Casper, D. 2008. PEMF potentiates the Induction of Nitric Oxide by Glutamate and 6-Hydroxydopamine in a Neuronal Cell Line. Bioelectomagnetic Society Meetings June, Davos, Switzerland.

Introduction: Nitric oxide (NO) is essential for neuronal viability, but it can also be toxic in high concentrations. Generally, neuronal nitric oxide synthase (nNOS) produces NO that participates in survival signaling pathways. During inflammation, inducible NOS (iNOS) produces 10-fold greater amounts, resulting in toxicity. Using a cell line of rodent dopaminergic neurons, we used glutamic acid, a neurotransmitter and excitotoxin, and 6-hydroxydopamine (6-OHDA), an oxidative stressor, to model the initiating events of the neurodegenerative process. Both of these insults cause a transient increase in cytosolic free Ca2+, which activates resting calmodulin (CaM), a calcium binding protein. This is known to rapidly activate the Ca2+-dependent NO cascade as the body attempts to control inflammation. In this study we tested the effects of specific PEMF signals configured to enhance Ca2+ binding to calmodulin (Ca/CaM) to activate the synthesis of NO in the presence of both neurotoxins.

Materials And Methods: N9D, a rodent dopaminergic hybrid cell line, was provided by Dr. Alfred Heller at the University of Chicago. Cells were dissociated, plated in 35 mm culture dishes, and allowed to attach for at least 24 hours before treatment. For experiments, cultures were equilibrated in Krebs buffer for 10 minutes, after which 30 μM 6-OHDA, 100 μM glutamic acid, or buffer alone were added. A PEMF signal, configured a priori to accelerate the kinetics of Ca2+ binding to CaM, and consisting of 5 msec bursts of 27.12 MHz sinusoidal waves at 5 bursts/sec and 0.05 Gauss peak amplitude, was administered beginning two minutes prior to the addition of toxin. Control cultures were subjected to "null" signals under identical conditions. NO levels were measured by an electrochemical detection system (WPI) in real time from equilibration in Krebs buffer until the effects of the added toxins or buffer were no longer detectible. NOS inhibitors were added at least 30 minutes prior to toxins and PEMF signals. NO levels were calculated from the maximum change in current induced by toxin ± PEMF treatment minus the values obtained by adding buffer alone. Data were compared by ANOVA and Fischer's PLSD post-hoc tests. Results: The selective dopaminergic neurotoxin 6-OHDA produced a rapid 500 nM increase in NO within 10 seconds. NO production decayed over 2 minutes, returning to baseline levels. When PEMF signals were applied to culture dishes prior to and during the addition of toxin, this increase was potentiated more than 200%, with measured NO concentrations of 900 nM. To confirm that this increase was due to NO synthesis, treatments were repeated in the presence of 3.3 mM L-NAME, a non-selective NOS inhibitor. Results indicate that LNAME diminished NO levels to 33% of cultures treated with 6-OHDA alone, and to 24% of NO levels induced by 6-OHDA + PEMF. Similar results were obtained in the presence of glutamate, an established endogenous neurotransmitter that acts on dopaminergic neurons through both ionotropic and metabotropic receptors. With this stimulant, the increase in NO production was lower in magnitude, increasing NO levels less than 100 nM. PEMF signals potentiated this increase by133%. Neither 6-OHDA nor glutamate were toxic over this time course. Conclusions: Our results demonstrate very rapid and transient effects of PEMF signals on NO synthesis in a neuronal cell line in two models of dopaminergic cells death. While higher concentrations of toxins and longer incubations may result in toxicity, the effects observed here are immediate early events in response to toxic challenge. While 6-OHDA causes oxidative stress, there is also an inflammatory

response to this compound in vivo and in vitro that may be inherent to dopaminergic neurons themselves even in the absence of other cell types. In contrast, glutamate-mediated cell death involves the influx of calcium ions through receptor ion channels. Importantly, relatively low-level increases in NO production have been shown to initiate neuroprotective signaling cascades that could increase resistance to both of these insults. Experiments to distinguish the role of inflammatory events and calcium influx in the mechanism by which PEMF affects NO signaling are in progress.