COVER PAGE

Study Official Title: Effects of Huperzine A in Treatment of Moderate to Severe TBI

NCT #: 01676311

Version Date of the Document: 4/11/19

ORGANIZATION OF DETAILED PROTOCOL

Title: Huperzine A for the Treatment of Cognitive, Mood, and Functional Deficits after Moderate and Severe TBI
Protocol #: 2012-p-002490
Date: 04/11/19

I. BACKGROUND AND SIGNIFICANCE

Traumatic Brain Injury (TBI) is a major public health issue, with 1.7 million new cases reported annually in the US [1, 2]. Public awareness of TBI is further heightened by the increase in combat-related TBI (cTBI) in soldiers returning from Iraq and Afghanistan [3]. However, many of the cognitive and psychological sequelae following TBI, especially combat related TBI, are difficult to differentiate from other disorders, and the chronic symptoms remain a vexing problem for those affected and for the military. Indeed, the most frequent problems of military personnel with TBI and their families relate to cognitive and executive disorders, pain, behavioral deficits, and post-traumatic seizures, which in the "real military world" present as a constellation of symptoms. Thus, we plan to evaluate symptoms as such. In order to meet the unmet medical needs in combat-related TBI, an ideal agent would provide multiple mechanisms of action relevant to the pathophysiology of TBI. Further, therapies for those with TBI should have low risk and side effect profiles and should preferably be already approved or available. Unfortunately, there are few if any such therapies in development.

Based on Huperzine-A's multiple mechanisms of action, we believe it will benefit patients with significant TBI. In this study, we will focus on Huperzine A's potential to improve cognitive and functional deficits in the chronic setting since this is a gap, and a necessity for the military. We therefore propose a dose-escalating phase II study. In this study, we also plan to link treatment to neurophysiologic metrics, which may help guide future work for the military.

Huperzine A

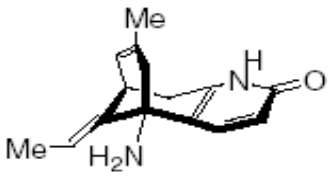

Figure 1: Structure of Huperzine A

Huperzine A (see figure 1) is purified from Chinese club moss and has been traditionally used in China for the treatment of swelling, fever, inflammation, blood disorders, and schizophrenia,[4]. In China, it is approved for use in the treatment of Alzheimer's disease (AD). Huperzine A was classified as a dietary supplement by the FDA in 1997. As a nutraceutical, it is available in American health food stores or via the Internet, labeled as a memory aid.

Huperzine A has multiple mechanisms of action that are relevant to the underlying pathophysiology of TBI. Huperzine A has been shown to be neuroprotective in several models. It is a non-competitive antagonist of N-Methyl-D-aspartate (NMDA) receptors at one of the polyamine binding sites [5] at or near the PCP and MK-801 ligand sites but without the psychotomimetic side effects of PCP or MK-801. [6] In addition to neuroprotection, this mechanism of action may also have importance for treating symptoms of depression within this

patient population. Furthermore, Huperzine A attenuates oxidative stress; regulates the expression of apoptotic proteins Bcl-2, Bax, P53, and caspase-3; protects mitochondria; upregulates nerve growth factor and its receptors; and interferes with amyloid precursor protein metabolism [7] [8] with favorable pharmacokinetics [9] [10].

Huperzine A produces dose-dependent increases of norepinephrine and dopamine in rat cortexes when administered chronically via the intra-peritoneal (i.p.) route, or locally through a microdialysis probe [11], which may have relevance to frontal lobe functioning in the TBI population.

Huperzine A inhibits acetylcholinesterase, which suggests a potential benefit for memory dysfunction in patients with TBI. Indeed, short-term memory loss is a frequent complication of TBI and often a barrier to returning to work. Because there are no specific pharmacological strategies for treating memory dysfunction in patients with TBI, other than avoiding drugs that worsen memory, studies are beginning to explore the potential usefulness of acetylcholinesterase inhibitors with promising results [12]. Huperzine A is a potent, highly specific, and reversible inhibitor of acetylcholinesterase, with comparable potency to physostigmine, galantamine, donepezil, and tacrine [4].

