1/ISI proportion (Other stages vs Base)

         Groups |Stabilization|     Twisting|    Retaining|     Recovery|
----------------------------------   low-frequency ----------------------------------
         AA+ACU |     0.97304 |     0.04937 |     0.15329 |     0.45168 |
  AA+Saline+ACU |     0.71477 |     0.01699 |     0.07748 |     0.52461 |
    AA+DSCG+ACU |     0.38714 |     0.88632 |     0.52282 |     0.19289 |
----------------------------------   mid-frequency ----------------------------------
         AA+ACU |     0.47077 |     0.02183 |     0.17990 |     0.19487 |
  AA+Saline+ACU |     0.57660 |     0.54599 |     0.56893 |     0.41102 |
    AA+DSCG+ACU |     0.11576 |     0.15433 |     0.37639 |     0.31116 |
----------------------------------  high-frequency ----------------------------------
         AA+ACU |     0.61683 |     0.00958 |     0.06614 |     0.21670 |
  AA+Saline+ACU |     0.81209 |     0.04956 |     0.39114 |     0.84384 |
    AA+DSCG+ACU |     0.25062 |     0.48604 |     0.76070 |     0.33148 |
```

```
folders = {'ACU','SALINE','DSCG'};
ISIp = zeros(3,7,5,3); % group, animal, stage, frequency_band
for fidx = 1:length(folders)
    load(['source_data\',folders{fidx},'_ISIp.mat']);
    ISIp(fidx,:,:,:) = ISIprop;
end
dI = zeros(3,7,4,3); % group, animal, stage, frequency_band
for stage = 1:4
    dI(:,:,stage,:) = ISIp(:,:,stage+1,:)-ISIp(:,:,1,:);
end
% to plot the result
figure(2); set(gcf,'position',[0,0,750,600]);
tiledlayout(2,2,"TileSpacing","compact","Padding","compact");
```

```
title_stage = {'Stablization-Base','Twisting-Base','Retaining-Base','Recovery-
Base'};
for stage = 1:4
    dIstage = dI(:,:,stage,:);
    d_mean = squeeze(mean(dIstage,2)); % group, frequency
    d_se = squeeze( std(dIstage,[],2)/sqrt(size(dIstage,2)) );
    nexttile(stage);
    d = 0.25; colors = hot(3)*0.75;
    x = [linspace(1-d,1+d,3),linspace(2-d,2+d,3),linspace(3-d,3+d,3)];
    barhandle = [];
    for i = 1:3 % group
        barhandle(i) = bar(x(i:3:end),d_mean(i,:), 'barwidth',0.18,...
            'facecolor',colors(i,:)); hold on;
        errorbar(x(i:3:end),d_mean(i,:),d_se(i,:),'k.','capsize',10,'linewidth',1);
    end
    % cope with Pvalues
    psigs = {'','*','**','***','****'};
    for i = 1:3 % group
        for j = 1:3 % fband
            pvalue = Pvalues(i,j,stage);
            p_threshold = [0.05,0.01,0.001,0.0001,0];
            tmp = find(pvalue>p_threshold); tmp = tmp(1);
            psig = psigs{tmp};
            mean_value = mean(dI(i,:,stage,j));
            se_value = std(dI(i,:,stage,j))/sqrt(size(dI,2));
            if mean_value>0
                yloc = mean_value+se_value+0.01;
            else
                yloc = mean_value-se_value-0.05;
            end

text(x(3*(j-1)+i),yloc,psig,'fontname','arial','fontsize',20,'color','r',...
                'HorizontalAlignment','center','VerticalAlignment','middle');
        end
    end
    %
    title(title_stage{stage});  set(gca,'fontsize',11, 'ytick', [-0.5:0.1:0.5]);
    set(gca,'xlim',[0.45,3.6],'ylim',[-0.5,0.5]);
    xlabel('1/ISI (Hz)','fontsize',12); ylabel('Proportion
difference','fontsize',12);
    set(gca,'xtick',[1,2,3],'xticklabel',{'low freq','mid freq','high
freq'},'fontsize',12);
    legend(barhandle,{'AA+ACU','AA+Saline+ACU','AA+DSCG+ACU'},...
        'FontSize',10,'box','off','location','best');
end
```


Stablization-Base
Twisting-Base
Retaining-Base
Recovery-Base
Proportion difference
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
AA+ACU
AA+Saline+ACU
AA+DSCG+ACU
low freq
mid freq
high freq
1/ISI (Hz)
*
**