Network synchronization across the longitudinal axis of the developing rat hippocampus

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Abstract:

Epilepsy is a neurological disorder characterized by spontaneous recurrent seizures. Researching epilepsy requires animal models, as the brain manipulation required to understand epileptogenic activity is not possible in humans. Extracellular recordings measure network level activation in slice models of epilepsy. A magnesium-free solution is often used in slice physiology to increase excitation through enhanced glutamate receptor activity. While the mechanisms underlying the no Mg2+ model are well established, the effects of this model across the longitudinal axis of the hippocampus and throughout development have not been clearly quantified. Using the no Mg2+ model, we aim to elucidate the mechanisms and development of a dorsal neuro-protective system, which reduces the excitability of dorsal hippocampal tissue with age. We studied the effects of the magnesium-free model through extracellular field recordings of CA1 pyramidal neurons from dorsal and ventral hippocampal slices throughout development, and found juvenile, dorsal hippocampal tissue to be the most excitable. Additionally, our data suggests a shift occurring during development. In adult rodents, the ventral hippocampus seems to be hyperexcitable compared to dorsal. These data support the dorsal protection system being critical in the reduction of excitability seen with age.

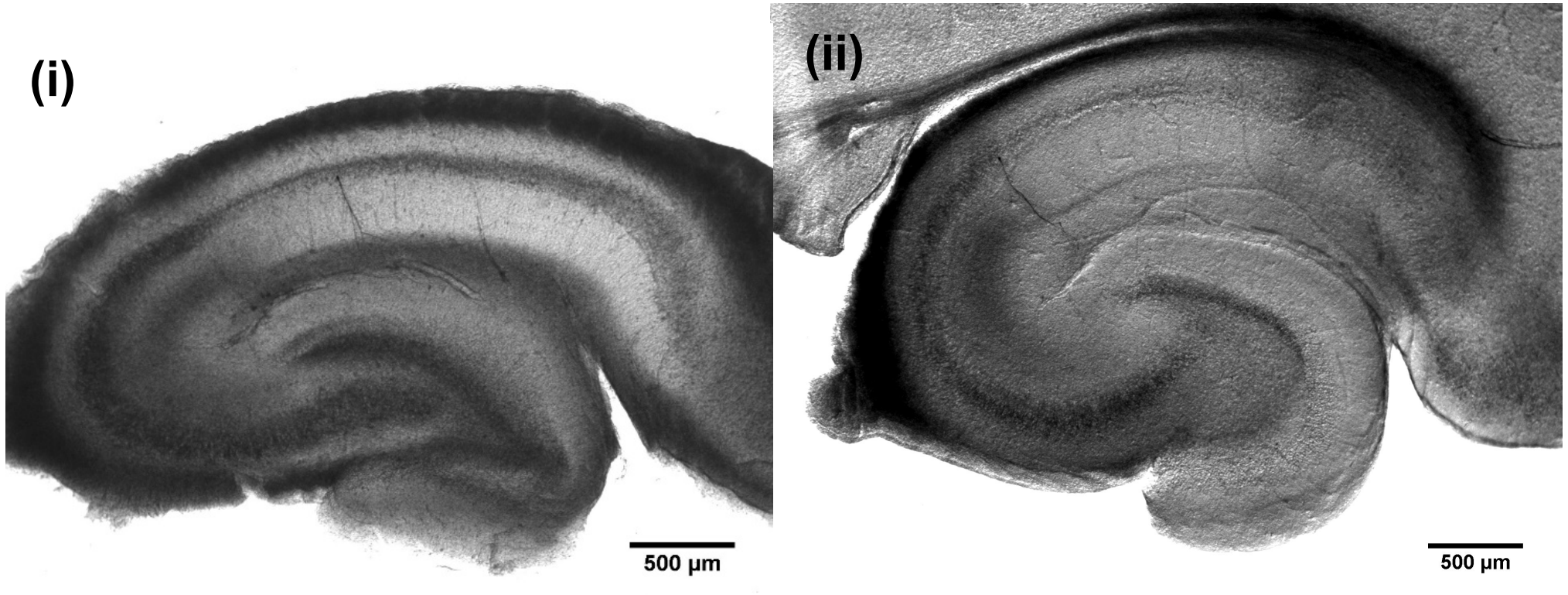
Introduction:

Epilepsy is a neurological disorder characterized by recurrent seizures, impacting over 50 million people worldwide. Researching epilepsy requires animal models, as the brain manipulation required to understand epileptogenic activity is not possible in humans. In vitro models of epilepsy examine seizure activity by chemically or electrically altering the physiology of hippocampal slices. Using these models, it has been well characterized that immature hippocampi are more susceptible to seizures (Kesslak et al., 1995; Haut et al., 2004). There is also evidence to suggest, in mature animals, the ventral hippocampus is more excitable (Iseava et al. 2015).

