REDUCTION OF SOD AGGREGATION USING IN VITRO AND IN VIVO ALS MODELS

By
John Shea

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ABSTRACT

Many neurodegenerative diseases are characterized by the presence of misfolded proteins and proteotoxicity. While the dysfunctional proteins may differ from disease to disease, the resultant neuronal stress can create debilitating cognitive and motor deficits. In our lab, we are working toward ways to reduce these symptoms in Amyotrophic Lateral Sclerosis (ALS) through experiments on cell, mouse, and in vitro models. Two efforts were used to reduce the proteotoxicity resulting from aggregation of a mutated superoxide dismutase (SOD1): screening for a UBE4B inhibitor and treatment with an inhibitor of Heat Shock Protein 90 (HSP90). An assay able to model UBE4B auto-ubiquitination in vitro was found to be reliable, scalable, and useful for finding potential inhibitors of UBE4B. Meanwhile, cellular exposure to the HSP90 inhibitor, NVP-BEP 800, was shown to reduce SOD aggregation, but a drug trial in mice did not show significant results. The UBE4B assay and mouse drug injection results have provided valuable information for optimizing future experiments, with the assay in particular showing unique potential.
ACKNOWLEDGEMENTS

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I also am thankful for the help of technical support for the UBE4B assay - the researchers and technicians at Biogen, Boston Biochem, Life Technologies, Biotek, and Corning helped guide our efforts with their expertise. Dr. Kerri Spilker at Biogen isolated UBE4B Variant 2 and thankfully shared with us the enzyme used primarily in our assays.
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INTRODUCTION

The themes of protein aggregation and protein misfolding are prevalent in neurodegenerative diseases. In amyotrophic lateral sclerosis (ALS), one of the most common causes of neuronal death responsible for motor deficits is the misfolding of mutant forms of super oxide dismuase 1 (SOD1). One mutant form, G93A SOD1, results in symptoms of ALS and is studied widely in ALS models (Pandya et al., 2013). Findings have shown that inhibiting ubiquitination from UBE4B (an E3/E4 ligase) and inhibiting heat shock protein (HSP) 90 result in less G93A SOD1 protein aggregates (Zeinab et al., 2012; Cha et al., 2013).

UBE4B Molecular Mechanisms

UBE4B is an E3/E4 ubiquitin ligase that is part of the E1-E2-E3 ubiquitination system (Wu and Leng, 2011). The first step of this system involves an E1 enzyme binding to Mg-ATP and ubquitin. Once a high-energy thioester bond has formed between ubiquitin and AMP-E1, the E2 conjugating enzymes can engage the E1 and transfer the ubiquitin to E2’s to an E3 or target proteins (Schulman and Wade Harper, 2009). UBE4B participates in this system by receiving ubiquitin from an E2 ligase and transferring it to target proteins. UBE4B’s ability to create multi-ubiquitin chains on target proteins because of its unique HFD2-homology domain (U-box) is characteristic of an E4 enzyme, a special type of E3 activity (Hoppe, 2005; Wu and Leng, 2011). By creating
polyubiquitin chains on its targets, UBE4B labels proteins for 26S proteasome degradation (Pickart and Fushman, 2004). In the case of UBE4B, one primary target is p53. The E3 ligase MDM2 is required for UBE4B to poly-ubiquitinate p53 (Zeinab et al., 2012). Findings from our lab (data unpublished) have indicated that UBE4B inhibition is able to reduce aggregates of G93A SOD1 in HEK cells.

**UBE4B Variants**

UBE4B has three variants, two of which are important in our assays. Transcript variant 1 (RefSeq Accession NM_001105562.2) contains exon ex7, while transcript variant 2 (NM_006048.4) is missing this exon. Variant 1 is therefore longer than variant 2, and while variant 1 is expressed briefly in striated muscle undergoing regeneration, variant 2 is expressed throughout the body, especially in the brain (2002; Mammen et al., 2011). As such, variant 2 is preferred for in vitro studies on ubiquitination. In our initial assays, we were working with a recently purified variant 1 (Boston Biochem), and as variant 2 (Biogen) became available, we switched our assays to include that variant. Transcript variant 3 (XM_005263422) has two exons more than variant 2, ex7 and ex7a. It is the longest of the three variants, and it only expressed in striated muscle (Mammen et al., 2011).
HSP90 Molecular Mechanisms

HSP90, like UBE4B, also plays a role in the proteasome system that is responsible for proper handling of mutant protein aggregates. HSP90 sequesters heat shock factor, HSF1, a factor that activates heat shock genes like HSP70 and HSP40 when it is not bound by HSP90 (Voellmy, 2004). Following induction of this heat shock response with HSP90 inhibitors like geldanamycin, G93A SOD1 inclusions were reduced as well, due to increased HSP70 and HSP40 levels (Batulan et al., 2006).

