PFC Neurons While Learning:

The Role of Pyramidal Neurons in Outcome-Dependent Synchronization

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Abstract

Medial Prefrontal Cortex (mPFC) is an important region for a healthy dynamic to shape our actions in response to changing conditions also for desired outcomes. Neural populations in mPFC and their behaviours were examined to understand how do animal brains do use these populations in tasks that examine the reactions to a outcome-dependent task. Neural populations include two major types of neurons are glutamatergic pyramidal neurons and GABAergic interneurons (Ferguson and Gao, 2018). Studies shown that GABAergic inhibition has a significant position in the regulation of excitation and inhibition as well as helping pyramidal neurons to maintain the accuracy. This project aimed to investigate any effective role of pyramidal neurons in outcome-dependent synchronization during learning with a Y- maze task. The data of serial sessions of mPFC population behaviour of four rats learning rules in the Y-maze (Peyrache et al., 2009) is examined and compared the results all types of neurons with the results of only pyramidal neurons. Fifty trials where examined, figures of analysis and statistics are reported. Results of statistical analysis showed that no significant difference in delta recall when pyramidal neurons were subordinated was observed. Replication of the study with greater sample size and addition of subtypes of interneurons is advised for a deeper understanding.

Keywords: Pyramidal Neurons, Interneurons, Medial Prefrontal Cortex, Outcome-dependent Synchronization, Learning

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Thank you

List of Contents

List of Figures

List of Abbreviations

- CC = Correlation Coefficient
- EPSPs = Excitatory Post-Synaptic Potentials
- ITIs = Inter-trial Intervals
- mPFC =The Medial Prefrontal Cortex
- PFC = Prefrontal Cortex

1.Introduction

1.1 Background Work

Brain has evolved with specialized functions to regulate our cognitive and behavioural progressions. Prefrontal Cortex (PFC) has major role In cognitive flexibility, working memory and decision-making which is also observed to be affected in various neurological abnormalities. Many cognitive abilities that regulates our behaviours consistently impaired when PFC is damaged. The main cognitive abilities that are crucial for learning and impaired in a presence of abnormalities in PFC are working memory (Kolb et al., 1974) and cognitive flexibility (Bissonette et al., 2008).

The neocortex, including PFC, include two major types of neurons which are glutamatergic excitatory pyramidal neurons and GABAergic interneurons (Ferguson and Gao, 2018). Pyramidal neurons obtain excitatory synaptic input or excitatory post-synaptic potentials (EPSPs), and are responsible for giving the decision of whether or not to fire an action potential (Ferguson and Gao, 2018). On the other hand, GABAergic interneurons are necessary to control our excitatory signalling as well as inhibition of neighbouring pyramidal neurons and prevents our brain to do overwork. Interneurons are also the neurons that helping to maintain this balance between excitation and inhibition. The help of GABAergic interneurons to pyramidal neurons gives the brain an incredible flexibility and potential rather than a simple all or none progression. Without interneurons, pyramidal neurons are working without any shot downs, they get active when there is enough input and activate other neurons. Interneurons are the ones that regulate and modulate this behaviour and stops whenever its needed. GABAergic interneurons in prefrontal cortex has crucial responsibility for feedforward and feedback processes as well as synchronisation in cortical circuits (Hu et al., 2014).

Studies has shown that GABAergic inhibition has a key role in the regulation of working memory (Ferguson and Gao, 2018). In vivo recordings from primates indicated that with pharmacological manipulation in prefrontal cortex, interneurons that were not associated with any task for spatial working memory started to show spatial tuning (Rao et al., 2000). Moreover, pyramidal neurons that showed directional sensitivity before started to respond to random directions (Rao et al., 2000). The link between interneurons and pyramidal neurons on various cognitive abilities like working memory in PFC, might be also the case for learning abilities.

The medial prefrontal cortex (mPFC) is a specialized area in PFC regarding to its roles in adaptive behaviours like adapting to a dynamic environment (Euston et al., 2012) and errors in performance (Laubach et al., 2015). Studies shown that animals with damaged mPFC also likely to have problems in adaptation to new strategies with respect to changes in environment and outcome (Guise and Shapiro, 2017; Benchenane et al., 2010).

Learning ability requires on both memory and evaluation of current situation. As the literature shown, both memory and adaptational skills are related to PFC, specially mPFC (Ferguson and Gao, 2018). It's relationship with outcome-dependent adaptation of behaviour are shown (Euston et al., 2012). The relationship of outcome-dependent learning tasks and mPFC and the impact of interneurons on cognitive abilities in PFC brings the interest of this research.