These models have not been characterized in light of recent studies that have observed changes in morphological and electrophysiological properties across the longitudinal axis of the hippocampus (Malik et al., 2016; Strange et al., 2014). Additionally, developmental changes in excitability and morphology have been noted, but not thoroughly characterized (Papatheodoropoulos and Kostopoulos, 1996). **Based on the combination of these observations, we hypothesize juvenile rodents, regardless of location of recording, will be hyperexcitable compared to adult rodents. Additionally, as development progresses, we hypothesize ventral slices will become hyperactive compared to dorsal slices.**

A common rodent model of epilepsy used to generate spontaneous seizure activity involves manipulating magnesium concentration. The magnesium-free model is often used in extracellular recordings of hippocampal slice physiology to increase excitation, measured via interictal activity, through enhanced glutamate receptor activity. Thus, the model uses 0mM Mg2+ artificial cerebrospinal fluid (aCSF) solution to create field hyperexcitability in hippocampal slices, representative of the pathological hyperexcitability of epilepsy (Anderson et al., 1986). While the mechanisms underlying the magnesium-free model are well established (Antonio et al., 2016), the effects of this model across the longitudinal axis of the hippocampus and throughout development have not been clearly characterized. We studied the effects of the magnesium-free model of epilepsy in dorsal and ventral hippocampal slices throughout development using extracellular field recordings of CA1 pyramidal neurons, and found a marginal difference in excitability between juvenile and adult dorsal slices, as well as a marginal difference between juvenile dorsal and juvenile ventral sources.

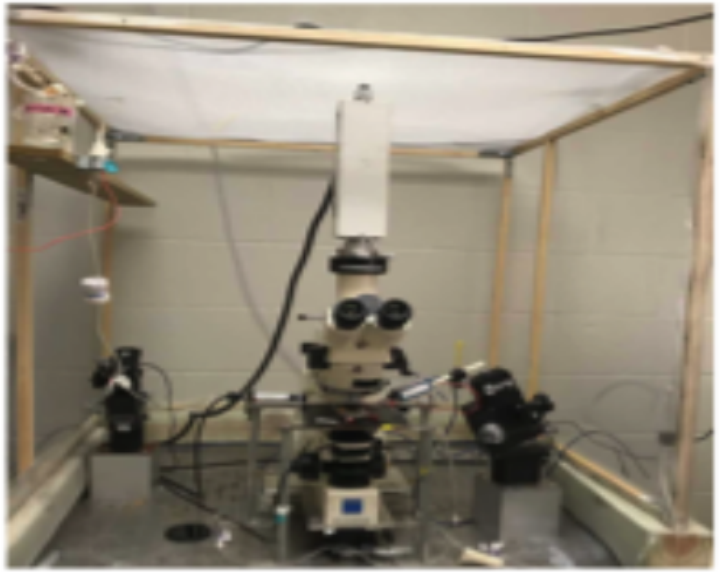
Figure 1. Hippocampal slice physiology



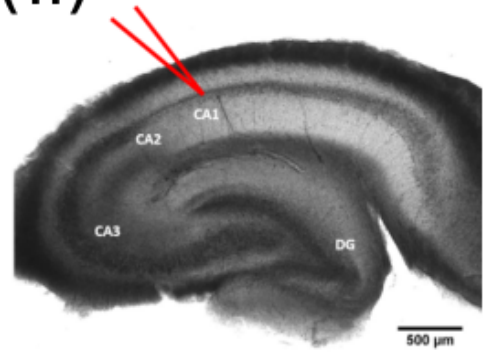
Hippocampal slices were prepared following transcardial perfusion of rats. Blocking cuts were made to isolate the dorsal (i) and ventral (ii) hippocampus prior to mounting on a vibrating microtome for 350 um slices which were kept in cutting saline of the following composition (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 12.5 dextrose, 1.3 ascorbic acid, and 3 sodium pyruvate (continuously bubbled with 95% O_2 / 5% CO_2).