Therefore, given its multiple mechanisms of action, Huperzine A might be ideal to modulate the primary as well as secondary injury mechanisms that occur during the first several weeks following TBI as well as chronic phases. Thus, a link to neurophysiologic data could help us develop a biomarker for the role of Huperzine A in chronic recovery.

Experience in humans:

Huperzine A has been administered to humans orally, intravenously, and intramuscularly, and has been well studied in dementia. There is one published study on the use of Huperzine A for mild to moderate TBI, conducted in China and published in Chinese [13]. According to Wang et al [14], who discussed this TBI study in English, thirty patients were treated with standard therapies (0.8 gram piracetam and 20 milligrams nimodipine, twice per day, combined with functional rehabilitation), and another 30 patients were treated with 100 micrograms Huperzine A BID in addition to standard therapies. Both groups demonstrated significant improvement in memory and cognition after both 1 and 3 months, but the improvement in memory and cognition in patients treated with Huperzine A was more dramatic than was the improvement in patients treated with standard therapies only. As an example of a study in AD, Xu and colleagues [15] evaluated the efficacy and safety of Huperzine A in patients with AD using a multicenter, prospective, double-blind, parallel group, placebo controlled, randomized design. Fifty patients received 200 micrograms of HUP A for 8 weeks and a well-matched group received placebo. Statistically significant improvements in memory ($P < 0.01$), cognitive functioning ($P < 0.01$), and behavioral measures ($P < 0.01$) were noted in Huperzine A-treated subjects compared to placebo. No severe side effects were reported.

Regarding pharmacokinetics, published data in humans are limited but suggest rapid absorption and widespread distribution. The pharmacokinetics of a single 0.99 milligram oral dose of Huperzine A in six Chinese volunteers are consistent with a one-compartment open model with a

first order absorption, with T 1/2ka = 12.6 minutes, T 1/2ke = 288.5 minutes, Tmax = 79.6 minutes, Cmax = 8.4 micrograms L-1, and AUC = 4.1 milligrams L-1 minutes [16]. A plasma steady state would likely be established within 2 days. Interactions with other drugs are unlikely, with the possible exception that CYP1A-inducing drugs, which may increase Huperzine A clearance.

Other Huperzine properties of interest:

Antinociception

Nociception is the ability to feel pain and antinociception is reduction in pain sensitivity. In the mouse formalin pain model, Huperzine A 1 mg/kg i.p. produced complete inhibition of pain behavior in all treated animals at all time points, and 0.5 mg/kg i.p. (60% of the TD_{50}) produced near complete inhibition [17]. Both sets of results demonstrated statistically significant differences from controls ($P < 0.01$). Similar results were obtained with Huperzine A 1 mg/kg i.p. in the sciatic ligature model of pain [17].

The ED_{50} of HupA when given intrathecally (IT) in the rat thermal escape model was 0.57µg/kg (95% CI [0.25, 1.30]) [17]. Likewise, IT Huperzine A resulted in a dose-dependent decrease in flinching in both phase I and phase II of the formalin test in rats. The observed antinociceptive effects of IT Huperzine A in both rat models were largely blocked by pre-treatment with IT atropine (15 µg/µL). Thus, this suggests the potential efficacy of this agent in combating nociception, a common phenomenon in our nation's heroes with TBI.

Anticonvulsant profile

Huperzine A was active against subcutaneous (s.c.) pentylenetetrazole, but not maximal electroshock-induced seizures, following oral (p.o.) administration to Swiss-Webster mice, with peak anticonvulsant activity at one hour [18]. At doses of 1, 2, and 4 mg/kg, a maximum of 62.5% protection was observed. Impairment on the rotarod test was observed in 75 and 100% of mice tested at doses of 2 and 4 mg/kg, respectively. The TD_{50} was 0.83 mg/kg.

