# ANALYSIS OF LIGHT RESPONSES IN SMH 2P

Data obtained by Percival D'Gama at 5dpf. HuC:h2B-GCamp6s smh controls (heterozygous and wt) and smh (homozygous) mutants

Codes updated July 2024 during revision process

See readME file for more information about steps performed before this analysis

Codes used to plot Figure3 panel A, D1-F4

```
 %%Set path to where your codes are
addpath(genpath('X:\Nathalie\data\github\DGama_et_al_CR_2024'))
```

## STEP1 LOAD ALL DATA INTO ONE CELL ARRAY-------------------------------------------

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

```
cfg.pathData='X:\Manuscripts\Dgama etal 2024 smh\data\Figure 3\Panel D1-E1\';
```

load the data into few matrix

```
[ctrlON,ctrlOFF,mutON,mutOFF,time]=nj_load2pdata_v3(cfg);
```

The matrix contain the following information, example for ctrlON

column 1: experimental animal

column 2: the DFF trace of cells that are activated by the ON stimulus

column 3: the DFF trace of cells that are inhibited by the ON stimulus

column 4: the position,x,y,z,brain region of cells that are activated by the ON stimulus

column 5: the position,x,y,z,brain region of cells that are inihibited by the ON stimulus

column 6: the DFF traces of all cells

column 7:  the position,x,y,z,brain region of all cells

NB: the DFF traces are the average traces for the 5 stimuli

calculate the number of dataset

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

```
numCTRL = 8
```

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

```
numMUT = 14
```

## 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" "OT" "hind" "habenula"];
cfg.brainRegion=char('telencephalon','TeO/thalamus','Hindbrain', 'Habenula');
cfg
```

```
cfg = struct with fields:
          MutID: 'SMH'
       Mutcolor: 'm'
        numCTRL: 8
         numMUT: 14
          label: ["tel"    "OT"    "hind"    "habenula"]
    brainRegion: [4×13 char]
```

## PLOT CELL DETECTION FOR  A REPRESENTATIVE EXAMPLE

generate panel A2

Representative example is 20220408_11_52_04_20220408volsmhctrlF01, which is saved in row 3 of ctrlON

```
toplot.xyzb=ctrlON{3,7};
toplot.dff=ctrlON{3,6};
```

set some parameters

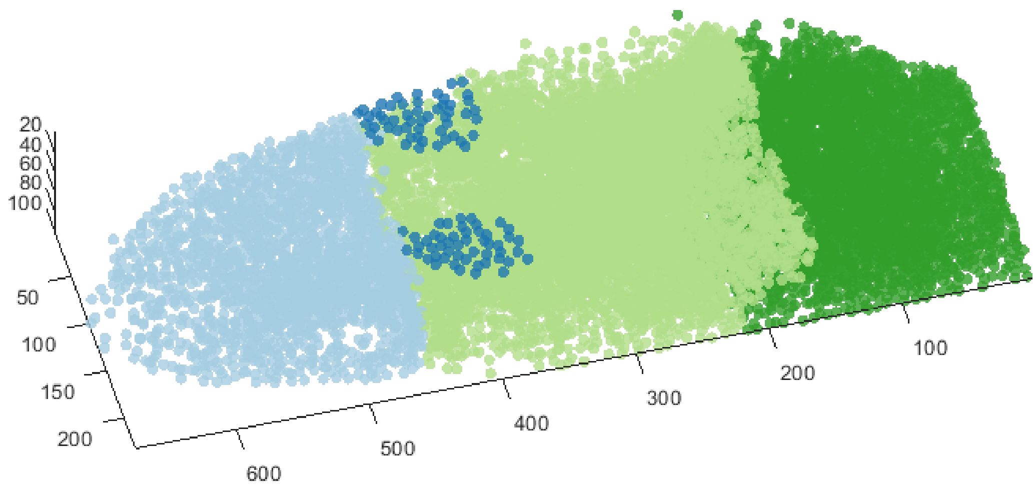
```
% pixel size of 2p recording
voxelSize=0.455729000000000;

% introduce colorscale to be used
colorM=[166 206 227;
178 223 138;
51 160 44;
31 120 180]/256;
```

plot 3D scatter of the identified cells, axis are in microns

```
figure
set(gcf,  'Position',[50 50 800 500])
hold on
for k=[1,3,4,2]
index=toplot.xyzb(:,4)==k;
```

```
scatter3(toplot.xyzb(index,1),toplot.xyzb(index,2),toplot.xyzb(index,3),30,toplot.xyzb(index,4)
set (gca,'Zdir','reverse'),  set (gca,'Ydir','reverse'),  set (gca,'Xdir','reverse')
colormap (colorM), caxis([1 4])
alpha(.8)
end
axis image
%axis off
view (-15,45)
```




# PLOT THE HEATMAP OF THE REPRESENTATIVE WT EXAMPLE

generate figure A3

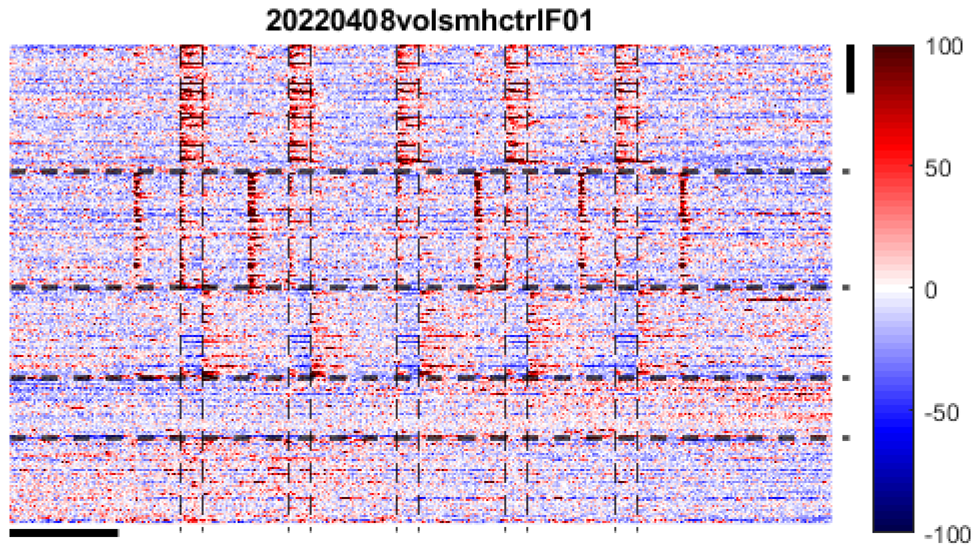
PLOT THE HEATMAP OF THE REPRESENTATIVE CTRL, dataset: 20220408_11_52_04_20220408volsmhctrlF01

load the data

```
load('X:\Manuscripts\Dgama etal 2024 smh\data\Figure 3\Panel A\20220408volsmhctrlF01Results_CLU
```

plot the heatmap

```
plot_heatmap(results)
```




To plot all the data that are included in the manuscript, please use the code SupplementaryF_activity_allsamples.mlx.

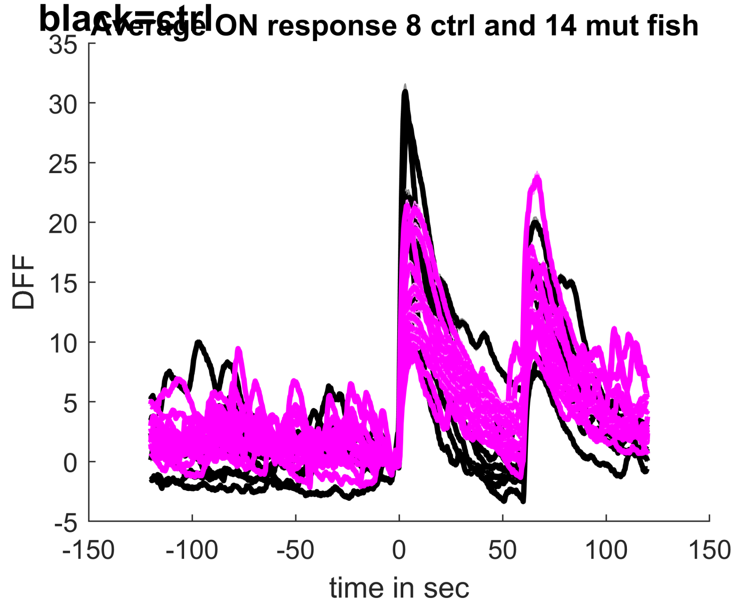
These are now included in Figure S

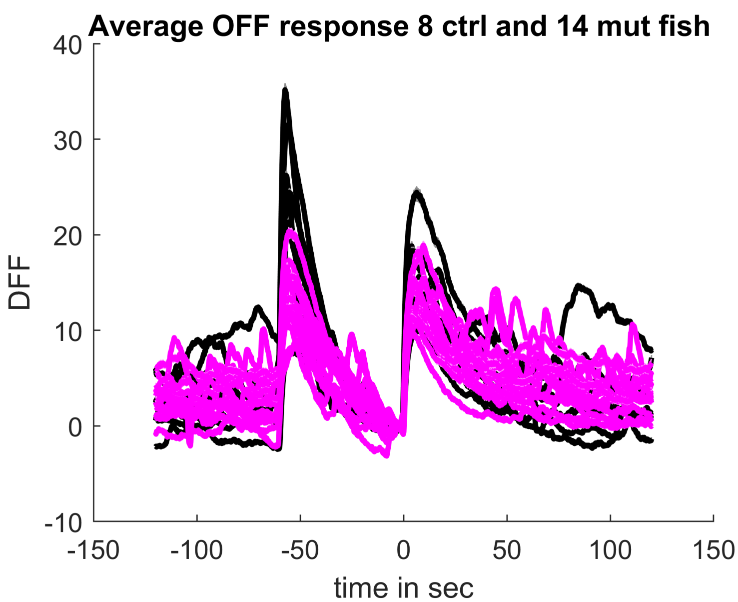
## PLOT THE AVERAGE RESPONSE FOR ALL CELLS PER FISH

plot all response to all fishes

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

**SMH Amplitude of ALL responses black=ctrl**



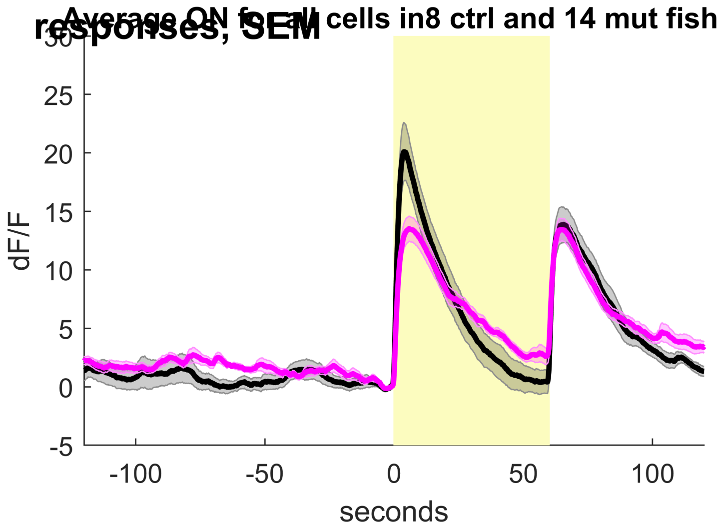



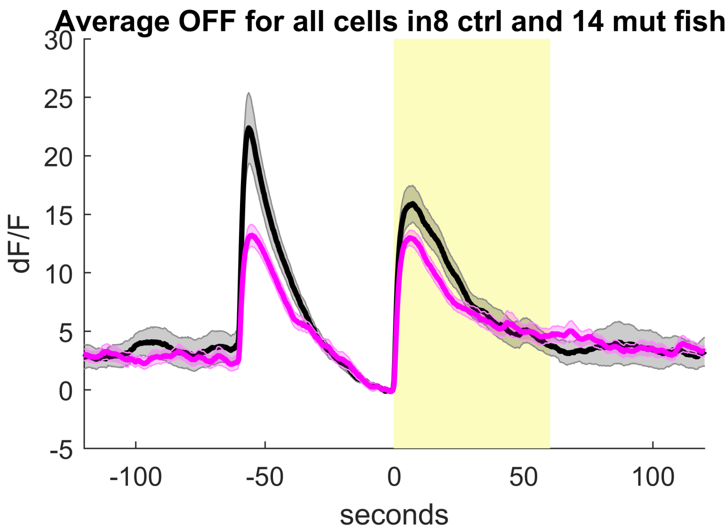

## PLOT THE AVERAGE RESPONSE FOR ALL CELLS FOR ALL FISH

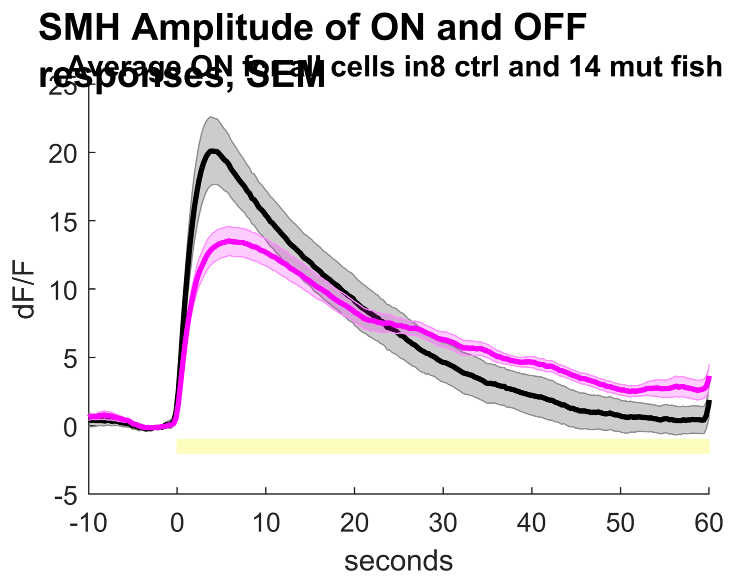
generate panel D1-E1

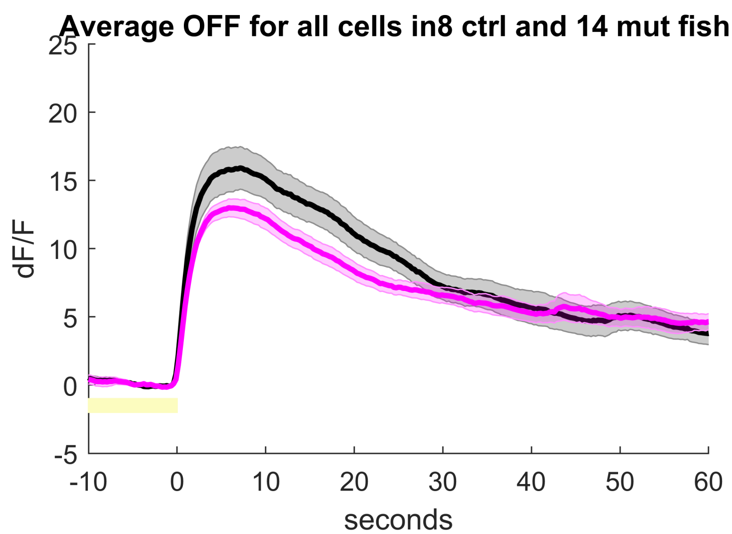
```
plot_responseALL_average(averageON_ALL,averageOFF_ALL,time,cfg);
```

## SMH Amplitude of ON and OFF responses; SEM





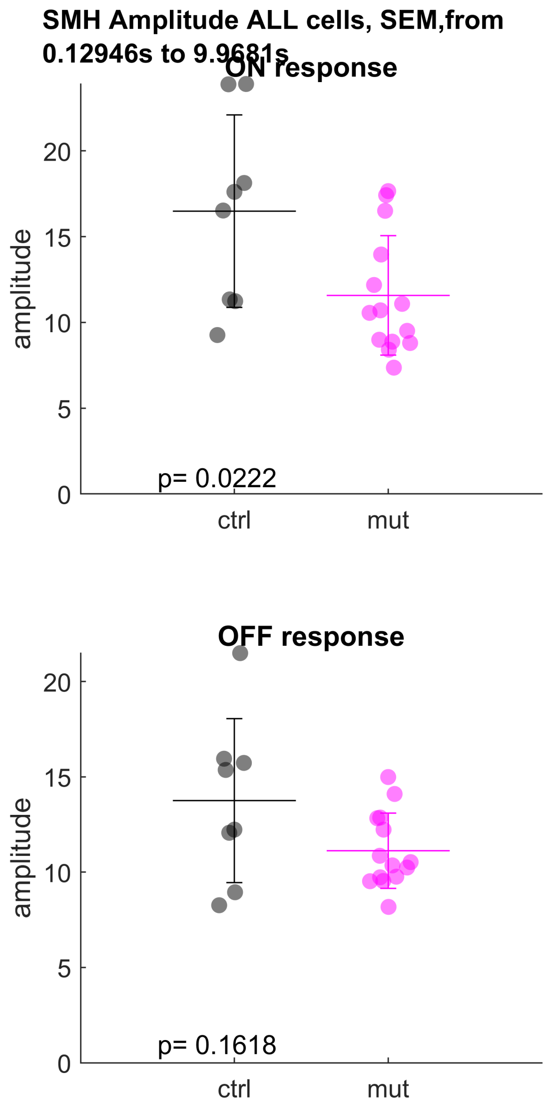




## PLOT THE AVERAGE RESPONSE FOR ALL CELLS PER FISH

generate statistics for panel D1-E1