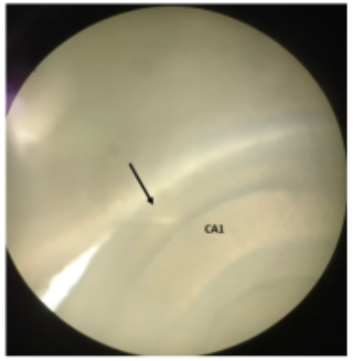
Figure 2: Extracellular recording from stratum pyramidale of CA1

(i)

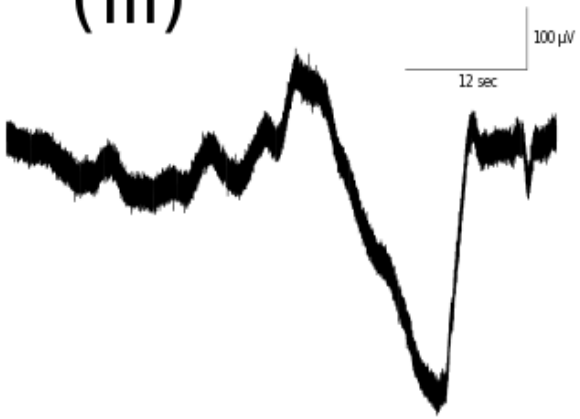


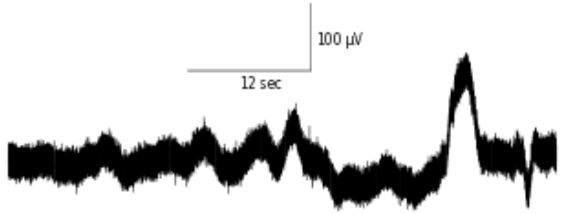
(ii)





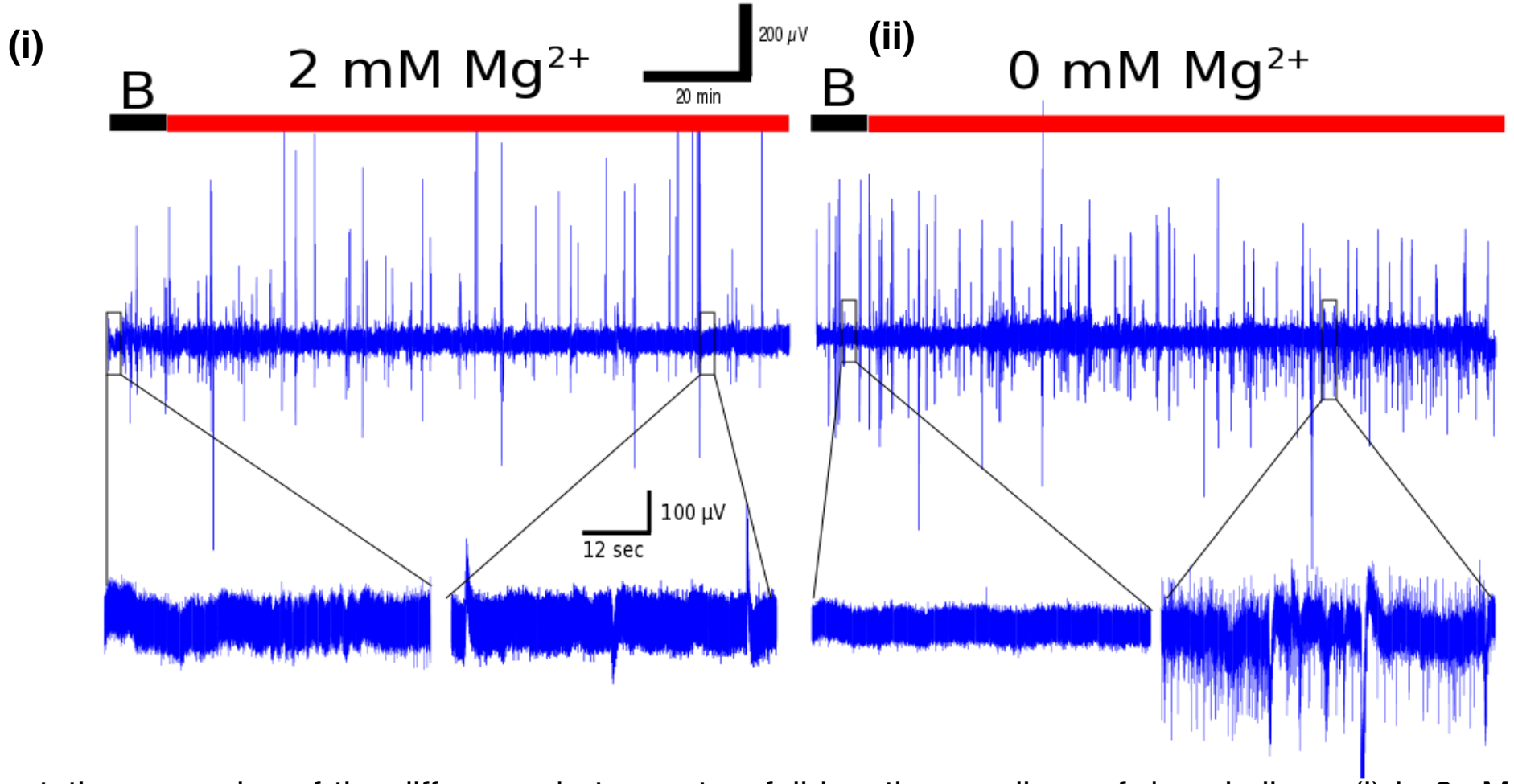
(iii)