In the 6-Hz model, ED_{50} values for i.p. Huperzine A were 0.28, 0.34 and 0.78 mg/kg for 22, 32, and 44 mA, respectively, suggesting a possible advantage over phenytoin, carbamazepine, lamotrigine and topiramate, each of which display limited efficacy in this model at doses devoid of behavioral toxicity [19]. The less than 2-fold ratio of dosages effective across the range of stimulations suggests a further possible advantage over other drugs active in this model such as levetiracetam. Atropine 30 mg/kg i.p. completely blocked the anticonvulsant effect in the 6-Hz model (32 mA) and nearly completely blocked the toxic effects of Huperzine A 1 mg/kg i.p. When given intravenously (i.v.) the ED_{50} of Huperzine A in the 6-Hz model at 32 mA was 0.21 pmol / 5 µL injection volume and the TD_{50} was 36.15 pmol / 5 µL (TD_{50}: ED_{50} = 172).

There are no available published data of efficacy or tolerability in patients with epilepsy or chronic neuropathic pain. Huperzine A has been studied in patients with myasthenia gravis and AD [4]. Efficacy was favorable and cholinergic side effects, such as dizziness, diarrhea, and nausea were generally infrequent and mild in intensity. Additionally, a multicenter, NIH-funded, placebo-controlled Phase II trial of Huperzine A has recently been published, testing 200 and 400 micrograms twice daily in patients age 55 and older with mild-to-moderate AD [20].

In this current study in patients with TBI, Huperzine A's pleuripotential mechanisms will be of great value. Specific to this study, Huperzine A's potency of ACh inhibition has been found to rival those of tacrine and donepezil [21]. Huperzine A appears to have no tolerance effect, as repeated doses appear to demonstrate similar AChE inhibition as that of a single dose in a rodent model [22]. Compared with agents such as tacrine and physostigmine, Huperzine A appears to result in the longest lasting boost in AChE levels. The dosage selected for this study is based on safety and efficacy data noted in several studies of AD in China. In these studies, the side effects are reported as being comparable to placebo. Subjects will receive study medications (either Huperzine A or Placebo) for 12 weeks while inpatient. They will undergo evaluations while on study drug (6 and 12 weeks), and post-dosing (24 and 52) weeks.

II. RATIONALE FOR EXPANDING THE STUDY TO INCLUDE OUTPATIENTS

The process of brain injury after a traumatic insult occurs in three different stages: an acute phase, characterized by a progressive path of cell degeneration, initiated by unrestrained neuronal depolarization. This initial phase is followed by the subacute and chronic stages of brain injury, which share common pathophysiologic mechanisms: cell repair, resolution of edema and inflammation, excess GABA-mediated inhibition in networks, changes in synaptic strength in networks: dysfunctional long-term potentiation (LTP) and long-term depression (LTD), structural modifications, and plastic changes and new connections [23].

Recovery of function after TBI can also be divided into three stages. During the initial phase, activation of cell repair is the main compensation mechanism, leading to resolution of edema and inflammation. This stage takes place mainly over the first three weeks after the injury. The second stage is characterized by substantial changes in previously existing neuronal networks that are reflected both in functional and anatomical cell plasticity leading to the formation of new connections. Plasticity and remyelination are the most important mechanisms governing the final stage of recovery and are usually most prominent within the first three months after the insult. Therefore, it is clear that most changes and recovery occur in the subacute and chronic stages, which share a common underlying physiologic pathway [23, 24].

It is clear that neuroplasticity plays a crucial role in promoting recovery after brain injury, and it can be viewed as a mechanism to compensate for the injury and reestablish function. Thus, understanding the functional and dysfunctional aspects of this ongoing process, as well as the main pathophysiologic events occurring after a TBI, is necessary to create optimal therapeutic approaches [23]. Due to the similar mechanisms subacute and chronic stages share for cell reparation and plasticity, strategies that promote reorganization of neural networks should allow for improvement of cognitive and functional deficits for patients in either phase of recovery [24].

