INTRODUCTION

In this report, we outline the results obtained from running a MatLab function (Appendix 1) analysing a series of electrophysiology experiments recording synaptic responses from hippocampal slices stimulated under different drug conditions: CADO, CADO + DPCPX and CADO+DPCPX+NBQX (and a control condition). Our analysis have focused particularly on the peak amplitude of the field excitatory post-synaptic potentials (fESPS), evaluating how the peak fESPS amplitude changes across the different drug conditions as well as assesing whether any differences were statistically significant.

RESULTS

Data analysis methods

The electrophysiology data we analysed shows synaptic recordings from 2 stimuli (the first at 10 ms and the second at 60 ms), of which we only analysed the response from the first. To do this we defined a time window (12-25 ms) where only the first fESPS amplitude would be analysed and where any stimulus artifacts would be ignored. Because of the nature of the recordings, the baseline before the stimuli are not always set to 0, and we have corrected this in our analysis by calculating the mean response from the first 80 samples across all recordings and subtracting the value from the raw data. We have also reduced the levels of noise of the recordings by applying a lowpass Butterworth filter.

To analyse how peak fESPS amplitude throughout the different drug conditions, we plotted a timecourse graph from 1 experiment showing how peak fESPS amplitude changes over the series of recordings (10 s between each) as well as plotted ‘bars’ showing the time each drug was added. To analyse whether these differences were significant, we used a one-way ANOVA test on the peak amplitude data across all 6 experiments, as well as a multiple comparisons test to further analyse which specific groups showed significant differences.

Peak amplitudes increased when DPXPX were added but decreased with CADO and NBQX

As seen in Fig. 1, baseline peak fESPS amplitude in the control was about -0.5 mV, which started to decrease at around 400 s following addition of CADO, reaching around -0.1 mV by 1000 s. Addition of DPCPX at around 1000 s increased the peak amplitude to a maximum of about -0.6 mV and addition of the final drug NBQX decreased it again until the peak amplitude was nearly 0. From the example traces plots below the time-course plot, we can

see that the peak amplitude was greatest in the presence of DPCPX and lowest in the presence of NBQX.

This is confirmed in Table 1 where the mean peak amplitudes in the DPCPX condition is about 0.1 mV higher than the control, while the mean peak amplitude in the NBQX condition is four times lower than control.

Differences in the CADO, DPCPX and NBQX conditions were statistically significant from each other but not from control

Statistical analysis using the one-way ANOVA resulted in a p-value of 0.0078076, warranting further multiple comparisons analysis. As seen in Fig. 2a, none of the drug conditions had statistically significant differences in peak amplitude from control, while both CADO and NBQX showed statistically significant differences from DPCPX (Fig. 2c). CADO and NBQX however, did not show any significant differences from each other (Fig 2b,d).

SUMMARY CONCLUSION

In conclusion, the drugs CADO and NBQX seem to decrease peak fESPS amplitude with NBQX being able to decrease it to a greater extent. DPCPX does the opposite and appear to increase peak fESPS above the control. However, since the peak amplitudes in the 3 drug conditions were only significantly different from each other and not from control, it is difficult to accurately determine whether each drug can considerably alter peak fESPS of normal un-drugged hippocampal slices. The result of this evaluation may be due to the fact that when each drug was added, it was present throughout the whole duration of the experiment, and may still be acting on the slices when subsequent drugs were added, thus introducing carry-over effects which may have affected the recorded peak amplitude values.

