ANALYSIS OF LIGHT RESPONSES IN GLIA IN SMH MUTANT

STEP1 SET PATHS AND LOAD ALL DATA INTO ONE CELL ARRAY---

set path to codes

```
addpath(genpath('\\forskning.it.ntnu.no\ntnu\mh\ikom\jurischyaksi\Nathalie\data\github\DGama_et
```

set the path for the 2p data and the number of cell data

```
cfg.pathData='\\forskning.it.ntnu.no\ntnu\mh\ikom\jurischyaksi\Nathalie\other\manuscript\in pre
```

load the data into few matrix

```
[ctrlON,mutON,time]=nj_load2pGliadata_v2(cfg);
```

calculate the number of dataset

```
numCTRL=size(ctrlON,1)-1
```

```
numCTRL = 10
```

```
numMUT=size(mutON,1)-1
```

```
numMUT = 11
```

STEP2 SET SOME PARAMETERS FOR THE PLOTS---

```
close all
clear cfg
```

enter some parameters for the plots into one variable called cfg

```
cfg.MutID='SMH' %enter the name of your mutant 'ELIPSA', this is for the title
```

```
cfg = struct with fields:
    MutID: 'SMH'
```

```
cfg.Mutcolor='m' %enter the color you want for your mutantd display, 'm'= magenta, 'c'=cyan
```

```
cfg = struct with fields:
        MutID: 'SMH'
    Mutcolor: 'm'
```
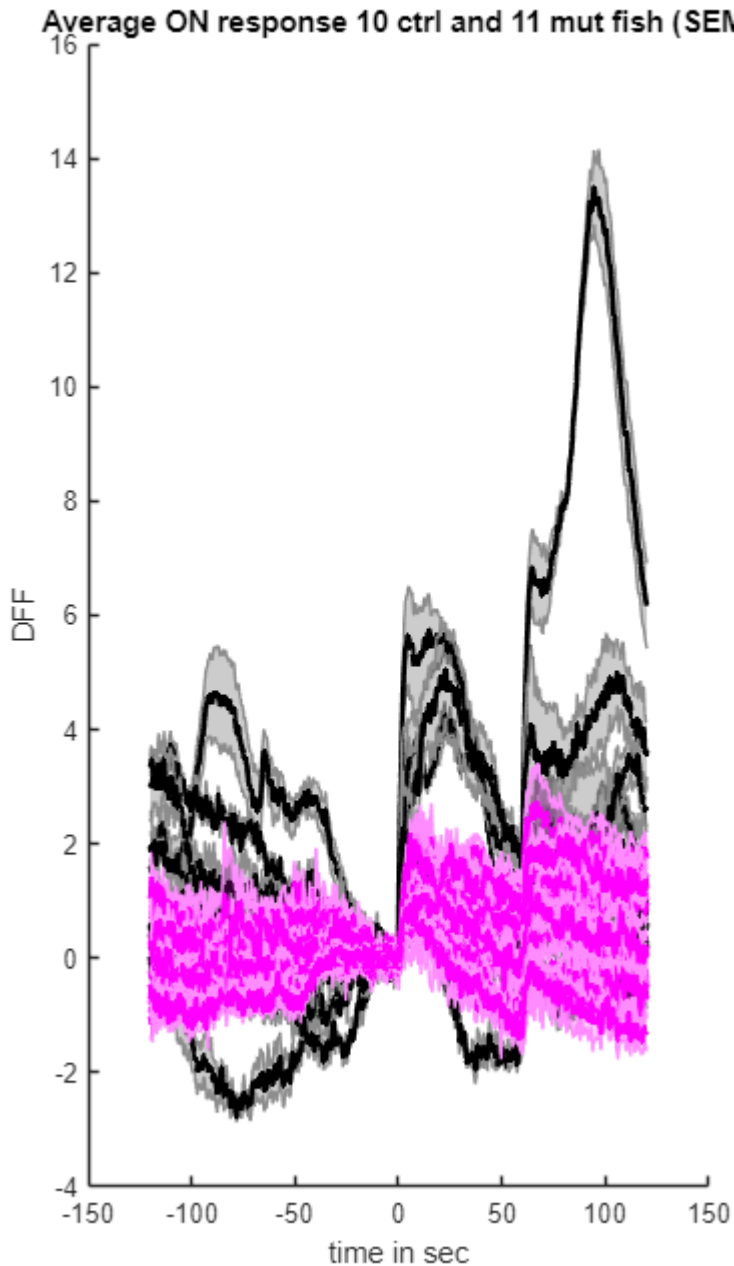
```
cfg.numCTRL=numCTRL;
cfg.numMUT=numMUT;
cfg.label=["tel" "mid/hb" "hind"];
cfg.brainRegion=char('Telencephalon','Midbrain/Habenula','Hindbrain');
cfg
```
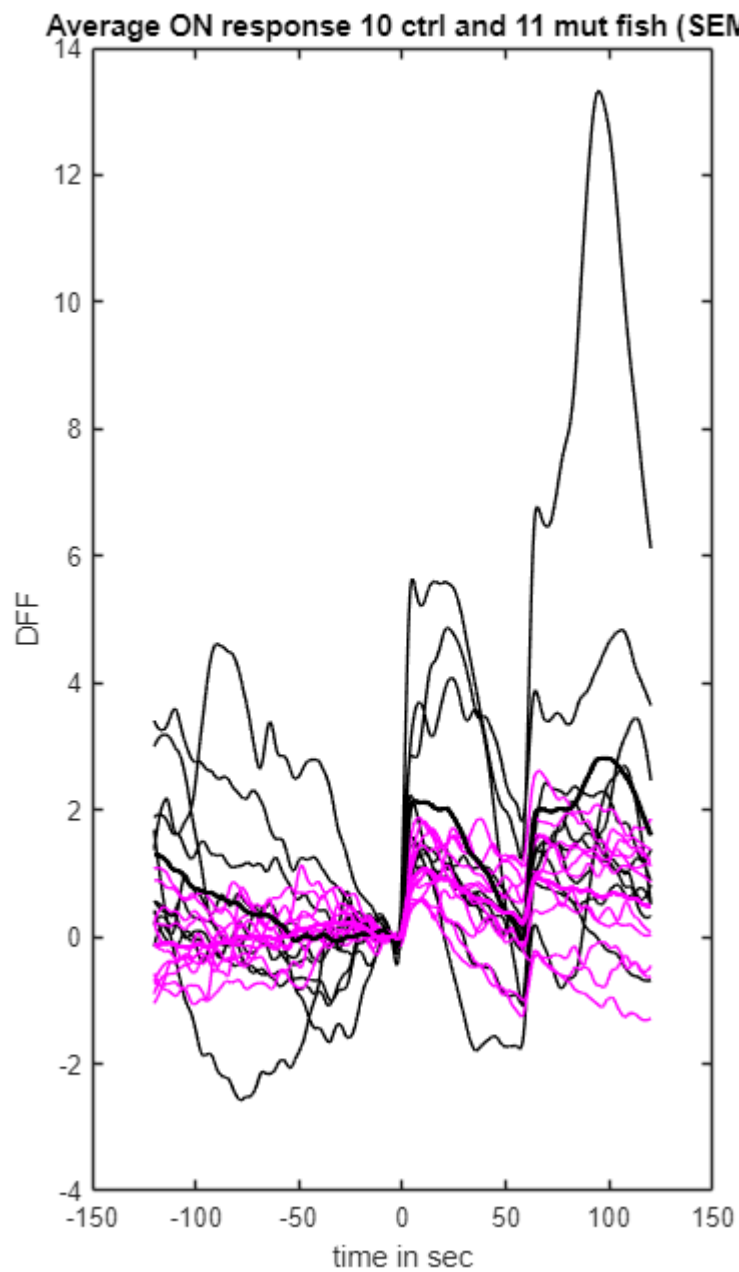
```
cfg = struct with fields:
```

```
      MutID: 'SMH'
   Mutcolor: 'm'
    numCTRL: 10
     numMUT: 11
      label: ["tel"    "mid/hb"    "hind"]
 brainRegion: [3×17 char]
```
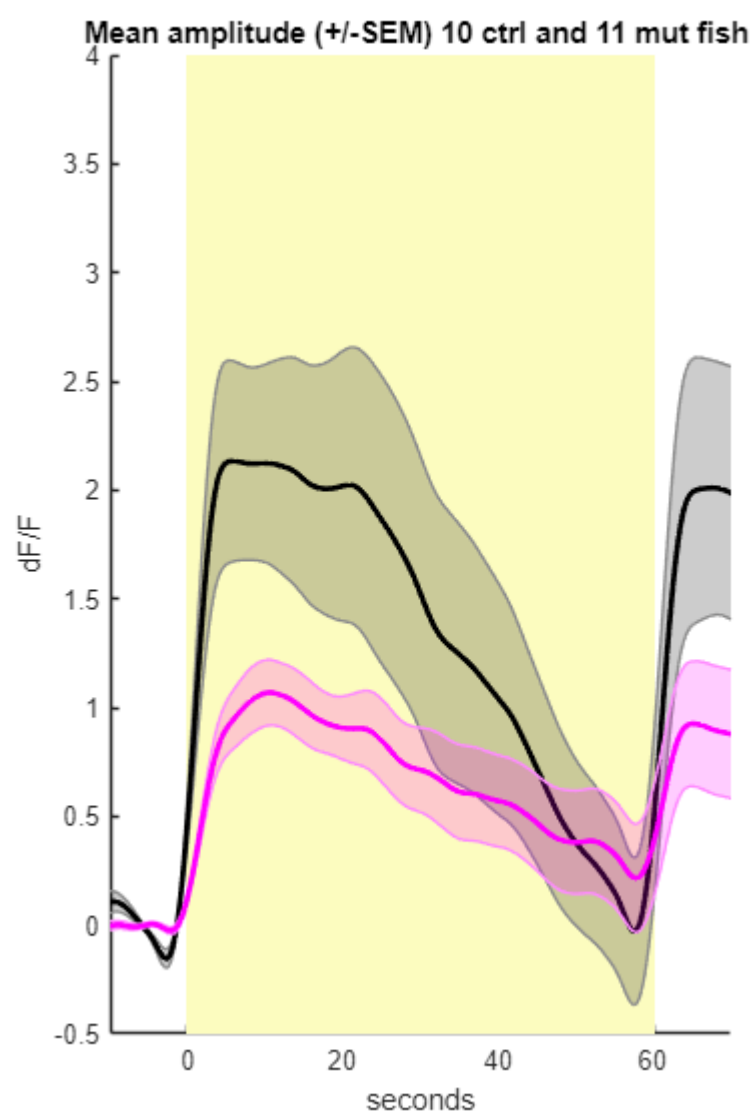
STEP3 PLOT THE AVERAGE RESPONSE TO LIGHT STIMULI IN THE WHOLE BRAIN OR IN SPECIFIC REGIONS------------------------------

```
[averageON_ALL]=plot_responseALL_perfish(ctrlON,mutON,time,cfg);
```


