Hippocampal Ripples Disengaged from Neocortex During High-Voltage Waves in Rats

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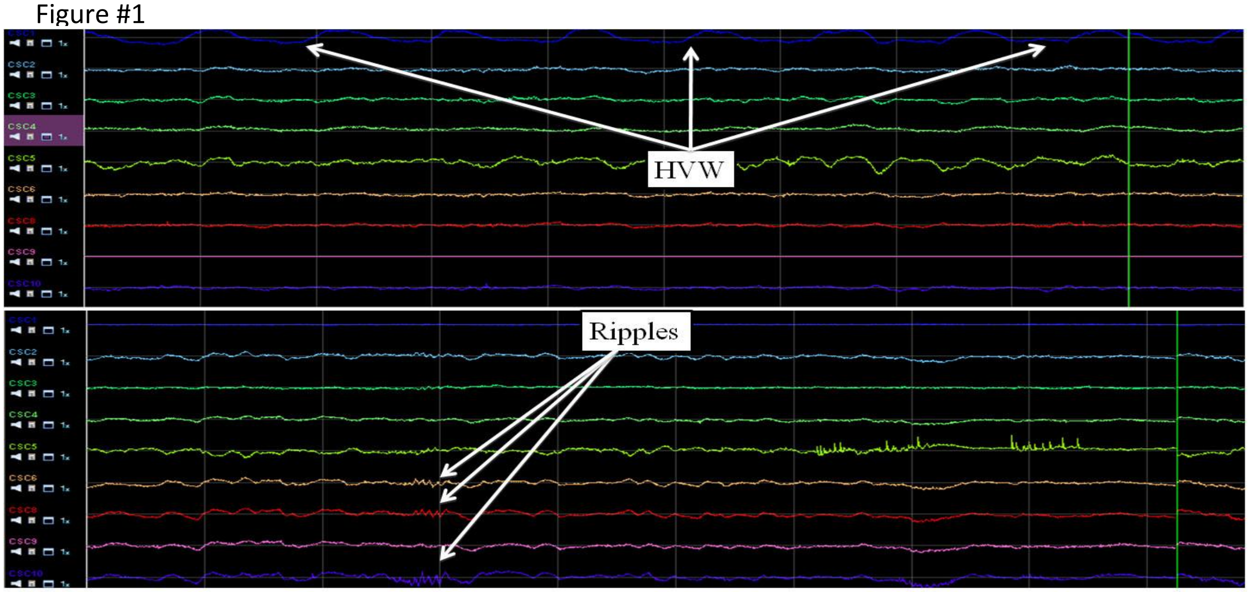
Introduction

The current theory of learning and memory consolidation requires communication between the hippocampus and the neocortex. Sleep Spindles, 7-14 Hz, and hippocampal ripples, 150-250 Hz, have been found to facilitate this communication during non-REM sleep. Activation of the hippocampus during the learning/awake process is assimilated for long-term store in the cortex during non-REM sleep. Cortical spindles, identified during slow-wave sleep, are the mark of assimilation. Another type of spindle called a high-voltage wave (HVW) activates during the onset of drowsiness in rats. This type is different because it has higher amplitude, occurs immediately before slow-wave sleep, and requires highly synchronized firings of cortical neurons. The relationship between HVW and the hippocampus is not understood.

Figure #1



Time Interval 1 second

Identifying significant events in the rats' brain is difficult. Beginners always miss lots of information when watching 96 traces of activity at 1 Hz. **Figure #1** is a template that can be used to identify HVW and ripple events. The HVW event is obvious and easy to distinguish from other types of activity. The Ripples on the other hand are difficult to isolate because their duration is short and have low amplitude.