```
plot_scatterAll(averageON_ALL,averageOFF_ALL,ctrlON,ctrlOFF, mutON, mutOFF, time,cfg)
```

```
ans =
'0.022228'
```

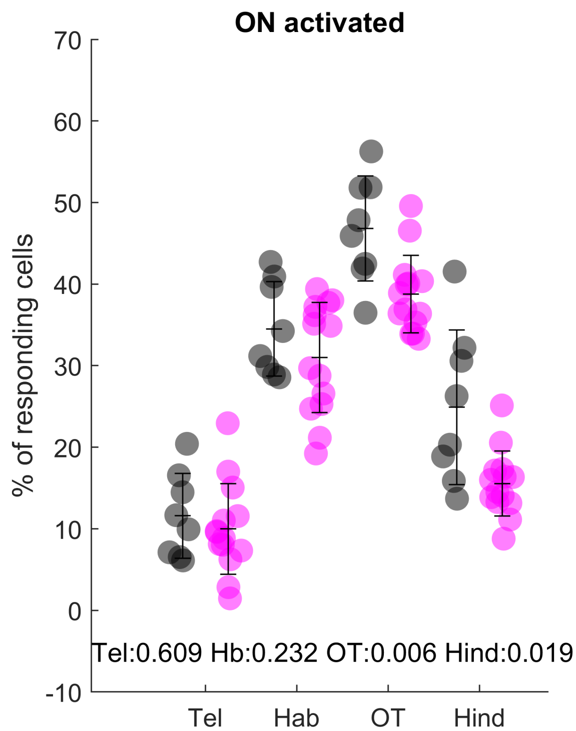


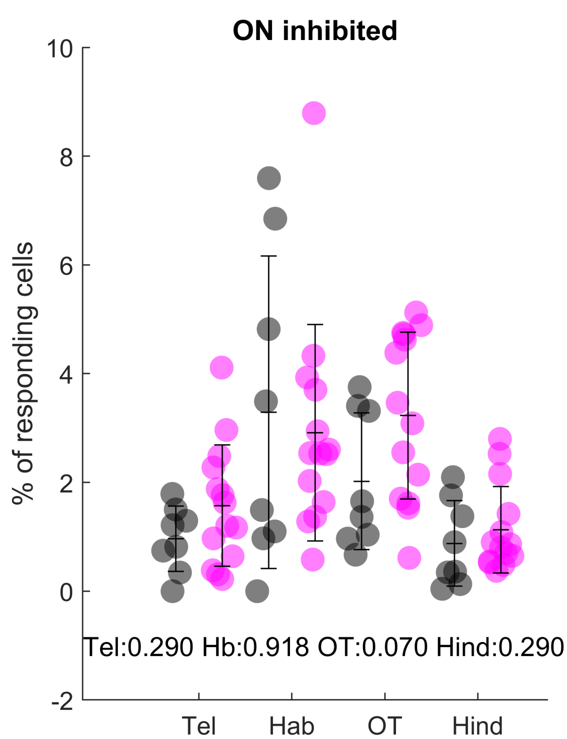

```
ans =
'0.16176'
```

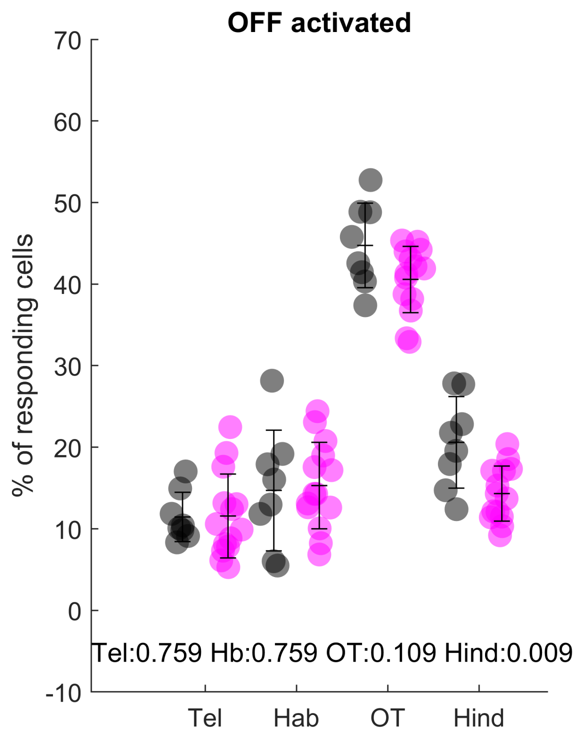
# CALCULATE % RESPONDING CELLS PER BRAIN REGION

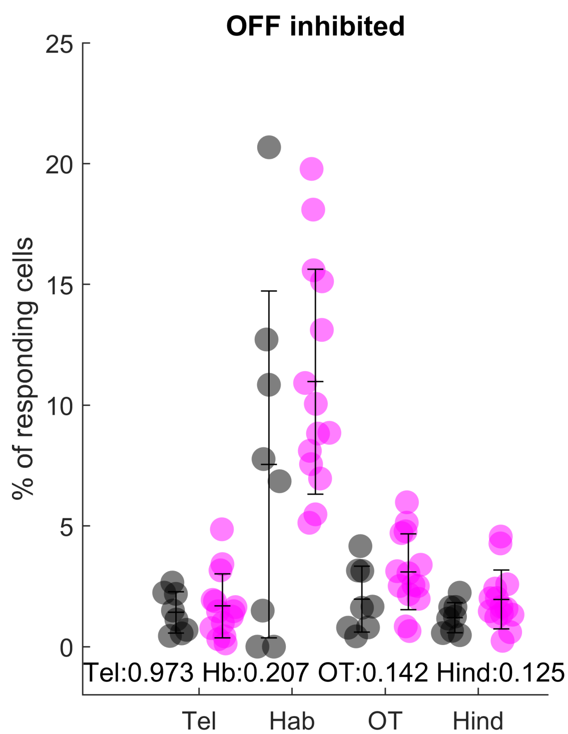
generate panel D3-D4-E3-E4

```
plot_response_perregion_v2(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)
```


ON activated
% of responding cells
70
60
50
40
30
20
10
0
-10
Tel:0.609 Hb:0.232 OT:0.006 Hind:0.019
Tel
Hab
OT
Hind


ON inhibited
% of responding cells
10
8
6
4
2
0
-2
Tel:0.290 Hb:0.918 OT:0.070 Hind:0.290
Tel
Hab
OT
Hind

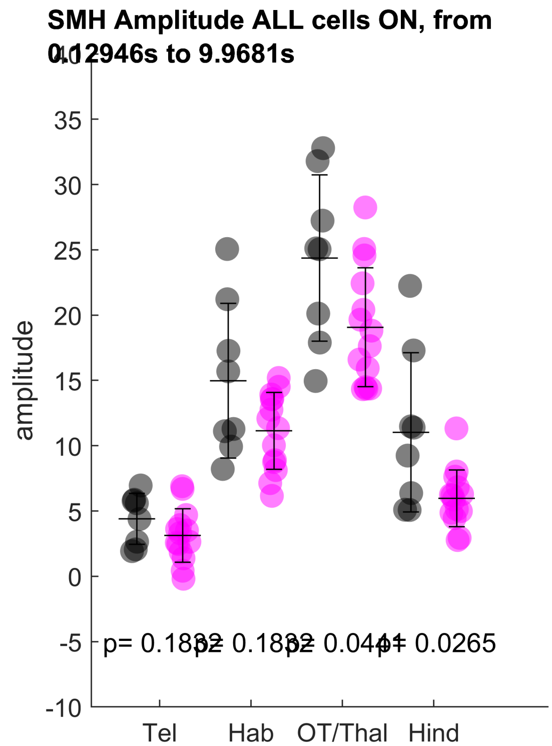


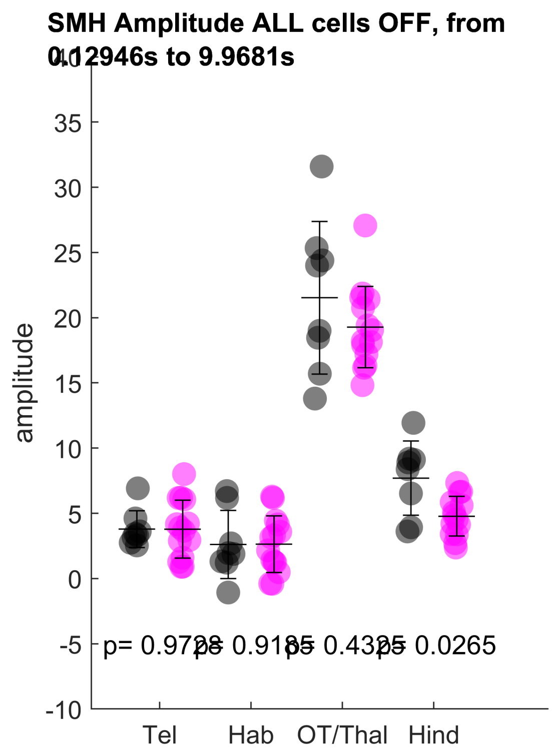




## CALCULATE AMPLITUDE PER BRAIN REGION

generate panel D2-E2

```
plot_amplitude_perregion(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)
```




## CALCULATE % ON RESPONDING CELLS BASED ON location of cells in x,y,z

chose ON dataset for the analysis

