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Investigating age-related changes of medial prefrontal cortex neural responses to ventral hippocampal stimulation

Sahana Srivathsa[1 2], Abhilasha Vishwanath[1 3], Stephen L. Cowen[1 3], Carol A. Barnes[1 3]

[1]Evelyn F. McKnight Brain Institute, [2]Neuroscience Graduate Interdisciplinary Program, [3]Department of Psychology, University of Arizona

INTRODUCTION

Neural ensembles in the hippocampus (HC) and medial prefrontal cortex (mPFC) play a crucial role in spatial working memory, a process susceptible to decline during aging in mammals. These regions are connected via a monosynaptic, unidirectional projection from the CA1 layer of intermediate (iHC) and ventral (vHC) hippocampus to the mPFC (Jay and Witter, 1991). Damage or inhibition to this connection leads to impairments in spatial working memory tasks. Performance on spatial working memory tasks is known to correlate with increased synchrony of hippocampal theta (8-12 Hz) rhythms to mPFC neural activity. The temporal offset of mPFC neurons phase-locked to hippocampal theta corresponds to the conduction delay between HC and mPFC neurons, suggesting that the HC-mPFC synchronization is a direct result of this projection. Little is understood about how monosynaptic iHC and vHC inputs engage mPFC neural activity along the dorso-ventral axis of the mPFC or how these change with age.

To investigate these questions, we delivered a single biphasic electrical pulse of varied intensities (100-600µA) with a 30s interval between pulses to the CA1 layer in iHC and vHC of anesthetized rats while we simultaneously recorded evoked neural activity along the dorsoventral length of the mPFC using Neuropixels probes. Recordings were obtained from neurons spanning the mPFC regions the prelimbic and infralimbic regions (areas 24b and 25). As iHC and vHC projections also vary across the different layers of the mPFC, we also compare evoked neural responses across different layers of mPFC in response to HC stimulation – by recording first from layer 2/3 and then from layer 5 in mPFC. Stimulating both the iHC and vHC, we observed responses in the mPFC at only very specific depths across all stimulation magnitudes for a given rat.
Anatomical studies suggest that there are more projections from the HC to the Infralimbic areas (IL) when compared to the Prelimbic (PL) areas of the mPFC. Corroborating this, we observe a stronger response from the IL regions in both L2/3 and L5. We also look at differences in iHC vs vHC stimulation to both L2/3 and L5. Our preliminary findings allow for a comparison of the effect of hippocampal axonal input, monosynaptic or otherwise, along the dorsoventral length of the mPFC and functional connectivity changes with respect to age.

METHODS

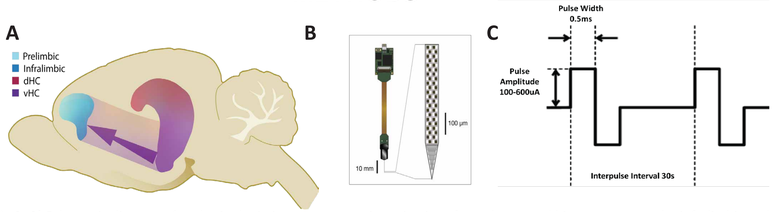


Fig 1: A) Schematic diagram of rat brain depicting monosynaptic projections from the ventral hippocampus to the infralimbic and prelimbic regions of the mPFC with no direct projections from the dHC. **B)** (Top) Schematic of the Neuropixel Probes that were used to record from 385 sites simultaneously spanning 3.84mm. (Bottom) An example of the square symmetric biphasic pulse of 0.5ms that was delivered at 30s intervals of varying amplitudes by a bipolar stimulating electrode that was 1mm apart.

Animal Subjects: Anesthetized male F344 rats that were n=4 Young (9-11 mo) and n=3 Old (23-25 mo) were used for this study.
Electrical Stimulation: Using a bipolar stimulation electrode in the HC of 1mm distance, we delivered 26 single biphasic electrical pulses 0.5ms wide with amplitudes ranging from 100-600µA in a randomized order at a 30s interval. The same randomized order was used at different locations for a given rat.
Neural Recordings: Using Neuropixel 1.0 probes, we were able to record neural activity in the mPFC from 385 sites that spanned 3.84mm. The observed voltage traces were bandpass filtered (0.1-300Hz).
Anatomical Locations: A total of 4 different conditions were tested with the stimulation electrode in the iHC and vHC. The neural recordings were from L2/3 and L5 in the mPFC.

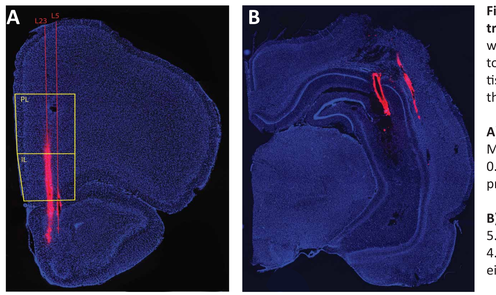


Fig 2: Histological verification of probe and electrode placement in the mPFC and HC. The probes were coated with the red fluorescent stain Di-I prior to insertion in the brain. Post-fixation, the coronal tissue sections were stained with DAPI to visualize the neuronal cell bodies.