Given the anatomical connection between the cortex and hippocampus, we hypothesized that the neocortical synchronization associated with the high-voltage waves enhances the synchrony of hippocampal neurons and thus correlates with hippocampal ripples. It is generally accepted that if ripples are absent from the hippocampus then no new memories are being formed. Girardeau and Buzsaki electrically suppressed ripples in rats during post-training and recorded significant learning deficits. Upon analysis of the EEG trace in the hippocampus and neocortex we found the opposite of our expectations. HVW and Ripples are exclusive events. We suspect that when an HVW is present the conduit of new memory formation is suppressed.

Materials & Methods

Animal and surgery

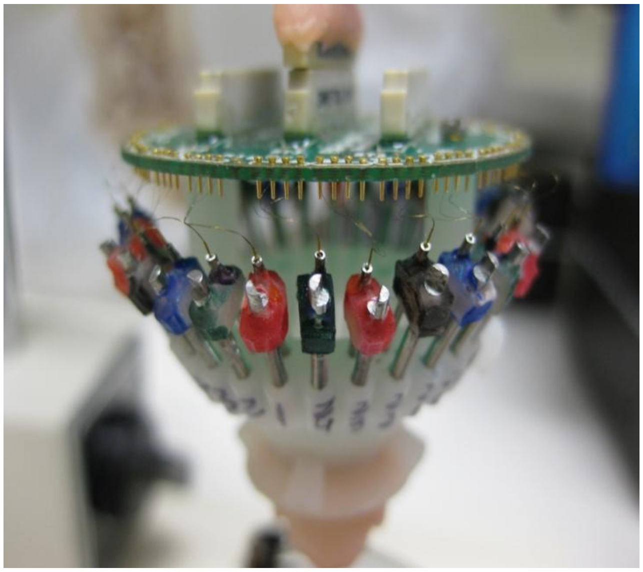
One Long–Evans rat (male; 700g) was housed in a transparent Plexiglas cage. The animal was deeply anesthetized with isoflurane and was implanted with a tetrode micro-drive (Figure #3) aimed to record from the dorsal hippocampus (CA1). All 24 tetrodes in the drive were composed of 12.5 µm nichrome wires. The tetrode tips were gold-plated to reduce impedance to 200kΩ. Tetrodes aimed for hippocampus recordings were inserted 3.8 mm anteroposterior from bregma, 2.4mm mediolateral. Over the course of 34 days, the tetrodes were lowered until pyramidal cells in the CA1 layer with spike bursts were isolated at appropriate depths. Three tetrodes were not sunk past the medio-parietal cortex in order to record the High Voltage Waves (HVW).

Figure #2

Data acquisition, processing, and analysis

During the recording session, the rat was connected to a counterbalanced cable that allowed the animal to move freely in the apparatus. Spike charts and EEG traces for every tetrode are visible on the recording GUI. There is also an auditory device that crackles when a cell spikes. The information that comes from the speaker is often the most useful when distinguishing specific brain events. The independently movable tetrodes, visible in figure #3, are lowered by rotating the screw head (one turn is approx. 250μm). Using the visual and auditory cues as a guide, the tetrodes were lowered into areas with activity corresponding to figures in the literature.

Figure #3



Local Field Potentials were pre-amplified and digitized at 2 kHz sampling rate at 24 bit resolution and stored for off-line analysis (DigiLynx system; NeuraLynx). Raw data were preprocessed using custom program mindcracker written in

Matlab by Daoyun Ji Ph.D. After examining raw data, one trace each from the hippocampus and the cortex were selected for correlation. 28 HVW and 560 ripple events were identified and cross-correlated.