FIGURES & TABLES

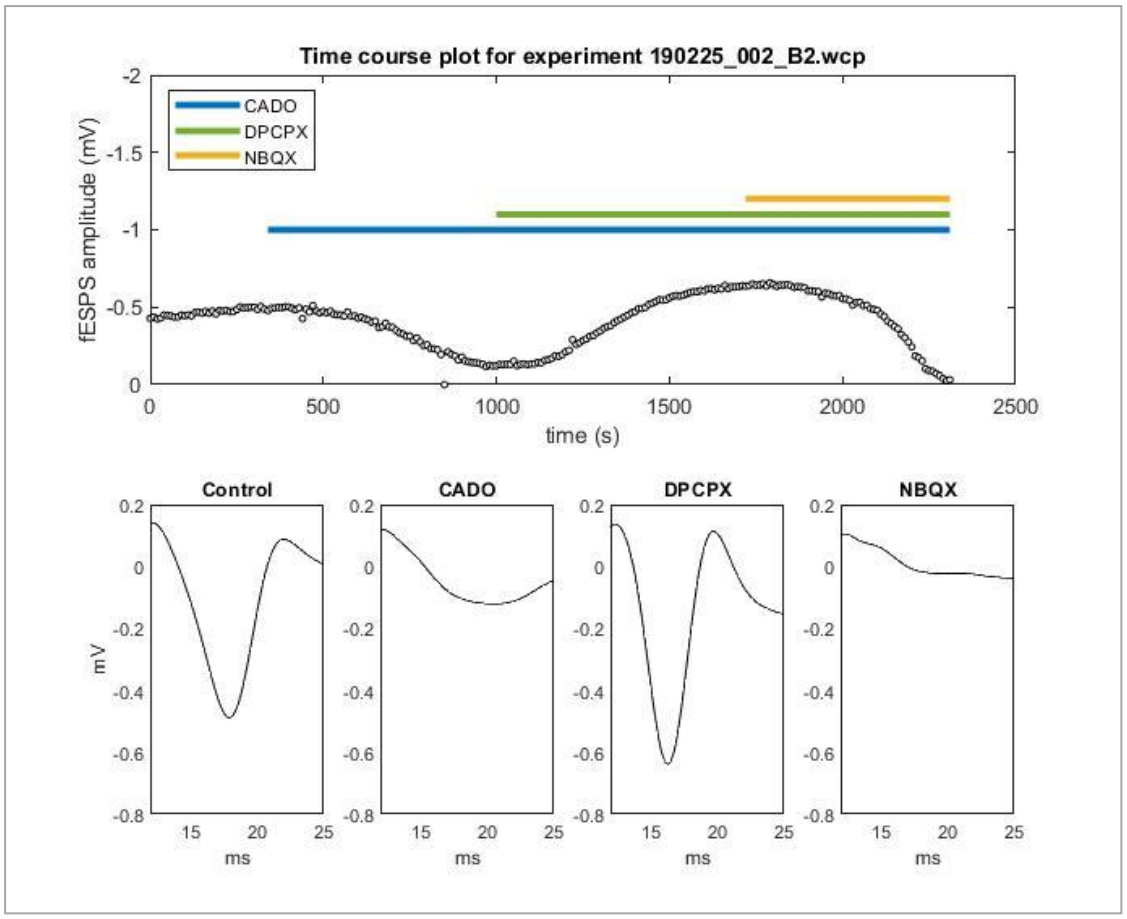

Figure 1 showing a timecourse plot and example traces of each experimental condition (control, CADO, DPCPX and NBQX) in experiment 1 (filename 190225_002_B2.wcp). The timecourse plot shows how peak fESPS amplitude in mV (y-axis) changes over time in s (x-axis). The plot also shows bars showing when each drug was added and how long they were present throughout the duration of the experiment. Each datapoint in the timecourse plot represents the peak fESPS amplitude of a single recording, with a total of 232 recordings in the experiment corresponding to 232 datapoints. The example traces plot shows how fESPS amplitude changes over time in ms, zoomed in to the specified 12-25 ms time window, and shows the peak amplitude for each condition.

	Control	CADO	DPCPX	NBQX
190225_002_B2.wcp	-0.488	-0.121	-0.639	-0.024
190225_002_X1.wcp	-0.072	-0.018	-0.107	-0.007
190225_003_A2.wcp	-0.123	-0.041	-0.163	-0.037
190228_002_A1.wcp	-0.218	-0.055	-0.338	-0.038
190228_002_X2.wcp	-0.397	-0.216	-0.505	-0.24
190228_006_X1.wcp	-0.192	-0.004	-0.264	-0.029
Mean	-0.248	-0.076	-0.336	-0.062

Table 1 showing the peak fESPS amplitudes (in mV) in each experimental condition across the 6 experiments (identified by their filenames shown on the left-hand side). The last row shows the mean peak amplitude of the 4 experimental conditions calculated from the 6 experiments above.