1.2 Aim

In this study, the main focus is to examine the role of pyramidal neurons in outcome-dependent synchronization while learning. With respect to that, the research question is what is the impact of pyramidal neurons in shaping the outcome-dependent activity in mPFC. The analysis were carried out with subordinating pyramidal neurons from all types of neurons to identify whether there would be any change in the role of pyramidal neurons. Silvia Maggi and her colleagues has observed an outcome-dependent change in the mPFC population activity and this was specific to learning (Maggi et al., 2019). Based on their findings, the current research aims to investigate whether pyramidal cell might be the major contributor to this phenomenon.

2. Methods

The data in this project comes from previously published data (Peyrache et al., 2009). Experiments were carried out according to institutional and international standards and legal regulations regarding the use and care of animals (details can be found in Peyrache et al., 2009).

Four male rats were implanted tetrodes in prelimbic cortex and trained for a Y-maze learning task. The Y-maze had three symmetrical arms (85 cm long, 8 cm wide, separated by 120 degrees) which were connected in a central point that is represented as the decision point. In the learning session, animals were asked to learn one of the following 4 rules in sequence; go to the right arm, go to the lit arm, go to the left arm and go to the dark arm (Figure 1) (Maggi and Humphries, 2019). Each rat was trained at the task phase by starting the trial by their own where they were left in the beginning of the start arm. Whenever the rats reached the finish of the aimed arm, trials were finished. In the end of the trials, the rats were rewarded if they manage to choose the correct arm determined by the rule. As soon as each trial finished, an inter-trial intervals (ITIs) recorded until the rat return to the beginning point (Figure 2)(Maggi et al,, 2018). Each session was a series of trials with inter-trial intervals after each trial.

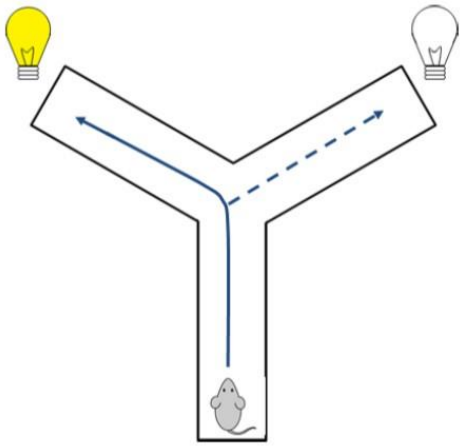

Figure 1. Graphical representation of the Y maze. Each rat was taught to go in a given rule to arrive correct arm so they could be rewarded. Each trial finished after they arrived to the correct arm and ITIs started at the end of each trial. Reprinted from " Independent population coding of the present and the past in prefrontal cortex during learning " by S. Maggi and M. Humphries, 2019, p. 3.

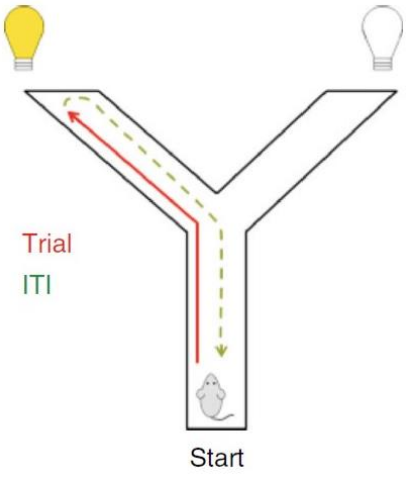

Figure 2. Graphical Representation of Inter-trial Intervals. Inter-trial intervals started from the end of a trial and finished when the rat manage to return to the beginning point of the maze. Reprinted from " An ensemble code in medial prefrontal cortex links prior events to outcomes during learning" by S. Maggi, A. Peyrache and M. Humphries, 2018, *Nature Communications, 9*(1), p. 2.