Results

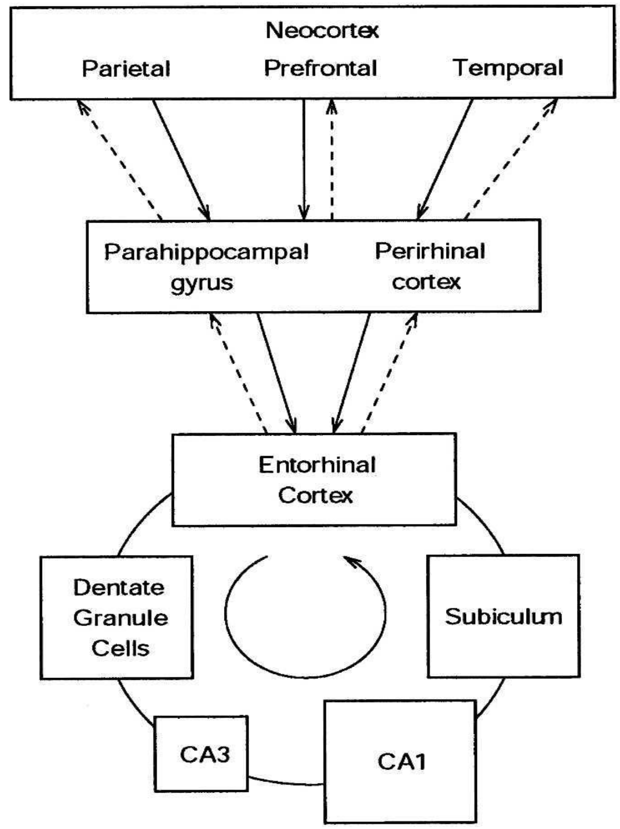
Our hypothesis treated the connection between the cortex and hippocampus as rigid. Therefore any activity in the cortex should trigger a corresponding event in the hippocampus. However, a priori examination of the recorded data revealed that **ripple events are suppressed during High-Voltage Waves.**

(**Appendix A**) Raw data appears to indicate the silencing of ripples during an HVW. Ripples can be clearly identified in the filtered hippocampal trace before and after the HVW, but not in its presence. There are only two traces in the appendix to save space, but these two are good representatives of how the other twenty-six events looked. The average HVW was just over five seconds and the longest was around fifteen. During analysis, these traces could not be filtered due to a bug in mindcracker. Any parameters set to identify these events were useless on an unfiltered trace. Instead, all events had to be manually identified by copying start and end times into a matrix. The ripple trace filtered with no problems and parameters found in literature were used to correctly identify all the events.

(**Appendix B**) Cross-correlation of HVW vs. ripple events confirmed the exclusion that is clearly visible in the raw data. The beginning of the HVW, set at zero, is correlated to hippocampal activity by plotting the summed ripple count for every time lag. The ripple count is high just before an HVW, but drops to zero during the event. Upon cessation of the HVWs the hippocampal ripple activity gradually returns.

Conclusion

Figure #5



Rolls E T Learn. Mem. 2007;14:714-731

In the context of memory consolidation, there is active communication between the cortex and hippocampus. While awake and especially when learning, information moves from the cortex to the hippocampus. During non-REM sleep, the flow of activity reverses in order to assimilate new memories. The reversal of activity is not 100 percent. The connection is like a high-way during rush hour; the net flow of traffic changes depending if it is morning or evening. However, between wake and sleep the hippocampus seems to disengage from the cortex. High-Voltage waves that mark the transition phase are exclusive to hippocampal ripples in the dorsal CA1. The temporal exclusion between High-Voltage waves and ripples is significant because it may provide a window for local circuits to operate independently and reverse in preparation for sleep.

The main purpose of HVW is not understood, but it is the largest and most synchronized event in the neocortex. The fact that the hippocampus becomes unsynchronized during the cortical event does not indicate a causal relationship. Knowing that ripples are suppressed is one thing, but individual cell activity reveals the hippocampus with higher resolution. Even if the EEG is quiet, a finer relationship can exist between CA1 pyramidal cells and neocortex cells.

References

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2. Clemens, Z., M. Molle, et al. (2007). "Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans." Brain: awm146.