The ability to assess both inpatient and outpatient cases will allow for a broader understanding of the effects Huperzine A has across various stages of recovery. Until now, the majority of studies assessing the effect of Huperzine A have been in outpatient settings, although only in mild to moderate TBI [25]. Thus, by evaluating Huperzine A in both an inpatient and outpatient setting we will have a comparative base by which to determine whether functional and cognitive recovery is in fact similar in subacute and chronic patients. Given that Huperzine A has been tested mainly in the outpatient/chronic setting and that the pathophysiology of the subacute and chronic stages

encompasses similar recovery mechanisms, we expect that benefits will be equally significant in these two populations. Expanding our study to outpatient population will also have clear benefits on enrollment, given that it will allow us to recruit TBI patients who did not receive direct care from our institution that would potentially otherwise be eligible.

III. OVERALL GOALS

We plan to conduct a phase II, 52 week, randomized, double-blind, placebo-controlled clinical trial that investigates the effect of Huperzine A on memory function in subjects who sustain moderate to severe traumatic brain injury. We will also explore the effects of Huperzine on transcranial magnetic stimulation (TMS)-induced neurophysiologic markers, EEG event related potentials (P50 and P300), seizure frequency/prevalence and incidence of adverse effects as secondary aims. Measures of mood, pain and subjective complaints of concussive symptoms, and length of time post-injury, will serve as covariates to address potential treatment confounders. The data generated from this study will be used to plan a subsequent phase II/III trial with Huperzine A.

IV. SPECIFIC AIMS

Primary Aim:

- To determine whether Huperzine A, as compared to placebo, has a differential effect on learning and memory functions after moderate to severe TBI. *We hypothesize that the changes in mean scores on tests of learning and free recall as measured by the California Verbal Learning Test-II (CVLT-II) will be significantly greater after 12 weeks of treatment with Huperzine A in individuals with moderate to severe traumatic brain injury relative to those treated with placebo.*

Secondary Aims:

- To determine whether administration of Huperzine A produces significant differences in neurophysiologic markers (as indexed by EEG event related potentials (P50 and P300) and TMS-indexed cortical excitability (cholinergic activity)) associated with cognition relative to a placebo. *We expect that patients who receive Huperzine, as compared to placebo, will have decreased latency and increased amplitude for P50 and P300 and, in addition, they will have increased short latency afferent inhibition (as indexed by TMS measurement), thus indicating increased cholinergic activity and cognitive processing at 12 weeks post-enrollment.*
- To determine whether Huperzine A reduces the prevalence/frequency of post-traumatic seizures after moderate and severe TBI as compared to placebo. *We hypothesize that the prevalence of seizure will be significantly lower at 12 weeks post-enrollment (immediate seizures prevalence) in subjects treated with Huperzine A relative to those treated with placebo.*
- To evaluate the safety and tolerability of Huperzine A in this patient population as compared to placebo. Safety and tolerability will be assessed by a comparison of the frequency and intensity of adverse effects. *We hypothesize that the incidence of clinically significant adverse effects will be no different in subjects treated with Huperzine A relative to those treated with placebo.*

V. INVESTIGATORS

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VI. SUBJECT SELECTION

We plan to enroll 30 subjects with moderate to severe TBI (Huperzine = 15, Placebo = 15).

Inclusion criteria:

- Males and females aged 18 to 65
- Meeting at least one of the following criteria for moderate or severe TBI:
    - PTA > 24 hours
    - Trauma related intracranial neuroimaging abnormalities
    - Loss of consciousness exceeding 30 minutes (unless due to sedation or intoxication)
    - GCS in the emergency department or ICU of less than 13 (unless due to intubation, sedation, or intoxication). If there is no GCS score available from the ED or ICU, the GCS from the scene can be documented.
- All subjects will be no more than 1 year post-injury, and will be symptomatic at enrollment (i.e. all subjects will exhibit evidence of ongoing posttraumatic amnesia via the Galveston Orientation Amnesia test (GOAT), OR score at least 1.5 SD below the mean for completion time on Part B of the Trail Making Test, OR score at least 1.5 SD below the mean on either the Learning and Memory short form of the Traumatic Brain Injury Quality of Life (TBI-QOL) scale or the Ruff Neurobehavioral Inventory (RNBI) Postmorbid Cognitive Domain scale.
- Agreement to undergo no changes in concomitant medications (including dietary supplements) or therapeutic interventions during the first 12 weeks of the study (that is, the 12 weeks of dosing with study drug), except where medically indicated. Intact enteral route
- English-speaking (since not all of the outcome metrics are normed outside of the English language)
- Patient can be on seizure medication.