```
cfg.data=1;
```

Identify midline, by plotting OT and using ginput

```
% % ONLY NEEDS TO BE DONE ONCE
% identify_midline(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)
% %
% % [midline_ctrl] = ginput() % select figure of ctrl
% [midline_mut] = ginput() % select figure of ctrl
% % copy values below
cfg.midline_ctrl = [352.102803738318    113.584829143338
370.917573872473    117.231685264341
456.775700934579    117.231685264341
391.951788491446    118.941323733066
391.355140186916    122.365919655480
369.634525660964    118.943096728054
418.118195956454    114.838067459285
423.403426791277    127.500154046618];
cfg.midline_mut = [376.401869158878    115.065469573066
374.183514774495    115.520273800628
444.626168224299    113.662232139248
266.757387247278    105.866776750983
426.401869158879    119.275181874520
393.856920684292    121.840388645827
447.429906542056    120.372432156119
304.276827371695    114.838067459285
422.313084112150    117.159232937755
400.388802488336    119.746625281139
387.1495            119.5109
401.788491446345    113.437603221977
377.803738317757    123.871136966938
467.573872472784    120.372432156118];
```

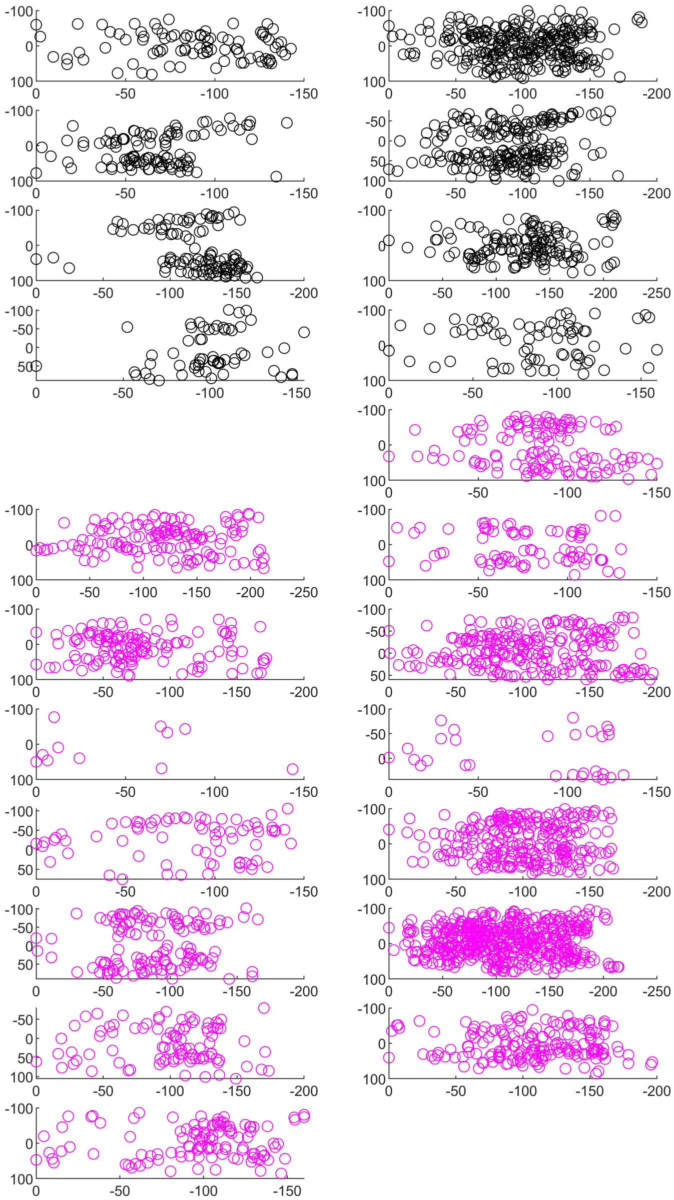
Location of the responding cells.

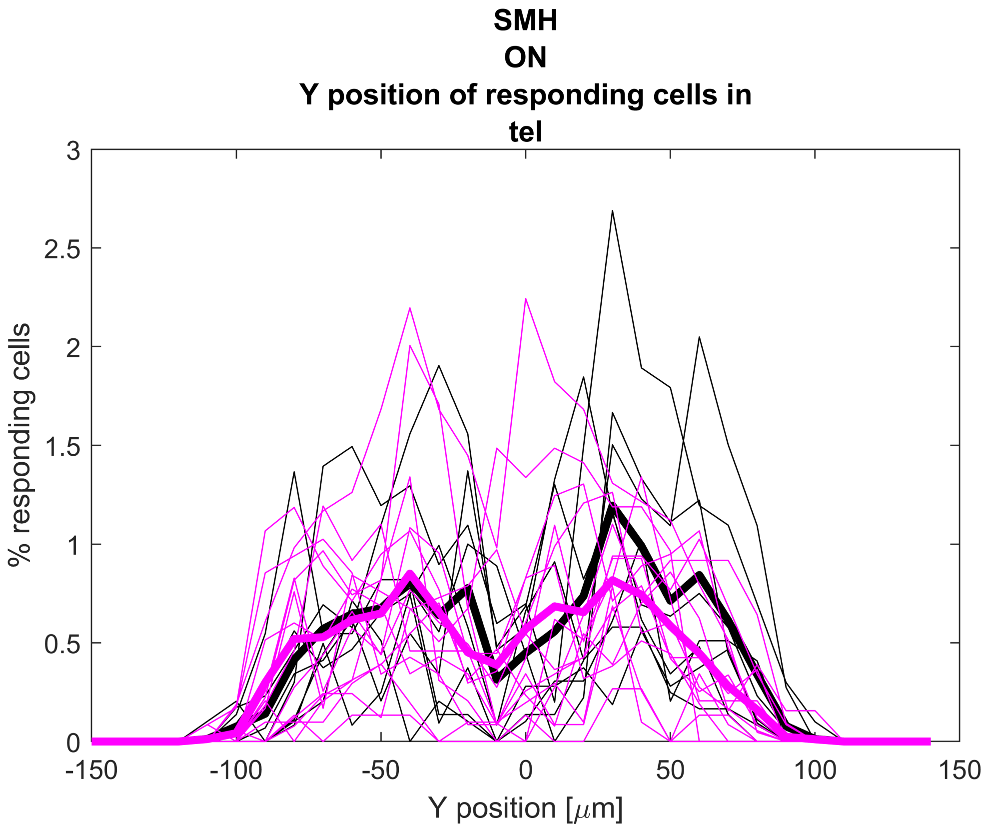
High Y axis value is left. In the scatter plots, left is anterior, right is poster, down is left, up is right

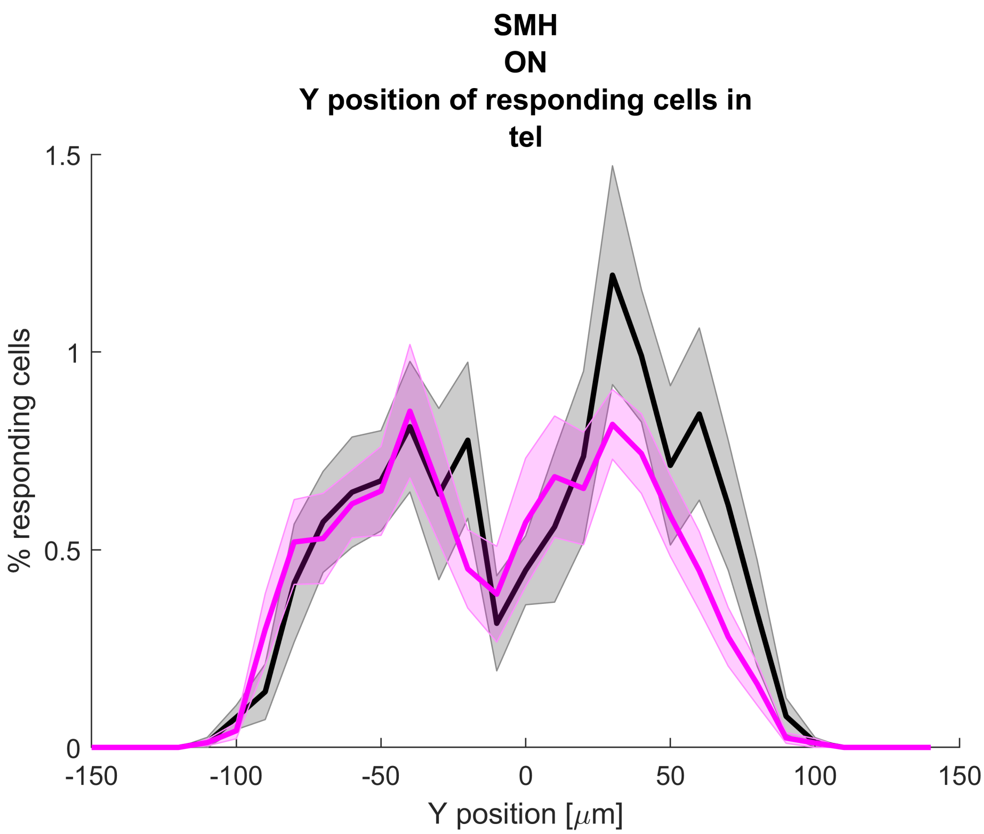
In teh shaded error bars are shown SEM

