Noninvasive and targeted interruption of the blood brain barrier for drug delivery using focused ultrasound in the treatment of CNS disorders

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Thesis

NONINVASIVE AND TARGETED INTERRUPTION OF THE BLOOD BRAIN BARRIER FOR DRUG DELIVERY USING FOCUSED ULTRASOUND IN THE TREATMENT OF CNS DISORDERS

by

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ABSTRACT

Despite the prevalence of CNS disorders, treatment options for CNS disorders fall woefully behind treatment options for other systemic disorders. This is due to the presence of the blood brain barrier (BBB) acting as an obstacle, preventing foreign substances from entering the brain. A newly developed and innovative biomedical procedure attempts to bypass the BBB in the delivery of therapeutics by using focused ultrasound (FUS) to disrupt and temporarily open the BBB. The use of FUS-facilitated BBB opening is able to target specific tissue for noninvasive, localized BBB penetration.

As the technique is experimental and in its nascent stage of development, there are only a few studies that investigate its abilities in delivering treatments directly to the brain. The studies involve delivery of large, hydrophilic molecules that traditionally would not be able to bypass the BBB and enter the brain, and analysis of CNS concentrations of the molecules after FUS treatment, as well as the therapeutic successes.

Results of FUS the studies are promising and the results demonstrate that the procedure is able to significantly increase drug concentrations in the brain, increase
survival rates in animal models, decrease tumor growth, and decrease tumor margins and volume. The potential and power of FUS should be further explored as the future of CNS disorder treatments.
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LIST OF ABBREVIATIONS

AAV2-GFP ........................................... Adeno-associated Virus-Green Fluorescence Protein
Aβ ........................................................................................................ Beta-Amyloid
AD ........................................................................................................ Alzheimer’s Disease
API ....................................................................................................... Active Pharmaceutical Ingredient
APP .................................................................................................... Amyloid Precursor Protein
BBB ..................................................................................................... Blood Brain Barrier
CNS ...................................................................................................... Central Nervous System
CSF ...................................................................................................... Cerebrospinal Fluid
DOX ...................................................................................................... Doxorubicin
GBM .................................................................................................... Glioblastoma Multiforme
GFP ...................................................................................................... Green Fluorescence Protein
IgG ...................................................................................................... Immunoglobulin G
IgM ...................................................................................................... Immunoglobulin M
MAb .................................................................................................... Monoclonal Antibody
MRI ...................................................................................................... Magnetic Resonance Imaging
MW ...................................................................................................... Molecular Weight
rAAV .................................................................................................. Adeno-associated Viral
TMZ .................................................................................................... Temozolomide
INTRODUCTION

Central Nervous System Disorders

Despite incredible advances in modern medicine, biotechnology, research, and pharmaceuticals, disorders of the central nervous system (CNS) remain one of the most elusive fields of treatment and one of the most prevalent afflictions with the highest rates of death. CNS disorders can be neurologic or psychiatric, and encompass a wide range of diseases affecting either the brain or the spinal cord, including, but not limited to ailments such as: epilepsy, Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, schizophrenia, multiple sclerosis, and brain cancer (National Institute of Neurological Disorders and Stroke, 2014).

Table 1 presents summary statistics for five of the most prevalent CNS disorders in the United States, afflicting a staggering number of 9,050,000 total patients (Brain Institute, 2014). According to the U.S. Department of Health and Human Services, Alzheimer’s and Parkinson’s disease were respectively the 6th and 14th leading causes of deaths in the United States in 2011, and brain cancer was the 8th leading cause of cancer-related deaths (Hoyert, 2012).
Table 1. Summary statistics for five of the most prevalent CNS disorders in the United States (Table taken from Hoyert, 2012).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Patients Diagnosed in United States</th>
<th>Number of deaths in 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>4,500,000</td>
<td>84,691</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>2,300,000</td>
<td>50,000</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>1,500,000</td>
<td>23,107</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>400,000</td>
<td>2,844</td>
</tr>
<tr>
<td>Tumor (brain and NS)</td>
<td>350,000</td>
<td>14,492</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9,050,000</strong></td>
<td><strong>175,134</strong></td>
</tr>
</tbody>
</table>

As demonstrated in Table 1, the need for increased therapeutic potency for CNS disorders is real, but pharmaceutical developmental progress is severely hindered by the physiological structure of the CNS.

**Blood Brain Barrier**

The existence of the tight junctions between endothelial cells in the cerebral vasculature creates the blood brain barrier (BBB), a tight protective seal against the entry of proteins into the brain (Barrett, 2012). The BBB serves to maintain the environment of the brain and acts as a protective mechanism against the various circulating pathogens and foreign bodies in the blood (University of Texas Medical School, 2013).

At a cellular level, the BBB (seen in Figure 1) is comprised of cerebral endothelial cells joined together by tight junctions (Abbott, 2009). Tight junctions are created by a network of transmembrane proteins such as claudin and occludin, which are
anchored to the actin of the cytoskeleton on each protein end, creating a tight seal between the two plasma membranes. Endothelial tight junctions, as well as a basement membrane directly under the endothelial cells, protect the cerebral vasculature. In addition, pericytes are also present intermittently along the capillaries, with astrocytic processes surrounding the entire BBB structure. While complex, these protective structures serve to prevent foreign particles from penetrating the brain.

Figure 1. Cellular structure of the blood brain barrier. The blood brain barrier is comprised of endothelial cells linked by tight junctions, a basement membrane, as well as the occasional pericyte (Figure taken from Abbott, 2009).
The efficacy of the BBB in preventing hydrophilic protein infiltration into the brain can be demonstrated by the constant intravenous infusion of urea and comparing the relative concentrations of urea in the tissues of the muscle, brain, and cerebrospinal fluid (Figure 2). In this figure, it can be seen that the concentration of urea in the muscle tissues increases rapidly in the minutes following the beginning of urea infusion, and then eventually equilibrates with the plasma urea concentration after an hour of constant infusion. However, while the brain and cerebrospinal fluid concentrations of urea rise slightly during the course of infusion, their concentrations never equilibrate with that of the plasma. The existence of the tight junctions of the BBB prevents the entry of urea into the brain, effectively creating a barrier that discourages change to the local environment.