Exclusion criteria:

- Patients taking acetylcholinesterase inhibitors and other cholinergic and anticholinergic drugs (e.g., tacrine, physostigmine, velnacrine, donepezil, rivastigmine, metrifonate) and CYP1A inducing/inhibiting drugs (ex. ciprofloxacin, enoxacin, fluvoxamine, methoxsalen, mexiletine, oral contraceptives, phenylpropanolamine, thiabendazole, zileuton, acyclovir, allopurinol, caffeine, cimetidine, daidzein, disulfiram, Echinacea, famotidine, norfloxacin, propafenone, terbinafine, ticlopidine, verapamil, montelukast, phenytoin, moricizine, phenobarbital).
- **Evidence of more than 1 seizure in the past 4 weeks prior to enrollment:** Patients may not be enrolled if there is evidence of more than one seizure (clinical or electrographic, but not including epileptiform or other irritative discharges) during the 4 weeks prior to enrollment.
- **Premorbid history of epilepsy with seizure frequency >1 per month:** Patients with a history of idiopathic epilepsy may not be enrolled if their seizure frequency was > 1 per month **in the 3 months prior to injury**. If pre-injury seizure frequency was < 1 per month but there is documented evidence that post-injury seizure frequency is > 1 per month *or* there is documented evidence of an increase in the severity or duration of a single seizure relative to the premorbid history, the patient must be excluded.
- **Evidence of premorbid major CNS disorder, developmental disorder, psychiatric disorder or substance abuse:** Prior to sustaining TBI, patient was diagnosed and/or treated for a major neurologic condition, pervasive developmental disorder (e.g., mental retardation, autism), psychiatric disorder or substance abuse *that continued to produce functional disability up to the time of injury.* This would be demonstrated by active treatment, hospitalization, and/or documentation of active withdrawal within the 12-month period prior to enrollment. The PI will determine if the patient was a chronic substance user or simply had an overindulgence that was not maintained. If in the judgement of the PI and/or the study physician the patient exhibits no present evidence of substance withdrawal and has demonstrated a commitment to substance abstinence, the patient can be enrolled.
- Individuals with disorders of consciousness, as defined at the time of screening of having vegetative and/or minimally conscious state, will not be enrolled. However, these patients may be followed until they meet eligibility criteria
- Pregnancy, as determined by serum hCG testing before randomization
- Breast feeding females
- Significant hematologic, renal or hepatic dysfunction [Hepatic/renal dysfunction is generally identified as lab results > two times the upper limits of normal (ULN), and hematologic dysfunction is determined by clinically significant abnormal lab results], on baseline laboratory examination.
- Slow heart rate (bradycardia) or other heart conditions related to rate
- History of peptic ulcer disease
- History of asthma or emphysema as exemplified by active treatment with consistent use of disease modifying drugs within the 12-month period prior to enrollment
- History of GI/urinary tract blockages (i.e. ileus, IBS)
- History of glaucoma
- Current smoker (have smoked within 1 week of enrollment).

VII. SUBJECT ENROLLMENT

Potential subjects will be identified by the following sources:

1. Attending physicians may refer their TBI patients to the study. We will provide physicians with study information sheets and flyers.
2. Medical record review by physician on clinical treating team - of inpatients in Spaulding Rehabilitation Network facilities (Boston/Cambridge). If the patient is found to be eligible based upon this search, we will contact the primary rehabilitation physician to approach the patient and/or family about participation.
3. Outpatients will be referred from other healthcare facilities, the community, or the treating physician.

