

The Effects of *Hamamelis Virginiana* Extracts on Memory *In Vitro*

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Abstract

The brain has the ability to form memories in a region called the hippocampus. Neurons in the hippocampus communicate with each other at synapses and synaptic changes are proposed to underlie learning and memory. Oxidative Stress contributes to negative effects in the human body, ranging from a hindrance of memory formation to premature cell death and possibly many other neurological disorders. As one ages, memory diminishes and Oxidative Stress increases, this may be caused by several factors including environmental factors. Previous studies performed using a mouse model of low level of lead (Pb) exposure have shown that Pb levels here in El Paso have a negative effect on memory, which could be caused by elevated levels of free radicals resulting in Oxidative Stress(Nava). The range of ailments that come from unbalanced free radicals creates a need for research on antioxidants. This encouraged us to use electrophysiology to test the possible neuronal beneficial role of antioxidants extracted from the plant Witch Hazel (a plant indigenous to North America) and examine the effect it will have on neurological activity in mice hippocampal slices. We hypothesize that these antioxidants will enhance neuronal responses and contribute to enhance memory.

Materials and Methods

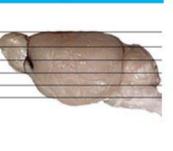
Tissue Sectioning:

For this experiment, we obtained hippocampal slices of brain tissue from Musmusculus C57BL/6 mice. Tissue was mounted onto a Leica Vibratome, horizontal slices were cut 300 μm thick in rostrocaudal extent of the hippocampus.

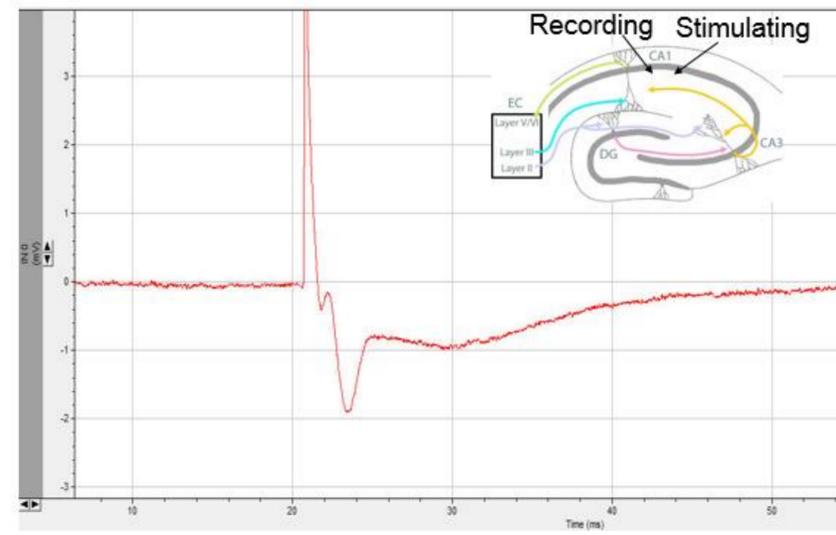
Open-Field Recordings:

Slices are placed in Harvard recording stage, and perfused in artificial cerebrospinal fluid, which is continuously oxygenated. Temperature is maintained at ~29°C, and recordings are taken in unbathed, bathed, and wash out conditions.

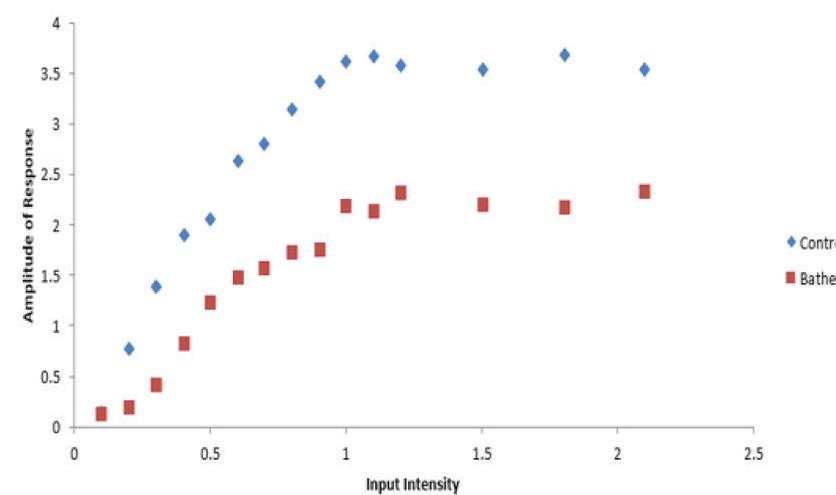
Procedure

<p>Step 1</p>  <p>Anesthetize and sacrifice mouse.</p>	<p>Step 2</p>  <p>Extract brain from mouse.</p>	<p>Step 3</p>  <p>Section brain into slices and separate hippocampus</p>	<p>Step 4</p>  <p>Stimulate & Record in the CA1 Region of the Hippocampus</p>
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Data / Observations