In each session, their brain activity was recorded for 20-30 minutes of sleep or rest period, then for another 20-40 minutes as training epoch which rats worked on the learning task (Maggi and Humphries, 2019). There were originally 53 trials in the data with learning and “other” sessions for

each rat with learning trials that the tasks were taken and which the rules were acquired (Peyrache et al., 2009). Sessions contained learning sessions, among all the sessions only 10 were considered learning sessions. This learning sessions satisfy the criteria of having 3 consecutive correct trials followed by a performance of at least 80% correct (Maggi et al, 2019). Each of the rats were taught at least two rules and three of them completed 14 sessions where only one of the rats completed 11 sessions. In the current study, only 50 sessions were used in analysis. The reason of exclusion of 3 sessions due to missing position data, problem in decoder analyses and missing spike data in some trials. Recordings from tetrodes were sorted by spikes within each session. Resolution of the spike recordings were 0.1 milliseconds and rat's position tracking recordings were 30 Hz (Maggi and Humphries, 2019).

Analysis were carried out with MATLAB (matrix laboratory) with R2019b version with modifying the code from the study of Maggi and Humphries in 2019 in respect to dissect the contribution of only pyramidal neurons compared to the entire population. Two syntax were used which one of them was run for both conditions by changing the cell type and another additional new one was for comparison of these two conditions.

3. Results

In this research, the aim was to identify the role of pyramidal neurons in outcome-dependent synchronization while learning. Analysis was conducted to see delta recalls in learning and other non-learning sessions for both pyramidal neurons and all type of neurons included and the comparison between these two groups are mentioned below. Learning sessions are ones with three sequential correct trials that comes after correct trials that gave 80% performance (Maggi and Humphries, 2019). Other sessions referred to mixed behaviours (Maggi et al., 2018).

Four rats were given a T shaped maze task with learned rules. They had tetrodes in their mPFC and the spikes were recorded in learning and other periods. Delta recall was computed from the recorded spike-train. Fifty sessions in the dataset was analysed. The delta recall refers to the difference between correct and error recall which means that a delta recall bigger than zero suggest more advanced synchronization in the network on average when a trial is correct compared to an error trial. The dataset was divided into subordinate to understand whether there is any difference that is linked with types of neurons in learning. In the first phase of analysis, all type of neurons included. Differences in correlation coefficients (CC) between learning sessions and other sessions for all type of neurons are shown (Figure 3 and 5). As the second phase of the analysis, same method was conducted for only pyramidal neurons (Figure 4 and 6).

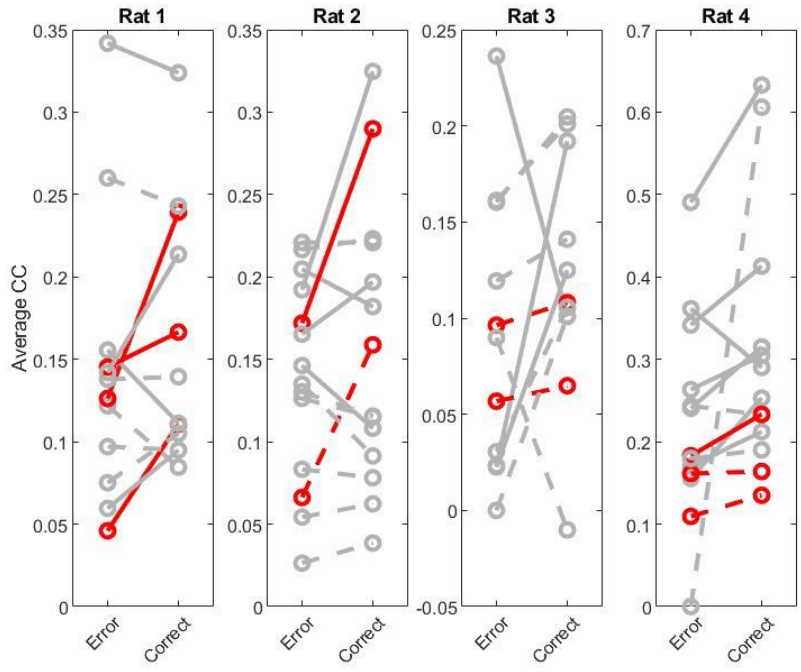

Figure 3. Average CC for each rat in the condition that all types of neurons were analysed. Representation of correlation coefficients for each rat in learning and other sessions. Red lines represents the learning trial as the three following correct trials after at least 80% correct trials. Grey lines represents other non-learning sessions.