Average ON response 10 ctrl and 11 mut fish (SEM)

```
plot_responseALL_perfish2(ctrlON,mutON,time,cfg);
```

Average ON response 10 ctrl and 11 mut fish (SEM)

Mean amplitude (+/-SEM) 10 ctrl and 11 mut fish
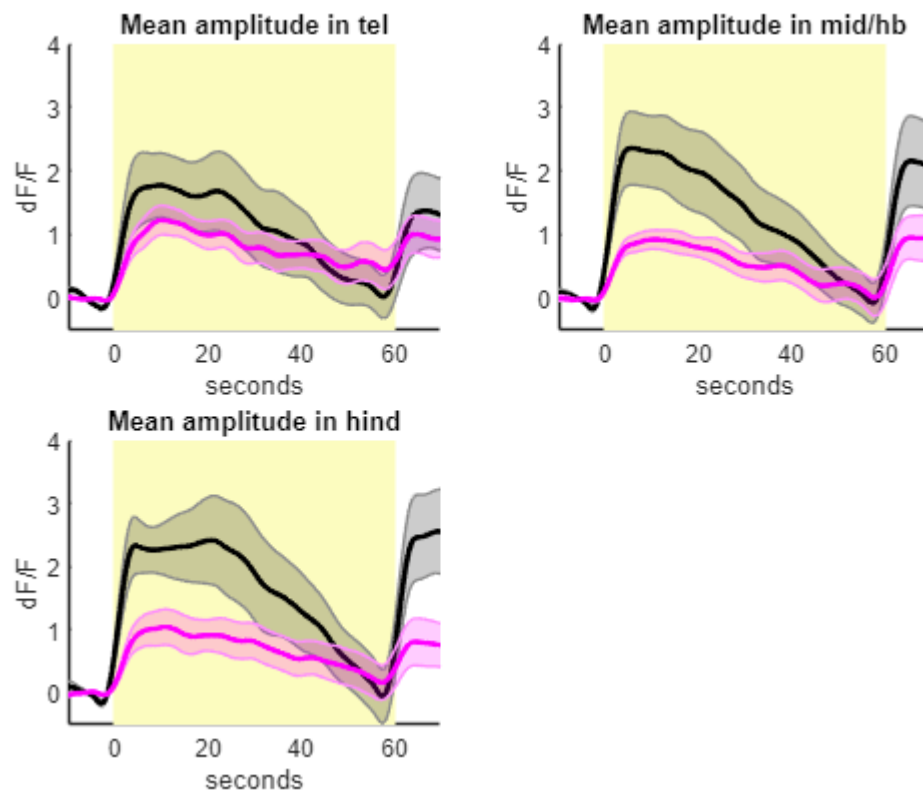
```
plot_responseALL_perfish3(ctrlON,mutON,time,cfg)
```

Mean amplitude in tel

Mean amplitude in mid/hb

Mean amplitude in hind

```
plot_scatterAll(averageON_ALL,ctrlON,mutON, time,cfg)
```

Mean amplitude ON response

p= 0.0083

amplitude dF/F

ctrl   mut
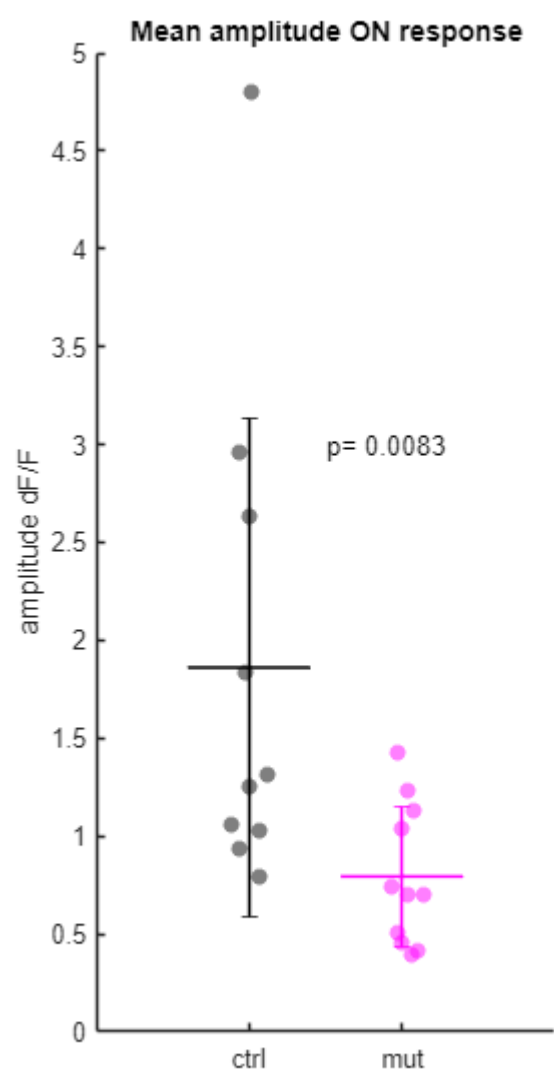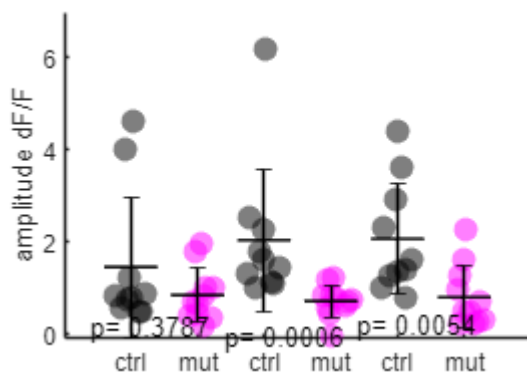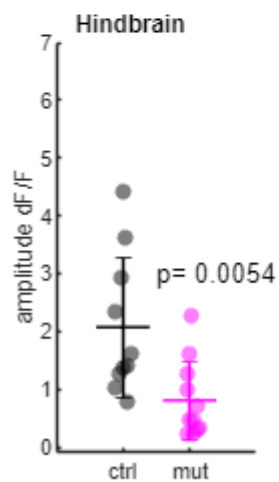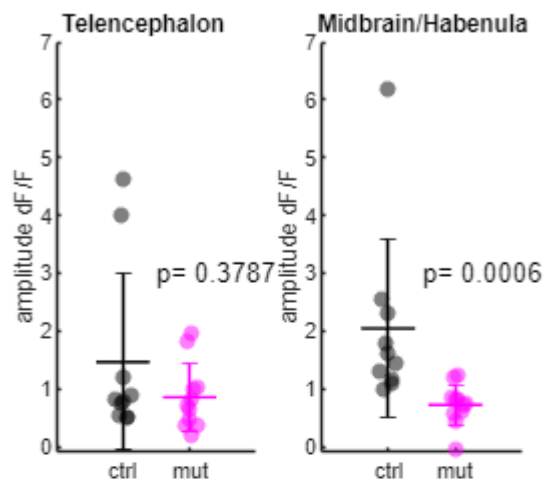
STEP4 PLOT THE OVERALL ACTIVITY FOR ALL CTRLS AND MUT AND BRAIN REGIONS---

```
plot_ALL_perfish(ctrlON,mutON,time,cfg)
```