```
plot_respondingXYZ(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg);
```

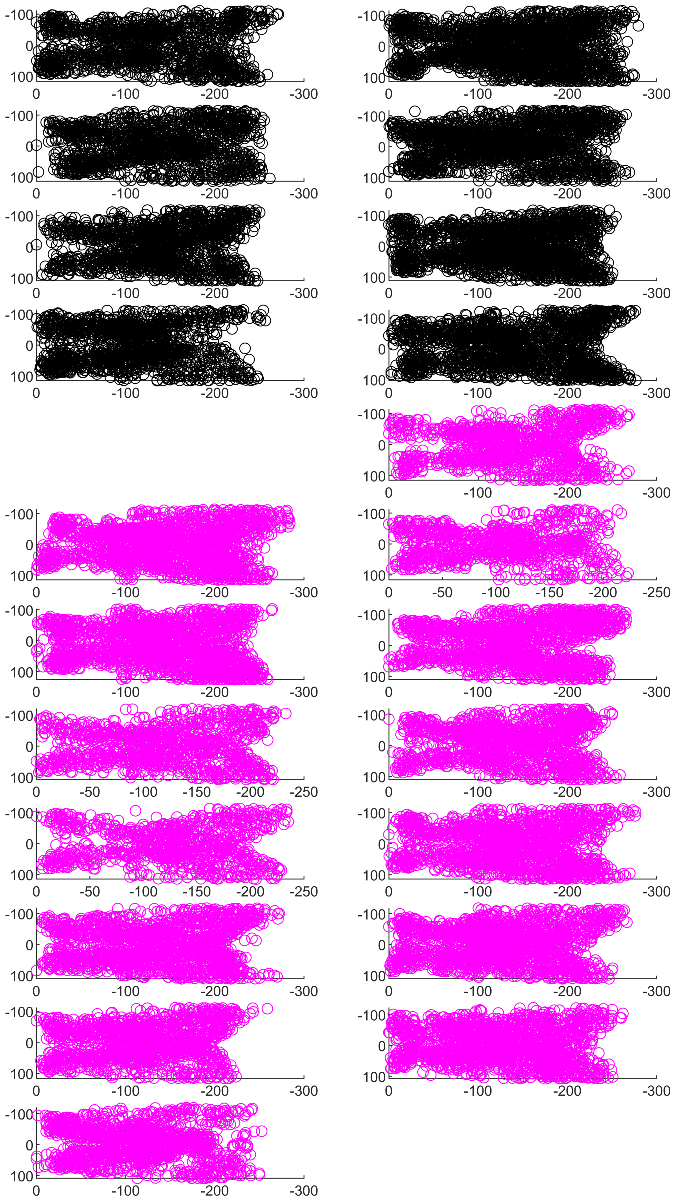
**SMH**
**ON**
**scatter of responding cells in tel**

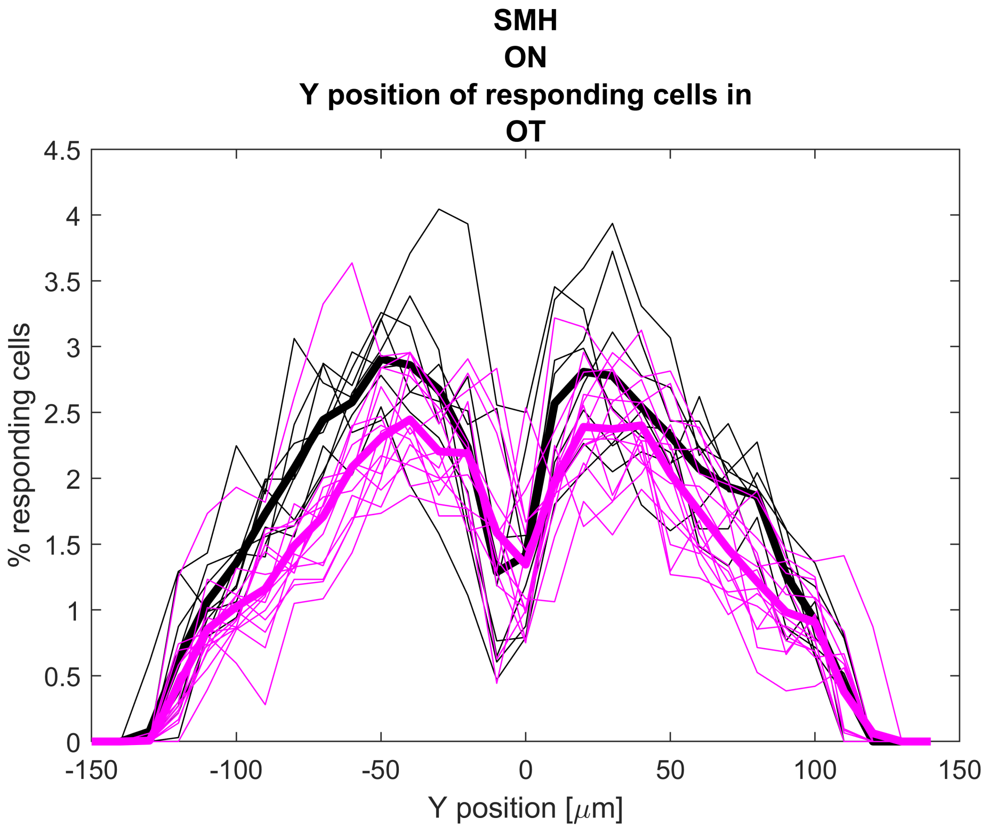


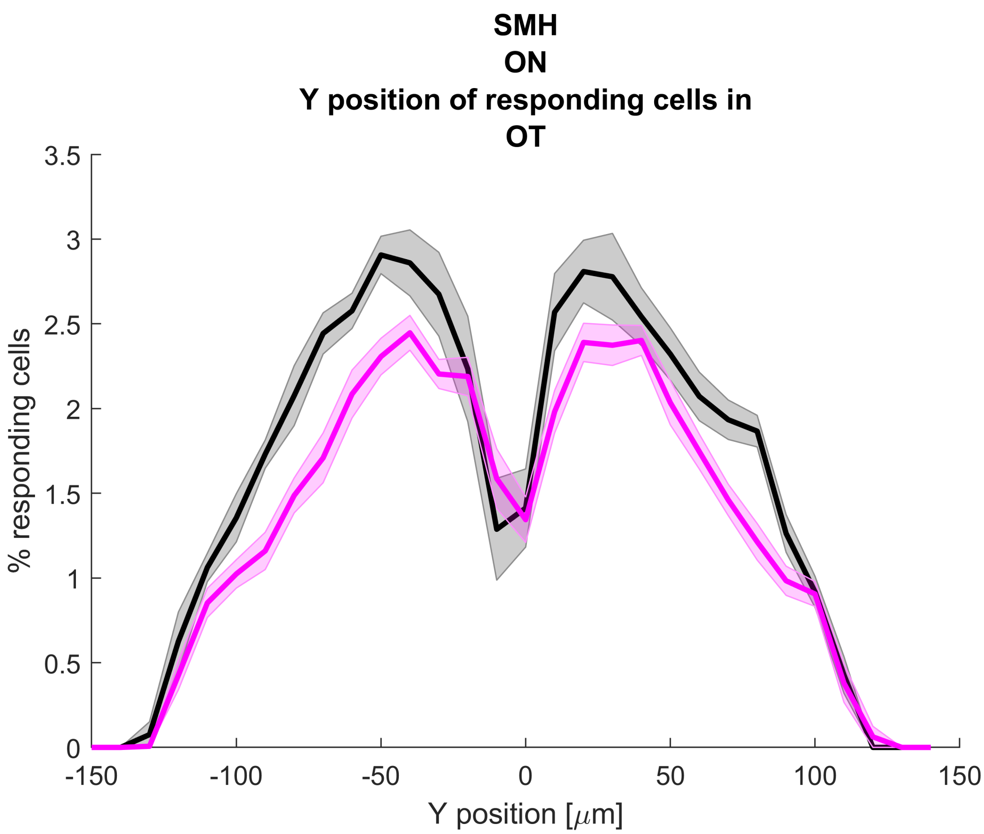

SMH
ON
Y position of responding cells in
tel
% responding cells
Y position [$\mu$m]
0
0.5
1
1.5
2
2.5
3
-150
-100
-50
0
50
100
150


SMH
ON
Y position of responding cells in
tel
% responding cells
Y position [$\mu$m]
0
0.5
1
1.5
-150
-100
-50
0
50
100
150

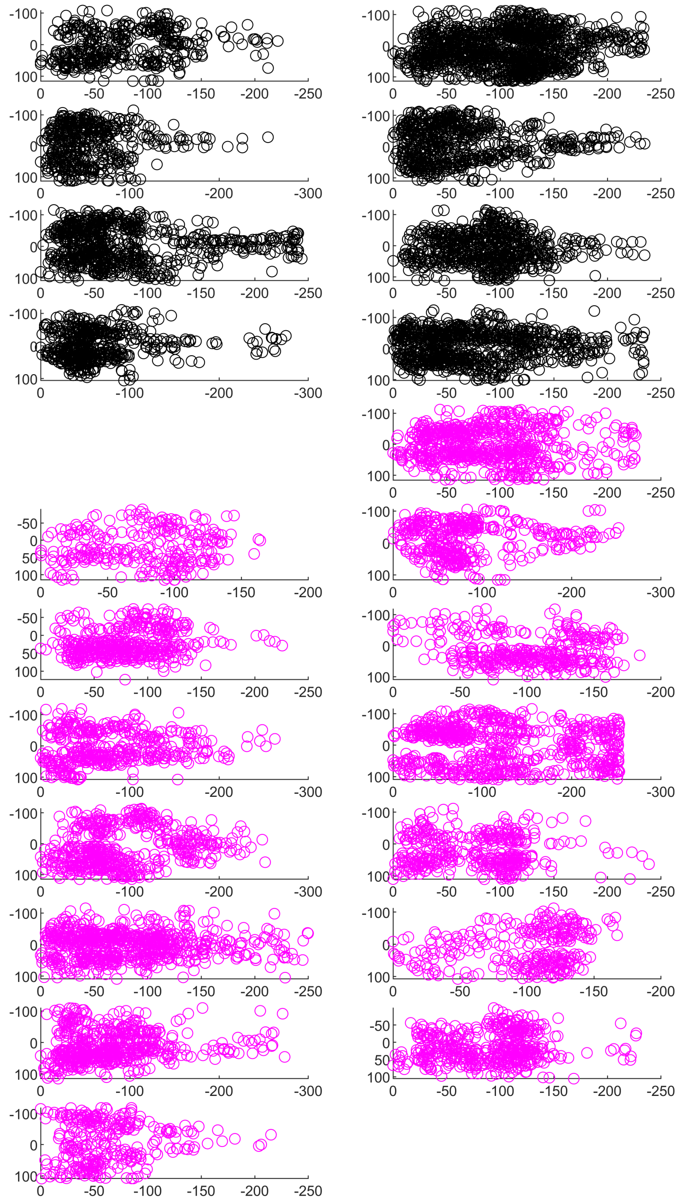
**SMH**
**ON**
**scatter of responding cells in**
**OT**

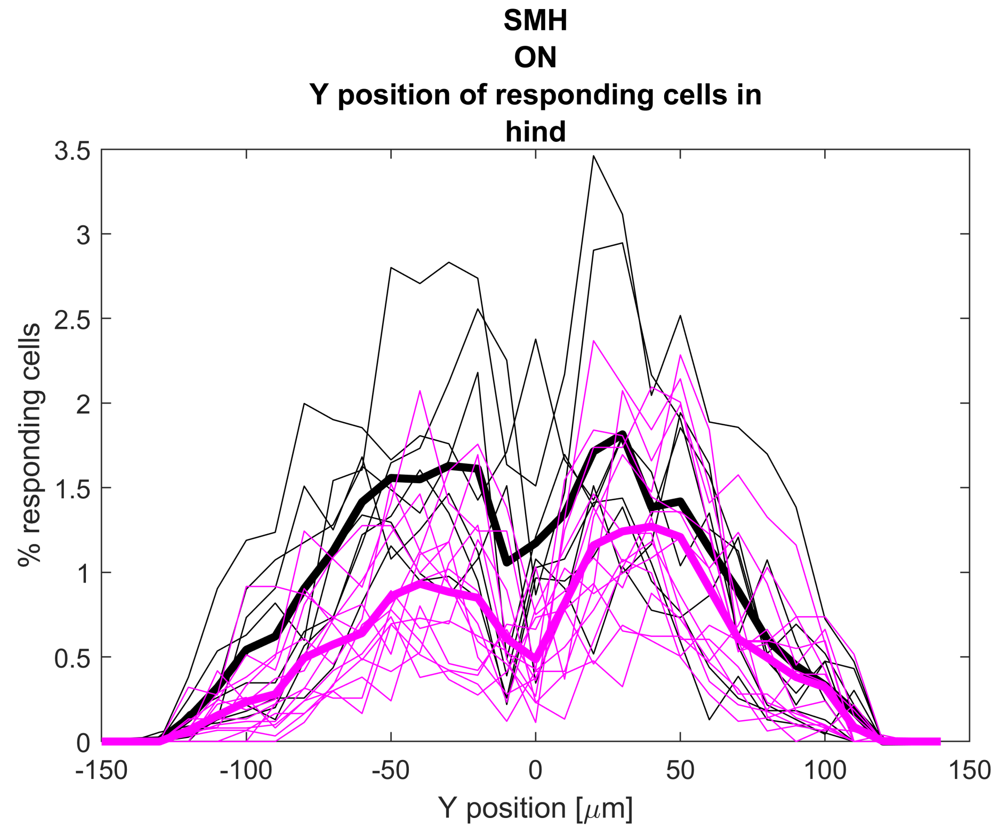


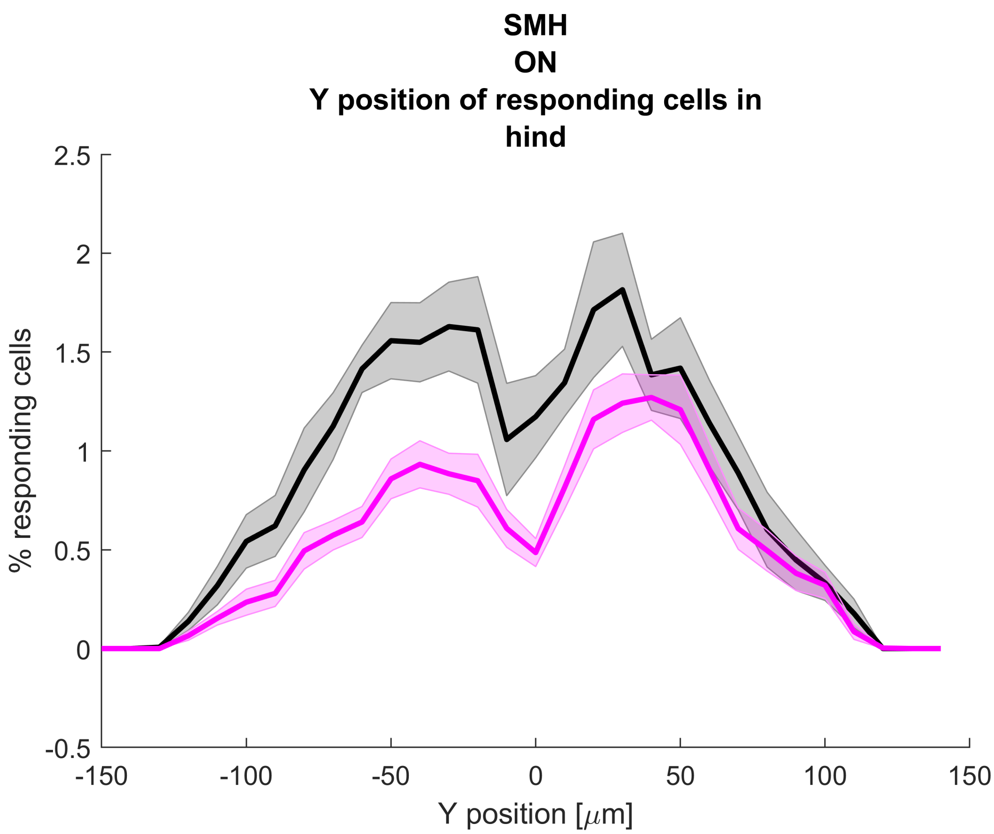

SMH
ON
Y position of responding cells in
OT
% responding cells
Y position [$\mu$m]
-150
-100
-50
0
50
100
150
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5


SMH
ON
Y position of responding cells in
OT
% responding cells
Y position [$\mu$m]
-150
-100
-50
0
50
100
150
0
0.5
1
1.5
2
2.5
3
3.5

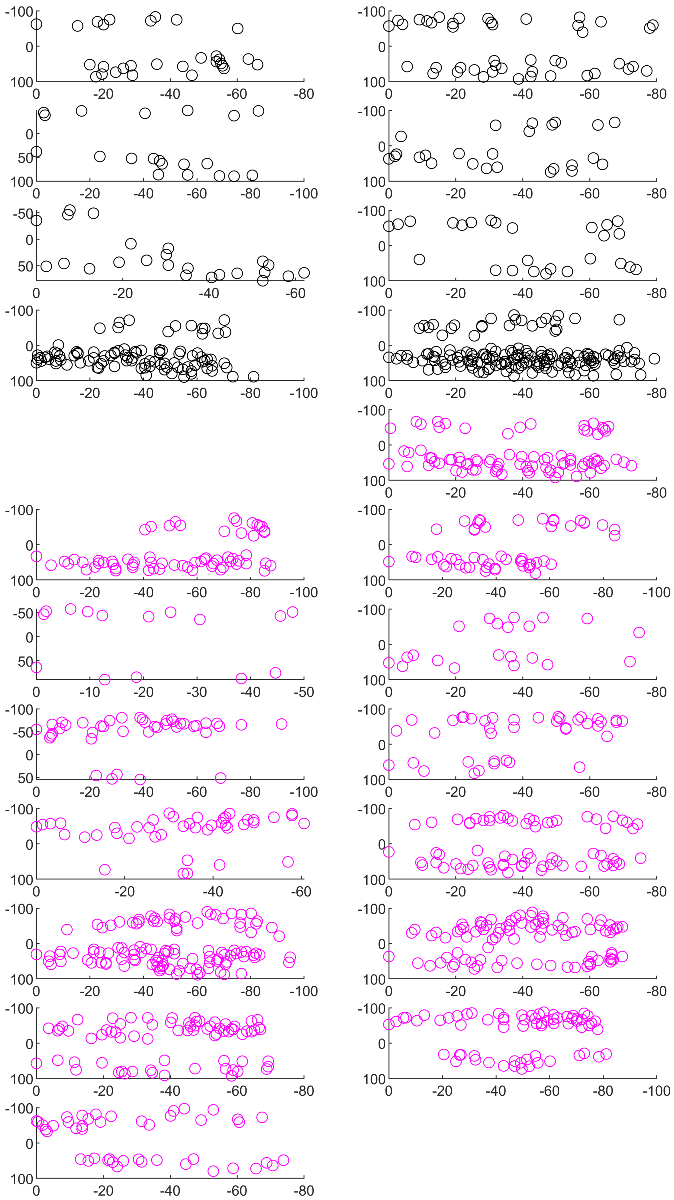
**SMH**
**ON**
**scatter of responding cells in hind**



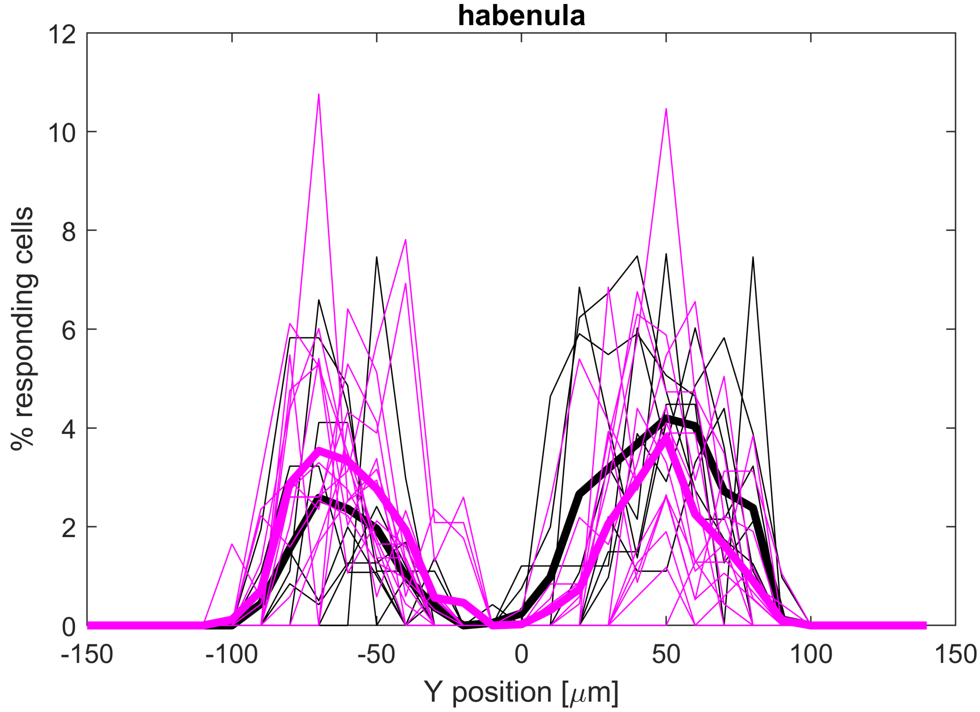

SMH
ON
Y position of responding cells in
hind
% responding cells
Y position [$\mu$m]


SMH
ON
Y position of responding cells in
hind
% responding cells
Y position [$\mu$m]

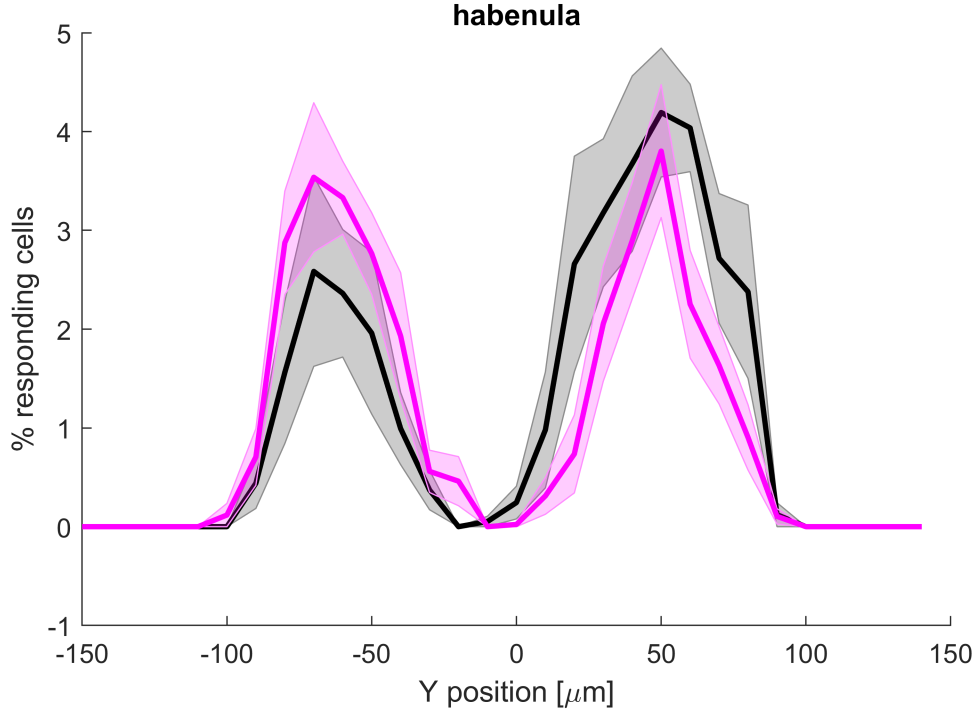
**SMH**
**ON**
**scatter of responding cells in habenula**



**SMH**
**ON**
**Y position of responding cells in habenula**




**SMH**
**ON**
**Y position of responding cells in habenula**




## CALCULATE % OFF RESPONDING CELLS BASED ON location of cells in x,y,z

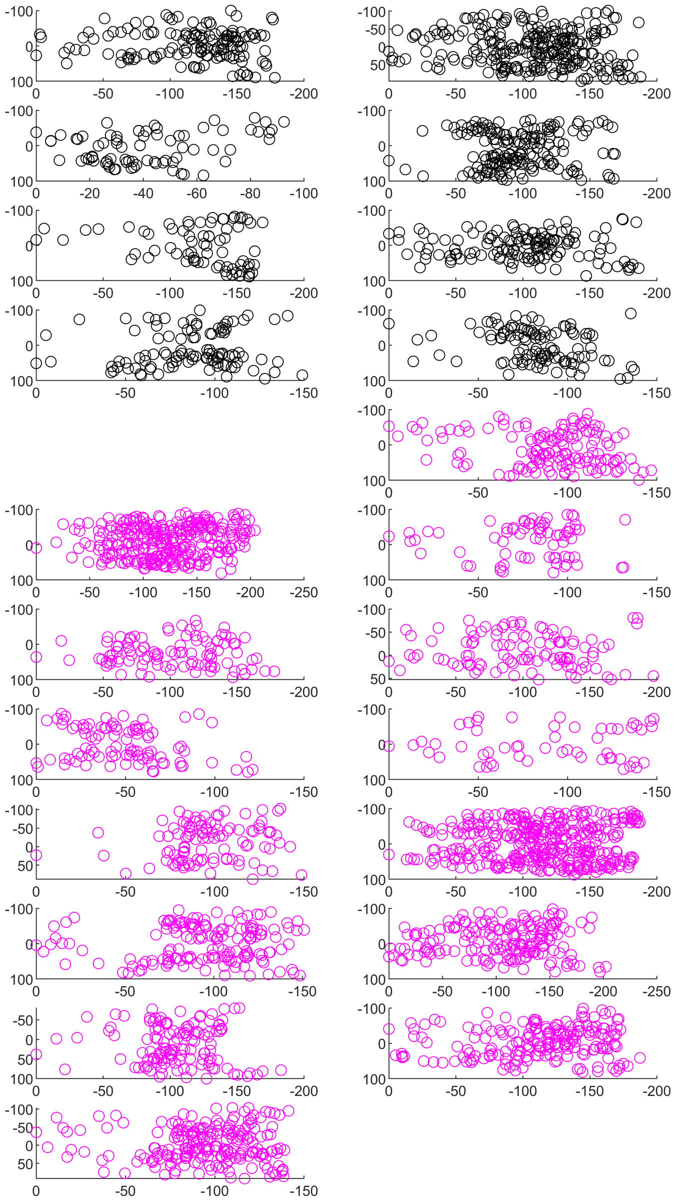
Choose OFF dataset for the analysis