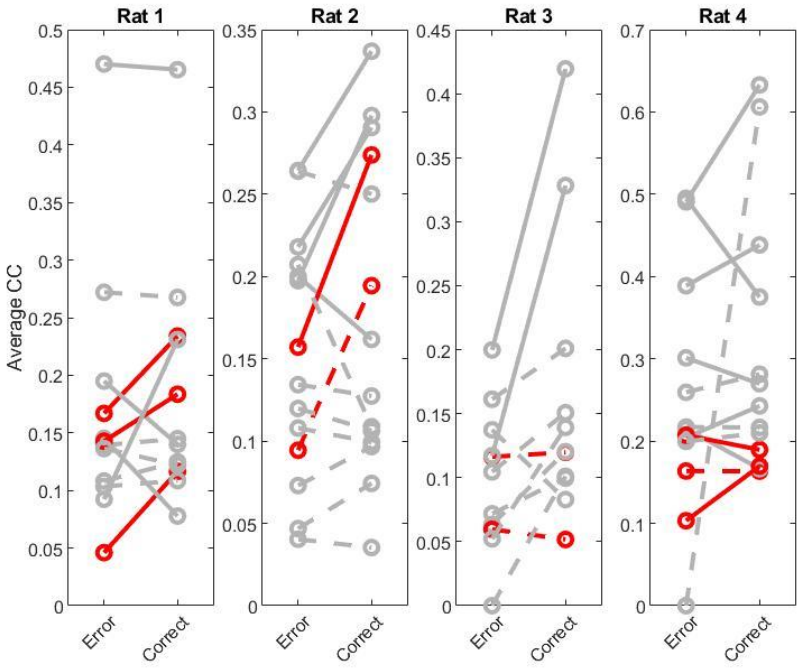

Figure 4. Average CC for each rat when only pyramidal neurons were included to the analysis. Correlation coefficient for each rat in learning (red) and other non-learning (grey) sessions for only pyramidal neuron subordinate.

As the figure 3 and 4 shows, there are some small changes in learning sessions for each rat. There are some correlation coefficient changes in both error and correct trials. Thus, we can say that there are some visible differences occur when we subordinate pyramidal neurons from other types of cells which includes interneurons. Delta recalls calculated from spike-trains differences for all neurons included condition and pyramidal neurons condition also carried out to examine any change between conditions with respect to the correlation coefficient changes.