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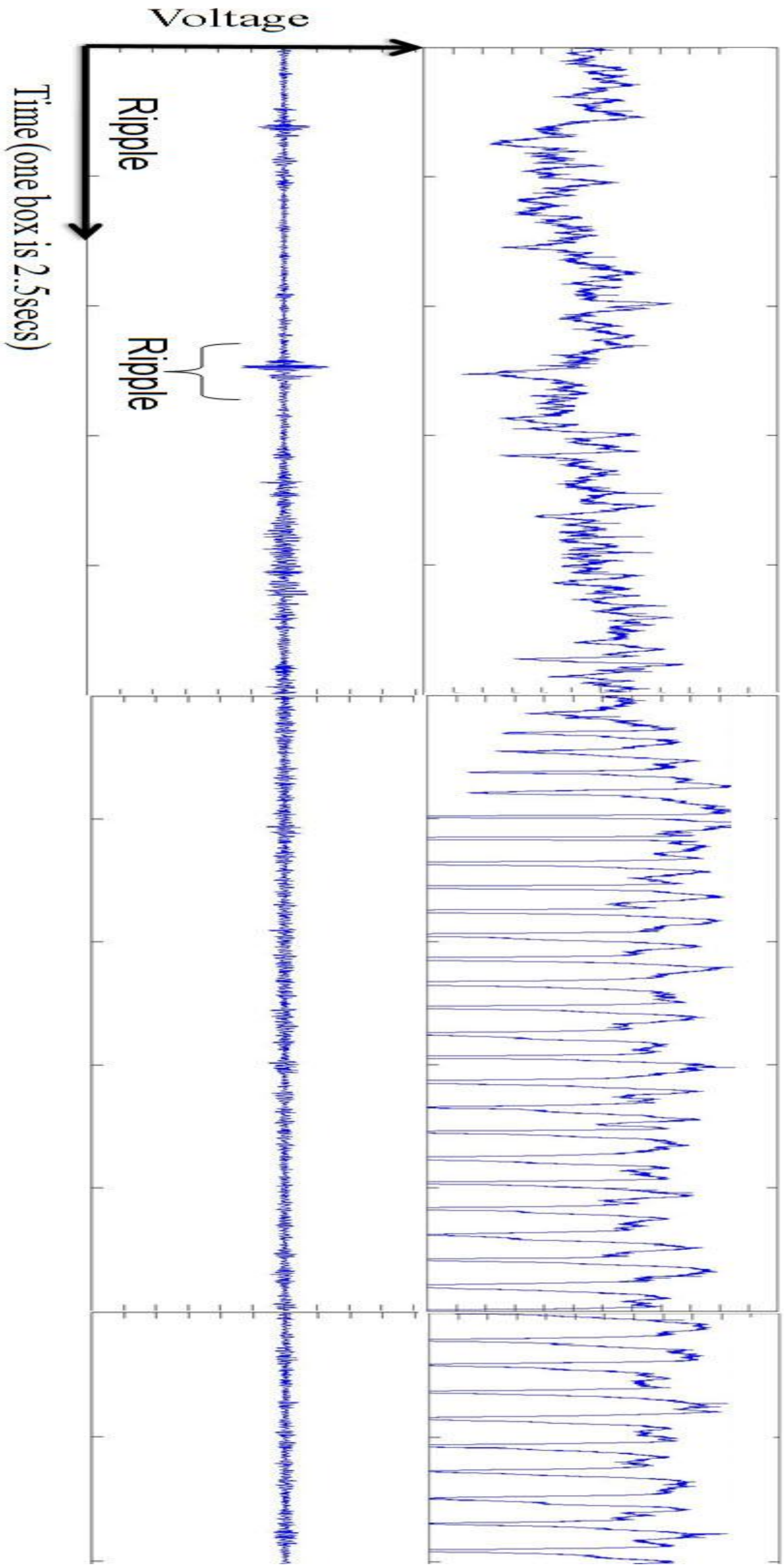