```
cfg.data=2;
```

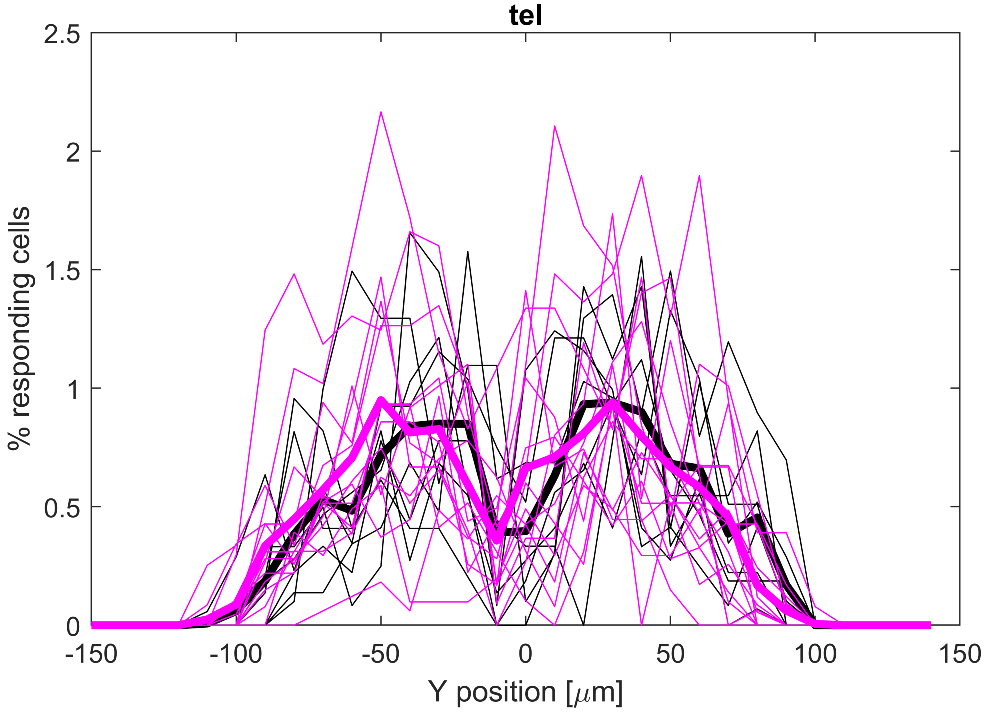
Location of the responding cells