A) The neuropixel probes placement in L23 (AP: 2.9, ML: 0.5-0.7, DV: 5.8-6.2)mm and L5 (AP: 3.1, ML: 0.8-1.1, DV: 5.8-6.2)mm. The yellow boxes mark the prelimbic (PL) and infralimbic areas (IL).

B) The stimulation electrode placement in iHC (AP: 5.8, ML: 4.8-5.8, DV: 3.5)mm and vHC (AP: 5.8, ML: 4.8-5.8, DV: 6.8)mm . The tips of the probe are on either side of the CA1 layer in the iHC and vHC.

RESULTS

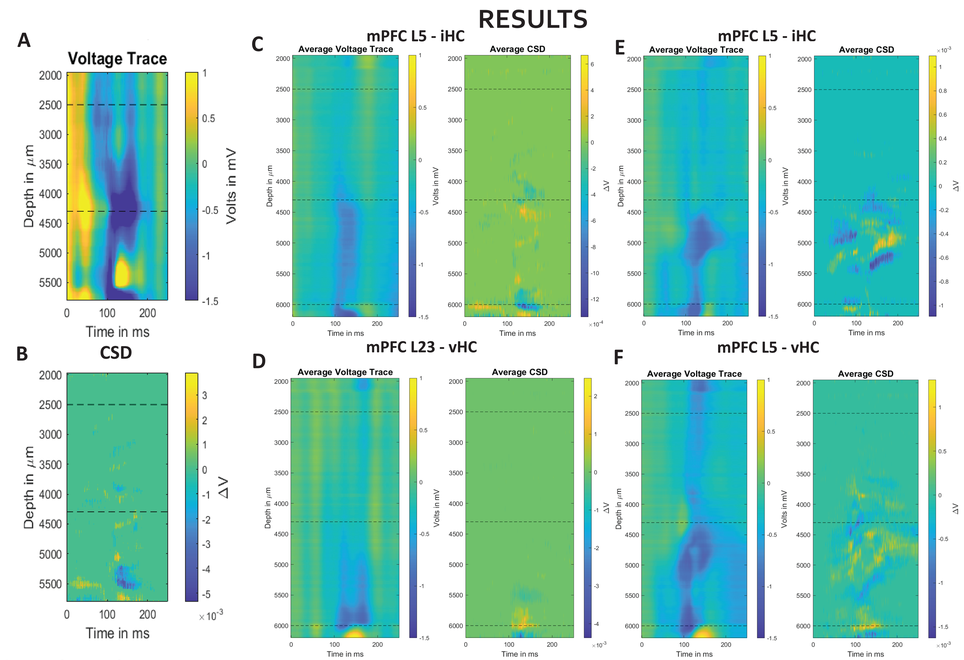


Fig 3 : Voltage and CSD Traces across mPFC.
A)The voltage trace for each channel for 250ms following a single stimulation pulse. The expected voltage responses are a negative deflection in the voltage as depicted in blue. he dashed lines represent the demarcation of PL and IL regions. **(B)** The current source density plot highlighting the areas of greatest change. In conditions **B)** L23-iHC **(C)** L23-vHC **(E)** L5-iHC **(F)** L5-vHC, the average voltage across different stimulation amplitudes across rats(n=7) is and aligned by depth and normalized to the maximum voltage response (negative) for that rat. The plot on the left shows the smoothed average voltage trace for each condition and the plot on the right is the average smoothed CSD. In L2/3 most of the response is seen only in the IL regions with a stronger and more consistent response with stimulation in the iHC compared to vHC. L5 shows responses in both PL and IL regions with the strongest and most consistent response with stimulation from the vHC not iHC. This suggests potentially different functional connectivity between iHC and vHC and L2/3 vs L5 of the mPFC.