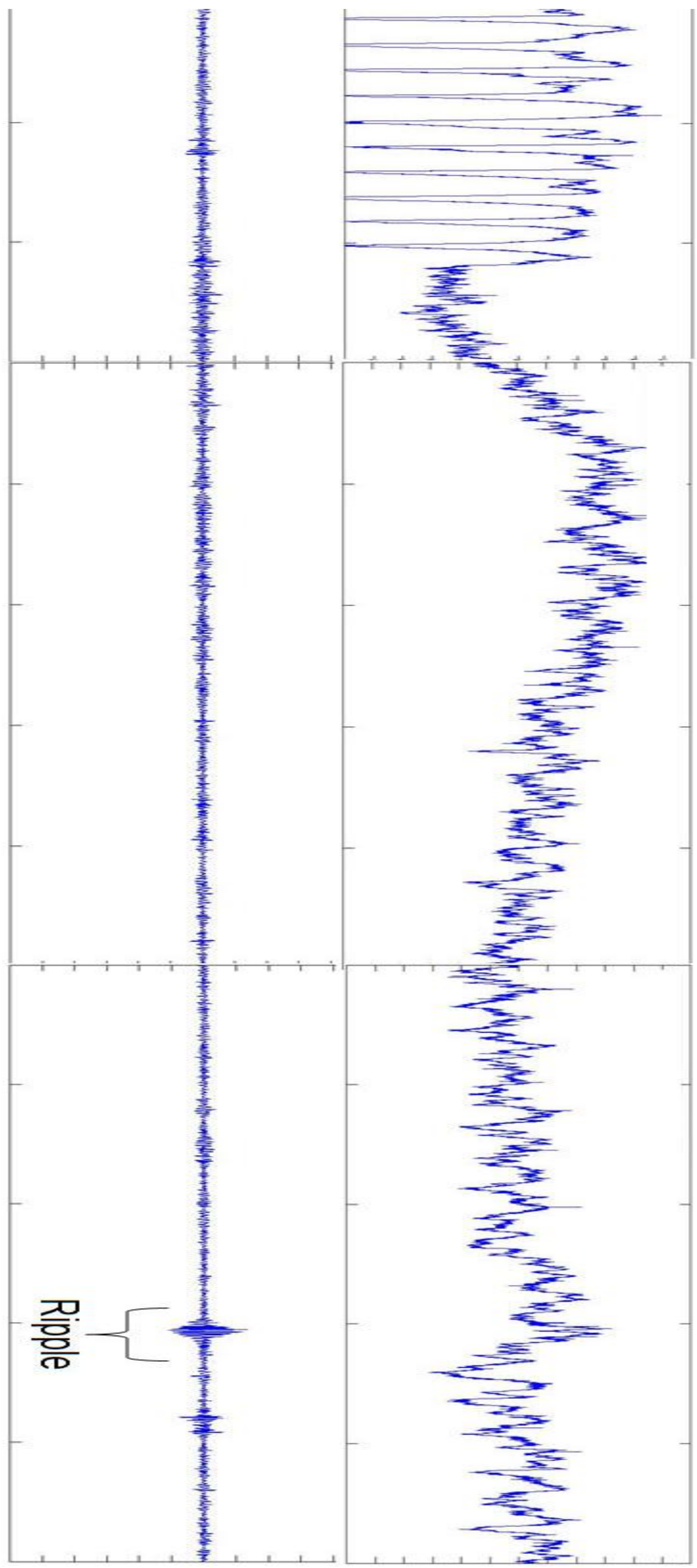
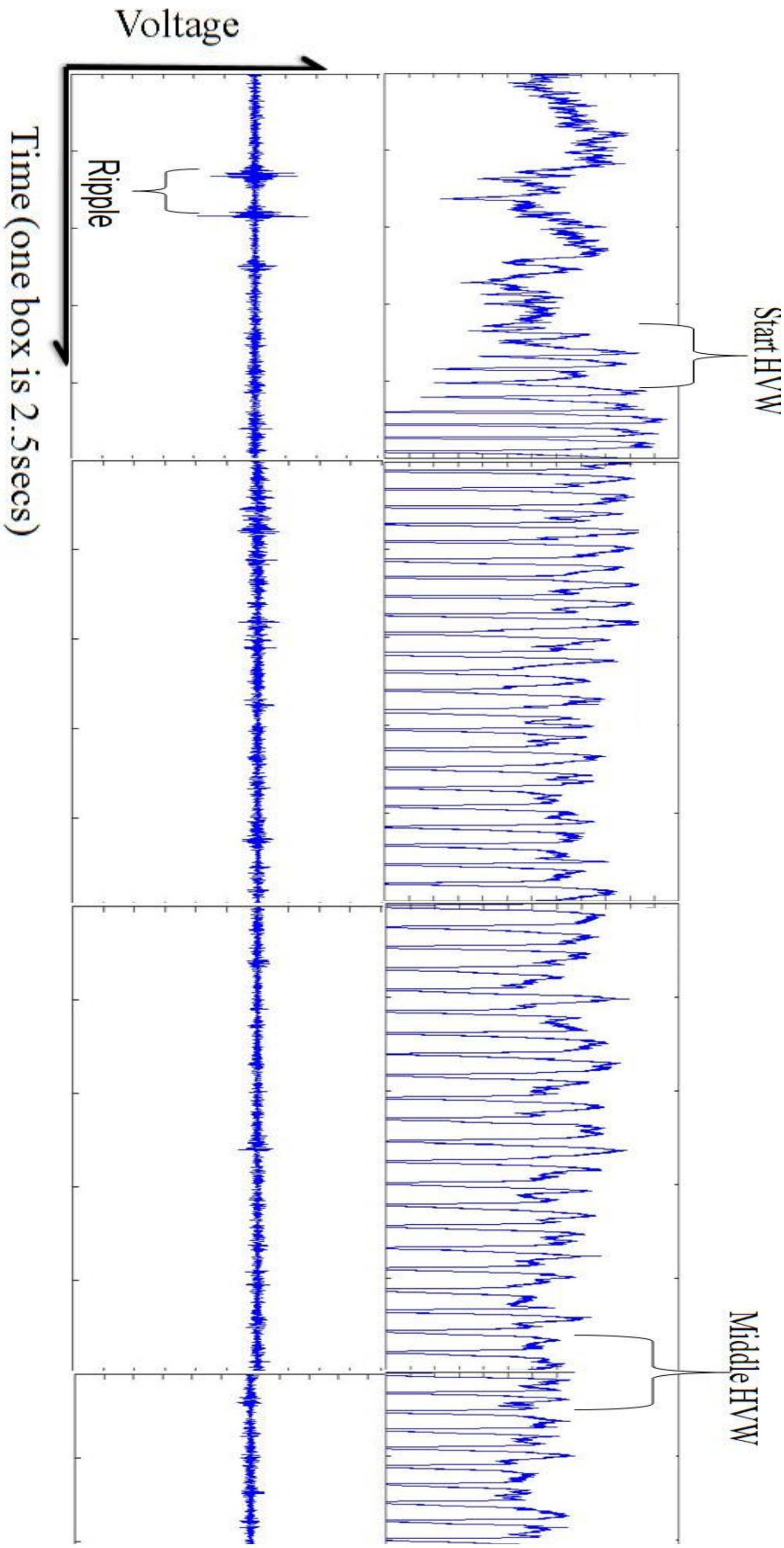