```
plot_respondingXYZ(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)
```

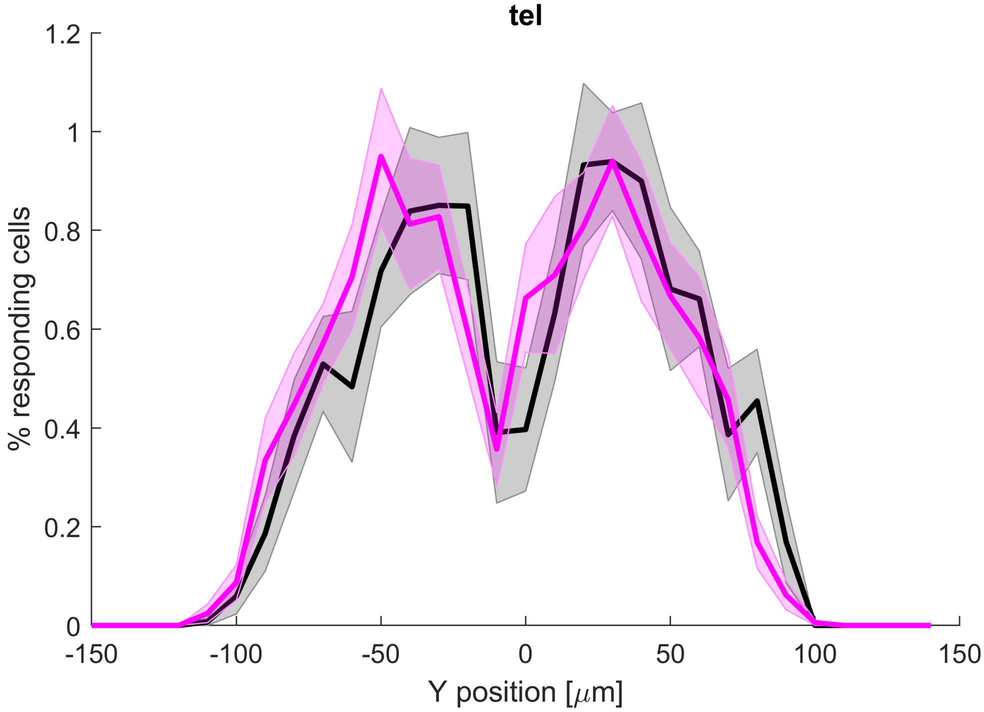
**SMH**
**OFF**
**scatter of responding cells in tel**

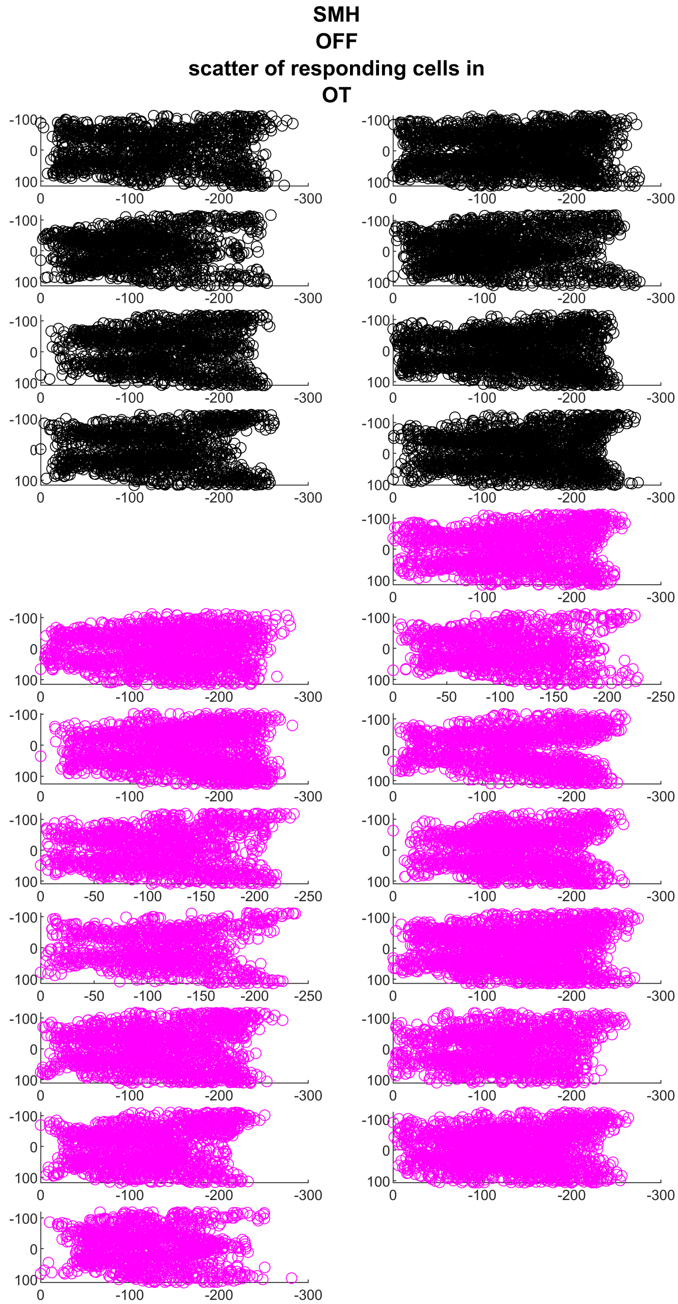


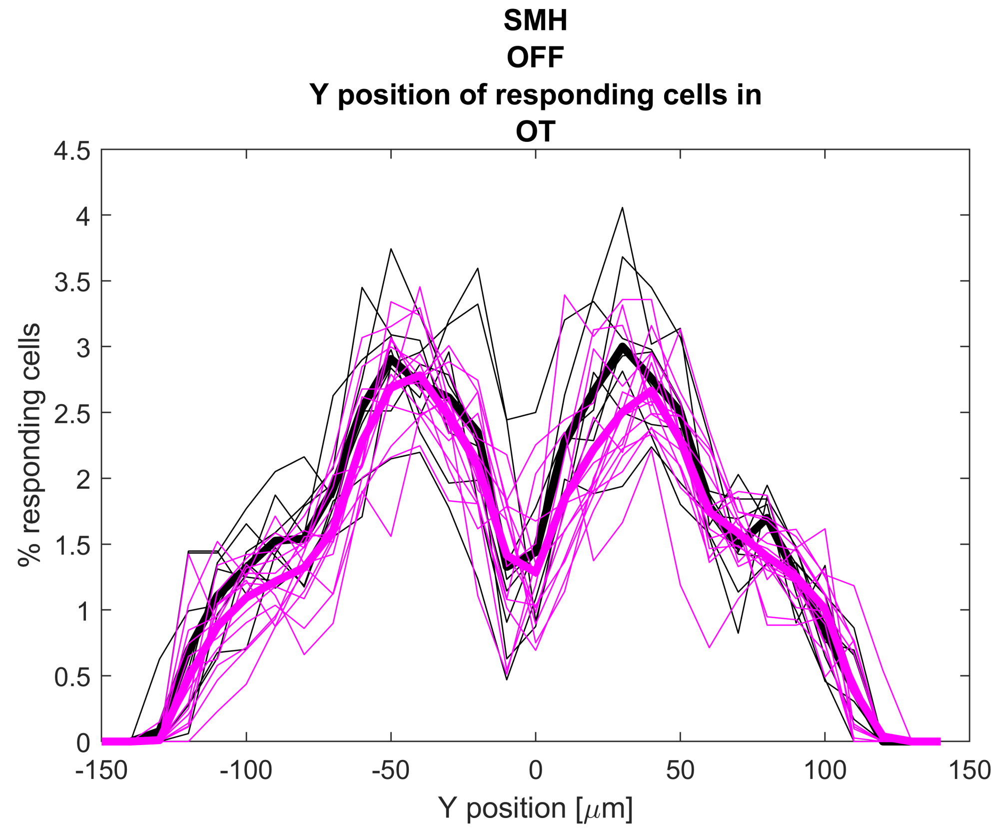
**SMH**
**OFF**
**Y position of responding cells in**
**tel**

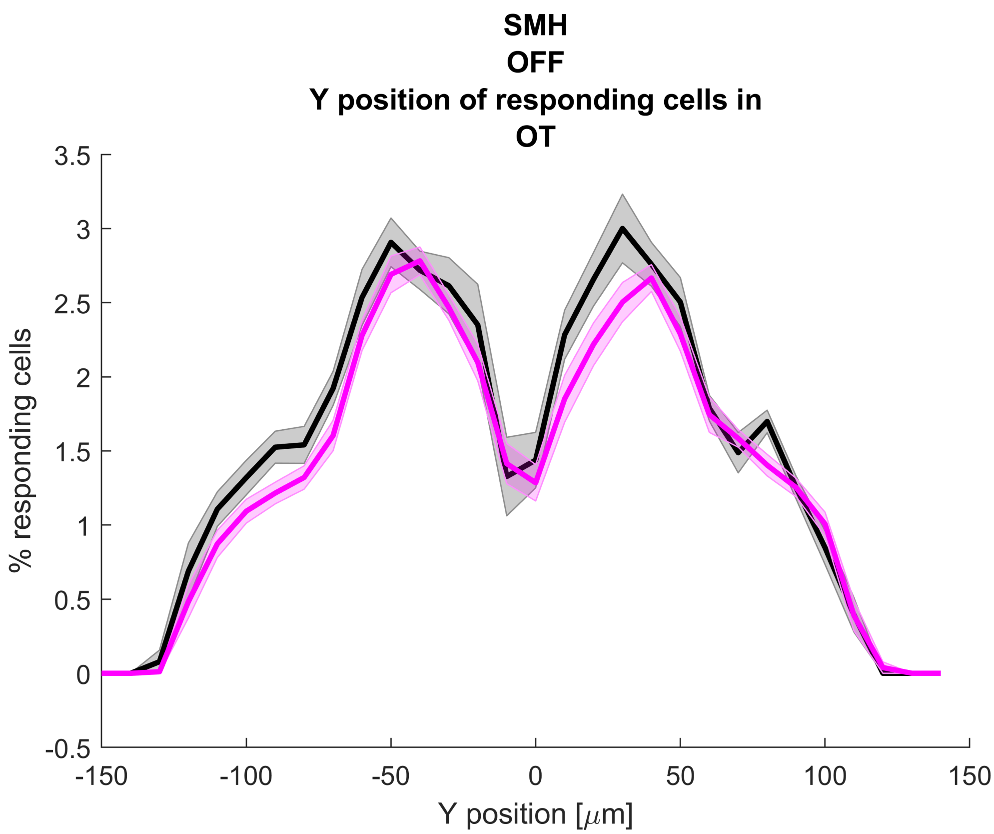



**SMH**
**OFF**
**Y position of responding cells in**
**tel**

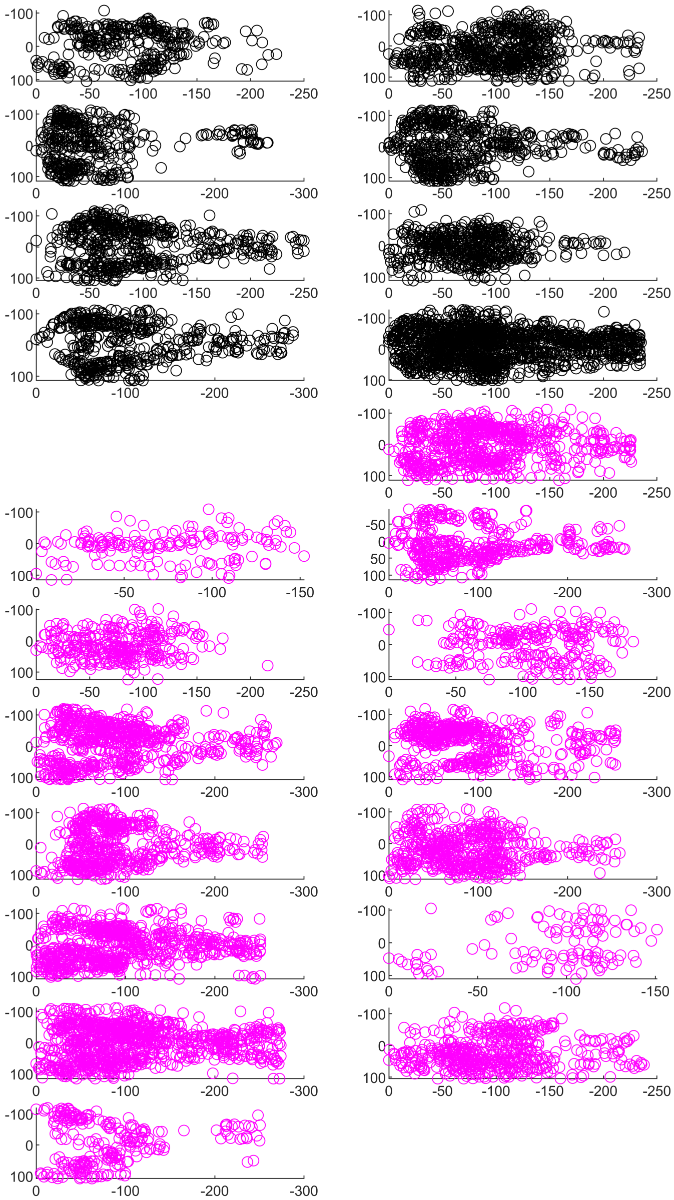


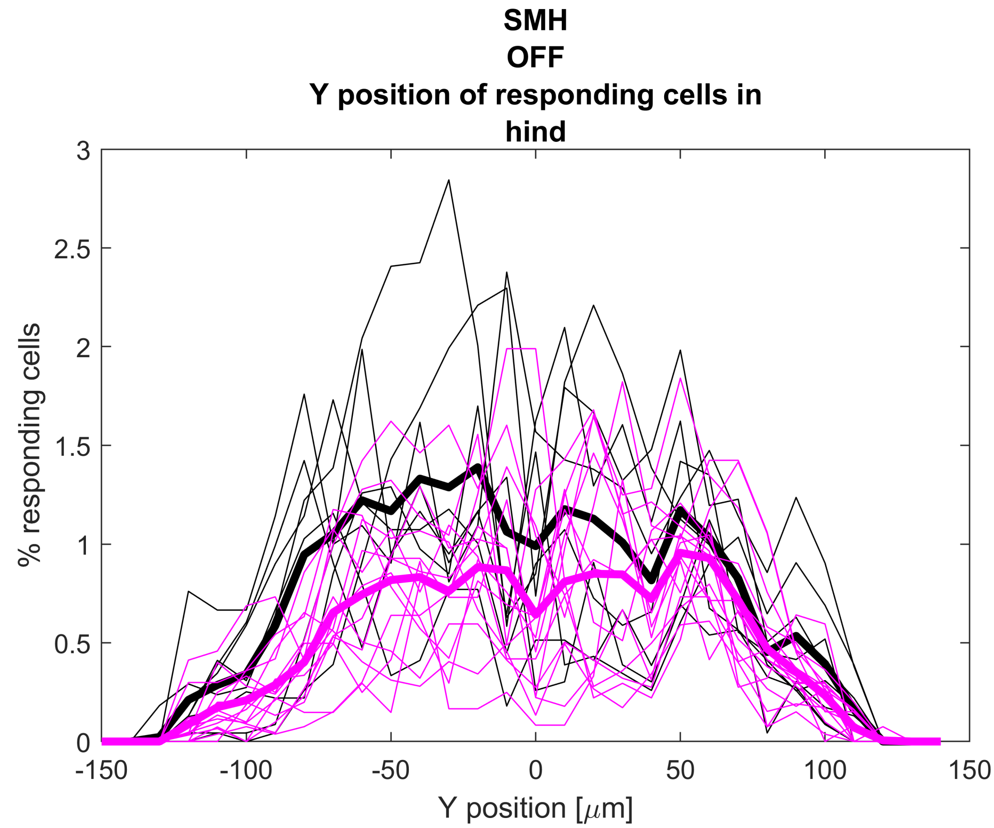

SMH
OFF
scatter of responding cells in
OT

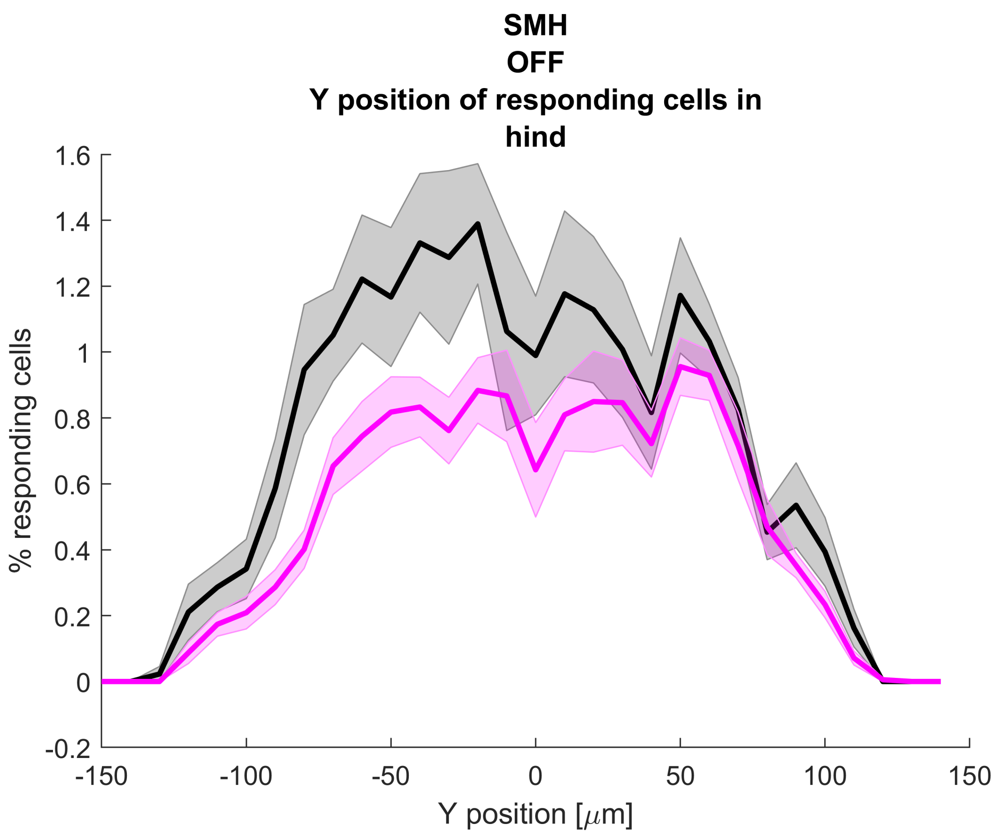

SMH
OFF
Y position of responding cells in
OT
% responding cells
Y position [$\mu$m]


SMH
OFF
Y position of responding cells in
OT
% responding cells
Y position [$\mu$m]

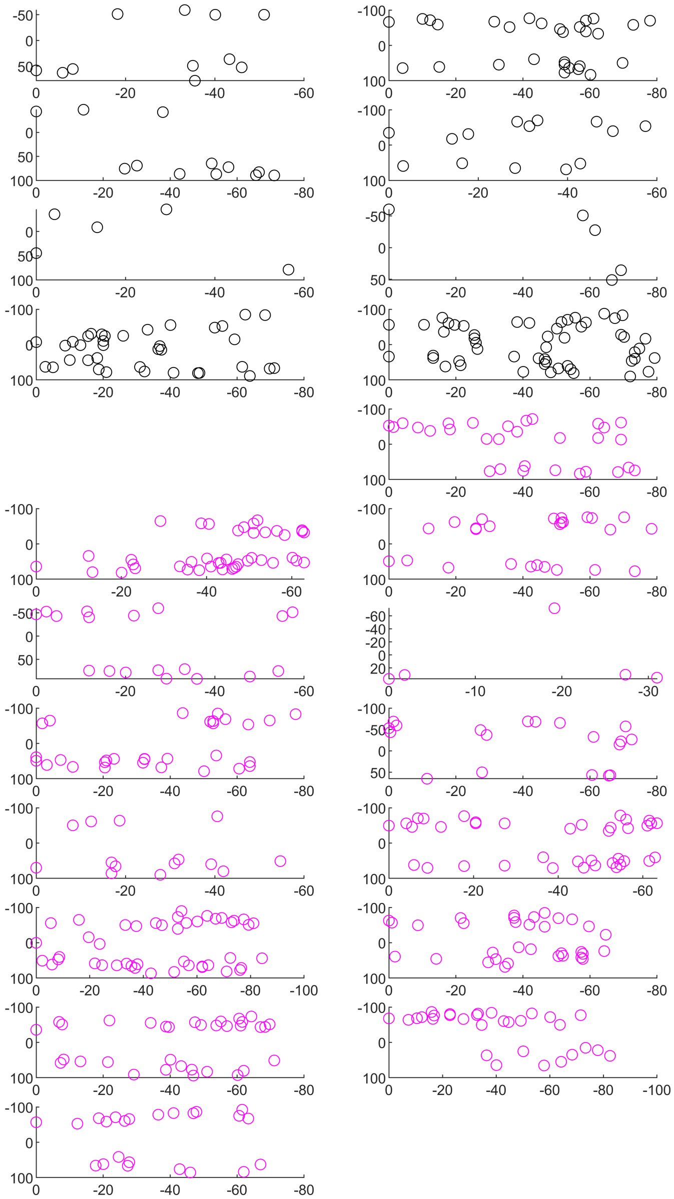
**SMH**
**OFF**
**scatter of responding cells in hind**

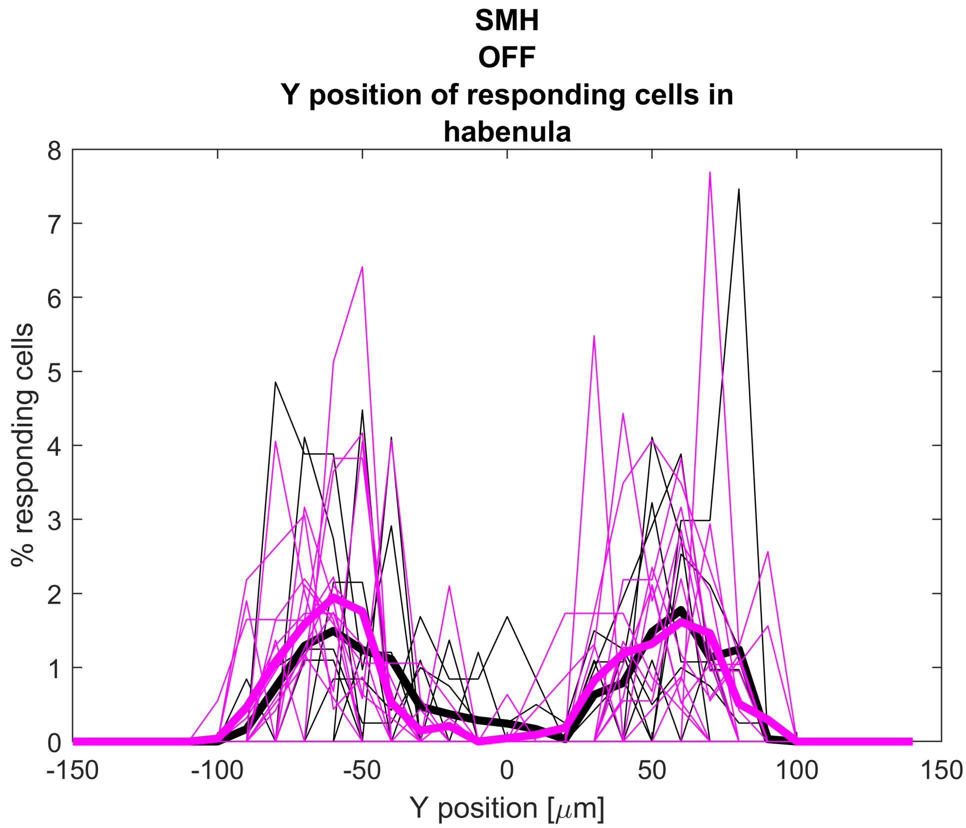


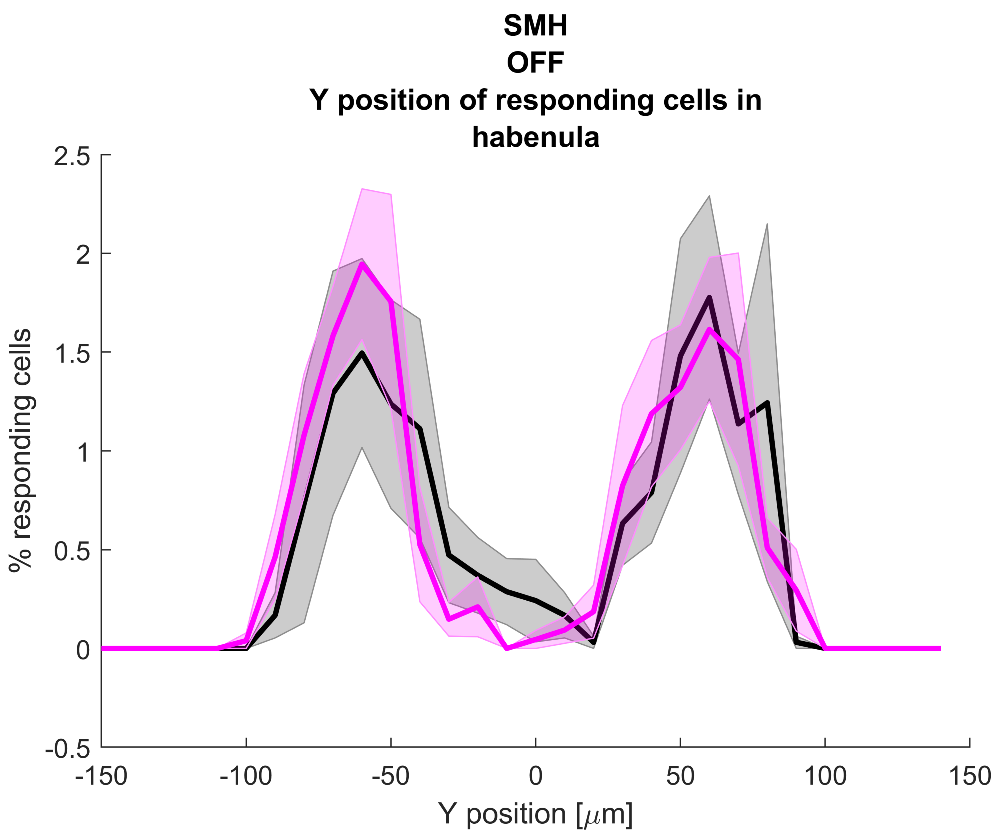

SMH
OFF
Y position of responding cells in
hind
% responding cells
Y position [$\mu$m]
3
2.5
2
1.5
1
0.5
0
-150
-100
-50
0
50
100
150


SMH
OFF
Y position of responding cells in
hind
% responding cells
Y position [$\mu$m]
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-150
-100
-50
0
50
100
150

**SMH**
**OFF**
**scatter of responding cells in habenula**






## PLOT ASSYMETRIC RESPONSE OF LIGHT IN HABENULA IN SMH

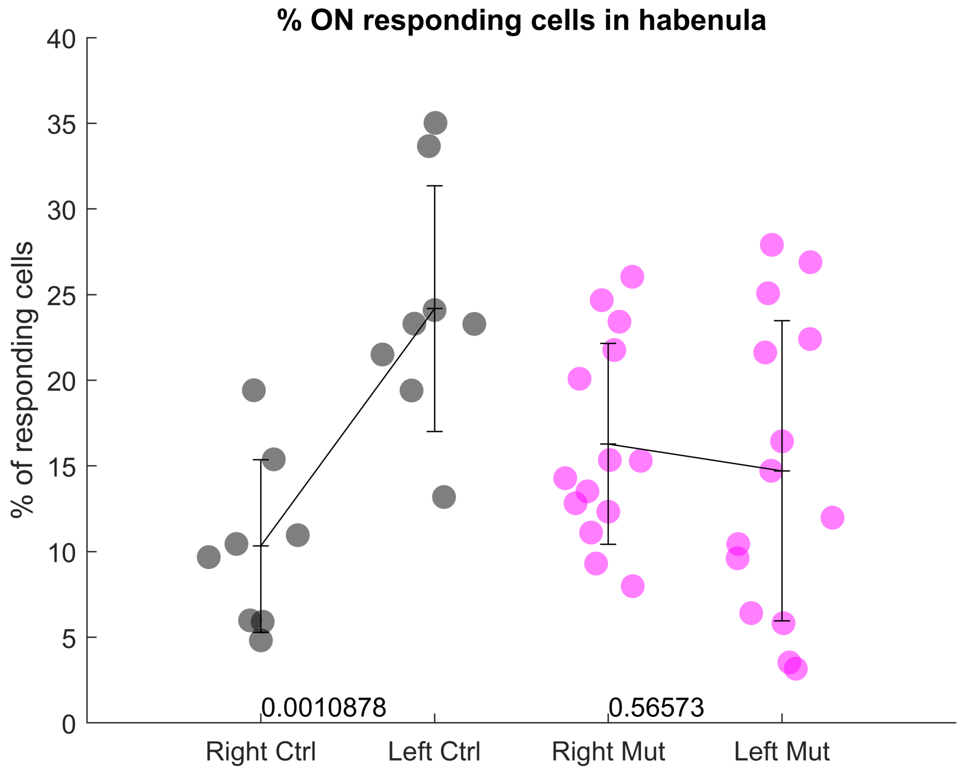
This is for the SUPPLEMENTARY INFORMATION=====================

p-value is ranksum