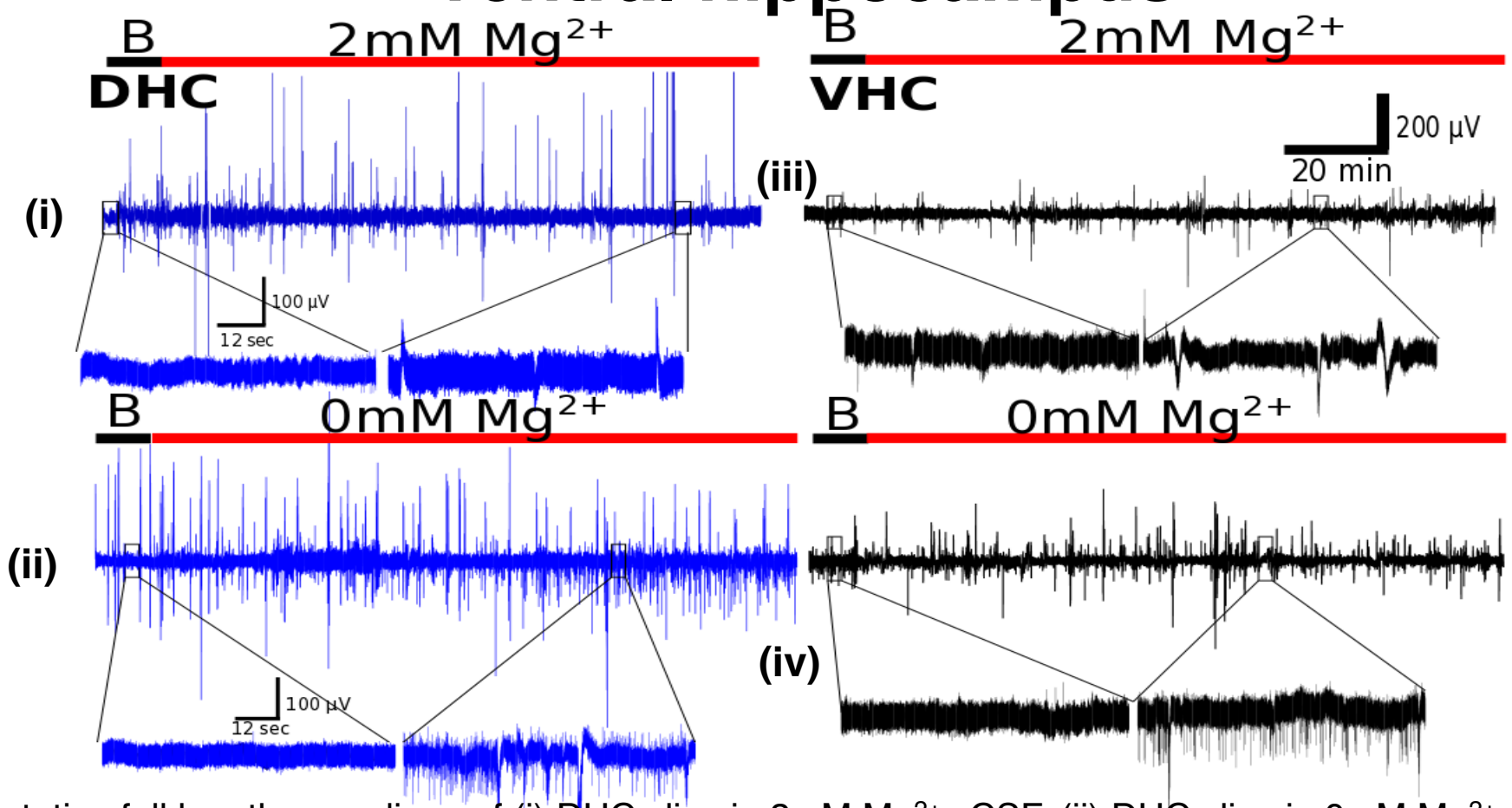
Visualization using the rig (i) allowed placement of the electrode in the CA1 region of the hippocampus (diagrammed in ii, above) to record synchronous network activity. The representative example in (ii, below) demonstrates a correctly placed electrode (indicated by arrow). Recording electrode collected data (iii, above) which was high-pass filtered at 0.2Hz post-collection (iii, below) to highlight fast synchronous events of network activity.