Applications of Protein Inhibition

Establishing the basic elements of this pathway opened the door for new ways to implement this knowledge. Two closely related applications of this pathway, drug screenings and mouse drug trials, target two different proteins and two different stages of treatment development. Whether we are testing our inhibitors using in vitro ubiquitination reactions, in cells, or in mice, our questions are the same - Are we manipulating the pathway as desired, and does this generate our desired result?

For the UBE4B ubiquitination assay, we wanted to make sure our E1-E2-E3 system was working, and that auto-ubiquitination of UBE4B could be isolated from assay noise. Furthermore, could we use this system to screen for inhibitors in a reliable, scalable screen? Other TR-FRET assays using the lanthascreen kits have shown promise in compound screening, and high-through put screening
using the E2 UbcH5c was shown to activate Ube4b, so in theory the assay would be able to accomplish our goal of an HTS for UBE4B inhibitors (2013; 2014). For the mouse drug trial, we wanted to make sure that the drug could inhibit SOD aggregation in HEK 293T cells. After the trial, we had to question whether the mice survived significantly longer, and was their motor behavior better with the drug. While both applications have a different focus in terms of the protein targeted, the main concept of testing a drug in various scenarios generated very useful information.
MATERIALS AND METHODS

UBE4B Assay Reagents

Our assay reagents were ordered according to the TR-FRET protocols found in the Lanthascreen Conjugation Assay from Life Technologies (Carlsbad, CA). This required Terbium-Ubiquitin and Fluorescein-Ubiquitin, also from Life Technologies. Also required was an E1, an E2, and ATP-Mg\(^{2+}\) for the reaction to occur. (Justification for using these enzymes). Boston Biochem (Cambridge, MA) supplied the E1 (His6-UBE1, E-304), E2 (GST-UbcH5c, E2-625), and the ATP-Mg\(^{2+}\). The E3 (His-UBE4B Isoform 1) was a gift from Boston Biochem, and was used initially in our assays. Later on, we obtained UBE4B Isoform 2 as a gift from Biogen. The fill-in buffer used was the “TR-FRET buffer” described in the protocols, composed of 50 mM Tris-HCl pH 8 and 4 mM MgCl\(_2\). In experiments with p53, Boston Biochem’s His6 p53 (SP-450) and GST MDM2/HDM2 (E3-202) were used.

UBE4B Small Scale Assay Protocols

Initial runs of the assay were based off of the protocols described for the lanthascreen conjugation assay from Life Technologies. Our final concentrations were 1 mM ATP-Mg\(^{2+}\), 50 nM E1, 200 nM E2, between 12 and 200 nM of the E3, 300 nM of Fluorescein-Ubiquitin, and 25 nM of Terbium-Ubiquitin. Initially, we loaded E1, E2, the ubiquitins, and UBE4B to a final volume of 20 µL in a 384 well
plate. Then ATP and inhibitors were added until a final volume of 30 µL, the plate was centrifuged briefly, and then plate reading occurred over 1 hour, reading once every minute. As the assay was optimized over time, various enzyme concentrations, incubation times, and assay volumes were tried. Our most current data contains a modified protocol. E1, E2, ATP, and the ubiquitins are incubated at room temperature for 30 minutes, while E3 and inhibitors were prepared separately. The E1-E2 reaction was dispersed into all the wells of a pre-chilled plate on ice at a volume of 5 µL and allowed to cool for 10 minutes before adding 5 µL of the E3 reaction and 5 µL of the inhibitor reaction. Brief centrifugation was followed by a 1 hour plate read, with reads occurring between one and seven minute intervals.