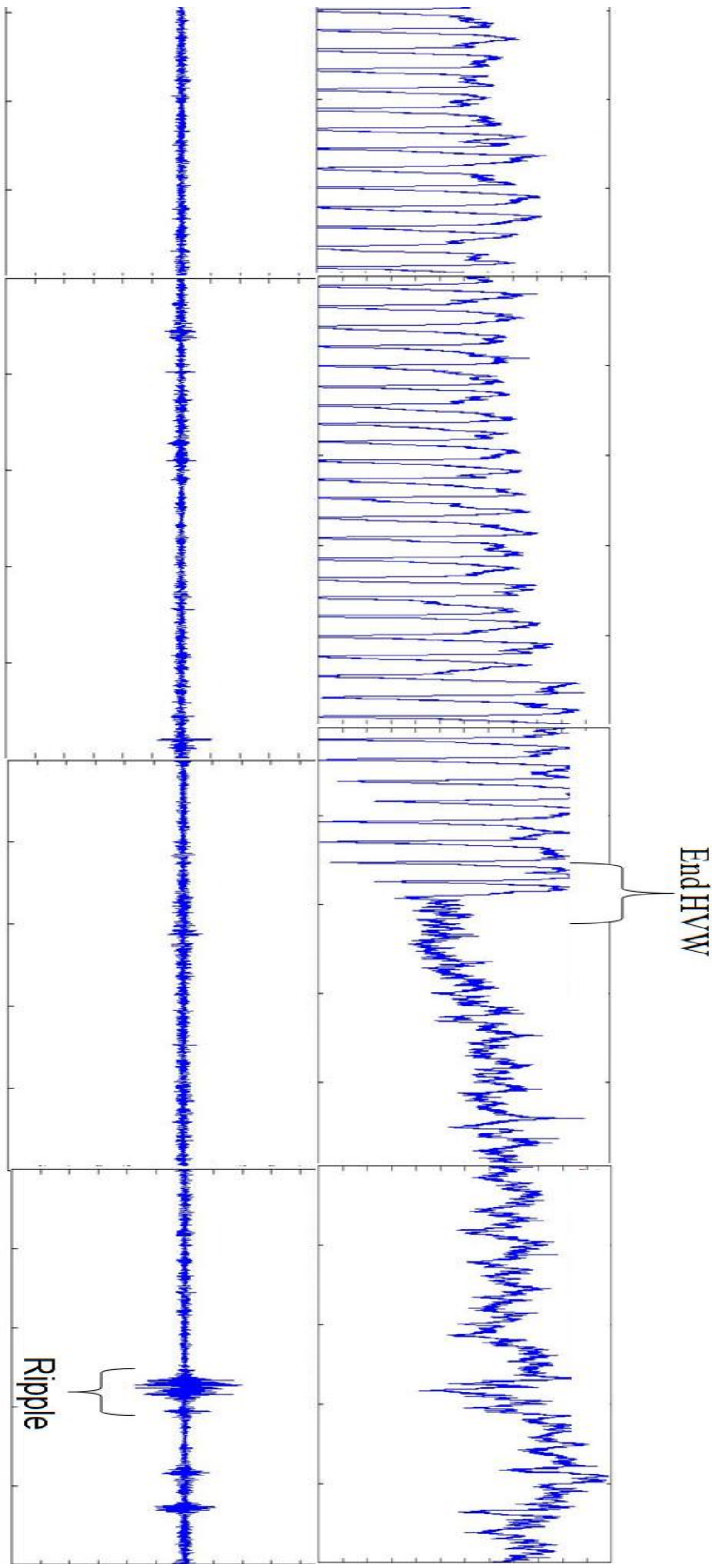
5. Molle, M., O. Yeshenko, et al. (2006). "Hippocampal Sharp Wave-Ripples Linked to Slow Oscillations in Rat Slow-Wave Sleep." J Neurophysiol **96**(1): 62-70.