Despite its necessary defensive properties, the BBB proves to be a cumbersome obstacle to drug delivery targeted to the brain and reduces the efficacy of such drugs immensely.
Figure 2. Infiltration of urea into various tissues. Constant intravenous infusion of urea demonstrates the relative impenetrability of the brain and cerebrospinal fluid (CSF) (Figure taken from Barrett, 2012).

Current CNS Disorder Therapeutic Options

The current development of therapeutics for CNS disorders lags woefully behind therapeutics for other system disorders, as the existence of the BBB creates a bottleneck against many potentially promising neurotherapeutics (Pardridge, 2005). The BBB proves selectively permeable for small (<400 Da), lipophilic molecules, and demonstrates exponential impermeability against molecules with increased surface area (Fischer, Gottschlich, & Seelig, 1998). In consideration of the physical limitations posed by the existence of the BBB, most therapeutics in the form of synthesized proteins, monoclonal antibodies (MAb), and enzymes are excluded from passage in the CNS (Pardridge, 2007).
While a given indication might prove efficacious in vitro, there is no delivery system capable of transporting the drug past the BBB in an adequate amount.

A look at the currently approved therapeutics for the treatment of CNS disorders such as depression (Table 2), Alzheimer’s disease (Table 3), and schizophrenia (Table 4) reveals a trend of developed therapeutics with a low molecular weight, lack of ionization at physiological pH, and high lipophilicity—qualifications that severely limit the options available for developing CNS disorder therapeutics. Strategies of temporarily manipulating the BBB include transient osmotic opening of the BBB, utilizing chemical transporters to increase endocytosis or uptake of drugs, and biodegradable implants—however, each method has proven to have limited success due to toxicity and safety concerns, or issues relating to low efficacy (Misra, 2003).

<table>
<thead>
<tr>
<th>API</th>
<th>MW (Da)</th>
<th>log p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>276.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>333.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>324.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>329.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Sertraline</td>
<td>306.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>319.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table 2. Approved drugs for the treatment of depression and their physical characteristics (Table taken from Banks, 2009).
Table 3. Approved drugs for the treatment of Alzheimer’s disease and their physical characteristics (Table taken from Banks, 2009).

<table>
<thead>
<tr>
<th>API</th>
<th>MW (Da)</th>
<th>log p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil</td>
<td>379.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Galantamine</td>
<td>287.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Memantine</td>
<td>179.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>250.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Tacrine</td>
<td>198.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 4. Approved drugs for the treatment of schizophrenia and their physical characteristics (Table taken from Banks, 2009).

<table>
<thead>
<tr>
<th>API</th>
<th>MW (Da)</th>
<th>log p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>448.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>318.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Clozapine</td>
<td>326.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>375.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Risperidone</td>
<td>410.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>312.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>383.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>412.9</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Another method for improving drug uptake is to create a prodrug precursor for the active compound (Han, 2000). A prodrug is an inactive form of the active species, and would be specifically biochemically designed to improve the lipophilicity through the addition of a nonpolar particle to the compound in order to increase its chances of bypassing the BBB. Once past the BBB, the nonpolar portion would be cleaved and the active compound released. Unfortunately, while the creation of the prodrug increases mobility of the compound across the BBB, it also increases uptake into other tissues, creating negative and unintended side effects. Additionally, prodrugs have the potential to undergo alternative cleavage and activation pathways, resulting in toxic metabolites that cause damage to surrounding tissue or tumorigenesis (Nelson, 1982).

Direct intracranial injection, an alternative method of CNS therapeutic delivery, has unfortunately been met with little success. The rapid clearance rate of cerebral fluids and the limited diffusion prevent effective drug distribution, and the invasive procedure of intracranial injection creates tissue damage at the area of therapeutic delivery (Krewson, Klarman, & Saltzman, 1995).

**Focused Ultrasound**

Focused ultrasound (FUS) is a procedure that utilizes the energy of supersonic waves to create local disturbances deep within the tissue without damaging the tissue surface. The physiological effects and biomedical potential of FUS were first explored by Lynn and colleagues in 1942 (Lynn, 1942). Lynn’s research demonstrates that the effects of the FUS are directly correlated with physical parameters such as pulse length and
frequency, and that FUS is capable of delivering localized and directed damage to tissue through the adjustment of the stimulating beam’s focal point. His results also show minimal damage to the tissue in the path of the FUS beam directly above the beam’s focal point, as well as in the tissue immediately surrounding the focal point (Table 5).

**Table 5. Beef liver changes produced by Focused Ultrasound.** The localized and contained effects of FUS can be observed. The surface tissue in the direct path of the stimulating beam remains free from damage, and the focusing effects of the beam is most effective when pulse length is minimized and power maximized (Table taken from Lynn, 1942).

<table>
<thead>
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<th>Liver block experiment......</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-200 plate, volts..........</td>
<td>600</td>
<td>1750</td>
<td>1750</td>
<td>2410</td>
<td>2410</td>
</tr>
<tr>
<td>T-200 plate, amp............</td>
<td>0.060</td>
<td>0.120</td>
<td>0.120</td>
<td>0.220</td>
<td>0.220~+</td>
</tr>
<tr>
<td>R.F. output, amp............</td>
<td>0.200</td>
<td>0.700</td>
<td>0.700</td>
<td>0.900</td>
<td>0.900~+1.00</td>
</tr>
<tr>
<td>Total exposure time, sec.</td>
<td>30</td>
<td>30</td>
<td>180</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Time from 0 to power indicated, sec......</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>Instantaneous</td>
</tr>
</tbody>
</table>

FUS’s ability to cause rapid coagulation necrosis in tissue has been used effectively in clinical cases against diseased tissue, such as in treatment of cancer (Blana, 2004). In a 5-year longitudinal study involving 146 patients with Stage T1-T2N0M0
prostate cancer, Blana was able to demonstrate the capability of FUS in the localized destruction of the targeted tissue, without damage to neighboring areas.