```
% plot ON response,
mut=mutON;
ctrl=ctrlON;
TEXT='ON';

j=4; %this decide on brain region, HABENULA

% calculate the position in the left and right
edges=[-150,0,150]; %positive values are LEFT
for k=1:cfg.numMUT
ID=find(mut{k+1, 4}(:,4)==j);
[N_mut(k,:),edges_mut(k,:)] = histcounts(mut{k+1, 4}(ID,2)-cfg.midline_mut(k,2),edges);%,'Norma
N_mutN(k,:)=100*N_mut(k,:)/sum(mut{k+1, 7}(:,4)==j); % normalize to number of cell per brain re
clear ID
end
for k=1:cfg.numCTRL
ID=find(ctrl{k+1, 4}(:,4)==j);
[N_ctrl(k,:),edges_ctrl(k,:)] = histcounts(ctrl{k+1, 4}(ID,2)-cfg.midline_ctrl(k,2),edges);%,'N
N_ctrlN(k,:)=100*N_ctrl(k,:)/sum(ctrl{k+1, 7}(:,4)==j);% normalize to number of cell per brain
clear ID
end
% Plot percentage response in left and right
figure,
swarmchart(repmat([1 1.5],cfg.numCTRL,1), N_ctrlN,80,'k','filled','MarkerFaceAlpha',0.5,'Marker
errorbar([1 1.5],mean(N_ctrlN,1), std(N_ctrlN,[],1),'Color','k', 'Marker','_')
swarmchart(repmat([2 2.5],cfg.numMUT,1), N_mutN,80,cfg.Mutcolor,'filled','MarkerFaceAlpha',0.5,
errorbar([2 2.5],mean(N_mutN,1), std(N_mutN,[],1),'Color','k', 'Marker','_')
xlim([0.5 3]), %ylim([-2 25])
ylabel('% of responding cells')
text(1,1,num2str(ranksum(N_ctrlN(:,1),N_ctrlN(:,2))))
text(2,1,num2str(ranksum(N_mutN(:,1),N_mutN(:,2))))
xticks([1 1.5 2 2.5])
xticklabels({'Right Ctrl', 'Left Ctrl','Right Mut', 'Left Mut'})
title('% ON responding cells in habenula')
hold off
```




## NESTED FUNCTIONS

FUNCTION 0

```
function plot_heatmap(data)
% HEATMAP--------------------------------------------------------------
% plot the clustering with a bar for 5 min --------------------------------
% re-orderd the cluster so that cluster 1-2 is ON, cluster 3 is OFF, cluster
% 4 and 5 is not showing large activity
idx2=data.idx;
idx2(data.idx==4)=12;
idx2(data.idx==2)=13;
idx2(data.idx==5)=10;
idx2(data.idx==1)=11;
idx2(data.idx==3)=14;
% x scalebar is 5 min, y scalebar is 1000 neurons
cmap = seismic();
figure('Position', [100 100 600 300])
imagesc(sortrows(cat(2, idx2, data.DV_DFFaveragetime ))), hold on
colormap(cmap)
caxis([-100 100])
%colorbar
xline(data.cfg.TimeVector.ON,'k--','LineWidth',0.1)
xline(data.cfg.TimeVector.OFF,'k--','LineWidth',0.1)
yline(1+find(diff(sort(idx2))==1),'k--', 'LineWidth',2)
xlim([500 size(data.DV_DFFaveragetime,2)+200])
ylim([0 size(data.DV_DFFaveragetime,1)+200])
```

```
line([500 round(500+data.cfg.fps*60*5)], size(data.DV_DFFaveragetime,1)+[200 200],'Color','k',
line(size(data.DV_DFFaveragetime,2)+[200 200], [0 1000],'Color','k','LineWidth',3)
box off
axis off
ax=gca;
set(ax,'XTick',[])
set(ax,'YTick',[])
%set(ax,'TickDir','out')
title(data.metadata.name)
colorbar
end
```

FUNCTION 1 TO PLOT THE AVERAGE DATA FOR ALL CELLS PER ANIMAL FOR ALL ANIMALS ON TOP OF EACH OTHER==========

```
function [averageON_ALL,averageOFF_ALL]=plot_responseALL_perfish(ctrlON,ctrlOFF,mutON,mutOFF,ti

figure (30)
clf
set(gcf,  'Position',[100 100 400 700])
str= {[cfg.MutID ' Amplitude of ALL responses black=ctrl']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',14, 'FontWeight', 'bold', 'L

% Average response ON
subplot(2,1,1)
for i=2:cfg.numCTRL+1
shadedErrorBar(time,mean(ctrlON{i,6},1),std(ctrlON{i,6},[],1)/sqrt(size(ctrlON{i,6},1)),'linepr
xlabel('time in sec')
ylabel('DFF')
averageON_ALL.ctrl(i-1,:)=mean(ctrlON{i,6},1);
end
for i=2:cfg.numMUT+1
shadedErrorBar(time,mean(mutON{i,6},1),std(mutON{i,6},[],1)/sqrt(size(mutON{i,6},1)),'lineprops
xlabel('time in sec')
ylabel('DFF')
averageON_ALL.mut(i-1,:)=mean(mutON{i,6},1);
end
title(['Average ON response ' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mut fish


% Average response OFF
subplot(2,1,2)
for i=2:cfg.numCTRL+1
shadedErrorBar(time,mean(ctrlOFF{i,6},1),std(ctrlOFF{i,6},[],1)/sqrt(size(ctrlOFF{i,6},1)),'lin
xlabel('time in sec')
ylabel('DFF')
averageOFF_ALL.ctrl(i-1,:)=mean(ctrlOFF{i,6},1);
end
for i=2:cfg.numMUT+1
shadedErrorBar(time,mean(mutOFF{i,6},1),std(mutOFF{i,6},[],1)/sqrt(size(mutOFF{i,6},1)),'linepr
```

```
xlabel('time in sec')
ylabel('DFF')
averageOFF_ALL.mut(i-1,:)=mean(mutOFF{i,6},1);
end
title(['Average OFF response ' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mut fish


end
```

% FUNCTION2 PLOT THE AVERAGE OF THE AVERAGE OF ALL CELLS FOR ALL ANIMALS ON TOP OF EACH OTHER==================

```
function plot_responseALL_average(averageON_ALL,averageOFF_ALL,time,cfg)

figure (40)
clf
set(gcf,  'Position',[100 100 400 600])
str= {[cfg.MutID ' Amplitude of ON and OFF responses, SEM']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',14, 'FontWeight', 'bold', 'L

subplot(2,1,1)
rectangle('Position',[0 -8 60 70],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]),
shadedErrorBar(time,mean(averageON_ALL.ctrl,1),std(averageON_ALL.ctrl,[],1)/sqrt(size(averageON
shadedErrorBar(time,mean(averageON_ALL.mut,1),std(averageON_ALL.mut,[],1)/sqrt(size(averageON_A
title(['Average ON for all cells in' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mu
xlim([-120 120])
ylim([-5 30])
xlabel('seconds')
ylabel('dF/F')

subplot(2,1,2)
rectangle('Position',[0 -40 60 80],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]),
shadedErrorBar(time,mean(averageOFF_ALL.ctrl,1),std(averageOFF_ALL.ctrl,[],1)/sqrt(size(average
shadedErrorBar(time,mean(averageOFF_ALL.mut,1),std(averageOFF_ALL.mut,[],1)/sqrt(size(averageOF
title(['Average OFF for all cells in' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' m
xlim([-120 120])
ylim([-5 30])
xlabel('seconds')
ylabel('dF/F')

figure (41)
clf
set(gcf,  'Position',[100 100 400 600])
str= {[cfg.MutID ' Amplitude of ON and OFF responses, SEM']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',14, 'FontWeight', 'bold', 'L

subplot(2,1,1)
rectangle('Position',[0 -2 60 1],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]), h
shadedErrorBar(time,mean(averageON_ALL.ctrl,1),std(averageON_ALL.ctrl,[],1)/sqrt(size(averageON
shadedErrorBar(time,mean(averageON_ALL.mut,1),std(averageON_ALL.mut,[],1)/sqrt(size(averageON_A
title(['Average ON for all cells in' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' mu
xlim([-10 60])
```

```
ylim([-5 25])
xlabel('seconds')
ylabel('dF/F')

subplot(2,1,2)
rectangle('Position',[-10 -2 10 1],'FaceColor',[0.99 0.99 0.75], 'EdgeColor',[0.99 0.99 0.75]),
shadedErrorBar(time,mean(averageOFF_ALL.ctrl,1),std(averageOFF_ALL.ctrl,[],1)/sqrt(size(average
shadedErrorBar(time,mean(averageOFF_ALL.mut,1),std(averageOFF_ALL.mut,[],1)/sqrt(size(averageOF
title(['Average OFF for all cells in' num2str(cfg.numCTRL) ' ctrl and ' num2str(cfg.numMUT) ' r
xlim([-10 60])
ylim([-5 25])
xlabel('seconds')
ylabel('dF/F')

end
```

FUNCTION3 PLOT AVERAGE AMPLITUDE FOR 10SEC AFTER FOR RESPONDING CELLS

```
function plot_scatterAll(averageON_ALL,averageOFF_ALL,ctrlON,ctrlOFF, mutON, mutOFF, time,cfg)

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

figure (51)
clf
set(gcf,  'Position',[100 100 300 600])
str= {[cfg.MutID ' Amplitude ALL cells, SEM,from ' num2str(time(onset)) 's to ' num2str(time(of
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',10, 'FontWeight', 'bold', 'L

subplot(2,1,1)
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)),'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)),'Color',cfg.Mutcolor)
xlim([0 3]), ylim([0 Inf])
ylabel('amplitude')
text(0.5,1,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1 2])
xticklabels({'ctrl','mut'})
title('ON response')

num2str(ranksum(mean(toplotC,2),mean(toplotM,2)))
clear toplotC toplotM


subplot(2,1,2)
toplotC=averageOFF_ALL.ctrl(:,onset:offset);
toplotM=averageOFF_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)),'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)),'Color',cfg.Mutcolor)
xlim([0 3]), ylim([0 Inf])
ylabel('amplitude')
text(0.5,1,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1 2])
xticklabels({'ctrl','mut'})
title('OFF response')

num2str(ranksum(mean(toplotC,2),mean(toplotM,2)))
clear toplotC toplotM
end
```

FUNCTION 4 plots % responding cells per brain region

```
function plot_response_perregion(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)

close all
%% calculate number of responding cells per region
NumCtrl=[];
NumMut=[];
jj=[1,4,2,3]; %reorder so that Hb is between Tel and OT, added 14.03.2023

% calculate responding cells per brain region
for k=1:cfg.numCTRL
for ik=1:4; %there are four brain region
    j=jj(ik);
NumCtrl.ON.activated(k,ik)=length(find(ctrlON{k+1, 4}(:,4)==j))/sum(ctrlON{k+1, 7}(:,4)==j)*100
NumCtrl.OFF.activated(k,ik)=length(find(ctrlOFF{k+1, 4}(:,4)==j))/sum(ctrlOFF{k+1, 7}(:,4)==j)*
NumCtrl.ON.inhibited(k,ik)=length(find(ctrlON{k+1, 5}(:,4)==j))/sum(ctrlON{k+1, 7}(:,4)==j)*100
NumCtrl.OFF.inhibited(k,ik)=length(find(ctrlOFF{k+1, 5}(:,4)==j))/sum(ctrlOFF{k+1, 7}(:,4)==j)*

end
end

for k=1:cfg.numMUT
for ik=1:4; %there are four brain region
    j=jj(ik);
NumMut.ON.activated(k,ik)=length(find(mutON{k+1, 4}(:,4)==j))/sum(mutON{k+1, 7}(:,4)==j)*100;
NumMut.OFF.activated(k,ik)=length(find(mutOFF{k+1, 4}(:,4)==j))/sum(mutOFF{k+1, 7}(:,4)==j)*100
NumMut.ON.inhibited(k,ik)=length(find(mutON{k+1, 5}(:,4)==j))/sum(mutON{k+1, 7}(:,4)==j)*100;
NumMut.OFF.inhibited(k,ik)=length(find(mutOFF{k+1, 5}(:,4)==j))/sum(mutOFF{k+1, 7}(:,4)==j)*100
end
end

figure (70),
clf
set(gcf,  'Position',[100 100 800 600])
```

```
str= {[cfg.MutID ' % responding cells per brain area black=ctrl']};
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',14, 'FontWeight', 'bold', 'L

subplot(2,2,1)
toplotC=NumCtrl.ON.activated;
toplotM=NumMut.ON.activated;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'Marker
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAl
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color',cfg.Mutcolor, 'Marker','_')
end
xlim([0 5]), ylim([0 80])
ylabel('% of responding cells')
for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('ON activated')
clear toplotC toplotM p
hold off

subplot(2,2,2)
toplotC=NumCtrl.ON.inhibited;
toplotM=NumMut.ON.inhibited;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'Marker
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAl
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color',cfg.Mutcolor, 'Marker','_')
end
xlim([0 5]), ylim([-1 10])
ylabel('% of responding cells')

for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('ON inhibited')
clear toplotC toplotM p
hold off
```

```
subplot(2,2,3)
toplotC=NumCtrl.OFF.activated;
toplotM=NumMut.OFF.activated;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'Marker
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAl
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color',cfg.Mutcolor, 'Marker','_')
end
xlim([0 5]), ylim([0 80])
ylabel('% of responding cells')
for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('OFF activated')
clear toplotC toplotM p
hold off


subplot(2,2,4)
toplotC=NumCtrl.OFF.inhibited;
toplotM=NumMut.OFF.inhibited;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,'k','filled','MarkerFaceAlpha',0.5,'Marker
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,cfg.Mutcolor,'filled','MarkerFaceAl
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color',cfg.Mutcolor, 'Marker','_')
end
xlim([0 5]), ylim([-1 25])
ylabel('% of responding cells')
for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('OFF inhibited')
clear toplotC toplotM p
hold off
end
```

FUNCTION 5 plots % active cells per region

```
function plot_response_perregion_v2(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)
%% calculate number of responding cells per region
%close all
NumCtrl=[];
NumMut=[];
jj=[1,4,2,3]; %reorder so that Hb is between Tel and OT, added 14.03.2023