Figure 3: Field Recordings in 2mM Mg^{2+} aCSF and 0mM Mg^{2+} aCSF



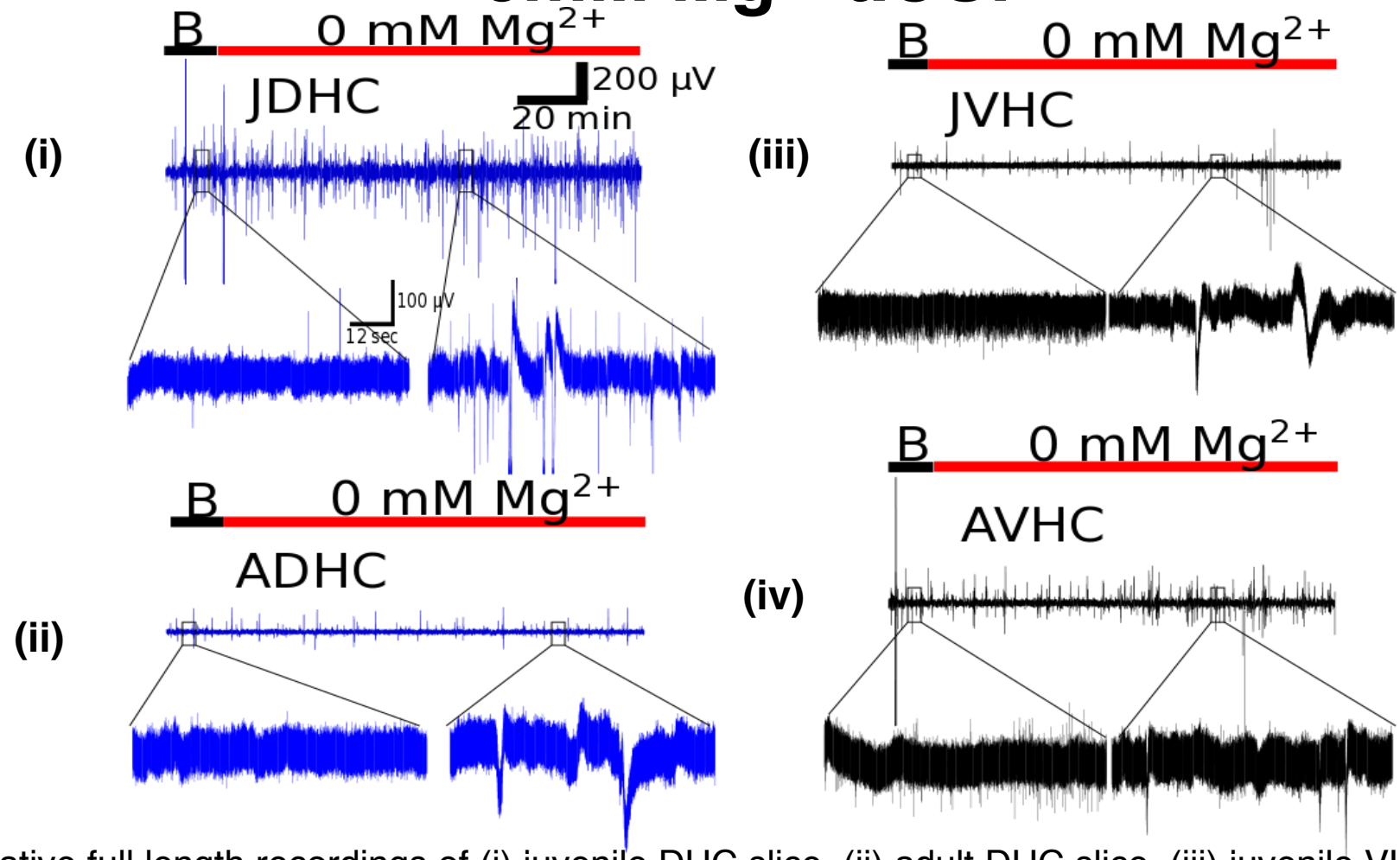
Representative examples of the difference between two full length recordings of dorsal slices, (i) in 2mM Mg^{2+} aCSF and (ii) 0mM Mg^{2+} aCSF, post filtering.