Average overall glia activity

10 ctrl fish

11 mut fish
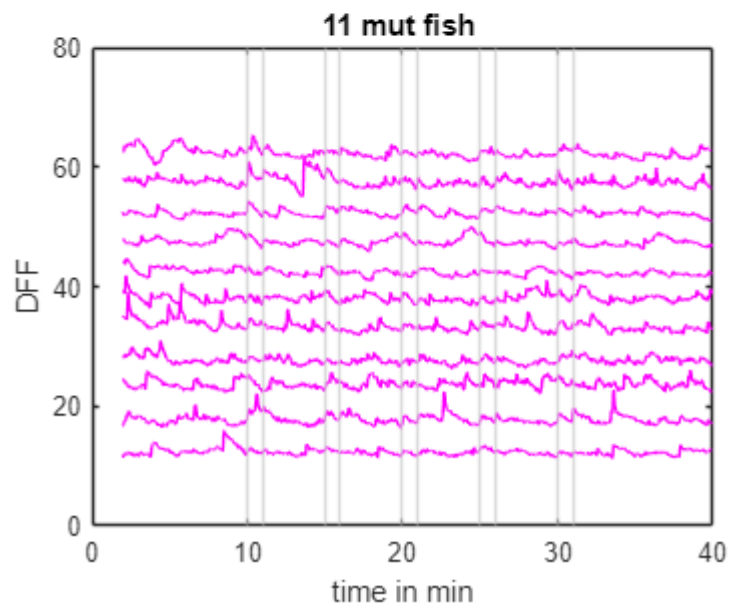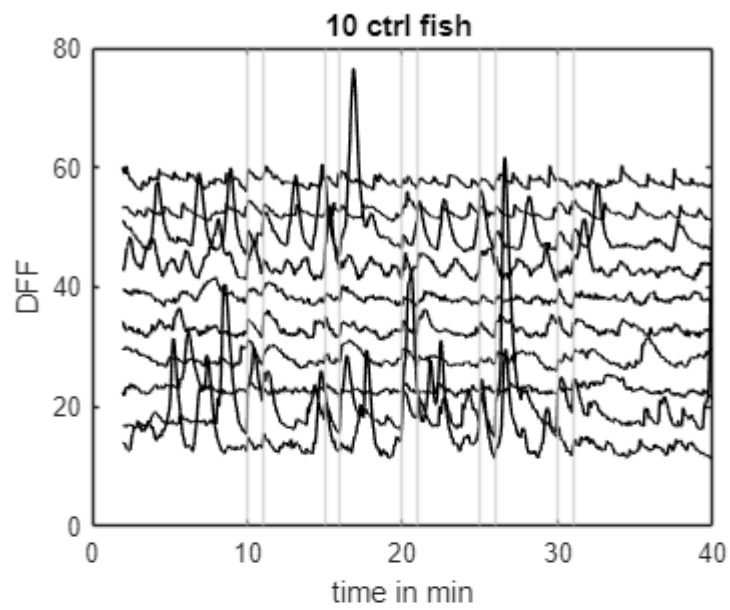

```
plot_ALL_perfish2(ctrlON,mutON,time,cfg)
```

counter = 1

Overall glia activity (std)


```
plot_scatter_allfish(ctrlON,mutON,cfg)
```

overall glial activity (std)

p= 0.0011
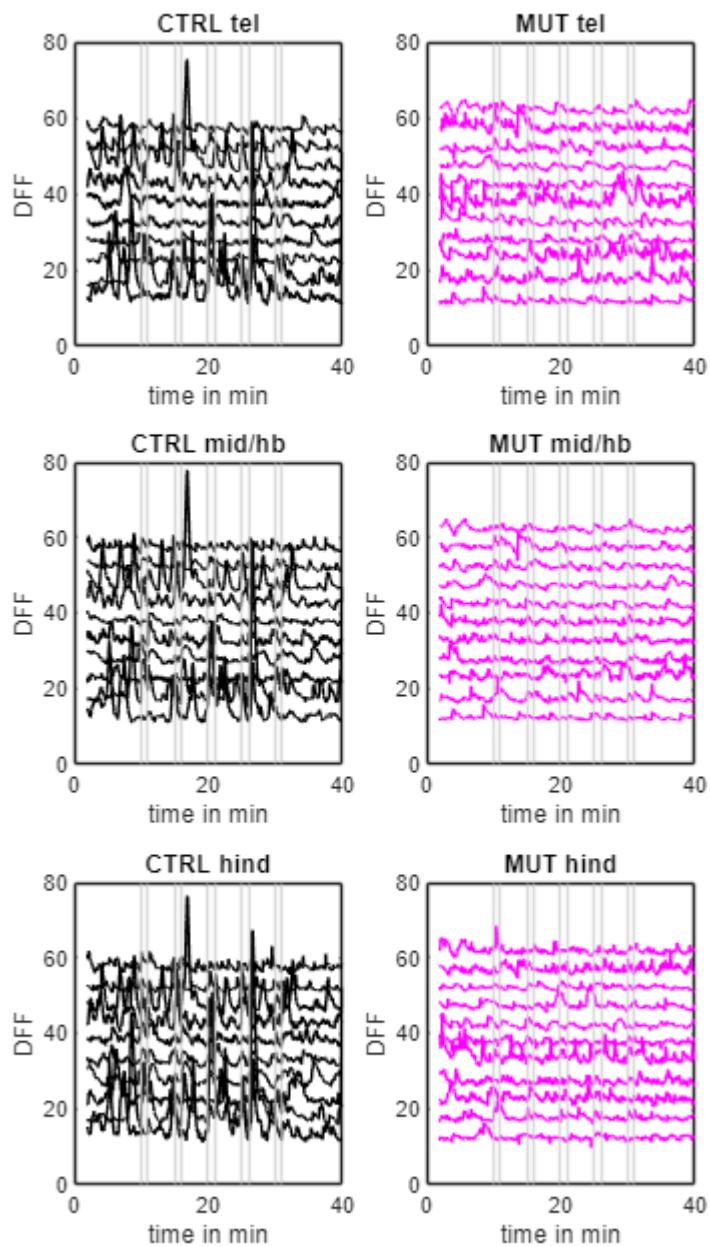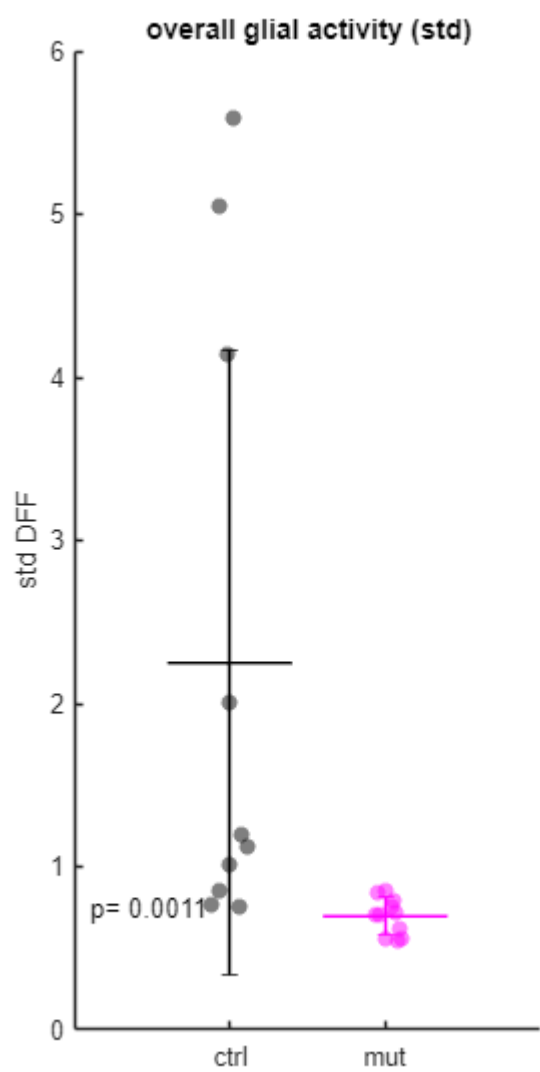
overall glial activity (std) per region