Input Output Curves



Expected Results

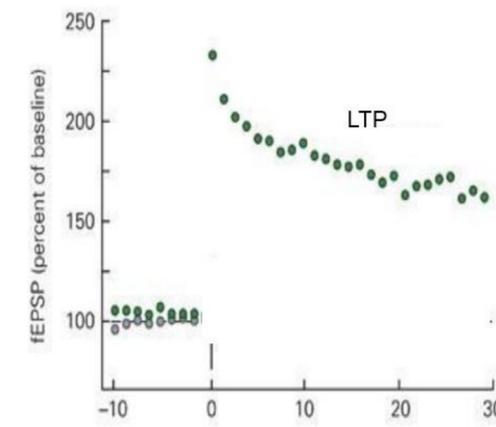


Figure 1:
Control Long Term Potentiation graph

HV- *Hamamelis Virginiana*

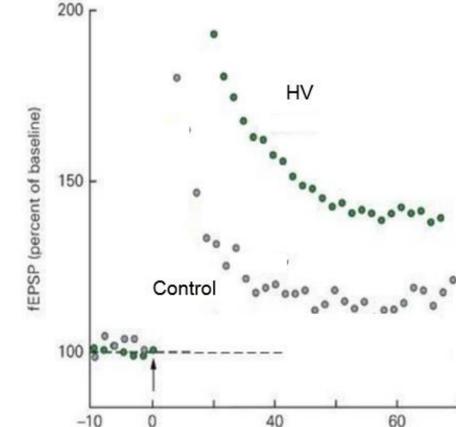


Figure 2:
Expected results of *Hamamelis Virginiana* in comparison with the control

Discussion

The *Hamamelis Virginiana* extract did not show any beneficial effects. Increasing the intensity of the stimulations showed us the relative strength of the neurons. Which did not really differ in the bathed experiment when compared to the control. This means that the extract did not exhibit statistically significant changes in the synaptic communications of the neuronal networks of the hippocampus.

Long Term Potentiation was tested in order to observe the effects of the plant extract on the formation of long term potentiation. We used completely normal healthy mice in order to test the normal development of Long Term Potentiation. When we compared the bathed slice to the control LTP formation we observed a diminished ability to form LTP, however this may be

caused by a number of factors and we cannot definitively say that this was due to the extract. Some of these effects may be due to the properties of the extract which could have been harmful towards the slice. We also did not have a dosage curve which did not allow us to determine an ideal dose for experiments run, which may have affected the viability of the slices. We were also only able to run one experiment for LTP which does not allow us to make any claims about the significance of the data.

Results

Stimulation and recording of the hippocampal CA1 region allowed us to characterize the neuronal properties and the synaptic strength between the networks of the stratum radiatum. The initial non synaptic fiber volley, whose amplitude is linked to the amount of recruited afferent fibers, was followed by a prominent synaptic response. The strength of the synaptic response was measured by taking the slope of the recorded response. Once a stable baseline was established, basal synaptic transmission was assessed by gradually increasing the stimulation intensity. Overall we found no differences in the amplitude of the fiber volley nor in the stimulus response curve.

In the LTP procedure, the brain slice was stimulated every thirty seconds for ten minutes, followed by two 100Hz stimulations with twenty seconds in between. After the two 100Hz stimulations, the brain slice was again stimulated every thirty seconds for an hour long. With this procedure, we can see how well the development of Long Term Potentiation occurs.

The outcomes of the procedures showed that the plant extract antioxidants had no beneficial effect on Long Term Potentiation. In the input/output and Paired Pulse tests we found no significant changes in the synaptic responses. However in the LTP test, although only one experiment was done, we observed a decrease in Long Term Potentiation.

Future Directions

In the future, we would like to run a dosage curve to find a concentration that would work best for the experiments. Performing the experiments in triplicates to ensure that the data is valid. Lastly, we would like to purify the plant extract more or even focus on one specific antioxidant from the extract.