Once referred, study staff will approach subjects who retain decision-making capacity, or the authorized representative of a patient who lacks decision-making capacity, for permission to complete a screening for possible enrollment. Please note that clearance for consent will follow standard clinical procedures. A co-investigator/coordinator will review with the potential subject/authorized representative the informed consent form, and clearly explain all the procedures and risks of the testing. The subject/authorized representative will be encouraged to ask questions, both during the initial interview and throughout the study. The PI or a co-investigator/coordinator will answer any questions regarding the study during the consent process. During the enrollment interview, the subject/authorized representative will be instructed not to take any prescribed or over-the-counter herbal supplements or nutraceuticals during participation in this study to avoid potentially confounding study results. Once enrolled, the subject may terminate his/her participation at any time during the study.

VIII. STUDY PROCEDURES

Study Design:

This is a randomized, double-blind, placebo-controlled phase II study. We plan to enroll 30 subjects with moderate to severe TBI (Huperzine = 15, Placebo = 15). Subjects may be inpatients or outpatients. Subjects will receive study drug or placebo for 12 weeks, and follow-up will continue through Week 52. Subjects enrolled in this study will receive standard acute and rehabilitative care. All subjects will be enrolled at Spaulding. Spaulding inpatient subjects who are discharged from Spaulding prior to closure of the active treatment window (at week 12) will continue to be followed either by telephone or in-person. Weekly phone calls between the study coordinators and subjects (or their authorized representative) will ensure that, for each subject actively enrolled, the dosage of study medication is correct, any new adverse events (including seizure) have been captured and any concerns raised by the subject or study staff have been addressed.

Recruitment and Consent

SRH Inpatients

Subjects will be recruited from the inpatient brain injury rehabilitation units at Spaulding Rehabilitation Network facilities. Medical records will be pre-screened by study staff to identify potentially-eligible subjects and relevant inclusion/exclusion criteria. The patient or patient's legal surrogate will be approached for consent after basic information about the study is provided. The patient/authorized representative will review study information and discuss the study with the coordinator/co-investigator. During the enrollment interview, the subject/authorized representative will be instructed not to take any prescribed or over-the-counter herbal supplements or nutraceuticals during participation in this study to avoid potentially confounding study results. The coordinator/co-investigator will answer all questions and provide all study information to the patient/authorized representative prior to obtaining consent. After consent has been provided, the screening process will take place.

Community-Dwelling Outpatients

Potential participants will be recruited from the Spaulding Rehabilitation Hospital Network, and MGH psychiatry, and Home Base clinics. Clinic staff will be informed about the study with an information sheet and asked to speak to patients fitting the inclusion/exclusion criteria. If a potential participant gives permission to be contacted, the research staff will review available medical records to determine preliminary eligibility prior to contacting them to discuss the study and their potential participation. During the enrollment interview, the subject/authorized representative will be instructed not to take any prescribed or over-the-counter herbal supplements or nutraceuticals during participation in this study to avoid potentially confounding study results.

Additional recruiting will be done through local healthcare providers, PatientsLikeMe, BIA chapters, mailings to databases such as RPDR, SHIP and TBIMS, support groups, health fairs, community programs for TBI patients and families, treating physician letters to patients, flyers posted in clinics, newsletters and local and regional presentations. Subjects will also be recruited through postings to electronic bulletin boards, internet websites (i.e. ClinicalTrials@Partners), and Spaulding's intra/internet web pages. Newspaper, TV, magazine, and radio ads as well as press releases will also be utilized.

A recruitment log of potential subjects screened using the study's inclusion/exclusion criteria will be maintained. The log will contain PHI such as the potential participant's name, date of admission and, if excluded, the rationale, which may be medical. Persons on the log include individuals that are being followed by study staff, pending consent, participating, refusing to participate or have been screened out. The log will be maintained during the study for tracking purposes to avoid re-approaching or re-screening potential participants, thereby, decreasing potential participant burden. This recruitment log will be destroyed at study completion.