**UBE4B Assay Instrument Settings**

For our assays, we measured the 495 nm and 520 nm emissions after a 340 nm excitation using a Lanthascreen TR-FRET filter cube (Biotek, PN:8040506) in a Biotek Synergy H1 plate reader (Winooski, VT). We used delay time of 100 µs, an integration time of 200 µs, a gain of 137 for both filters, and a 7 mm read height. Temperature was set to 25ºC. Assays were performed in black, low-volume 384-well plates (Corning No. 3677). Pipettes used were Rainin Pipet-Lite XLS 2-20 µL multichannel (Columbus, OH) with Rainin RT-L10 tips, an Eppendorf L28886C 10-100 µL multichannel (Hamburg, Germany) with Denville Scientific 200 µL polypropylene tips (South Plainfield, NJ), and a 96-well Benchtop Pipettor.
**Intraperitoneal Injections**

Intraperitoneal (IP) injections of SOD G93A mice with NVP-BEP 800 began once the mice reached 60 days old. Injections were administered intraperitoneally twice a week for three months. NVP-BEP 800, an Heat Shock Protein 90 (HSP90) inhibitor (Selleck), was dissolved in DMSO to a concentration of 2mM for stock solutions. Mice receiving the drug were injected with 29 gauge insulin syringes containing 300 µl of a solution containing half NVP-BEP800 stock solution and half of 10% 2 - hydroxypropyl - Beta- cyclodextrin (obtained from Sigma-Aldrich [St. Louis, MO]) dissolved in PBS. Control mice received 300µl of a solution containing half DMSO and half 10% 2 - hydroxypropyl - Beta-cyclodextrin dissolved in PBS.

**Rotarod**

The Rotarod machine (LE8200) was obtained from Harvard Apparatus (Holliston, MA). Mice were trained on the rotarod at a constant speed of 12 rpm for 5 minutes, three times in one day, at one hour intervals, the day prior to testing. Mice that fell off during training were placed back on the rod. During testing day, the mice were placed on the rod at an accelerating speed, from 4 rpm to 40 rpm, during a span of 5 minutes. As mice fell off the rod, the time was recorded and they were removed from the machine. Before each run, the rod and trip plates were wiped with 70% ethanol. The mice underwent training and testing once a week. Rotarod testing and training never occurred directly
following injections. Testing and training ended for all mice once any mouse could not stay on the rod for more than one second.

**Grip Test**

Mice were administered the grip test using the Bioseb Grip Test (Vitrolles, France) machine using the slanted mesh or a rod attachment. Three strategies were tried. Forelimb strength was tested by lowering the mouse by its tail and allowing it to grasp a slanted mesh or a rod. Hindlimb strength was tested by grasping a rod while being pinched behind the head (Parone et al., 2013a). Total limb strength was tested by holding the mouse by its tail and lower until all four limbs were grasping the slanted mesh.

**Mouse Perfusion**

At age 20 weeks, 8 mice (1 male and 3 females from the control and treated groups) were transcardially perfused with 4% paraformaldehyde in PBS. The mice were euthanized with carbon dioxide, then rinsed with normal heparanized saline, then flushed with about 100 mL of physiological rinse until the liver became pale, and then perfused with about 100 mL PFA or until tail rigidity was seen. The brain and spinal cord were removed, post-fixed for 24 hours, and cryopreserved in 30% sucrose.
Mouse Survival

Disease endpoint was defined as the point when a mouse could not right itself 10 seconds after being placed on its back (Dardiotis et al., 2013). This was measured as the average of three successive tests. Survival measured using 10 seconds allowed to better differentiate between mice at different stages than measurements using 30 seconds (Parone et al., 2013b; Aggarwal et al., 2014; Mancuso et al., 2014)
UBE4B ASSAY RESULTS

**UBE4B Variant 1 Undergoes ATP-dependent Reaction In Vitro**

![Figure 1: UBE4B Variant 1 Reaction](image)

The “Instrument Control Set Up”, as described by Life Technologies, was initially tried to establish a Z factor (Z’) for our assays, but troubleshooting this test proved too time-consuming for our particular plate-reader machine. Instead, we chose to run a positive and negative control reaction, complete E1-E2-E3 reactions with and without ATP, to test whether there was a significant difference among these controls. Figure 1 shows a complete reaction in 15 µL using 200 nM of UBE4B Variant 1, measured during the first half hour, without incubating any of the enzymes beforehand. The response ratio is the value of the
520 nm signal divided by the 495 signal. Since fluorescence energy transfer (FRET) occurs between the terbium-ubiquitin and the fluorescein-ubiquitins that have randomly paired during auto-ubiquitination of UBE4B, we will get the 520 nm signal during FRET, but only the background 495 signal when ubiquitination does not occur. The terbium-ubiquitin receives 320 nm light and either transfers to fluorescein-ubiquitin, which itself emits at 520 nm, or itself emits at 495 nm (if fluorescein is not in close proximity). By taking the ratio of the 520 nm emission to that at 495 nm, we are able to obtain a “response ratio” that accounts for slight differences in volume between wells and quenching effects of colored compounds.