In a safety and efficacy study conducted by McDannold on nonhuman primates, he was able to establish that BBB opening using FUS did not result in cerebral tissue damage, or have visual or behavior effects on the rhesus macaques. All the monkeys recovered completely after BBB opening, supporting the safety of this procedure (McDannold, 2013). The noninvasive procedure and low-morbidity risk of the FUS makes it an invaluable tool in treating patients unable to undergo dramatic and invasive surgical options.

The shortcomings of current CNS therapeutic options combined with the advantages of the FUS technique contribute to making FUS a prime candidate for the future of CNS therapeutic treatment.

**Microbubble Enhancement**

The efficacy of the drug delivery aided by FUS can be further enhanced by the addition of microbubbles, synthesized shell structures with short half-lives (Liu, 2014). Current microbubble designs incorporate materials such as albumin, phospholipids, or polymers as its biodegradable shell, which allow the microbubbles to be more stable (Frinking, 2000). Microbubbles are routinely used to perfuse organs for diagnostic purposes, due to their ability to enhance the contrast between the vasculature and neighboring tissues during ultrasound imaging. Commercial microbubbles are typically 2-4 μm in size and have a life span of 5-10 minutes.
When used in conjunction with FUS, microbubbles have been found to enhance the spread of the drug at its delivery target. Microbubbles, when stimulated by ultrasound, can interact with the local environment through stable or inertial cavitation (Figure 3). Through stable cavitation, the energy of the FUS causes repeated microbubble expansion and contraction of the microbubble, the pressure of which, when applied to cell surfaces, causes changes in cell permeability through adjustments in ion channel permeability (Sboros, 2008). Through inertial cavitation, the energy of the FUS causes such rigorous microbubble expansion and contraction that it implodes, agitating the local cell membranes enough to increase permeability due to the release of shock waves and local heat (Dalecki, 2004).

Figure 3. Schema of acoustic pressures and their effects on microbubble behavior. Stable cavitation is the constant but stable oscillation in size of the microbubble, and inertial cavitation is the extreme agitation of a microbubble using acoustic pressures such that it collapses and generates shock waves (Figure taken from Lentacker, 2009).
The size of the microbubbles and the frequency of the FUS were found to be critical factors in the opening of the BBB. In a study conducted by Choi, microbubbles with larger diameters, resonated at the same frequencies, were found to be the more effective in BBB penetration than smaller microbubbles (Choi, 2010). This observation is most likely due to the higher mechanical stress larger microbubbles place on cell membranes compared to smaller microbubbles.

With the combinatory use of microbubbles in the CNS vasculature and FUS, the efficacy of BBB opening can increase significantly.
FUS TECHNOLOGIES

While FUS has been shown to noninvasively and locally penetrate the BBB, the procedure is still very novel, mostly conducted in the last three years, and research on its potential in relation to the treatment of CNS disorders is still in its nascent stages. Very few experiments have been conducted that does not simply prove that FUS is able to facilitate the temporary opening of the BBB, but actually attempts to locally deliver medications to the brain and assess the success of the delivered therapeutics for the given indication.

The goal of this study is to assemble and examine the range of experiments completed that has aimed to deliver a therapeutic substance, one that would have been unable to penetrate the BBB by traditional methods of administration, to the brain in treatment of a CNS disorder. This study organizes experiments by intended indication in order to discuss current methodologies and to assess the feasibility, efficacy, and future potential of the novel and experimental procedure of FUS-facilitated penetration of the BBB in the delivery of CNS disorder therapeutics.
PUBLISHED STUDIES

The use of FUS has been shown to increase the permeability of the BBB to proteins and other hydrophilic components. One of the prime advantages of FUS over other therapeutic techniques is its localized and reversible effect on the BBB, which is in stark contrast to previous methods of intracranial injections or removal of skull pieces, an invasive and high-risk method of drug administration (Hynynen, 2003; Jordao, 2010).

FUS contains the BBB to a circumscribed area by centering the ultrasonic beam onto the region of the brain for which the therapeutic agents are formulated. Administration of microbubbles prior to FUS treatment further improves the efficacy of BBB opening by minimizing damage to neighboring tissues and containing the damages to the cerebral vasculature (Choi, 2008; Hynynen 2003). The current studies addressing the usage of this technique demonstrates its delivery of a range of therapeutics in the treatment of a myriad of CNS disorders.

**Glioblastoma**

Glioblastoma multiforme (GBM) is a common and destructive form of brain tumor with a median survival length of 4 ½ months without treatment (Johnson, 2012). Incredibly fast-acting and aggressive, GBM is pathologically characterized by necrotizing tissues surrounded by anaplastic cells, which are in turn nurtured by hyperplastic blood vessels. GBMs form very quickly in the cerebral white matter, and can be visualized by a CT scan (Figure 4).
Figure 4. CT scan of the brain of a patient with GBM. The tumor can be seen as a dark mass representing necrotic brain tissue in the right hemisphere, with irregular thick margins (Figure taken from Weerakkody, 2014).

The current modes of treatment for GBM are combinatory methods of surgery, radiation therapy, and chemotherapy. Despite the available treatment options, the prognosis of GBM is still poor, with the medial survival time post-diagnosis being 15 months (Huse, 2013). While surgical resection is the first line of treatment, it is incredibly invasive and, due to the tumor’s tendency to be widely diffused, may not
completely remove the cancerous cells. In most cases, residual tumor cells are still left after surgery, and can only be removed through radiation therapy or chemotherapy. Temozolomide (TMZ) is a DNA alkylating agent offered to GBM patients, which has increased the 5-year survival rate from a dismal 2% to 10% (Johnson, 2012). Despite the increased survival rate TMZ has been able to provide for GBM patients, it still demonstrates high inefficiency as its blood concentrations must remain low due to the toxic side effects of the drug, yet much of its therapeutic effects are inhibited by the BBB, preventing the drug from even entering the CNS.

