Introduction: Therapeutic hypothermia and histone deacetylases inhibitors (HDACi), such as valproic acid (VPA), have independently been shown to have neuroprotective properties in models of cerebral ischemic and traumatic brain injury. However, the depth of hypothermia and the dose of VPA needed to achieve the desired result are logistically challenging. It remains unknown whether these two promising strategies can be combined to yield synergistic results. We designed an experiment to answer this question by subjecting hippocampal-derived HT22 cells to severe hypoxia in vitro. Hypoxic cells were treated with 1 mM of VPA, a concentration within the typical therapeutic range, and incubated at 32 or 37 degree C for 30 hours (n=4/condition). Cellular viability was evaluated by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and lactate dehydrogenase (LDH) release assays. Levels of acetylated histone H4 were measured by Western blotting 6 and 24 hours following the VPA treatment as a marker of biologic effectiveness (increase in histone acetylation). Results: High levels of acetylated histone H3 were detected in the VPA treated cells. Release of LDH (figure) was greatly suppressed following the combined hypothermia + VPA treatment (0.269 ± 0.003) versus VPA (0.836 ± 0.026) or hypothermia (0.451 ± 0.005) treatments alone (p < 0.05). MTT assay showed that the number of viable cells increased by 29.3% when VPA and hypothermia were used in combination (p < 0.05). Conclusions: This is the first study to demonstrate that the neuroprotective effects of VPA and hypothermia are synergistic. This novel approach can be used to develop more effective therapies for the prevention of neuronal death.