Given that E1 is an ATP-dependent enzyme, this separation between reaction with and without ATP makes sense (Variant 1 was used here because it was the first available enzyme purified for our use. Variant 1 is expressed in development in muscle cells, but is not the variant commonly used in research. Variant 2, the isoform present in neurons and most relevant to our studies, was available later (Mammen et al., 2011)).

From this data, we can calculate a Z-factors (Z’) at any point along the time frame, which is displayed in Figure 2. Since Z’ > 0.5 is characteristic of a reliable screen, our assay should be able to accurately assess ubiquitination changes using the response ratio in this manner. Also a measure of proper separation of positive and negative controls, the signal to background ratios (S/B) for our assay at its peak is around 6, which is within a very acceptable
UBE4B Variant 1 Shows Concentration-Sensitive Kinetic Reactions

In order to establish a concentration of UBE4B that would be highly sensitive to the effects of inhibitors, a range of concentrations were tested, from 195 pM to 200 nM (the protocols suggested a range from 50 to 200 nM). Figure 3 highlight a range of 50 to 200 nM. There is hardly a difference at these concentrations, suggesting saturation levels of UBE4B at this high nanomolar concentrations. In data not shown, control wells that contained the same concentration range of UBE4B, but without ATP, all showed the same values. This suggests that the influence of UBE4B concentration is negligible when ATP

Figure 2.

![Z' Factor Variant 1](image-url)
is absent from the reaction. Figure 4 illustrates a lower concentration range, from 1.56 nM to 50 nM.

**Figure 3.**

![50-200 nM Variant 1](image)

**Figure 4.**

![0-50 nM Variant 1](image)
Within this range, we start to see concentrations that saturate more slowly and contain slower reaction rates. From here, it seemed that 25 nM was one of the sensitive concentrations going forward, due to its sensitivity to two-fold decreases in concentration.

**E1 Undergoes ATP-Dependent Reaction In Vitro**

The other aspect notable about Figure 3 and Figure 4 is that there is a consistent rise in response ratio from wells that contain everything but UBE4B (at 0 nM UBE4B curves). This means that over one hour, a steady signal is coming from just E1 and E2 reactions. This could be due to the fact that E1 contains two ubiquitin binding pockets that are used when transferring to E2 (Schulman and Wade Harper, 2009). A rare cause could be contamination of E3s in the purification of E1 or E2s. This phenomenon was a repeatable occurrence, which precludes contamination from pipetting errors.

**Variant 2 Acts Similar to Variant 1**

Obtaining access to Variant 2 allowed us to produce data more relevant to our research. As demonstrated in Figure 5, variant 2 performs similarly to variant 1 in an assay run over an hour.
Figure 5.

UBE4B Variant 2

![Graph showing response ratio over time for UBE4B Variant 2 with symbols for + ATP and - ATP.]

Figure 6.

0-50 nM Variant 2

![Graph showing response ratio over time for 0-50 nM Variant 2 with different concentrations indicated.]

Measuring Reaction Rate Isolates UBE4B Activity in Variant 2

In establishing a measure of activity and its inhibition, we chose to focus on the initial slope of the reaction rather than the endpoint response ratio at an arbitrary time point. Since inhibitors affect the rate of reaction, reaction rate derived from the initial slope of a kinetic reaction would be a more direct measure of inhibition. Looking at the past data such as that found in Figure 4, this type of slope changes does indeed correlate with concentration. After optimizing the assay to better capture this initial slope, through extended incubation times and assay cooling, we were able to isolate the slope of a sensitive concentration of UBE4B while reducing the slope of the negative control wells without UBE4B (Figure 6).