Wei, in a preclinical study, demonstrated the use of FUS opening of the BBB to deliver TMZ to the brain was an improvement of current GBM treatments (Wei, 2013). In a three-part experiment, Wei first established that FUS was effective in the penetration of the BBB with Evans Blue dye.

The nonreactive Evans Blue dye would allow for easy visual confirmation of BBB opening by dying the brain tissue a bright blue once the BBB was penetrated. Figure 5 shows that brain tissue sonicated with FUS allows for a statistically significant increase in concentration of Evans Blue dye compared to unsonicated brains. Thus, the results reveal that FUS had a significant effect in BBB opening, through the visualization of the infiltration of Evans Blue dye into the brain tissue of rats treated with FUS.
Figure 5. Evans Blue dye concentration in the brains of rats with and without FUS treatment. The concentration of dye deposited in the brain without FUS treatment is significantly lower for both normal and tumor rats. FUS mediated BBB opening demonstrated a 3.8 times increase in dye concentration in normal rats (p < 0.001), and a 2.1 times increase in dye concentration in tumor rats (p = 0.09) (Figure taken from Wei, 2013).

In the second arm of the experiment, control (rats not receiving any treatment procedures) and experimental (rats receiving treatment procedures) rats were given TMZ orally and divided into without FUS-treatment and with FUS-treatment groups. The concentration of TMZ in the CSF and blood plasma was then measured for all rats. The results are presented in Figure 6.
The results demonstrate that while FUS treatment elevated CSF concentrations of TMZ, the differences were not significant (p = 0.225), and the blood plasma concentration differences between rats with and without FUS treatment did not differ significantly at all (p = 0.909). However, when the CSF/Plasma ratio was determined, it showed that the ratio was 22.7±3.9% in the group without FUS treatment and 38.6±16.8% in the group with FUS treatment, showing that FUS opened the BBB, and increased the TMZ concentrations in brain tissues.
In the final part of the experiment, the efficacy of combinatory TMZ and FUS treatment was evaluated by measuring the growth of tumor volume between day 10 and day 17, for control and experimental rats. The experimental rats were orally administered low (50 mg), medium (75 mg), and high (100 mg) doses of TMZ, and only rats undergoing medium doses of TMZ were concurrently treated with FUS. The results are presented in Figure 7.

![Figure 7](image)

**Figure 7. Tumor progression amongst rats undergoing various level of TMZ and FUS treatment.** a) Tumor volume (mm$^3$) in rats of various treatments on day 10 and day 17. Combined TMZ and FUS treatment was most effective in tempering tumor growth. b) Ratio of tumor volume from day 17 to day 10, derived from a) (Figure taken from Wei, 2013).

The results clearly show that the combined TMZ and FUS treatment was far more effective in tempering tumor growth than the sole oral administration of TMZ. In
addition, a lower dosage of TMZ treatment in conjunction with FUS was able to produce slower tumor progression than just the higher TMZ dose alone, which reduces the necessity of taking higher doses of TMZ, a substance with toxic effects when administered in high quantities. Wei’s study shows that combined drug and FUS treatment has great potential in increasing drug CSF concentrations, increasing drug efficacy, and reducing the need for higher drug dosages.

A similar experiment conducted by Yang and colleagues revealed similar results (Yang, 2012). Also in an attempt to treat GBM using chemotherapy in conjunction with FUS, Yang explores the anti-tumor effects of another therapeutic agent, doxorubicin (DOX). DOX has been shown to be effective in the treatment and targeting of GBM, both extending the survival time as well as reducing the growth of the brain tumor (Lesniak, 2005). However, the use of DOX is tempered by its tissue toxicity at the necessary therapeutic level, as well as the physiological barrier that the BBB poses.

Yang’s experiment involved the creation of liposomal DOX. Liposomes were created using hydrogenated soybean L-α-phosphatidylcholine, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000], extruded through polycarbonate membranes of a pore size of 0.05 μm, creating a suspension of liposomes. The liposome suspension was then mixed with DOX and intermittently shaken until the final product was liposomal DOX. The lipid bilayer of the liposomal DOX is meant to assist in the penetration of the BBB due to its high lipophilicity.
Two groups of mice with GBM were used in the experiment—one group of mice was treated with combined liposomal DOX and FUS, while another group did not receive any treatment, acting as a control group. FUS treatment varied in acoustic power, from 1.43 W, 2.86 W, and 4.29 W. Figure 8 shows that DOX concentrations in the brain is directly correlated with acoustic power, which demonstrate that level of BBB opening and infiltration of chemotherapies can be manipulated with acoustic power.

![Graph showing brain tissue DOX concentration](image)

Figure 8. **Brain tissue DOX concentration amongst mice undergoing various level of liposomal DOX and FUS treatment.** Higher DOX concentrations were seen in mice undergoing FUS treatment with higher acoustic powers (# p < 0.05; ** p < 0.01) (Figure taken from Yang, 2012).
In addition, a group of mice received repeated sonication, and were sonicated a second time at an acoustic power of 2.86 W. Figure 9 shows the DOX concentrations in brain tissue in the surrounding tissues (contralateral brain), in the brain tumor of the non-FUS treated GBM mice, as well as in the brain tumor of the double-sonicated GBM mice. There is a significantly higher concentration of DOX in the double-sonicated brain tumor, indicating that not only was FUS able to successfully penetrate the BBB, it was able to deliver more than 400% more therapeutics to the tumor tissue than the lack of FUS treatment.

**Figure 9. DOX concentration measurement amongst different brain tissue treatments.** Compared with contralateral brain tissue and unsonicated brain tumor tissue, the repulsed brain tumor shows significantly higher concentrations of DOX (* p < 0.05; *** p < 0.001; ### p < 0.001) (Figure taken from Yang, 2012).
Furthermore, the control and treated brains were imaged using magnetic resonance imaging (MRI), and the tumor margins assessed for growth. Figure 10 demonstrates a greatly retarded tumor growth between the two groups, indicating the success of the dual FUS and DOX treatment in reducing tumor margins.