overall activity (std) tel / overall activity (std) mid/hb

p= 0.0448

p= 0.0124
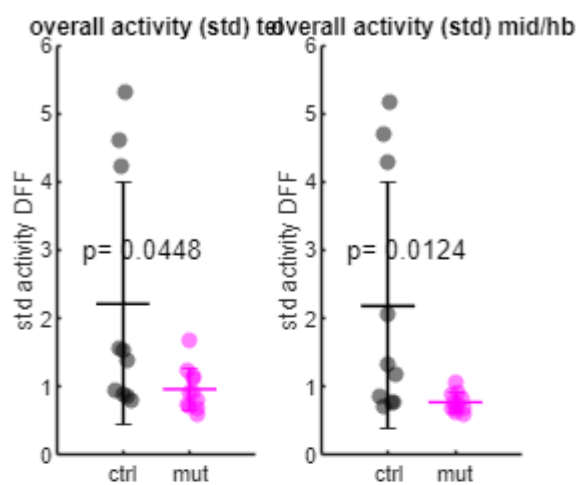
overall activity (std) hind

p= 0.0035
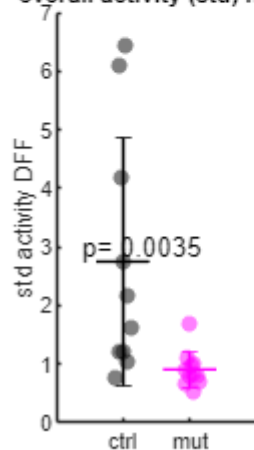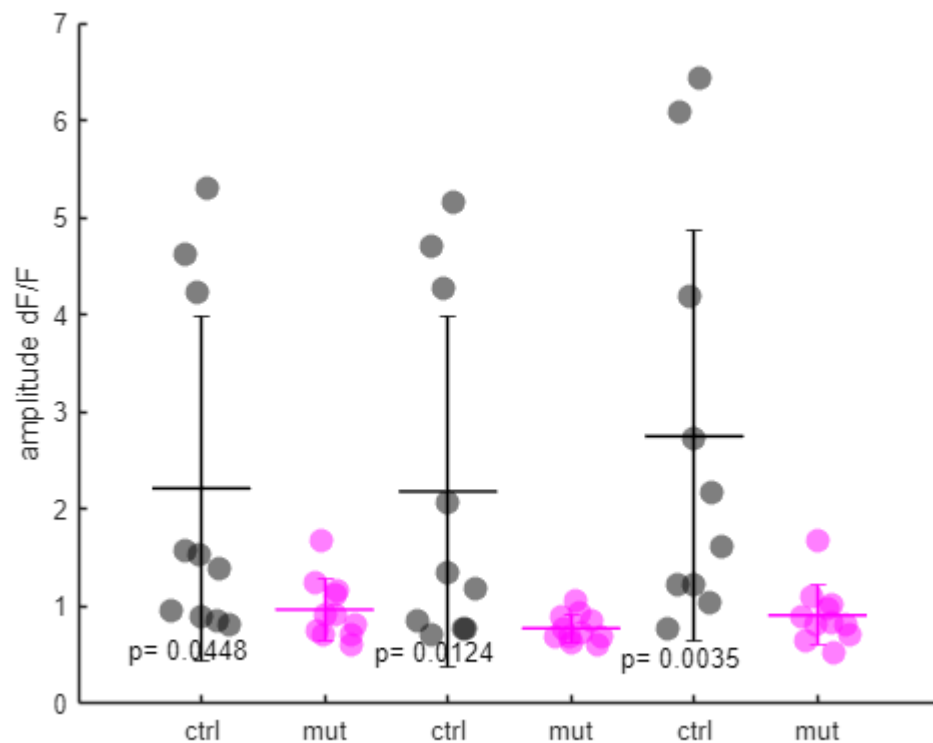
NESTED FUNCTIONS

FUNCTION 1: IMPORT ALL DATA INTO THE LIVE EDITOR SCRIPT

```
function [ctrlON,mutON,time]=nj_load2pGliadata_v2(cfg)

% STEP 1 LOAD THE RESPONDING CELL DATA
path=cfg.pathData;
cd(path)

% STEP1.1 LOAD THE CTRL DATA INTO ONE CELL ARRAY-----------------------------------------

stk_files = dir(fullfile(path, '*ctrl*' ));

% makes a cell array called ctrlON for ctrlON datasets and ctrlOFF for ctrlOFF datasets
% raw one is just the title of the columns in the array
ctrlON=cell(1,3);
ctrlON{1,1}='name';
ctrlON{1,2}='averageONresponse';
ctrlON{1,3}='averageactivity';

% I need a counter so that tehre is no empty rows
countON=2;

for i=1:length(stk_files)
load(stk_files(i).name);
fps=amplitude.cfg.originalfps;
    ctrlON{countON,1}=stk_files(i).name;
```