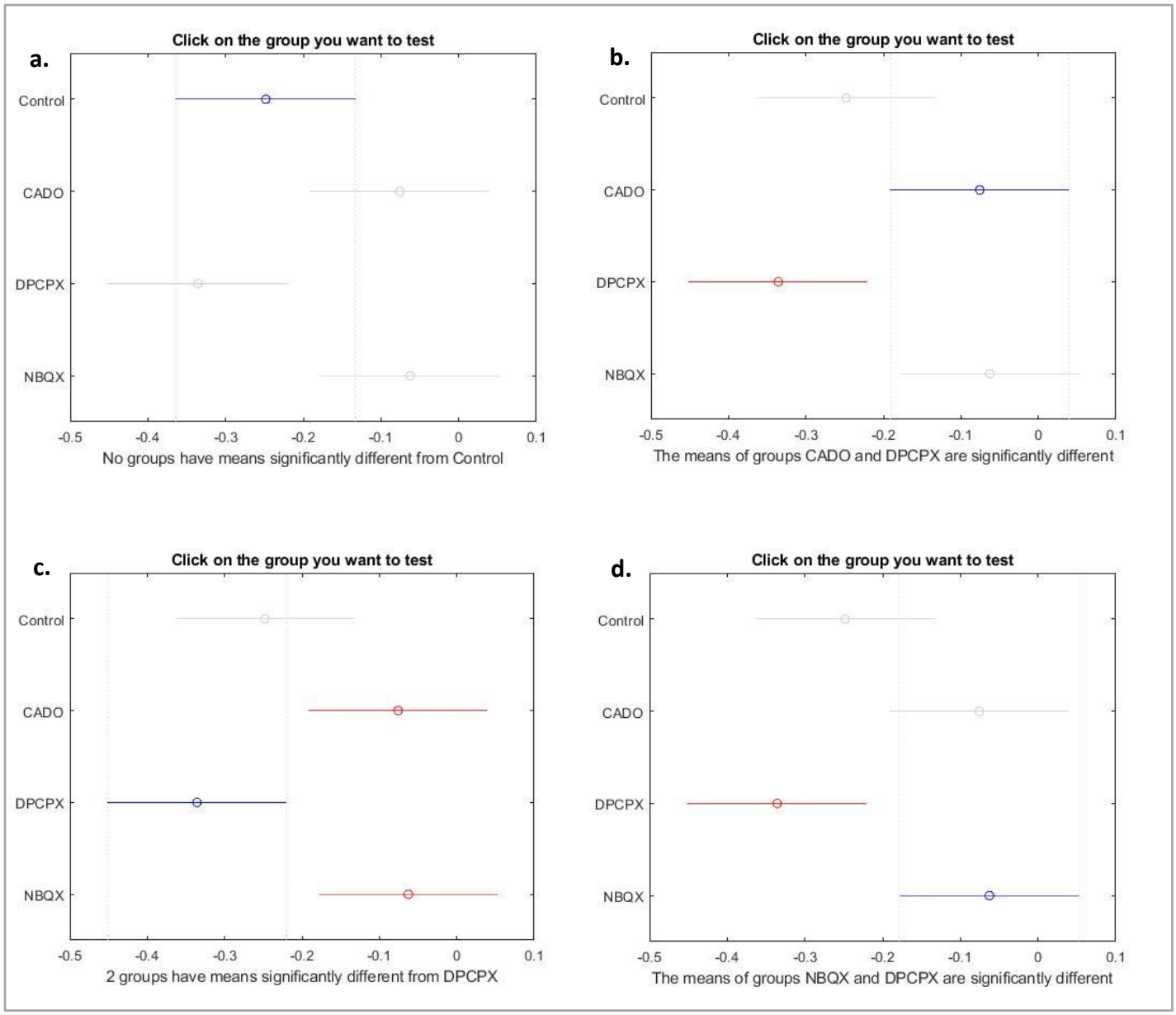

Figure 2 showing the output table from the multiple comparisons test showing which groups had statistically significant differences in fESPS amplitude, with the the y-axis showing the different experimental conditions and the x-axis showing the range of peak fESPS amplitudes in each condition. (a) shows which groups were significantly different from control. (b) shows which groups were significantly different from CADO. (c) shows which groups were significantly different from DPCPX and (d) shows which groups were significantly different from NBQX.