Figure 10. MRI assessed brain tumor margins between the control group and the experimental group. a) Coronal view of the brain. b) Axial view of the brain. Both views show a significant reduction in the growth of the GBM tumor for the mice injected with liposomal DOX and double treated with FUS (Figure taken from Yang, 2012).
An experiment conducted by Kovacs explores the longitudinal effects of GBM mice treated with DOX and subsequently sonicated with FUS (Kovacs, 2014).

Survival rates were assessed as mice that lost less than 15% of their body weights or did not develop neurological symptoms (characterized by behavioral deterioration or increased intracranial pressures). “Survival” is defined by the endured absence of these symptoms.

Figure 11 shows the cerebral DOX concentrations of the contralateral versus the sonicated tissue. The sonicated tissue has a significantly higher concentration of DOX compared to the contralateral tissue, emphasizing the success of the FUS facilitated BBB penetration by DOX (p = 0.0004).

![Figure 11. Cerebral DOX concentration in sonicated and unsonicated brain tissue.](image)

The unsonicated contralateral cerebral tissue shows significantly lower levels of DOX concentration than the sonicated and MB treated tissue (Figure taken from Kovacs, 2014).
The results of the survival analysis can be seen in Figure 12. The DOX and FUS treated mice saw a 68.18% higher average survival rate (p = 0.001) compared to DOX-only treated mice and control mice, demonstrating the ability of FUS to not only aid in the local and specified delivery of therapeutics, but to affect the longitudinal survival patterns of mice with GBM.

The combinatory administration of DOX and FUS serve to delivery a higher concentration of DOX to the brain, which subsequently provides a higher survival rate for mice with GBM. This animal model holds great promise for current patients with GBM, potentially extending the life spans of patients post-diagnosis.

Figure 12. Survival rates of the four groups of GBM mice, in days after tumor implantation. The untreated mice, as well as the DOX-only and FUS-only treated mice, demonstrate a quick drop in survival at the Day 22 mark. The DOX + FUS treated mice demonstrate a 68.18% longer survival rate (Figure taken from Kovacs, 2014).
Through the experiments of Wei, Yang, and Kovacs, it can be proven that chemotherapy efficacy in the treatment of GBM can be significantly increased through the concurrent administration of FUS and chemotherapies (Wei, 2013; Yang, 2012; Kovacs, 2014). In addition to the advantage of temporary localized BBB opening for increased drug delivery to the brain, FUS treatment also allows for lowered drug doses, which decreases the harmful tissue side effects usually observed with the administration of drugs such as TMZ and DOX. FUS treatment also alleviates the physical burden for patients unable to undergo aggressive and invasive procedures, such as surgical resection of the tumor or intracranial drug injection.

Given the discouraging statistics regarding GBM survival lengths and treatment success rates, FUS treatment, conjointly administered with current treatment options, could be the key to a more optimistic prognosis and a higher survival rate for GBM patients.

*Alzheimer’s Disease*

One of the most common forms of senile dementia, Alzheimer’s disease (AD) afflicts the elderly population above the age of 65, while early-onset AD can afflict people well before the age of 65. Early symptoms are characterized by short-term memory loss, absent mindedness, and confusion in new situations (Selkoe, 2001). In late stage AD, patients lose self-awareness, lose cognitive functions and abilities, and eventually lead to death. In the United States alone, there are an estimated 5.2 million
people with AD in 2014, and it is the 6th leading cause of death (Alzheimer’s Association, 2014).

Physiologically, it is caused by the irregular cleavage of the neuronal transmembrane amyloid precursor protein (APP) (Selkoe, 2001). In the normal pathway, enzymes alpha-secretase and gamma-secretase cleave APP into the fragment sAPPα and another fragment that remains in the neuron (Figure 13).

![Diagram of normal cleavage of APP by alpha-secretase and gamma-secretase](image)

**Figure 13. Normal cleavage of APP by alpha-secretase and gamma-secretase.** In a normal pathway, the neuronal transmembrane protein APP is cleaved into sAPPα by the enzymes alpha-secretase and gamma-secretase (Figure taken from Rodgers, 2008).

However, in the case of AD, beta-secretase instead cleaves APP, into the fragment sAPPβ, which is released from the cell. Gamma-secretase then cleaves the resulting fragment, and releases a free beta-amyloid (Aβ) peptide (Figure 14).
Figure 14. Abnormal cleavage of APP by beta-secretase and gamma-secretase. In an abnormal pathway, the neuronal transmembrane protein APP is cleaved into sAPPβ and Aβ peptide by the enzymes beta-secretase and gamma-secretase (Figure taken from Rodgers, 2008).

The free Aβ peptides begin to aggregate and create oligomers, which clump with other oligomers to create extracellular Aβ plaques. These plaques interfere with cerebral cells and synapses, preventing normal brain functions, which lead to the characteristic symptoms of AD (Figure 15).
Figure 15. The biochemical pathway of Alzheimer’s disease. The transmembrane protein APP is improperly cleaved and leads to the synthesis of Aβ plaques (Figure taken from Rodgers, 2008).

While anti-Aβ antibodies have been proven to be efficacious against Aβ plaques, the BBB poses as a formidable obstacle against their delivery into the cerebral cortex (Kotilinek, 2002). The size and hydrophilicity of the anti-Aβ antibodies inhibit them from passing the BBB, thus preventing the CSF concentrations of the anti-Aβ antibodies from
reaching therapeutic levels. Procedures involving direct delivery of anti-Aβ antibodies to the cortex have been developed, but the invasive and aggressive nature of the procedure makes this an impractical option for most elderly patients of AD (Wilcock, 2003).