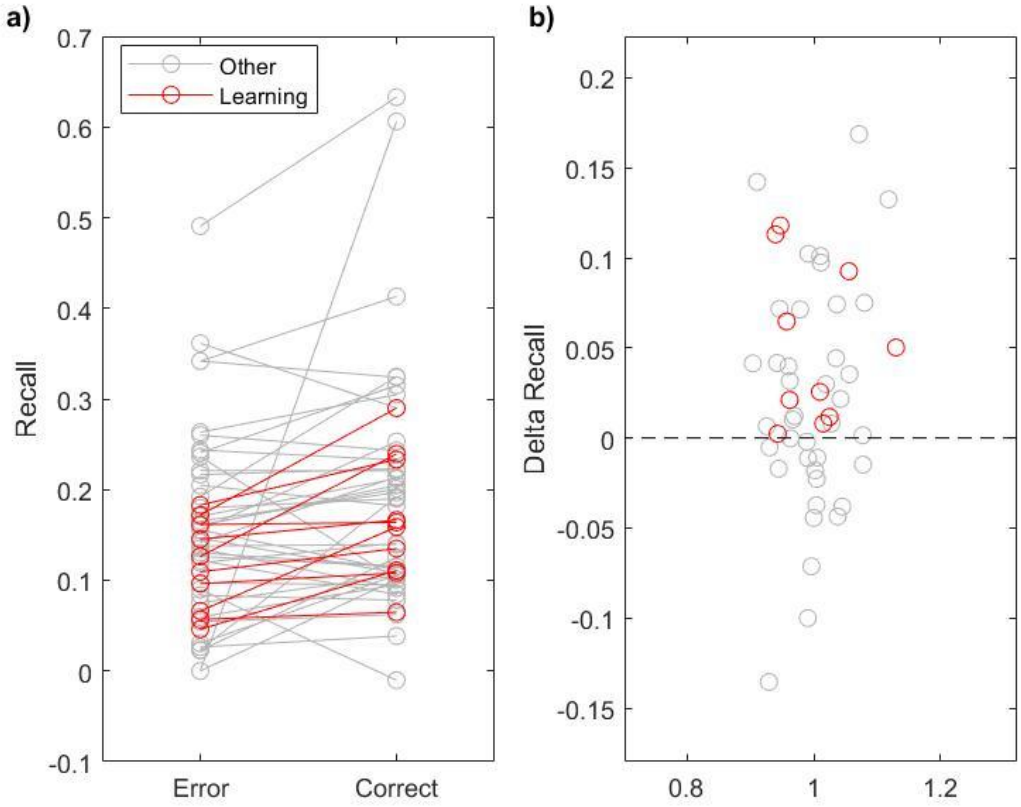

Figure 5. Delta recall changes for all neurons. (a) Representation of the average recall across the error (grey) and correct (red) trials for each session. (b) The Delta Recall is the difference between correct and error recall (Delta recall = correct recall – error recall). This means that a Delta recall > 0 suggest higher synchronization in the network when a trial is correct compared to an error trial (on average).

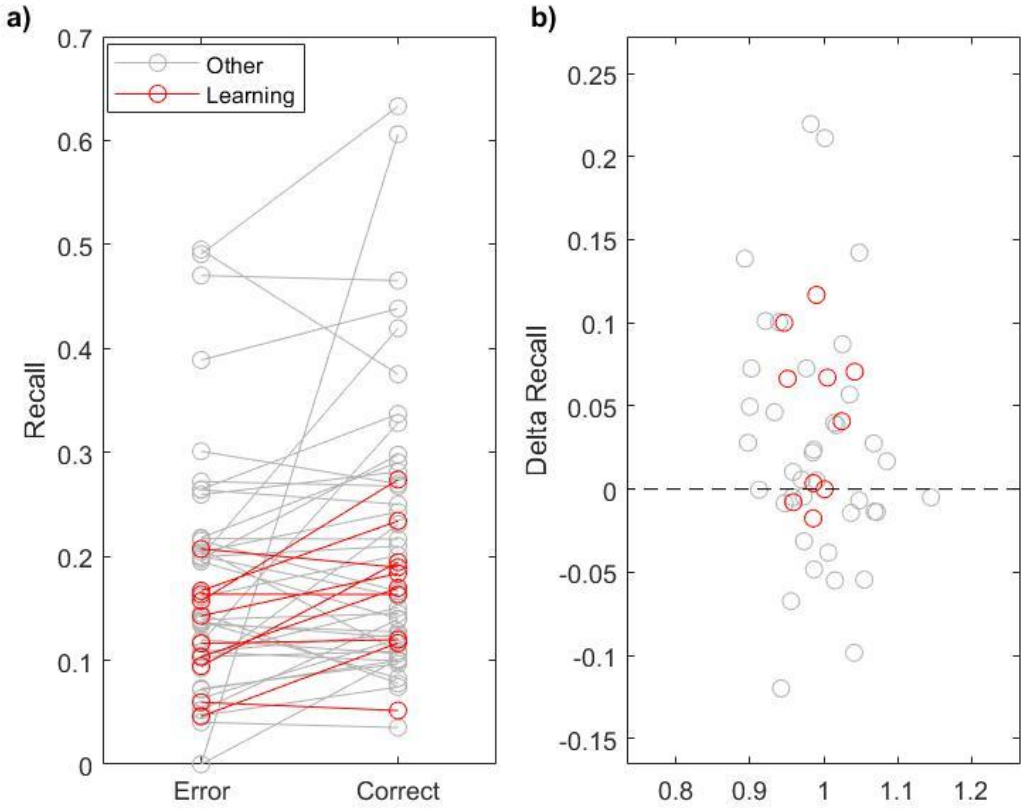

Figure 6. Delta recall changes for pyramidal neurons. (a) Representation of the average recall across the error (grey) and correct (red) trials for each session including only pyramidal neurons. (b) The Delta Recall is the difference between correct and error recall (Delta recall = correct recall – error recall).

The difference in delta recalls between all neurons included condition compared to only pyramidal neurons included condition is shown (Figure 5 and 6). When we eliminate the role of other neurons and leave the role of pyramidal neurons alone, we see that small decrease in the level of synchronization occurs in delta recalls. There are some trials that could not get higher than zero means that errors were greater than correct sessions in learning and non-learning other sessions. Especially for learning sessions, we see that trials there is no trial get below zero in all neurons included condition. Thus, we can claim that when we investigate the analysis with all neurons, synchronization is higher compared to the synchronization by only pyramidal neurons. Comparison of these conditions according to delta recall scores, a new analysis was conducted and mentioned below.