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```matlab
    ctrlON{countON,2}=amplitude.DFFstim_meanRegion;
    ctrlON{countON,3}=amplitude.DFF_meanRegion;
    countON=countON+1;

time.stim=amplitude.timestim;
time.overall=amplitude.time;
amplitude=[];
end
stk_files=[];


% STEP1.2 LOAD THE MUTANT DATA INTO ONE CELL ARRAY----------------------------------------

stk_files = dir(fullfile(path, '*mut*' ));

% makes a cell array called mutON for mutON datasets and mutOFF for mutOFF datasets
% raw one is just the title of the columns in the array
mutON=cell(1,3);
mutON{1,1}='name';
mutON{1,2}='averageONresponse';
mutON{1,3}='averageactivity';

% I need a counter so that tehre is no empty rows
countON=2;

for i=1:length(stk_files)
load(stk_files(i).name);
fps=amplitude.cfg.originalfps;
    mutON{countON,1}=stk_files(i).name;
    mutON{countON,2}=amplitude.DFFstim_meanRegion;
    mutON{countON,3}=amplitude.DFF_meanRegion;
    countON=countON+1;
amplitude=[];
end

stk_files=[];

end
```

FUNCTION 2 PLOT THE AVERAGE DATA FOR ALL ROI PER ANIMAL FOR ALL ANIMALS, NOTE THE DATA IS NOT SMOOTHENES

```matlab
function [averageON_ALL]=plot_responseALL_perfish(ctrlON,mutON,time,cfg)

figure
set(gcf,  'Position',[100 100 400 700])

% Average response ON
for i=2:cfg.numCTRL+1
shadedErrorBar(time.stim,mean(ctrlON{i,2},1),std(ctrlON{i,2},[],1)/sqrt(size(ctrlON{i,2},1)),'
xlabel('time in sec')
ylabel('DFF')
averageON_ALL.ctrl(i-1,:)=mean(ctrlON{i,2},1);
end
```

```
    for i=2:cfg.numMUT+1
    shadedErrorBar(time.stim,mean(mutON{i,2},1),std(mutON{i,2},[],1)/sqrt(size(mutON{i,2},1)),'line
    xlabel('time in sec')
    ylabel('DFF')
    averageON_ALL.mut(i-1,:)=mean(mutON{i,2},1);
    end
    title(['Average ON response ' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mut fish
    hold off

    end
```

FUNCTION 3: PLOT THE AVERAGE RESPONSE OF ALL FISHES, DATA IS SMOOTHENED

```
    function plot_responseALL_perfish2(ctrlON,mutON,time,cfg)

    figure

    set(gcf,  'Position',[100 100 400 700])

    % Average response ON
    for i=2:cfg.numCTRL+1
    plot(time.stim,smooth(mean(ctrlON{i,2}),0.05,'loess'), 'k'), hold on
    xlabel('time in sec')
    ylabel('DFF')
    averageON_ALL.ctrl(i-1,:)=smooth(mean(ctrlON{i,2}),0.05,'loess');
    end
    for i=2:cfg.numMUT+1
    plot(time.stim,smooth(mean(mutON{i,2}),0.05,'loess'),'Color', cfg.Mutcolor), hold on
    xlabel('time in sec')
    ylabel('DFF')
    averageON_ALL.mut(i-1,:)=smooth(mean(mutON{i,2}),0.05,'loess');
    end
    plot(time.stim,mean(averageON_ALL.ctrl,1), 'k','LineWidth',2)
    plot(time.stim,mean(averageON_ALL.mut,1), 'Color', cfg.Mutcolor,'LineWidth',2)
    title(['Average ON response ' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mut fish
    hold off


    figure

    set(gcf,  'Position',[100 100 400 600])

    rectangle('Position',[0 -8 60 70],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]),
    shadedErrorBar(time.stim,mean(averageON_ALL.ctrl,1),std(averageON_ALL.ctrl,0,1)/sqrt(size(avera
    shadedErrorBar(time.stim,mean(averageON_ALL.mut,1),std(averageON_ALL.mut,0,1)/sqrt(size(average
    title(['Mean amplitude (+/-SEM) ' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mut
    xlim([-10 70])
    ylim([-0.5 4])
    xlabel('seconds')
    ylabel('dF/F')
    hold off
    end
```

FUNCTION 4: PLOT MEAN RESPONSE ACCROSS FISH, DATA IS SMOOTHENED

```matlab
function plot_responseALL_perfish3(ctrlON,mutON,time,cfg)

for k=1:3
% Average response ON
for i=2:cfg.numCTRL+1
averageON_ALL.ctrl(i-1,:)=smooth(ctrlON{i,2}(k,:),0.05,'loess');
end
for i=2:cfg.numMUT+1
averageON_ALL.mut(i-1,:)=smooth(mutON{i,2}(k,:),0.05,'loess');
end
averageON_ALL.ctrl_BR(k,:,:)=averageON_ALL.ctrl;
averageON_ALL.mut_BR(k,:,:)=averageON_ALL.mut;
averageON_ALL.ctrl=[];
averageON_ALL.mut=[];
end


figure
for i=1:3
    toplotC=squeeze(averageON_ALL.ctrl_BR(i,:,:));
    toplotM=squeeze(averageON_ALL.mut_BR(i,:,:));
subplot (2,2,i)
rectangle('Position',[0 -8 60 70],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]),
shadedErrorBar(time.stim,mean(toplotC,1),std(toplotC,0,1)/sqrt(size(toplotC,1)),'lineprops', 'k
shadedErrorBar(time.stim,mean(toplotM,1),std(toplotM,0,1)/sqrt(size(toplotM,1)),'lineprops', ct
title(['Mean amplitude in '  num2str(cfg.label(i))])
xlim([-10 70])
ylim([-0.5 4])
xlabel('seconds')
ylabel('dF/F')
hold off
clear toplotC toplotM
end
end
```

FUNCTION 5: PLOT SCATTER OF AVERAGE AMPLITUDE FOR 10SEC AFTER LIGHT ON FOR THE
WHOLE BRAIN AND INDIVIDUAL ROIS

```matlab
function plot_scatterAll(averageON_ALL,ctrlON,mutON, time,cfg)