Jordao’s experiment combining anti-Aβ antibodies with MRI-guided FUS addresses both these limitations by avoiding an invasive therapeutic delivery procedure and creating a method of bypassing the BBB (Jordao, 2013).

In this experiment, mice exhibiting abundant plaque load were injected with a MB and anti-Aβ antibody mixture of immunoglobulin G (IgG) and immunoglobulin M (IgM), along with an MRI contrasting agent. MRI-guided FUS was administered to the right cortex of each mouse, with the MRI acting as a targeting mechanism to allow for precise positioning and target control. With each mouse’s left cortex acting as a control, the treated right cortex demonstrates the effects of FUS in aiding IgG and IgM delivery (Figure 16).

Jordao’s results demonstrate that not only was MRI guided FUS capable of aiding the delivery of the anti-Aβ antibodies into the cerebral cortex, the increased presence of the IgG and IgM was directly able to decrease plaque number, size, and total surface area of the plaques.
Figure 16. The effects of MRI-guided FUS on Aβ plaque pathology. A) Visible plaque reduction in histologic stain comparisons of the right and left cortex. B) Mean plaque size, C) total surface area of the plaques, and D) number of plaques have also been reduced. E) At the right cortex, looking at the plaques treated with FUS and antibodies, F) IgG and G) IgM are found to be bound to the plaques. H) At the left cortex, looking at the plaque treated with antibodies but no FUS, I) IgG is found only slightly bound to the plaque, and J) no IgM is found bound to the plaque (Figure taken from Jordao, 2013).
Histological staining for each anti-Aβ antibody (IgG and IgM) was able to demonstrate the heightened presence of each antibody in the right cortex, which was treated with MRI guided FUS, compared to the left cortex, which was left untreated (Figure 17). The darker stain in the right cortex clearly demonstrates that MRI guided FUS was able to open the BBB and allow for higher antibody infiltration into the brain tissue.

![Image of histological staining](image)

**Figure 17. Histological staining of cortex treated with MRI guided FUS and untreated cortex.** A) Staining for IgG presence in the cortex. B) Staining for IgM presence in the cortex (Figure taken from Jordao, 2013).

Currently, AD is without treatment, and its high prevalence in the global elderly population emphasizes a pressing need to develop a treatment that doesn’t just combat symptoms. As a noninvasive and localized procedure, FUS allows for specified and targeted delivery of therapeutics to the brain, which is a distinct advantage over other treatment options such as surgery and oral medications.
**Tumor Metastasis in CNS**

Frequently in cases of advanced cancer stages, patients will develop cancer metastasis in the CNS, which remains impervious to current cancer chemotherapies and can be a point of cancer relapse. For example, 10-16% of breast cancer patients develop CNS metastasis, and the five-year survival rate of breast cancer patients with CNS metastasis is a feeble 1.3% (Pienkowski, 2010). The high mortality rates for patients with tumor metastasis in the CNS is due to the low efficacy of chemotherapies in the CNS, as the BBB acts as a barrier against drug entry.

Trastuzumab (herceptin) has been used with success in patients with breast cancer, but has not been of benefit for breast cancer patients with CNS metastasis. In a study conducted by Park, FUS is used to open the BBB to deliver trastuzumab to the brains of rats, in a breast cancer brain metastasis model (Park, 2012). To achieve the breast cancer brain metastasis model, 41 rats were inoculated by direct surgical implantation of HER2-positive human breast ductal carcinoma cells into the right frontal lobe of the brain. The tumors were subsequently monitored with MRI for 14 days to ensure proper growth to 2-3mm in size. In rats that received FUS, MBs were also intravenously administered to facilitate BBB penetration.
Figure 18. Tumor volume analysis for each breast cancer brain metastasis experimental group. a) Tumor volume over time for each experimental group. b) Tumor volume normalized to tumor volume at week 1. c) Measurement for each rat’s normalized tumor volume at week 7 (Figure taken from Park, 2012).
Over the subsequent 13 weeks, each rat’s tumor volume was measured with MRI and analyzed (Figure 18). The results show that the concurrent trastuzumab and FUS treatment was significantly more efficacious in reducing tumor volume over the weeks (p < 0.05). Rats that received only FUS treatment and rats that received only trastuzumab treatment observed tumor volume growth similar to rats that received no treatment at all. Only rats that received concurrent trastuzumab and FUS treatment were able to observe a decline in tumor volume, and 4 rats in the this group appears to have complete tumor regression as measured with MRI. At the end of the study (week 13), only 4 rats survived, and they were all from the trastuzumab and FUS treatment group.

Looking at the survival rates for up to 83 days, mice that did not receive any treatment had the lowest survival rate, and had all died by day 80 (Figure 19). However, the trastuzumab and FUS treatment group were able to live the longest of the 4 groups, at 17% longer than the trastuzumab only group, and 32% longer than the group without treatment. These results demonstrate that FUS was able to increase therapeutic delivery to the tumor region, as well as significantly influence the survival rates of the combinatory FUS and trastuzumab treatment group.
Figure 19. Survival rates for each breast cancer brain metastasis experimental group, up to 83 days. The group without treatment had the lowest survival rate, while the trastuzumab and FUS treatment group was the longest surviving, at 32% longer than the group without treatment (Figure taken from Park, 2012).

**Gene Therapy**

Gene therapy holds great potential in the treatment of genetic CNS diseases, such as Parkinson’s and AD, and is in the clinical trial stages of research and development (Christine, 2009; Mandel, 2010). Recombinant adeno-associated viral (rAAV) vectors are advantageous in its lack of pathogenicity, low immunopathology, continual removal of viral genes, and its longitudinal effects (Mingozzi, 2011).
The principle is illustrated in Figure 20, in which a new genome is introduced into a cell via an adenoviral vector. The viral vector contains an engineered genome that includes a new gene, to be expressed by the cell receiving the vector. The adenoviral vector binds to the cell membrane, undergoes endocytosis, the viral vector is broken down, and the gene is injected into the nucleus, where it proceeds to be translated.