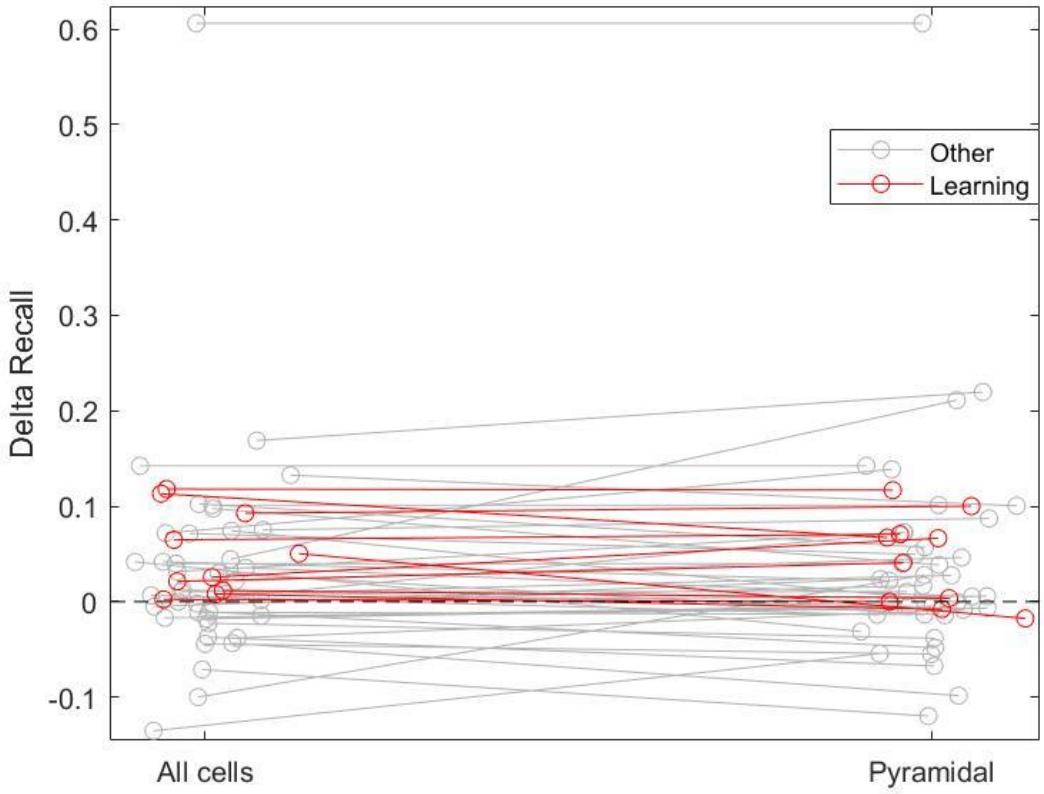

Figure 7. Comparison of delta recalls between all cells and pyramidal cells. Comparison between all types of cells condition and only pyramidal neurons condition was made regarding to the calculated delta recalls for each session. Difference between learning (red) and other (grey) sessions are also visible for all trials in both conditions.

The comparison between all type of neurons condition and pyramidal neurons condition showed that in learning sessions, a small decrease in delta recalls is visible for most of the trials. Pyramidal neurons by itself seem to not able to give as strong recall as they do when other types of neurons also included. Statistical analyses were carried out to identify whether any change occurred in the results were significant. However, two-sample *t*-test results showed that there was no significant change between recall results for only pyramidal neurons compared to all types of neurons in learning sessions (p= 0.9448). Moreover, due to the similar mean and standard deviation in the dataset, two-sample Kolmogorov-Smirnov test also conducted to examine any difference between conditions. However, Kolmogorov-Smirnov test also could not detect any significant difference between all types of neurons and pyramidal neurons conditions (p= 0.9541). Wilcoxon rank sum test also conducted but also failed to find any significant difference between conditions (p= 0.8335).

As a summary, results of statistical analysis showed that there was no significant change between all types of cells and only pyramidal neurons conditions. Even though there were small changes in figure 7 when the analysis was made for comparison of all neurons and only pyramidal neurons, statistical analysis showed that none of this changes where significant. Thus, we can claim that there is no significant difference when we exclude interneurons.