APPENDIX

1. Published MatLab code for the function

```
function [T]=epanalysis(notebookname)
```

```
%INTRODUCTION

%This function outputs 2 things:
%1: A figure showing a timecourse plot of peak fESPS amplitude from an
%electrophysiology experiment.
%The figure is setup in a way that the first row shows the time-course plot
%and the bottom rows show 4 example peak fESPS traces from each of the
%4 drug conditions.

%2: A table outlining the peak fESPS amplitudes for each experimental
%condition from all experiment trials + their means

%This function also runs a statistical analysis on the data using
%one-way ANOVA,and will display a character array stating whether the
%test found any significant difference.

%If the test finds a significant difference, it will also output the
%calculated p-value and run a multiple comparisons test,
%which will output an additional figure showing which groups were
%significantly different from each other


%FUNCTION INPUT/OUTPUT

%Output[T]= the summary table containing mean fESPS amplitudes
%Input(notebookname)= the name of the .xlxs file containing the names of
%the electrophysiology experiment files
```

```
%LOOP FUNCTION TO BATCH IMPORT DATA
notebook=readtable(notebookname);
num_exp=height(notebook); % finding number of experiments

%Preallocating some values
baselinedata=[]; % cell containing data that has been set to baseline=0
filterdata=[]; %cell containing data that has been filtered
windowdata=[]; %cell containing data from only the specified time window
peakdata=[]; %cell containing peak fESPS amplitudes

for n=1:num_exp

    %Creating character array for each filename
    filename=char(notebook.Filename(n));
    alldata(n)=import_wcp(filename); %importing data

    %Setting baseline to 0
    baseline=mean(alldata(n).S{1}(1:80,:));
    baselinedata{n}=alldata(n).S{1}-baseline;

    %Filtering data using lowpass Butterworth filter
    Fc=[250 250 250 250 150 250]; %cutoff values (see note at the end of
                                  %the section
    Fs=1/alldata(1).t_interval;
```

```
    [b,a]=butter(4,Fc(n)/(Fs/2),'low');
    filterdata{n}=filtfilt(b,a,baselinedata{n});
```

```
    %Creating time axis
    timeaxis=0:alldata(1).t_interval:((height(alldata(1).S{:,1}))-1)...
        *alldata(1).t_interval;
    timeaxis=timeaxis'*1000; %converting to ms

    %Finding data from time window
    %time window for 1st fESPS was determined to be 12-25 ms
    %We started at 12 because the stimulus was applied at 10 ms and we
    %added an extra couple of ms so our analysis would ignore any
    %stimulus artifacts

    index=find(timeaxis>12 & timeaxis<25);
    windowdata{n}=filterdata{n}(index,:);

    %Finding peak amplitudes within time window
    peakdata{n}=min(windowdata{n});


    %*note: Cutoff values for the butterworth filter were determined
    %manually via trial and error by plotting the filtered data using
    %various cutoff values (ranging from 100-1000) on top of the raw data
    %and seeing which value resulted in the most suitably filtered line
    %ie. filtered enough but not so much that it distorts the original
    %shape of the raw data

end
```

```
%PLOTTING TIME COURSE FOR EXPERIMENT 1
%Using the timecourse subfunction
timecourse(1)
```

```
%CREATING TABLE OUTPUT
%For loop to find peak amplitudes from all experiments

%preallocating values
control_peak=zeros(1,6); %array containing all peak amplitudes for control
cado_peak=zeros(1,6); %array containing all peak amplitudes for CADO
dpcpx_peak=zeros(1,6); %array containing all peak amplitudes for DPCPX
nbqx_peak=zeros(1,6); %array containing all peak amplitudes for NBQX

for n=1:num_exp
    control_peak(n)=peakdata{n}((notebook.CADO(n))-1);
    cado_peak(n)=peakdata{n}((notebook.CADO_DPCPX(n))-1);
    dpcpx_peak(n)=peakdata{n}((notebook.CADO_DPCPX_NBQX(n))-1);
    nbqx_peak(n)=peakdata{n}((width(alldata(n).S{1,:}))-1);
end