Testing of known E3 Inhibitors

Figure 7.
Five known E3 inhibitors, labeled #619-623, were tested alongside two p53 activators, Tenovin-1 and CP31398, and NVP-BEP 800, an HSP90 inhibitor. The non-E3 inhibitors were used as controls. A range of concentrations was tried (data not shown), and 10 µM was chosen as the ideal concentration based on the S/B ratio. Inhibitor concentrations in this range are found other TR-FRET assays as well (Madiraju et al., 2012; Mooiman et al., 2013). In an assay ran over an hour,
with reads every 7 minutes (the minimal amount of time to scan a full 384-well plate), kinetic data for these compounds was obtained (Figure 7). In order to capture the reaction rate, the window between 7 and 28 minutes was chosen because over this reading period the slope was most stable and representative of a constant reaction rate. Linear regression was used to obtain a value for the slope of this window (Figure 8). The slope best represents the reaction rate, and any compounds inhibiting UBE4B will have a decreased slope compared to controls. Our results demonstrate that control drugs, and some known E3 inhibitors, show minimal effect on the slope compared to UBE4B with no inhibitors. No UBE4B reactions showed a near-zero slope, which further confirmed that the negative controls showed the absolute absence of a reaction rate. In data not shown, the final reaction ratios present at 1 hour showed the same trends seen in Figure 8, but the S/B was significantly higher. Compound 623 shows a significant decrease in UBE4B auto-ubiquitination, and these trends are present in repeated assays.

Assay Optimizations:

Assay Volume

Before embarking on the task of measuring a full compound library, changes were made to the protocols to make the assay more reliable at the small scale, in about 32 wells, or 2 columns in a 384 well plate. While prior reactions ranged in volume from 10 -30 µL, the final well volume used was changed 15 µL,
which made sense for the use of a range of multichannel pipettors in pipetting three 5 µL reactions (R) into each well: E1, E2, ATP, and Ubiquitins (R1), UBE4B (R2), and inhibitors (R3). Lower volumes would be more prone to error, and any less than three master reaction mixes would be too cumbersome and time-sensitive. 15 µL was also small enough to cheaply run large screens while large enough to generate an accurate S/B ratio.

**Read Height**

The S/B ratio was also further optimized for read height. Automatic read height for our machine was set at 7.0 mm, generating an S/B ratio of 2.55. Read heights at 1.0, 4.0, 8.0, and 10.68 (maximum read height allowed) were also tried, generating S/B ratios of 1.9, 2.18, 1.9, and 1.9, respectively. 10.68 mm was rejected due to read signal overflow issues on some of the wells. Given the highest S/B ratio at 7.0 mm, this data shows manual confirmation that existing instrumentation settings were optimized for our assay.

**Incubation Times**

Another improvement came from incubation time, a factor largely impacting our assay. Initial runs were done with no incubation times, as we wanted to capture the reaction in real-time and see what happened to begin with. Over time, after learning about how only E1 and E2 (R1) signals behave and the possible of incubating the inhibitors with UBE4B (R2+R3), various incubation times were tried. R1 reactions were tried at 0, 30, and 45 minutes. R2+R3 incubation was tried at 0, 30, and 60 minutes. After running through some
possible combinations of incubations times that generated consistent results, the final protocol involved a 30 minute incubation of R1 and no incubation time for R2+R3. R2+R3 incubation times resulted in reaction well response ratios that were much higher than controls.

**Scaling**

Assays at this small scale were excellent for optimizing various assay conditions unrelated to scaling up. Also, it could be performed quickly enough with multichannel pipettes. Control compounds were tested on this scale as well. Running a whole 384 plate for a compound screen, however, would require different conditions. Preparation time for 384 wells cannot be accomplished in a timely manner using just the multichannel pipettes: with three steps required for each well, it would take about 30 minutes of pipetting to cover a full plate for just the addition of the last reaction, during which the reaction rate window would have already passed. However, using a 96-well bench-top pipettor solves this issue by reducing pipetting times by a factor of 12. It also reduces errors that can occur between different expulsions of a multichannel pipettor. The fixed nature of a 96-well electronic pipettor further ensures that reactions are dispensed accurately in each well. Future assays can be scaled up in this manner.
MOUSE TRIAL RESULTS

Figure 9.