% enter the frames.
onset=465;%this is time 0
offset=503;% this is for 10sec

figure
set(gcf,  'Position',[100 100 300 600])

toplotC=averageON_ALL.ctrl(:,onset:offset);
toplotM=averageON_ALL.mut(:,onset:offset);
swarmchart(ones(cfg.numCTRL,1), mean(toplotC,2),'k','filled','MarkerFaceAlpha',0.5,'MarkerEdgeA
line([0.6 1.4], repmat(mean(mean(toplotC,2)),1,2),'Color','k')
```

```
errorbar(1,mean(mean(toplotC,2)), std(mean(toplotC,2),0,1),'Color','k')
swarmchart(2*ones(cfg.numMUT,1), mean(toplotM,2),cfg.Mutcolor,'filled','MarkerFaceAlpha',0.5,'M
line([1.6 2.4], repmat(mean(mean(toplotM,2)),1,2),'Color',cfg.Mutcolor)
errorbar(2,mean(mean(toplotM,2)), std(mean(toplotM,2),0,1),'Color',cfg.Mutcolor)
xlim([0 3]), ylim([0 5])
ylabel('amplitude dF/F')
text(1.5,3,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1 2])
xticklabels({'ctrl','mut'})
title('Mean amplitude ON response')
%clear toplotC toplotM
hold off


figure
for k=1:3
set(gcf,  'Position',[100 100 300 600])

subplot(2,2,k)
for i=1:cfg.numCTRL
matrixC(i,:)=mean(ctrlON{i+1,2}(k,:),1);
end
for i=1:cfg.numMUT
matrixM(i,:)=mean(mutON{i+1,2}(k,:),1);
end
toplotC=matrixC(:,onset:offset);
toplotM=matrixM(:,onset:offset);
swarmchart(ones(cfg.numCTRL,1), mean(toplotC,2),'k','filled','MarkerFaceAlpha',0.5,'MarkerEdgeA
line([0.6 1.4], repmat(mean(mean(toplotC,2)),1,2),'Color','k')
errorbar(1,mean(mean(toplotC,2)), std(mean(toplotC,2),0,1),'Color','k')
swarmchart(2*ones(cfg.numMUT,1), mean(toplotM,2),cfg.Mutcolor,'filled','MarkerFaceAlpha',0.5,'M
line([1.6 2.4], repmat(mean(mean(toplotM,2)),1,2),'Color',cfg.Mutcolor)
errorbar(2,mean(mean(toplotM,2)), std(mean(toplotM,2),0,1),'Color',cfg.Mutcolor)
xlim([0 3]), ylim([-0.1 7])
ylabel('amplitude dF/F')
text(1.5,3,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1 2])
xticklabels({'ctrl','mut'})
title([cfg.brainRegion(k,:)])
clear toplotC toplotM matrixC matrixM
hold off
end
hold off

figure
for i=1:3
set(gcf,  'Position',[100 100 300 200])

for k=1:cfg.numCTRL
matrixC(k,:)=mean(ctrlON{k+1,2}(i,:),1);
end
for k=1:cfg.numMUT
```

```
    matrixM(k,:)=mean(mutON{k+1,2}(i,:),1);
    end
    toplotC=mean(matrixC(:,onset:offset),2);
    toplotM=mean(matrixM(:,onset:offset),2);
    swarmchart((i-1)+ones(size(toplotC,1),1), mean(toplotC,2,'omitnan'),80,'k','filled','MarkerFace
    line((i-1)+[0.8 1.2], repmat(mean(mean(toplotC,2,'omitnan'),'omitnan'),1,2),'Color','k')
    errorbar((i-1)+1,mean(mean(toplotC,2,'omitnan'),'omitnan'), std(mean(toplotC,2,'omitnan'),'omit
    swarmchart((i-1)+1.5*ones(size(toplotM,1),1), mean(toplotM,2,'omitnan'),80,'m','filled','Marker
    line((i-1)+[1.3 1.7], repmat(mean(mean(toplotM,2,'omitnan'),'omitnan'),1,2),'Color','k')
    errorbar((i-1)+1.5,mean(mean(toplotM,2,'omitnan'),'omitnan'), std(mean(toplotM,2,'omitnan'),'or
    ylabel('amplitude dF/F')
    xlim([0.5 4]), ylim([-0.1 7])
    text((i-1)+0.7,0.9*min(cat(1,toplotC,toplotM)),['p= '...
        num2str(ranksum(mean(toplotC,2,'omitnan'),mean(toplotM,2,'omitnan')),'%.4f')]);
    xticks([1 1.5 2 2.5 3 3.5])
    xticklabels({'ctrl','mut','ctrl','mut','ctrl','mut'})
    clear toplotC toplotM matrixC matrixM
    end
    hold off



    end
```

FUNCTION 6: PLOT THE DFF FOR THE WHOLE RECORDING FOR EACH FISH, DATA IS SMOOTHEND

```
function plot_ALL_perfish(ctrlON,mutON,time,cfg)

figure
set(gcf,  'Position',[100 100 400 700])
str= {['Average overall glia activity']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',12, 'FontWeight', 'bold', 'l

% Average response ON
subplot(2,1,1)
for i=2:cfg.numCTRL+1
plot(time.overall/60,smooth(mean(ctrlON{i,3},1),0.005,'loess')+i*5, 'k'), hold on
xlabel('time in min')
ylabel('DFF')
averageON_ALL.ctrl(i-1,:)=smooth(mean(ctrlON{i,3},1),0.005,'loess');
end
ylim([0 80])
title([num2str(cfg.numCTRL) ' ctrl fish '])
xline([10,11,15,16,20,21,25,26,30,31],'Color', [0.8 0.8 0.8],'LineWidth', 0.5)
hold off
subplot(2,1,2)
for i=2:cfg.numMUT+1
plot(time.overall/60,smooth(mean(mutON{i,3},1),0.005,'loess')+i*5,'Color', cfg.Mutcolor), hold
xlabel('time in min')
ylabel('DFF')
averageON_ALL.mut(i-1,:)=smooth(mean(mutON{i,3},1),0.005,'loess');
end
ylim([0 80])
title([num2str(cfg.numMUT) ' mut fish '])
```