% calculate responding cells per brain region
for k=1:cfg.numCTRL
for ik=1:4; %there are four brain region
    j=jj(ik);
NumCtrl.ON.activated(k,ik)=length(find(ctrlON{k+1, 4}(:,4)==j))/sum(ctrlON{k+1, 7}(:,4)==j)*100
NumCtrl.OFF.activated(k,ik)=length(find(ctrlOFF{k+1, 4}(:,4)==j))/sum(ctrlOFF{k+1, 7}(:,4)==j)*
NumCtrl.ON.inhibited(k,ik)=length(find(ctrlON{k+1, 5}(:,4)==j))/sum(ctrlON{k+1, 7}(:,4)==j)*100
NumCtrl.OFF.inhibited(k,ik)=length(find(ctrlOFF{k+1, 5}(:,4)==j))/sum(ctrlOFF{k+1, 7}(:,4)==j)*

end
end

for k=1:cfg.numMUT
for ik=1:4; %there are four brain region
    j=jj(ik);
NumMut.ON.activated(k,ik)=length(find(mutON{k+1, 4}(:,4)==j))/sum(mutON{k+1, 7}(:,4)==j)*100;
NumMut.OFF.activated(k,ik)=length(find(mutOFF{k+1, 4}(:,4)==j))/sum(mutOFF{k+1, 7}(:,4)==j)*100
NumMut.ON.inhibited(k,ik)=length(find(mutON{k+1, 5}(:,4)==j))/sum(mutON{k+1, 7}(:,4)==j)*100;
NumMut.OFF.inhibited(k,ik)=length(find(mutOFF{k+1, 5}(:,4)==j))/sum(mutOFF{k+1, 7}(:,4)==j)*100
end
end

figure (57),
clf
set(gcf,  'Position',[100 100 300 400])
toplotC=NumCtrl.ON.activated;
toplotM=NumMut.ON.activated;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,80,'k','filled','MarkerFaceAlpha',0.5,'Mar
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,80,cfg.Mutcolor,'filled','MarkerFac
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color','k', 'Marker','_')
end
xlim([0 5]), ylim([-10 70])
ylabel('% of responding cells')
for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
```

```
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('ON activated')
clear toplotC toplotM p
hold off

figure (58)
clf
set(gcf,  'Position',[100 100 300 400])
str= {[cfg.MutID ' % ON inhibited']};
toplotC=NumCtrl.ON.inhibited;
toplotM=NumMut.ON.inhibited;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,80,'k','filled','MarkerFaceAlpha',0.5,'Mar
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,80,cfg.Mutcolor,'filled','MarkerFac
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color','k', 'Marker','_')
end
xlim([0 5]), ylim([-2 10])
ylabel('% of responding cells')

for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-1,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('ON inhibited')
clear toplotC toplotM p
hold off


figure (59)
clf
set(gcf,  'Position',[100 100 300 400])
str= {[cfg.MutID ' % OFF activated']};
toplotC=NumCtrl.OFF.activated;
toplotM=NumMut.OFF.activated;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,80,'k','filled','MarkerFaceAlpha',0.5,'Mar
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,80,cfg.Mutcolor,'filled','MarkerFac
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color','k', 'Marker','_')
end
xlim([0 5]), ylim([-10 70])
ylabel('% of responding cells')
for i=1:4
```

```
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-5,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('OFF activated')
clear toplotC toplotM p
hold off


figure (60)
clf
set(gcf,  'Position',[100 100 300 400])
str= {[cfg.MutID ' % OFF inhibited']};
toplotC=NumCtrl.OFF.inhibited;
toplotM=NumMut.OFF.inhibited;
swarmchart(repmat([1 2 3 4], cfg.numCTRL,1), toplotC,80,'k','filled','MarkerFaceAlpha',0.5,'Mar
for ii=1:4
errorbar(ii,mean(toplotC(:,ii)), std(toplotC(:,ii)),'Color','k', 'Marker','_')
end
swarmchart(repmat([1.5 2.5 3.5 4.5], cfg.numMUT,1), toplotM,80,cfg.Mutcolor,'filled','MarkerFac
for ii=1:4
errorbar(ii+0.5,mean(toplotM(:,ii)), std(toplotM(:,ii)),'Color','k', 'Marker','_')
end
xlim([-0 5]), ylim([-2 25])
ylabel('% of responding cells')
for i=1:4
p(i)=ranksum(toplotC(:,i),toplotM(:,i));
end
text(0,-1,num2str(['Tel:' sprintf('%.3f',p(1))...
    ' Hb:' sprintf('%.3f',p(2)) ' OT:' sprintf('%.3f',p(3))...
    ' Hind:' sprintf('%.3f',p(4))]))
xticks([1.25 2.25 3.25 4.25])
xticklabels({'Tel', 'Hab','OT', 'Hind'})
title('OFF inhibited')
clear toplotC toplotM p
hold off
end
```

FUNCTION calculate amplitude per brain region

```
function plot_amplitude_perregion(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)

onset=465;
offset=503;

jj=[1,4,2,3]; %reorder so that Hb is between Tel and OT, added 14.03.2023


figure (61)
clf
for k=1:4
```

```
    j=jj(k);
set(gcf,  'Position',[100 100 300 400])
str= {[cfg.MutID ' Amplitude ALL cells ON, from ' num2str(time(onset)) 's to ' num2str(time(of
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',10, 'FontWeight', 'bold', 'L

for i=1:cfg.numCTRL
matrixC(i,:)=mean(ctrlON{i+1,6}(ctrlON{i+1,7}(:,4)==j,:),1);
end
for i=1:cfg.numMUT
matrixM(i,:)=mean(mutON{i+1,6}(mutON{i+1,7}(:,4)==j,:),1);
end
toplotC=matrixC(:,onset:offset);
toplotM=matrixM(:,onset:offset);
swarmchart((2*k-2)+ones(cfg.numCTRL,1), mean(toplotC,2),80,'k','filled','MarkerFaceAlpha',0.5,
line((2*k-2)+[0.6 1.4], repmat(mean(mean(toplotC,2)),1,2),'Color','k')
errorbar((2*k-2)+1,mean(mean(toplotC,2)), std(mean(toplotC,2)),'Color','k')
swarmchart((2*k-2)+2*ones(cfg.numMUT,1), mean(toplotM,2),80,cfg.Mutcolor,'filled','MarkerFaceA
line((2*k-2)+[1.6 2.4], repmat(mean(mean(toplotM,2)),1,2),'Color','k')
errorbar((2*k-2)+2,mean(mean(toplotM,2)), std(mean(toplotM,2)),'Color','k')
%xlim([0 3]),
ylim([-10 40])
ylabel('amplitude')
text((2*k-2)+0.25,-5,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1.5 3.5 5.5 7.5])
xticklabels({'Tel', 'Hab','OT/Thal', 'Hind'})
%title([cfg.brainRegion(k,:) 'ON response'])
clear toplotC toplotM matrixC matrixM
end

figure (62)
clf
for k=1:4
    j=jj(k);
set(gcf,  'Position',[100 100 300 400])
str= {[cfg.MutID ' Amplitude ALL cells OFF, from ' num2str(time(onset)) 's to ' num2str(time(o
annotation('textbox',[0.05 0.95 0.9 0.05],'String',str, 'FontSize',10, 'FontWeight', 'bold', 'L

for i=1:cfg.numCTRL
matrixC(i,:)=mean(ctrlOFF{i+1,6}(ctrlOFF{i+1,7}(:,4)==j,:),1);
end
for i=1:cfg.numMUT
matrixM(i,:)=mean(mutOFF{i+1,6}(mutOFF{i+1,7}(:,4)==j,:),1);
end
toplotC=matrixC(:,onset:offset);
toplotM=matrixM(:,onset:offset);
swarmchart((2*k-2)+ones(cfg.numCTRL,1), mean(toplotC,2),80,'k','filled','MarkerFaceAlpha',0.5,
line((2*k-2)+[0.6 1.4], repmat(mean(mean(toplotC,2)),1,2),'Color','k')
errorbar((2*k-2)+1,mean(mean(toplotC,2)), std(mean(toplotC,2)),'Color','k')
swarmchart((2*k-2)+2*ones(cfg.numMUT,1), mean(toplotM,2),80,cfg.Mutcolor,'filled','MarkerFaceA
line((2*k-2)+[1.6 2.4], repmat(mean(mean(toplotM,2)),1,2),'Color','k')
errorbar((2*k-2)+2,mean(mean(toplotM,2)), std(mean(toplotM,2)),'Color','k')
%xlim([0 3]),
ylim([-10 40])
```

```
ylabel('amplitude')
text((2*k-2)+0.25,-5,['p= '...
    num2str(ranksum(mean(toplotC,2),mean(toplotM,2)),'%.4f')]);
xticks([1.5 3.5 5.5 7.5])
xticklabels({'Tel', 'Hab','OT/Thal', 'Hind'})
%title([cfg.brainRegion(k,:) 'ON response'])
clear toplotC toplotM matrixC matrixM
end
%close all
end
```

FUNCTION IDENTIFY MIDLINE USING GINPUT

```
function identify_midline(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)

%% plot the scatter of the location of all responding cells
j=2; %this is OT only
% figure,
% suptitle('ctrl ON activated')
% for k=1:cfg.numCTRL
% ID=find(ctrlON{k+1, 4}(:,4)==j);
% subplot(ceil(size(ctrlON,1)/2),2,k)
% scatter(ctrlON{k+1, 4}(ID,1),ctrlON{k+1, 4}(ID,2))
% end


figure,
suptitle('mut ON activated')
for k=1:cfg.numMUT
ID=find(mutON{k+1, 4}(:,4)==j);
subplot(ceil(size(mutON,1)/2),2,k)
scatter(mutON{k+1, 4}(ID,1),mutON{k+1, 4}(ID,2))
end
end
```

FUNCTION PLOT % ACTIVE CELLS ACCORDING TO X,Y,Z

```
function plot_respondingXYZ(ctrlON,ctrlOFF,mutON,mutOFF,time,cfg)

% if you want to plot ON response,
if cfg.data==1
mut=mutON;
ctrl=ctrlON;
TEXT='ON';
elseif  cfg.data==2
mut=mutOFF;
ctrl=ctrlOFF;
TEXT='OFF';
end

for j=1:4; %this decide on brain region
```

```
clear edges N_ctrlN N_mutN N_ctrl N_mut edges_mut edges_ctrl
% plot the scatter of the location of all responding cells
figure ,
clf
set(gcf,  'Position',[100 100 600 1200])
for k=1:cfg.numCTRL
ID=find(ctrl{k+1, 4}(:,4)==j);
subplot(ceil(size(ctrl,1)/2)+ceil(size(mut,1)/2),2,k)
scatter(ctrl{k+1, 4}(ID,1)-max(ctrl{k+1, 4}(ID,1)),ctrl{k+1, 4}(ID,2)-cfg.midline_ctrl(k,2),'k
end
for k=1:cfg.numMUT
ID=find(mut{k+1, 4}(:,4)==j);
subplot(ceil(size(ctrl,1)/2)+ceil(size(mut,1)/2),2,size(ctrl,1)+k)
scatter(mut{k+1, 4}(ID,1)-max(mut{k+1, 4}(ID,1)),mut{k+1, 4}(ID,2)-cfg.midline_mut(k,2),cfg.Mut
end
suptitle([cfg.MutID, TEXT ' scatter of responding cells in ' ,cfg.label(j)])

% calculate the position in the width
edges=[-150:10:150];
for k=1:cfg.numMUT
ID=find(mut{k+1, 4}(:,4)==j);
[N_mut(k,:),edges_mut(k,:)] = histcounts(mut{k+1, 4}(ID,2)-cfg.midline_mut(k,2),edges);%,'Norma
N_mutN(k,:)=100*N_mut(k,:)/sum(mut{k+1, 7}(:,4)==j); % normalize to number of cell per brain re
clear ID
end
for k=1:cfg.numCTRL
ID=find(ctrl{k+1, 4}(:,4)==j);
[N_ctrl(k,:),edges_ctrl(k,:)] = histcounts(ctrl{k+1, 4}(ID,2)-cfg.midline_ctrl(k,2),edges);%,'N
N_ctrlN(k,:)=100*N_ctrl(k,:)/sum(ctrl{k+1, 7}(:,4)==j);% normalize to number of cell per brain
clear ID
end
%
figure,
plot(edges(1:end-1),N_ctrlN.','k'), hold on, plot(edges(1:end-1),mean(N_ctrlN,1),'k','LineWidth
plot(edges(1:end-1),N_mutN.',cfg.Mutcolor),plot(edges(1:end-1),mean(N_mutN,1),cfg.Mutcolor,'Lin
xlabel('Y position [\mum]')
ylabel('% responding cells')
title([cfg.MutID, TEXT ' Y position of responding cells in ' ,cfg.label(j)])

figure
shadedErrorBar(edges(1:end-1),mean(N_ctrlN,1),std(N_ctrlN,[],1)/sqrt(size(N_ctrlN,1)),'lineprop
shadedErrorBar(edges(1:end-1),mean(N_mutN,1),std(N_mutN,[],1)/sqrt(size(N_mutN,1)),'lineprops',
xlabel('Y position [\mum]')
ylabel('% responding cells')
title([cfg.MutID, TEXT ' Y position of responding cells in ' cfg.label(j)])


end
end
```

```
function c=seismic()
c = [
    0.0, 0.0, 0.3;
    0.0, 0.0, 0.35;
    0.0, 0.0, 0.4;
    0.0, 0.0, 0.45;
    0.0, 0.0, 0.5;
    0.0, 0.0, 0.55;
    0.0, 0.0, 0.6;
    0.0, 0.0, 0.65;
    0.0, 0.0, 0.7;
    0.0, 0.0, 0.75;
    0.0, 0.0, 0.8;
    0.0, 0.0, 0.85;
    0.0, 0.0, 0.9;
    0.0, 0.0, 0.95;
    0.0, 0.0, 1.0;
    0.05, 0.05, 1.0;
    0.1, 0.1, 1.0;
    0.15, 0.15, 1.0;
    0.2, 0.2, 1.0;
    0.25, 0.25, 1.0;
    0.3, 0.3, 1.0;
    0.35, 0.35, 1.0;
    0.4, 0.4, 1.0;
    0.45, 0.45, 1.0;
    0.5, 0.5, 1.0;
    0.55, 0.55, 1.0;
    0.6, 0.6, 1.0;
    0.65, 0.65, 1.0;
    0.7, 0.7, 1.0;
    0.75, 0.75, 1.0;
    0.8, 0.8, 1.0;
    0.85, 0.85, 1.0;
    0.9, 0.9, 1.0;
    0.95, 0.95, 1.0;
    1.0, 1.0, 1.0;
    1.0, 0.95, 0.95;
    1.0, 0.9, 0.9;
    1.0, 0.85, 0.85;
    1.0, 0.8, 0.8;
    1.0, 0.75, 0.75;
    1.0, 0.7, 0.7;
    1.0, 0.65, 0.65;
    1.0, 0.6, 0.6;
    1.0, 0.55, 0.55;
    1.0, 0.5, 0.5;
    1.0, 0.45, 0.45;
    1.0, 0.4, 0.4;
    1.0, 0.35, 0.35;
    1.0, 0.3, 0.3;
    1.0, 0.25, 0.25;
    1.0, 0.2, 0.2;
```

```
    1.0, 0.15, 0.15;
    1.0, 0.1, 0.1;
    1.0, 0.05, 0.05;
    1.0, 0.0, 0.0;
    0.95, 0.0, 0.0;
    0.9, 0.0, 0.0;
    0.85, 0.0, 0.0;
    0.8, 0.0, 0.0;
    0.75, 0.0, 0.0;
    0.7, 0.0, 0.0;
    0.65, 0.0, 0.0;
    0.6, 0.0, 0.0;
    0.55, 0.0, 0.0;
    0.5, 0.0, 0.0;
    0.45, 0.0, 0.0;
    0.4, 0.0, 0.0;
    0.35, 0.0, 0.0;
    0.3, 0.0, 0.0;
];
end
```