Justification of NVP-BEP 800 for Mouse Trial

Before testing the HSP-90 inhibitor NVP-BEP 800 on G93A mice, we tested its ability to reduce mutant SOD aggregation on HEK 293T cells expressing G85R SOD. In Figure 9, the supernatant and pellet fractions demonstrate reduction of G85R SOD1, with both showing a correlation between drug concentration and G85R reduction.

NVP-BEP 800 Trial with G93A Mice

Given NVP-BEP 800’s ability to perform well in cell assays, we decided to launch a drug experiment in mice using intraperitoneal (IP) injections of the drug. Based on previous IP injections of mice at 5 mg drug per kg of mouse, we chose to inject at this concentration as well (Hedrich, 2010; Massey et al., 2010).
The drug was dissolved in DMSO and cyclodextrin, and injected into mice divided into male and female groups, which were further divided into treated and untreated cohorts (Gad et al., 2006). We began with 3 mice in each cohort, but over time lost two males from each treated and untreated cohorts, resulting in a final count of 1 male in each cohort, and 3 females in each cohort.

*Treated Males and Females Show No Significant Increases in Motor Ability Over Controls*

A rotarod was used to test motor ability in the G93A SOD mice. Once per week, mice underwent a training day consisting of 3 rounds on the rotarod at constant speed, and a testing day consisting of 3 rounds of accelerating speeds. Testing days measured the seconds each mouse could stay on the rod. By training at constant speeds and testing at accelerating speeds, the test limited the effects of learned rotarod behavior on trying to measure raw motor ability (Abada et al., 2013). In Figure 10 we can see that for both males and females, the rotarod performance of the treated cohort did not significantly differ from that of the untreated group.

*Survival Was not Significantly Different Between Treated and Untreated Cohorts*

Mice that could not right themselves up in ten seconds after being placed on their back were considered at the endpoint of the disease. Survival to this disease endpoint was measured to try to establish drug efficacy. Comparing the males,
there is a three day longer survival of the treated male, but the degree to which
drug treatment played a role in this life extension is indeterminable given the
low sample size. The females had a larger sample size and an even greater
difference, 7 days, between the treated and untreated group. However, in this
trial we had not controlled for differences between litters, which can be a
significant factor (Scott et al., 2008). There was also significant variance in the
survival within each cohort that reduces the strength of the one-week difference
between cohorts.

_Grip Tests Did Not Provide Useful Data_

On our Bioseb Grip Test machine, a variety of strength tests were
implemented: forelimb strength, hind limb strength, and total limb strength.
None of these measurements provided any useful data, and were discontinued
after some initial trials. Firstly, mice grip strength varied wildly from trial to trial.
Secondly, much of this variability was due to inconsistencies that the
experimenter could not control, such as mice grasping with two limbs, but then
during pull, releasing one or both limbs and thus giving a grip strength reading
less than what the mouse actually possessed. Lastly, mice sometimes would
randomly pull very strongly or loosen their grip, skewing the reliability of this
measurement even more. Despite following the best practices for these three
measurements, none of them seemed reliable for our purposes. A better test of
strength might be the hanging grip test (Kim et al., 2013).
Figure 10.

Male Rotarod

- Male Untreated
- Male Treated

Seconds on Rod vs. Week

Female Rotarod

- Female Untreated
- Female Treated

Seconds on Rod vs. Week
DISCUSSION

By implementing an in vitro assay to model ubiquitination with UBE4B, I was able to assess a range of compounds for UBE4B-specific inhibition. Both isoforms of UBE4B showed concentration-dependent changes in the response ratio, allowing us to correlate activity with response ratio. In addition to being able to detect activity changes from concentration, I also detected activity changes from a small screen of known E3 inhibitors, one of which demonstrated robust knock down of UBE4B activity.

There is a great potential for the assay to be used in high-throughput screening of compound libraries in search of more UBE4B inhibitors. By taking advantage of the 384 well plates, the small 15 μL size of the reaction, and the advantages of large 96-well pipettors, we can quickly assess thousands of compounds in a relatively short time frame.

Meanwhile, the mouse drug trial gave us knowledge of what works and does not work for the type of trials we want to run. Though NVP-BEP 800 did not significantly increase mouse survival or motor ability compared to controls, we were able to confirm the amount of DMSO that the mice can tolerate, as well as the IP injection volume that G93A mice can absorb. Also, we discovered that these trials are more suited to females. In future drug trials, we will keep in mind the importance of large sample sizes that are comparing litter-matched mice to control for inter-litter differences.
REFERENCES


melatonin is not neuroprotective in the G93ASOD1 transgenic mouse model of familial ALS and may exacerbate neurodegeneration. Neuroscience Letters 548, 170–175.