Figure 4: Effects of 0mM Mg^{2+} aCSF in dorsal and ventral hippocampus



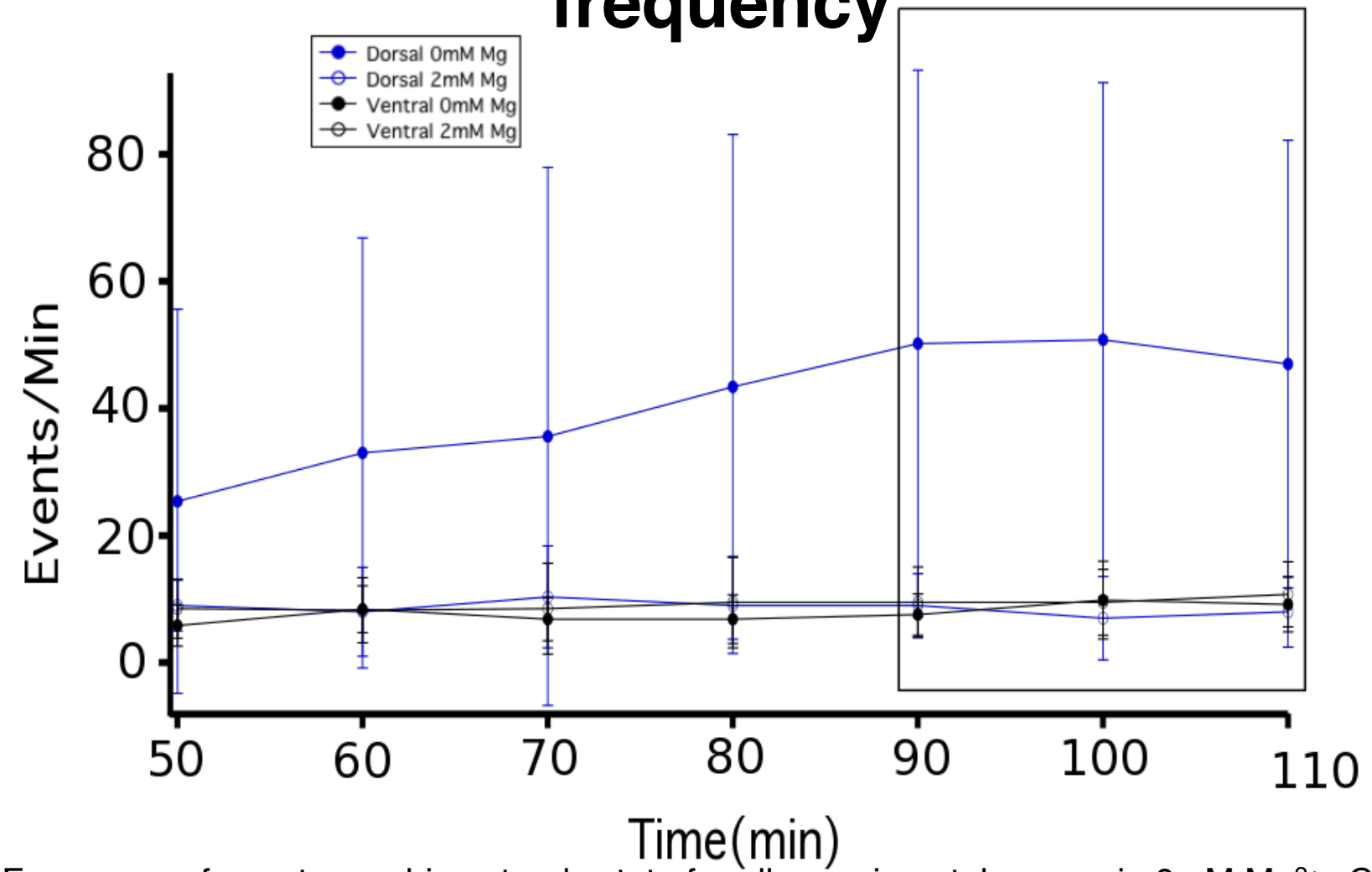
Representative full length recordings of (i) DHC slice in 2mM Mg^{2+} aCSF, (ii) DHC slice in 0mM Mg^{2+} aCSF, (iii) VHC slice in 2mM Mg^{2+} aCSF, (iv) VHC slice in 0mM Mg^{2+} aCSF. Representative baseline and stead-state activity expanded below.