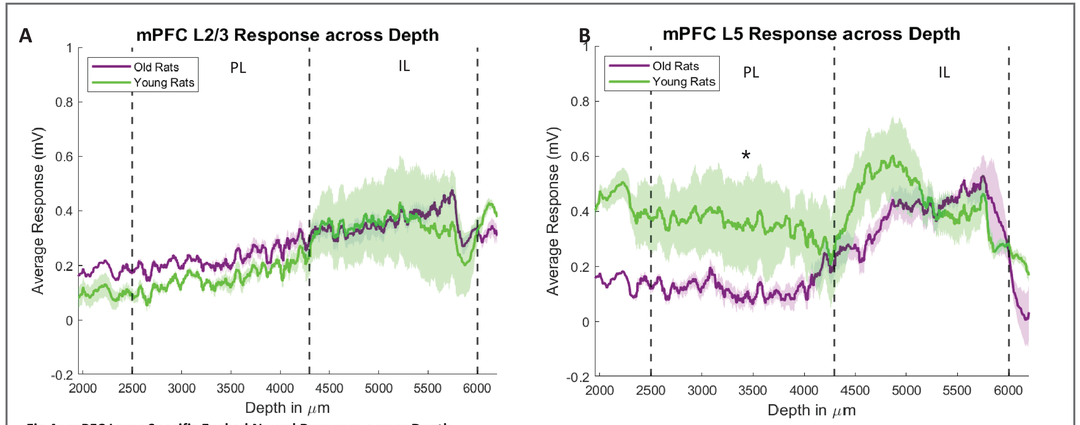
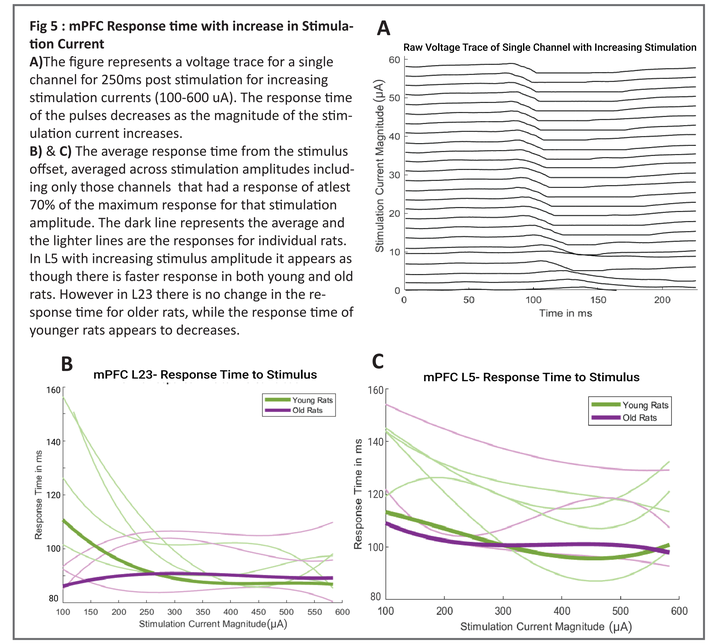


Fig 4 : mPFC Layer Specific Evoked Neural Response across Depth
The maximum voltage response (negative) for each depth was calculated and normalized to each stimulation amplitude for each rat. We show here the mean of the maximum response for each channel across different stimulation amplitudes (nstim=26) for young (n=4) and old (n=3) rats. The shaded lines represent the SEM. The dashed lines show the boundaries for prelimbic and infralimbic regions.The magnitude of voltage response overall is higher in L5 compared to L23.
A) L2/3: There is a greater voltage response in the infralimbic region compared to the prelimbic region for both old and young rats. The old rats show a slightly higher response in the prelimbic area compared to the young rats but it is not significant as shown by a Kruskal-Wallis Test: *H(32)=39.21, p=0.14*.
B) L5: There is a greater voltage response in the infralimbic region compared to the prelimbic region for old rats and slightly for young rats. The young rats have a much stronger response in the prelimbic region compared to old rats which is significant (*H(31)=48.34, p<0.05*. In the infralimbic region the depths for the max response are higher in young than old rats.

Fig 5 : mPFC Response time with increase in Stimulation Current
A)The figure represents a voltage trace for a single channel for 250ms post stimulation for increasing stimulation currents (100-600 uA). The response time of the pulses decreases as the magnitude of the stimulation current increases.
B) & **C)** The average response time from the stimulus offset, averaged across stimulation amplitudes including only those channels that had a response of atlest 70% of the maximum response for that stimulation amplitude. The dark line represents the average and the lighter lines are the responses for individual rats. In L5 with increasing stimulus amplitude it appears as though there is faster response in both young and old rats. However in L23 there is no change in the response time for older rats, while the response time of younger rats appears to decreases.



CONCLUSIONS

The vHC stimulation evoked neural activity in L2/3 of the mPFC for different stimulation amplitudes was predominantly in the infralimbic regions. In L5, while there was evoked neural activity in both the prelimbic and infralimbic areas, the strongest response was in the infralimbic region. This supports previous work by (Liu and Carter,2018) that show projections from the vHC to the IL regions of L2/3 but both PL and IL regions in L5.

Electrical stimulations of the CA1 layer of the iHC and vHC both elicit neural responses in L2/3 and L5 of the mPFC. However, the largest and most consistent evoked responses were seen in L2/3 through iHC stimulation and in L5 through vHC stimulation. It is possible that some of the lower amplitude signal was removed during filtering of 60Hz noise. However this suggests that there may be differences in how neurons in L2/3 vs L5 respond to iHC vs vHC input.

In old vs young rats we see that with increase in stimulation magnitude there appears to be an initial decrease in response time by the is a faster response by mPFC neurons in L5. In L23 while young rats seem to show the decrease in response times, there is no difference in the response times of old rats.

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ACKNOWLEDGEMENTS

We would like to thank Elizabeth Church for her help with histological sectioning and staining of the tissue and to Mauricio Serna for his help in spike sorting the collected data. This work was supported by McKnight Brain Research Foundation and RO1 1RF1AG081767.