For all potentially eligible patients, the patient, or authorized representative of a patient who lacks decision-making capacity, will be approached for consent. If the decision-making capacity of a subject is in question, the subject will be evaluated by a member of the clinical staff to determine if the subject understands the difference between treatment and research, the risks and benefits of the research protocol and its procedures, and the consequences of acting (or not acting), and can make the choice to participate. If the subject is determined to lack decision-making capacity, persons who might be proposed as appropriate authorized representatives are, in order of general

preference: (1) court-appointed guardians with authority to consent to participation in the proposed research or authority to make decisions for a class of health care decisions inclusive of the proposed research, (2) health care proxy with authority to make decisions for a class of health care decisions inclusive of the proposed research, (3) durable powers of attorney with authority to make health care decisions inclusive of the proposed research, or (4) a spouse, adult child, parent, adult sibling, or other close family member. If an authorized representative signature is required due to lack of decision-making capacity, the authorized representative will sign the consent form on the Guardian or Legal Representative for Adult line and the subject may also sign the Assent line. Documentation of the informed consent process will be recorded for each subject with the Partners QI Documentation of Informed Consent document.

If, in the opinion of the clinical staff or research team, a participant who was originally enrolled in the study by an authorized representative subsequently regains decision-making capacity (e.g., emerges from post-traumatic amnesia), he or she will be re-consented to determine the participant's preference for continued study participation.

During the consent process, participants/authorized representatives will be asked if they would like to be contacted regarding future research opportunities. If the participant/authorized representative chooses not to decide either "yes" or "no" to contact for future research at the time of signing, the co-investigator/coordinator will ask at subsequent follow-up visits, and their pending decision will be tracked. Completion of the study before obtaining an answer will result in that participant not being contacted regarding future research opportunities.

Screening and Baseline Assessments:
As part of the screening process, we will complete the following assessments:

- Neuropsychological examination (i.e., GOAT, Trails B)
- Learning and Memory short form of the Traumatic Brain Injury Quality of Life (TBI-QOL)
- Ruff Neurobehavioral Inventory (RNBI) Postmorbid Cognitive Domain
- Required lab studies (pregnancy test via serum hCG, hepatic, hematologic, and renal function) and physical examination (including vital signs and EKG to rule out cardiac arrhythmias)
- A medical history including seizure history will be obtained via the patient's medical records
- Baseline symptom checklist
- TMS screening checklist

If all eligibility criteria are met and the patient is enrolled in the study, randomization will take place. We will employ a computer-generated randomization scheme to determine whether the subject receives placebo or Huperzine A. Randomization will be performed in permuted blocks of four or eight with random variation of the blocking number to prevent potential unblinding. The randomization code will be kept by a third party with no access to subjects or study data.

Within 7 days of randomization, the following additional baseline assessments will be completed:

- Neurophysiological baseline measures (i.e. P50, P300 and SAI)
- Neuropsychological baseline measures (i.e. CVLT-II,)
- Learning and Memory short form of the Traumatic Brain Injury Quality of Life (TBI-QOL)

- Ruff Neurobehavioral Inventory (RNBI) Postmorbid Cognitive Domain
- Beck Depression Inventory
- Modified Brief Pain Inventory
- British Columbia Post Concussion Scale
- Eye Tracking baseline measures

Study Visit Timeline:

All study visits for inpatients and outpatients will take place in-person at Spaulding. During the course of this study, the subjects will be assessed at predetermined time points. These time points are: Screening/Baseline, Week 6, Week 12, Week 13, Week 24 and Week 52. In the table below, the study visits and assessments are outlined.

As we are investigating patients with Traumatic Brain Injury, there may be instances where the study timeline will need to be adjusted. This may occur if the patient is transferred during his/her hospital stay through clinical treatment, or during follow-up scheduling. Therefore, we will employ a window of **+/- 2 weeks** to complete the study visits, in order to accommodate for scheduling issues and changes in the subject's care.

Study Visit Summary:

Table 1 provides a summary of the study visits and study-related activities.