4. Discussion

4.1. Interpretation

Results indicated that there were no significant change on delta recalls for pyramidal neurons. Thus there were no significant difference between when only pyramidal neurons included and all types of neurons in learning. Thus, these results are enough to claim that as only pyramidal neurons are sufficient to carry all the outcome-dependent information observed during learning. Moreover, other types of cells are found to be helpful but not necessarily have to involve into outcome-dependent synchronization while learning. This study was contribution to the literature with its conclusion about the role of pyramidal neurons in outcome-dependent synchronization during learning that is enough and there is no other types of cells contribute as significant role as pyramidal neurons does to the process.

4.2. Limitations and Further Research

In this study, an already existing data was used. However, because of the size of sample, the data is respectively small. Even though the sample was 50 sessions, there were some small changes which could be larger with a lot more observations and trials. This can be a possible reason of the nonsignificant small changes in recordings for pyramidal neurons compared to all types of neurons. Higher amount of observations can be a good idea for a replication study to investigate for a better understanding whether the results are affected by the sample size. Greater number of observations are can be a good idea for more generalizable results in the future analysis. It is possible to collapse similar data in the literature and run the analysis. Another possible method could be a new data collection with higher amounts of observations.

Moreover, GABAergic interneurons are divided in into three broad types which are parvalbumin (PV), somatostatin (SST) and ionotropic serotonin receptor 5HT3a (5HT3aR) (Rudy et al., 2011). Types of interneurons were not recorded so it was not possible to control and examine effects of different kinds of interneurons. As a future research contribution, a dataset with the subordinates for each type of interneurons can be important to investigate any difference in the contribution of different types of interneurons.

Literature also links between some pharmacological manipulation on interneurons and behavioural outcomes (Rao et al., 2000). This model can be repeated in similar content with a special manipulation that can be linked to disorders with various levels of cognitive impairments such as schizophrenia. If the data can represent various steps of impairments, and having a better understanding of types of interneurons, the model can be helpful to use in such a content for a deeper understanding.

4.3. Data Availability

The data used for the findings of this study are available in CRCNS.org (DOI: 10.6080/K0KH0KH5) (ref. (Peyrache et al., 2009)).

5. References

Benchenane, K., Peyrache, A., Khamassi, M., Tierney, P. L., Gioanni, Y., Battaglia, F. P., and Wiener, S. I. (2010). Coherent theta oscillations and reorganization of spike timing in the hippocampal-prefrontal network upon learning. *Neuron, 66*(6):921–936.

Bissonette, G. B., Martins, G. J., Franz, T. M., Harper, E. S., Schoenbaum, G., and Powell, E. M. (2008). Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *J. Neurosci. 28*, 11124–11130. doi: 10.1523/JNEUROSCI.2820-08.2008

Euston, D. R., Gruber, A. J., and McNaughton, B. L. (2012). The role of medial prefrontal cortex in memory and decision making. *Neuron, 76*(6):1057–1070.

Ferguson BR and Gao W-J (2018). PV Interneurons: Critical Regulators of E/I Balance for Prefrontal Cortex-Dependent Behavior and Psychiatric Disorders. *Front. Neural Circuits*, 12:37. doi: 10.3389/fncir.2018.00037

Guise, K. G. and Shapiro, M. L. (2017). Medial prefrontal cortex reduces memory inter457 ference by modifying hippocampal encoding. *Neuron,* 94:183–192.

Hu, H., Gan, J., and Jonas, P. (2014). Interneurons. Fast-spiking, parvalbumin+ GABAergic interneurons: from cellular design to microcircuit function. *Science*, 345:1255263. doi: 10.1126/science.1255263

Kolb, B., Nonneman, A. J., and Singh, R. K. (1974). Double dissociation of spatial impairments and perseveration following selective prefrontal lesions in rats. *J. Comp. Physiol. Psychol. 87*, 772–780. doi: 10.1037/h0036970

Maggi, S., & Humphries, M. (2019). Independent population coding of the past and the present in prefrontal cortex during learning. doi: 10.1101/668962

Maggi, S., Peyrache, A., & Humphries, M. (2018). An ensemble code in medial prefrontal cortex links prior events to outcomes during learning. *Nature Communications, 9*(1). doi: 10.1038/s41467-018-04638-2

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., and Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuroscience*, 12:919–926.

Rao, S. G., Williams, G. V., and Goldman-Rakic, P. S. (2000). Destruction and creation of spatial tuning by disinhibition: GABAA blockade of prefrontal cortical neurons engaged by working memory. *J. Neurosci. 20*, 485–494. doi: 10.1523/jneurosci.20-01-00485.2000

Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev. Neurobiol. 71*, 45–61. doi: 10.1002/dneu.20853