Madiraju, C., Welsh, K., Cuddy, M.P., Godoi, P.H., Pass, I., Ngo, T., Vasile, S.,


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RESUME

John Shea
Permanent: 8 Union Hill Road, Denville, NJ 07834 | School: Apt #1003, 218 N. Charles Street, Baltimore, MD 21201
Cell: 201-602-6146 Home: 973-328-2027 E-Mail: johnshea88@gmail.com

Education
Johns Hopkins School of Public Health, Baltimore, MD 2012-2014
Master of Science, Biochemistry and Molecular Biology, expected June 2014 GPA: 3.4
Johns Hopkins University, Baltimore, MD 2008-2012
Bachelor of Arts, Neuroscience, May 2012
Minor: Entrepreneurship and Management, May 2012

Research Experience
ScM Student Researcher, JHU School of Public Health, Baltimore, MD 4/13-Present
• Worked on Master’s thesis project with Dr. Goran Periz in Dr. Jou Wang’s lab
• Examined how protein knock downs affect protein aggregation in ALS using in vitro assays and drug therapy in mice
Research Assistant, JHU School of Medicine, Baltimore, MD 9/11-5/12
• Assisted a postdoc with auditory research on marmosets in the Wang Auditory Neurophysiology Lab
• Monitored marmoset experiments, handled marmosets, kept equipment synchronized
Research Assistant, JHU School of Medicine, Baltimore MD 4/11-8/11
• Worked for a Johns Hopkins School of Medicine health study on gender roles and sexual risk behavior among adolescents
• Improved interviewing and interpersonal skills through door-to-door recruitment of research subjects
• Completed an NRB approved research project on the usability of an iPHONE-based alarm clock
• Based on our small student sample group, we found that those that used the app’s gyroscopic data to sense sleep movements felt more awake and alert than those that used a normal alarm clock
NJ Governor’s School of Engineering and Technology, New Brunswick, NJ 6/07-7/07
• Collaborated with Rutgers graduate students in the biomedical science program on a research project and paper
• Used knowledge of MATLAB computer language and prostate cancer diagnostic techniques to refine a computer program that could successfully rate prostate histology images on a 1-3 scale.

Work Experience
Volunteer at St. Clare’s Hospital, Denville, NJ 5/10-9/10
• Assisted disabled patients to hospital rooms and aided in the ER and Maternity Ward
• Performed various hospital errands, answered phones, and trained incoming volunteers
Nursery Attendant, Morris County Farms, Denville, NJ 5/08-8/08, 5/09-6/09
• Management and multi-tasking skills refined through assisting green house managers.
• Stocked shelves, transported plants and inventory, assisted customers
• Developed great relationships with customers, and fellow employees were consistently impressed with the quality of my work.

Activities
Salud 10/08-5/12
2008-09 Esperanza Project: Helped at the Esperanza Center offering pharmacy, financial, and health assistance
• General Wolfe Project- Tutored and mentored children at Wolfe Academy
• 2009-10 Help Ayacucho Project Coordinator- Raised $3,000 to get on ambulance wait list for Peruvian village
• 2010-12 Vice President of Salud- Helped refine current projects, boosted the size of the body, and directed the establishment of new projects to provide more open access to healthcare for Hispanics in Baltimore
• Used Facebook ad campaign to garner new members, and implemented a website to disseminate info
Hopkins Emergency Response 10/08-1/12
• Responded to campus emergencies and applied first aid knowledge and medical care
The Triple Helix Magazine, Writer 9/08-1/09
• Wrote a 2,000 word article highlighting the research on the medicinal, legal, and societal aspects of hypnosis
Baltimore Rescue Mission Clinic 11/08-5/12
• Practiced gathering medical histories and giving diagnoses to patients.

Skills
• Familiar with Python programming language
• Microsoft Office (Word, Excel, Access, Powerpoint)
• Biochemical lab techniques (PCR, qPCR, digital PCR, genotyping, cell culture, western and southern blots, TR-FRET, gel electrophoresis, mouse injection and perfusion)