```
xline([10,11,15,16,20,21,25,26,30,31],'Color', [0.8 0.8 0.8],'LineWidth', 0.5)
hold off
end
```

FUNCTION 7: PLOT THE DFF FOR THE WHOLE RECORDING FOR EACH FISH PER BRAIN REGION,
DATA IS SMOOTHEND

```
function plot_ALL_perfish2(ctrlON,mutON,time,cfg)

figure
set(gcf,  'Position',[100 100 400 700])
str= {['Overall glia activity (std)']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',12, 'FontWeight', 'bold', 'L

counter=1
for k=1:3
% Average response ON
subplot(3,2,counter)
for i=2:cfg.numCTRL+1
plot(time.overall/60,smooth(ctrlON{i,3}(k,:),0.005,'loess')+i*5, 'k'), hold on
xlabel('time in min')
ylabel('DFF')
end
ylim([0 80])
title(['CTRL ' num2str(cfg.label(k))])
xline([10,11,15,16,20,21,25,26,30,31],'Color', [0.8 0.8 0.8],'LineWidth', 0.5)
hold off
counter=counter+1;
subplot(3,2,counter)
for i=2:cfg.numMUT+1
plot(time.overall/60,smooth(mutON{i,3}(k,:),0.005,'loess')+i*5,'Color', cfg.Mutcolor), hold on
xlabel('time in min')
ylabel('DFF')
end
ylim([0 80])
title(['MUT ' num2str(cfg.label(k))])
xline([10,11,15,16,20,21,25,26,30,31],'Color', [0.8 0.8 0.8],'LineWidth', 0.5)
hold off
counter=counter+1;
end
end
```

FUNCTION 8: PLOT SCATTER FOR STD OF DFF FOR THE ENTIRE BRAIN AND BRAIN REGION, DATA IS
SMOOTHEEND

```
function plot_scatter_allfish(ctrlON,mutON,cfg)

% plot standard deviation of glial activity per fish for the entire bain
%smoothen the data
for i=1:cfg.numCTRL
    data=smooth(mean(ctrlON{1+i,3},1),0.005,'loess');
    dataCTRL(i,:)=data.';
end
```

```matlab
%
figure,
for i=1:cfg.numMUT
    data=smooth(mean(mutON{1+i,3},1),0.005,'loess');
    dataMUT(i,:)=data.';
end


figure
set(gcf,  'Position',[100 100 300 600])

toplotC=std(dataCTRL,0,2).';
toplotM=std(dataMUT,0,2).';

swarmchart(ones(length(toplotC),1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'MarkerEdgeAlpha
line([0.6 1.4], repmat(mean(toplotC,2),1,2),'Color','k')
errorbar(1,mean(toplotC,2), std(toplotC),'Color','k')
swarmchart(2*ones(cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAlpha',0.5,'MarkerEdg
line([1.6 2.4], repmat(mean(toplotM,2),1,2),'Color',cfg.Mutcolor)
errorbar(2,mean(toplotM,2), std(toplotM),'Color',cfg.Mutcolor)
xlim([0 3]), %ylim([-0.5 Inf])
ylabel('std DFF')
text(0.1,min(toplotC),['p= '...
    num2str(ranksum(toplotC,toplotM),'%.4f')]);
xticks([1 2])
xticklabels({'ctrl','mut'})
title('overall glial activity (std)')
%clear toplotC toplotM
hold off

% plot standard deviation of glial activity per fish for the entire bain
%smoothen the data
for k=1:3
for i=1:cfg.numCTRL
matCTRLs(k,i)=std(smooth(ctrlON{1+i,3}(k,:),0.005,'loess'),0,1);
end
for i=1:cfg.numMUT
matMUTs(k,i)=std(smooth(mutON{1+i,3}(k,:),0.005,'loess'),0,1);
end
end

figure
set(gcf,  'Position',[100 100 300 600])
str= {['overall glial activity (std) per region']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',10, 'FontWeight', 'bold', 'I

for k=1:3
toplotC=matCTRLs(k,:);
toplotM=matMUTs(k,:);
subplot(2,2,k)
swarmchart(ones(length(toplotC),1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'MarkerEdgeAlpha
line([0.6 1.4], repmat(mean(toplotC,2),1,2),'Color','k')
errorbar(1,mean(toplotC,2), std(toplotC,0,2),'Color','k')
swarmchart(2*ones(cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAlpha',0.5,'MarkerEdg
```

```matlab
    line([1.6 2.4], repmat(mean(toplotM,2),1,2),'Color',cfg.Mutcolor)
    errorbar(2,mean(toplotM,2), std(toplotM,0,2),'Color',cfg.Mutcolor)
    xlim([0 3]), %ylim([-2 Inf])
    ylabel('std activity DFF')
    text(0.4,3,['p= '...
        num2str(ranksum(toplotC,toplotM),'%.4f')]);
    xticks([1 2])
    xticklabels({'ctrl','mut'})
    title(['overall activity (std) ' num2str(cfg.label(k))])
    %clear toplotC toplotM
    hold off
    end

    figure
    for i=1:3
    toplotC=matCTRLs(i,:);
    toplotM=matMUTs(i,:);
    swarmchart((i-1)+ones(size(toplotC,2),1), toplotC,80,'k','filled','MarkerFaceAlpha',0.5,'Marker
    line((i-1)+[0.8 1.2], repmat(mean(toplotC,2),1,2),'Color','k')
    errorbar((i-1)+1,mean(toplotC,2), std(toplotC,0,2),'Color','k')
    swarmchart((i-1)+1.5*ones(cfg.numMUT,1), toplotM, 80 ,cfg.Mutcolor,'filled','MarkerFaceAlpha',0
    line((i-1)+[1.3 1.7], repmat(mean(toplotM,2),1,2),'Color',cfg.Mutcolor)
    errorbar((i-1)+1.5,mean(toplotM,2), std(toplotM,0,2),'Color',cfg.Mutcolor)
    ylabel('amplitude dF/F')
    %xlim([0.5 4]), ylim([-0.1 7])
    text((i-1)+0.7,0.9*min(cat(2,toplotC,toplotM)),['p= '...
        num2str(ranksum(toplotC,toplotM),'%.4f')]);
    xticks([1 1.5 2 2.5 3 3.5])
    xticklabels({'ctrl','mut','ctrl','mut','ctrl','mut'})
    clear toplotC toplotM matrixC matrixM
    end
    hold off

    end
```