Figure 5: Recordings of juvenile and adult slices in 0mM Mg^{2+} aCSF



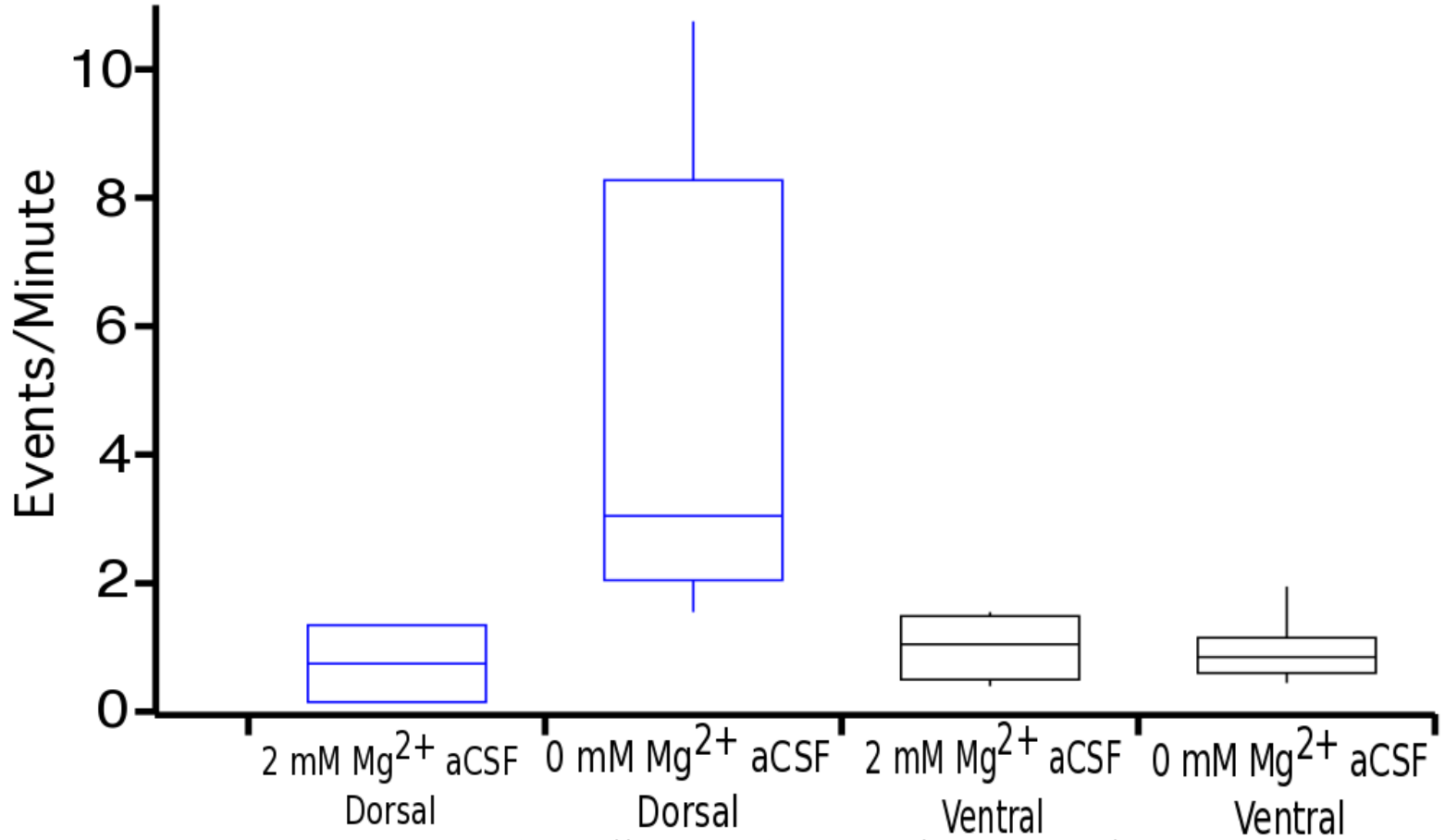
Representative full length recordings of (i) juvenile DHC slice, (ii) adult DHC slice, (iii) juvenile VHC slice, (iv) adult VHC slice in 0mM Mg^{2+} aCSF. Representative baseline and stead-state activity expanded below.