%Creating array with all the peak amplitude values
t=zeros(7,4);
t(1:6,1)=control_peak;
t(1:6,2)=cado_peak;
t(1:6,3)=dpcpx_peak;
t(1:6,4)=nbqx_peak;
t(7,:)=[mean(control_peak) mean(cado_peak) mean(dpcpx_peak)...
```

```
 mean(nbqx_peak)]; %last row contains all the means

T=array2table(t); %converting array to table

%Setting table properties
T.Properties.VariableNames={'Control' 'CADO' 'DPCPX' 'NBQX'};
T.Properties.RowNames={char(notebook.Filename(1))...
    char(notebook.Filename(2)) char(notebook.Filename(3))...
    char(notebook.Filename(4)) char(notebook.Filename(5))...
    char(notebook.Filename(6)) 'Mean'};
T.Variables=round(T.Variables,3); % rounding values to 3 decimal points
```

```
%STATISTICAL ANALYSIS%

%Creating character array with all experimental conditions to be used
%as grouping variable for the anova test
Drugnames=char('Control','CADO','DPCPX','NBQX');

[p,~,stats]=anova1(t(1:6,:),Drugnames,'off'); %one-way ANOVA test

if p<0.05
    x=['There is evidence that mean fESPS peak amplitude is affected'...
        ' by the various drug treatments with a p-value of '...
        num2str(p)];
    disp(x)

    %Multiple comparisons test if ANOVA test was positive
    c=multcompare(stats);

else
    x=['There is no evidence that mean fESPS peak amplitude is affected'...
        ' by the various drug treatments.'];
    disp(x)
end
```

```
%TIMECOURSE SUBFUNCTION

function timecourse(n)

%INFO
%This subfunction outputs a 2-by-4 timecourse subplot for
%experiment number n.
%The top row subplots are the timecourse plot with lines showing when
%each drug was added and the bottom row 4 subplots show example traces
%of each of the 4 experimental conditions


%Making time axis for timecourse plot
num_rec=width(peakdata{n})-1;
timeaxis2=[0:10:num_rec*10];

%Creating subplots
tc=subplot(2,4,[1,4]); %main time course plot
Control=subplot(2,4,5); %example trace for control
CADO=subplot(2,4,6); %example trace for CADO
DPCPX=subplot(2,4,7); %example trace for DPCPX
NBQX=subplot(2,4,8); %example trace for NBQX
```

```
%Time course plot
figure
plot(tc,timeaxis2,peakdata{n},'ok','MarkerFaceColor','w','MarkerSize',3)
set(tc,'YDir',"reverse")
ylabel(tc,'fESPS amplitude (mV)')
xlabel(tc,'time (s)')
ylim(tc,[-2 0])
title(tc,['Time course plot for experiment ' char(notebook.Filename(n))],...
    'Interpreter','none')

%Showing when each drug was added
hold(tc,"on")

x_cado=timeaxis2((notebook.CADO(n)):end); %creating X-axis for lines
x_dpxpc=timeaxis2(notebook.CADO_DPCPX(n):end);
x_nbqx=timeaxis2(notebook.CADO_DPCPX_NBQX(n):end);

y_cado=-1.*ones(1,length(x_cado)); %creating Y-axis for lines
y_dpxpc=-1.1.*ones(1,length(x_dpxpc));
y_nbqx=-1.2.*ones(1,length(x_nbqx));

p1=plot(tc,x_cado,y_cado,'LineWidth',3,'Color','#0072BD');
p2=plot(tc,x_dpxpc,y_dpxpc,'LineWidth',3,'Color','#77AC30');
p3=plot(tc,x_nbqx,y_nbqx,'LineWidth',3,'Color','#EDB120');

legend(tc,[p1 p2 p3],{'CADO','DPCPX','NBQX'},'Location',"northwest")

hold(tc,"off")

%Plotting the 4 example traces

%creating new time axis
num_samp=height(alldata(n).S{:,1})-1;
time=[0:alldata(n).t_interval:num_samp*alldata(n).t_interval];
time=time'*1000;

%Traces taken at the time the next subsequent drug was added minus 1
%with the exception of the trace for NBQX as it is the final drug
%so its trace was taken as the total number of recordings minus 1
plot(Control,time,filterdata{n}(:,(notebook.CADO(n))-1),'-k') %Control
plot(CADO,time,filterdata{n}(:,(notebook.CADO_DPCPX(n))-1),'-k') %CADO
plot(DPCPX,time,filterdata{n}(:,(notebook.CADO_DPCPX_NBQX(n))-1),'-k') %DPCPX
plot(NBQX,time,filterdata{n}(:,num_rec-1),'-k') %NBQX

%Setting plot params

%setting axes limits
ylim([Control,CADO,DPCPX,NBQX],[-0.8 0.2])
xlim([Control,CADO,DPCPX,NBQX],[12 25])

%setting labels
ylabel(Control,'mV')
xlabel([Control,CADO,DPCPX,NBQX],'ms')

%setting titles
title(Control,'Control')
title(CADO,'CADO')
```

```
title(DPCPX,'DPCPX')
title(NBQX,'NBQX')
end

end
```