Figure 20. Principle of gene therapy using an adenovirus vector. The adenovirus vector contains the modified DNA, which the cell proceeds to uptake. The vector is broken down, the DNA injected into the cell nucleus, and the packaged DNA is expressed (Figure taken from U.S. National Library of Medicine, 2014).
Gene therapy holds much promise and power for current untreatable genetic illnesses, as instead of treating symptoms, it would be able to direct the treatment to the genetic source and rectify the wayward gene. In addition, its persistence would eliminate the need for constant treatments or procedures. In the treatment of CNS diseases, gene therapies are tempered by the inaccessibility of the CSF to viral vectors due to the impenetrability of the BBB.

In an experiment conducted by Hsu, rAAV vectors were directly delivered to the brain using MB mediated FUS (Hsu, 2013). In this study, mice were injected with AAV2-GFP (adeno-associated virus-green fluorescence protein). GFP expression would not only demonstrate the success of the BBB opening, but the delivery of the vector to the target and its subsequent expression. Bright green regions on the brain slices when seen under the fluorescent microscope would easily visually affirm GFP expression.

Experimental mice received a MB facilitated FUS treatment, guided by MRI. The mice that received both vector and FUS treatment were gradually sacrificed on a weekly basis over the course of 6 weeks to observe longitudinal GFP expression (Figure 21).
Figure 21. Longitudinal GFP expression in 4 experimental groups of mice receiving rAAV vector treatment with FUS. a) The top panel demonstrates the target region of the brain and the bottom panel demonstrates the contralateral brain region, acting as control. b) Measurement of GFP signal increase at each point of the longitudinal study (Figure taken from Hsu, 2013).

In each experimental group, the target region of the brain is demonstrated on the top panel, and the contralateral brain region is on the bottom panel, acting as control. In both the group that only received FUS treatment and the group that only received viral
vector treatment, no GFP expression was visualized in both the target and control region. In the case of the FUS only treatment, there is a lack of AAV2-GFP gene to even express the GFP fluorescence. In the AAV2-GFP only treatment, the absence of fluorescence demonstrates that without FUS, there is no BBB opening, which prevents the vector from being delivered to the brain, thus no GFP was expressed in both target and control regions.

In the group receiving direct cranial injections of AAV2-GFP, the target region is highly fluorescent, and the control region without fluorescence. In the group receiving the viral vector concurrently with FUS treatment, there is a steady increase in GFP expression from day 7 to day 21, which is a peak, then a decrease in fluorescence until the end of the longitudinal study. The GFP expression in the group received dual treatment at the peak (day 21) is comparable to the GFP expression in the group that received direct cranial injection (122.8% increase in fluorescence compared to 148.7% increase), denoting that conjoined vector and FUS treatment can be comparably effective as direct cranial injection of the viral vector.

The results from this study show that FUS facilitated BBB opening can not only directly deliver viral vectors to the cranial target region, but can also bring about successful gene expression. This noninvasive manner of drug administration is comparable in efficacy to direct cranial injection of viral vectors, pairing high therapeutic effect with low levels of procedural aggression.
DISCUSSION

Due to the stagnant nature of current CNS disorder treatments and the pressing need for more advancement in the field of CNS disorder therapeutics, this study poses to explore the potential of FUS as a key role in the future treatments of CNS disorders.

In the treatment of GBM, Wei was able to demonstrate the successful delivery of the chemotherapy, TMZ, to the area of the tumor while using combinatory FUS and therapeutics (Wei, 2013). Not only was Wei able to use lower doses of TMZ to prevent tissue toxicity, the study showed that there was a statistically significant higher concentrations of TMZ in the sonicated tumor tissue than the untreated tissue, which also lead to a lower rate of tumor growth in the sonicated rats.

Yang’s experiment utilized DOX in the treatment of GBM, and similarly established that FUS was able to significantly boost DOX concentrations in the brain, which lead to decreased tumor margins (Yang, 2012). The double sonication of the tumor in this study further emphasized the efficacy of the FUS—when the cerebral cortex was double sonicated, the DOX concentration was able to increase to over 400% the levels of that in the unsonicated mice, suggesting that repeated sonication may be a valid course of action for patients who cannot tolerate high doses of chemotherapies.

Kovac’s experiment also analyzed the combinatory effects of DOX and FUS in the treatment of GBM, but looked further at the longitudinal effects of the dual treatment on survival rate (Kovac, 2014). He found that not only was FUS able to deliver DOX effectively to the GBM tumor, it was able to significantly increase the survival rates of
the experimental mice by 68%. In this study, the success of the DOX drug delivery was
directly correlated with increased survival rates, illustrating the powerful potential of
FUS in the treatment of brain tumors.

In the field of AD treatments, Jordao’s experiment combines anti-A\(\beta\) antibodies
with FUS (Jordao, 2013). Due to the hydrophilic nature and large size of anti-A\(\beta\) antibodies, they were unable to enter the brain and execute therapeutic effect due to BBB
impermeability. When combined with FUS, and with MRI acting as a targeting guide, the
anti-A\(\beta\) antibodies were able to be delivered to the cerebral cortex. Through histological
analysis, Jordao was able to ascertain that the A\(\beta\) plaques, which are primary contributors
to AD pathology, decreased in size, number, and surface area after treatment with anti-A\(\beta\) antibodies. With FUS as a tool, there could be a more optimistic outlook for patients with
AD, one with more aggressive forms of treatment.

Park’s experiment addresses the metastasis of other tumor cells into the CNS,
such as in the instance of breast cancer metastasis (Park, 2012). Park researched the
longitudinal impact of combined FUS and trastuzumab treatment, and observed that it
was able to greatly reduce tumor size, as well as increase survival rates up to 32%. A
number of rats in this experiment also experienced complete tumor remission, an
encouraging prospect in the treatment of tumor metastasis into the CNS.