Figure 6: Determination of steady state event frequency



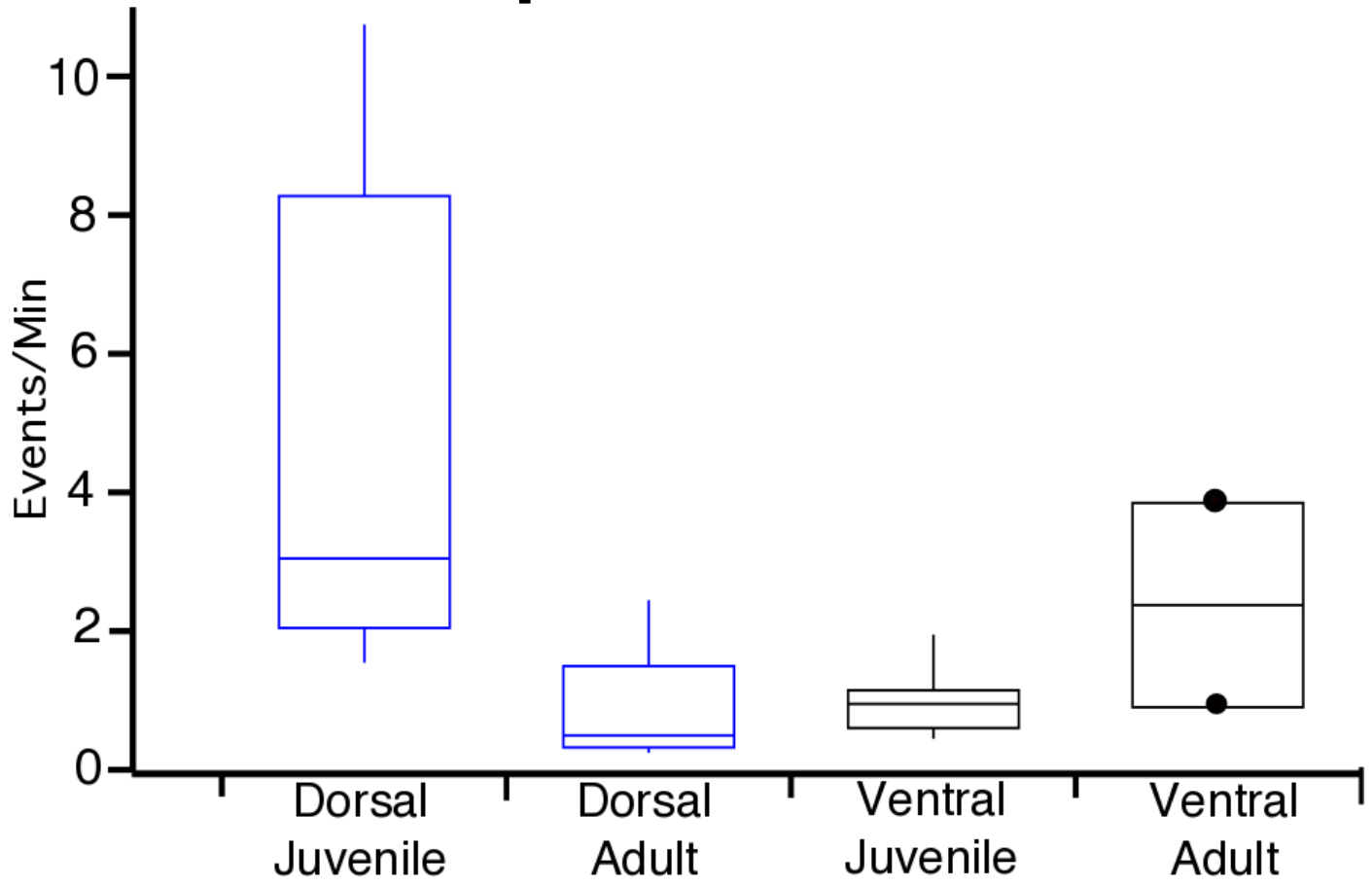
Frequency of events reaching steady state for all experimental groups in 0mM Mg^{2+} aCSF.

Figure 7: Frequency of events in 2mM Mg^{2+} aCSF and 0mM Mg^{2+} aCSF in DHC and VHC slices



A series of *Wilcoxon Rank Sum Tests* indicated that differences between frequency of events in DHC and VHC slices based on model type (0mM Mg^{2+} aCSF and 2mM Mg^{2}aCSF) were not significant, $p > 0.05$. This can be attributed to low sample sizes.

Figure 8: Event frequencies across development



A series of *Wilcoxon Rank Sum Tests* indicated that there was a marginal difference between event frequencies in the dorsal hippocampus over time and between juvenile DHC and juvenile VHC. Juvenile DHC tended to experience events more frequently than adult DHC (juvenile DHC 2.60 ± 3.11 events/min; adult DHC 0.77 ± 0.61 events/min; *Wilcoxon's RS Test* p = 0.08). Juvenile DHC experienced a marginally greater number of events than juvenile VHC (juvenile DHC 2.60 ± 3.11 events/min; juvenile VHC 0.83 ± 0.38 events/min; *Wilcoxon's RS Test* p = 0.053).

Summary

- The data is trending towards a decreased network synchronization in adult dorsal hippocampus compared to adult ventral hippocampus is consistent with previous reports (Papatheodoropoulos, 2012)

- In the juvenile hippocampus, 0mM Mg^{2+} aCSF showed more network level synchronization through increased event frequency

Conclusion

These observations suggest that there is a shift in the dorsal hippocampus from hyperexcitable synchronization to hypoexcitable synchronization over the course of development.

References:

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