Appendix A

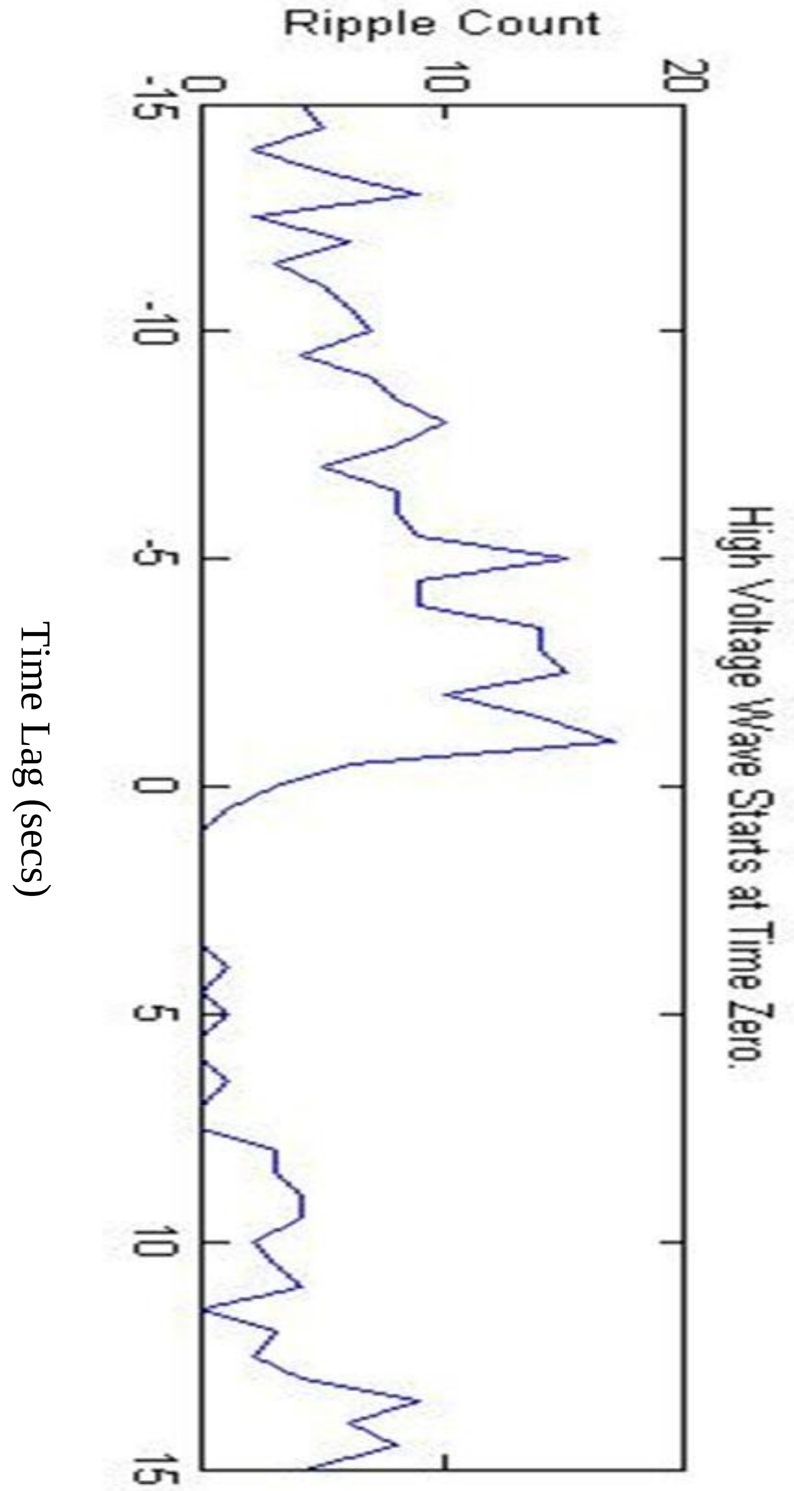



Ripple


Voltage
Time (one box is 2.5secs)
Ripple
Start HVW
Middle HVW


End HVW
Ripple

Appendix B


High Voltage Wave Starts at Time Zero.
Ripple Count
0
10
20
-15
-10
-5
0
5
10
15
Time Lag (secs)