In the use of gene therapy to treat genetic CNS disease, such as Parkinson’s and
AD, Hsu used rAAV vectors containing a AAV2-GFP gene to test the ability of FUS
ability to deliver rAAV vectors, then the ability of the delivered vectors to successfully
produce GFP in the cerebral cortex. The results of the experiment demonstrate that FUS
was capable of delivering the viral vector, and the viral vector was able to successfully express the GFP gene. The GFP expression from FUS-facilitated BBB opening was comparable to the level of GFP expression from direct intracranial injection of AAV2-GFP into the brain, which prove that FUS is highly efficacious in its localized delivery of therapeutics.

From the FUS studies conducted, the results demonstrate that the procedure is able to significantly increase drug concentrations in brain, increase survival rates of the animal models, decrease tumor growth, and decrease tumor margins and volume. The potential and power of FUS should be further explored as the future of CNS disorder treatments.

There are numerous distinct advantages of FUS-facilitated BBB opening over other methods of CNS disorder treatments. First, its noninvasive nature makes it a safer alternative to surgery, especially for elderly or more weakened patients unable to undergo an aggressive procedure such as brain surgery. Second, its localized treatment allows for specific, targeted drug delivery, which can reduce unnecessary tissue damage and prevent drug dispersion. Third, the application of FUS reduces the doses of drugs necessary to reach therapeutic levels, as its targeted delivery directs its effects. This would lower the chances of toxicity, especially in cases in which the chemotherapy is highly damaging to systemic tissues. Finally, the use of FUS would wide the options of drug development for CNS disorders, as therapeutics are no longer limited to small, hydrophobic molecules. Antibodies, viral vectors, and large chemotherapeutics can all be explored as potential drugs treatment options.
CONCLUSION/ FUTURE DIRECTION

Despite the advantages FUS can bring to CNS treatments, there is still much to improve on and consider. Primarily, the procedure as it applied to BBB opening is still in its beginning stages of research, and there are still many points to address. Currently, some researchers increase the BBB opening efficiency by using MBs, and guiding the FUS with MRI to help targeting. However, there could be better methods for increasing BBB permeability, one that does not include MB injections and double sonication. The process could be further researched to reach a more streamlined and standardized process for FUS-facilitated BBB opening.

Further research should also be conducted in the longitudinal safety and effects of temporary BBB opening, in the assessment of the extent of neuronal damage, cell necrosis, and any ensuing behavioral changed from this procedure. Also to be considered is whether the temporary BBB opening poses a danger to the CNS for any circulating foreign materials or pathogens in body that unintentionally enters the CNS during the treatment. Furthermore, the use of FUS in the treatment of a wider range of CNS disorders should be investigated, such as encephalitis, epilepsy, Tourette’s, Huntington’s, and multiple sclerosis.

Current animal models of FUS-facilitated BBB opening are all rodents; it would be beneficial to attempt a study on nonhuman primates, to analyze its effects on an animal with a thicker cranium. The acoustic pressures would change in order to penetrate the BBB, and the ensuing tissue damage could be more significant.
Due to the novel and experimental nature of this procedure, there are many issues to be considered and further researched, and it may be many years before the treatment can reach clinical stages. However, the advantages that FUS offers to the field of CNS disorders grant it a key role in the future of CNS treatments, potentially helping CNS treatment options to improve in efficacy and safety.
### LIST OF JOURNAL ABBREVIATIONS

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<td>American Association of Pharmaceutical Scientists: Pharmaceutical Science</td>
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REFERENCES


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EDUCATION

Robert Wood Johnson Medical School  
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Boston University  
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Masters of Medical Sciences  
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Columbia University  
New York, NY
Bachelor of Science in Applied Mathematics  
May 2012

EXPERIENCE

ProPhase, LLC  
New York, NY
Research Statistician  
Jul ’13 – Jul ’14

• Analyzed and collected client data fromm various pharmaceutical clinical trials relating to neurology and psychiatry
• Communicated to pharmaceutical clients the status of longitudinal clinical trials from a statistical perspective
• Created articles and materials for scientific publications and conferences relating to current statistical projects and clinical studies

MileWise  
New York, NY
Mathematic Analyst Intern  
Jun ’12 – Aug ’12

• Created models to estimate the value of a travel reward point depending on various parameters such as user status level, reward preference, and past history

Virginia Commonwealth University  
Richmond, VA
Statistics Consulting Intern - Biostatistics Department  
Jun ’11 - Aug ’11

• Worked on a 12 person statistical consulting team to analyze medical data for clients including US and international universities
• Performed statistical and numerical analysis using Excel, R, and Matlab to interpret brain imaging data for the University of British Columbia Department of Neuroscience
• Personally managed client relationships and communicated with researchers through meetings and calls. Created and drafted presentations for research conferences
• Generated research ideas with the team director. Research publication in international journal pending

St. Barnabas Medical Center
Livingston, NJ
Clinical Research Intern - Emergency Room
Jun ’10 - Aug ’10
• Interacted with patients to receive feedback and chronicle progress on an ongoing clinical trial
• Precisely recorded critical medical information and organized data for the clinical trial
• Provided support for patients on extended stays and shadowed

Columbia University School of Engineering
New York, NY
Engineering Intern - Biomedical Research Department
Jun ’09 - Aug ’09
• Quantified and analyzed the treatment of Alzheimer's disease in mice using Matlab and Excel
• Presented research to the engineering team. Published paper in Journal of Cerebral Blood Flow and Metabolism

ACTIVITIES

American Medical Students Association
New York, NY
Vice President, Treasurer
Sept ’08 – May ’12
• Organize programs and opportunities for over 1000 students, such as services abroad, panels, and workshops
• Arrange meetings between different committees and delegate responsibilities to executive members
• Directed and managed the finances of the organization, including accounting and budgeting of $3000

Community Impact
New York, NY
Volunteer, Member
Sept ’09 – May ’12
• Tutor disadvantaged individuals for the GRE in a range of subjects including mathematics, reading, and writing
• Educate families about health and nutrition and assist families applying for government support