Table 1: Study Visit Summary

			Hup-A Dose Administration Period				
	Screening	**Baseline**	**6 Wks**	**12 Wks**	**13 Wks**	**24 Wks**	**52 Wks**
History and Physical							
Baseline Symptom Checklist	X	X	X	X	X	X	X
Pregnancy Test (Serum hCG)	X	-	-	-	-	-	-
Medical History Review	X	-	-	-	-	-	-
TMS Screening Checklist	X	-	-	-	-	-	-
Laboratory Data (renal and liver function, hematology) including a general chemistry 6 panel at baseline	X	-	X	X	X	-	-
ECG	X	-	X	X	X	-	-
Physical Exam (to include vital signs)	X	-	X	X			
Neurocognitive							
GOAT	X	-	X	X	-	X	X
Trail Making Test A&**B**	X	-	X	X	-	X	X
TBI Quality of Life	X	X	X	X	-	X	X
Ruff Neurobehavioral Inventory	X	X	X	X	-	X	X
CVLT-II	-	X	X	X	-	X	X
Brief Pain Inventory	-	X	X	X	-	X	X
Suicidality Scale	-	X	X	X	X	X	X
Beck Depression Inventory	-	X	X	X	-	X	X
British Columbia Post-Concussion Scale	-	X	X	X	-	X	X
Neurophysiologic							
Eye Tracking (Pupil Activation)	-	X	X	X	-	X	X
P50/P300 (EEG)	-	X	X	X	-	X	X
SAI (TMS)	-	X	X	X	-	X	X
TMS Adverse Effects Checklist	-	X	X	X	-	X	X
Concomitant Safety Monitoring							
Drug Side Effects	Weekly				X	-	-
Seizure Log	Weekly				X	-	-
Medication Log (for all medications)	Weekly				-	-	-
Adverse Event Monitoring	Ongoing Monitoring						

On a weekly basis during the 12-week active treatment period, study staff will record medication changes that have occurred since the previous week. For inpatient subjects, study staff will conduct a structured chart review and speak with the treating physician/nursing staff to identify any medication changes. For outpatient subjects, medication monitoring will be conducted through weekly phone calls. In addition to recording medication changes that have occurred over the last week, changes in baseline symptoms, side effects from Huperzine-A, adverse events and episodes of seizure activity that may have occurred in the intervening week will also be recorded. Approximately every three months after the 12-week active treatment phase (i.e., week 24, week 36, week 52), subjects will be monitored for the occurrence of any new *serious* adverse events as well as any unresolved/unstable AEs that may have occurred during the 12-week treatment period. Subjects will be instructed to call the Study Coordinator if a serious adverse event occurs between scheduled follow-ups. Serious adverse events will be reported immediately, in accordance with DSMB and IRB policies.

Drug Administration and Dosing:

Within 7 days of completion of the baseline assessment, subjects will receive the initial dose of the study drug (Huperzine A or placebo). The study drug will be prepared and stored by a third-party vendor and will be shipped to the Spaulding pharmacy. Due to changes in Massachusetts State Laws compounding pharmacies are now required to prepare and label the study drug for each individual subject. The shelf life of the study drug has been reduced from one year to six months. The study drug will be couriered to SRH hopefully, within 24 hours of receiving the prescription. Thus, the window between randomization and first dose of study drug has been increased to seven days. The randomization list will be maintained by a Spaulding pharmacist with no access to subjects or study data. Please see Appendix I for more detailed drug administration guidelines. For inpatient subjects, the study drug will be administered by nursing staff. For outpatients, instructions will be given to the subject and/or caretaker on how to continue the dosage schedule. Weekly phone calls will be made to ensure compliance.

Dose Titration:

The starting dose of the study drug will be 100 micrograms once a day, administered in the morning. The starting dose will be continued for 4 days and will then be increased on a fixed titration schedule as follows: 100 micrograms twice a day for 4 days; 200 micrograms in the morning, 100 micrograms at night for 4 days; 200 micrograms twice a day for 4 days; 300 micrograms in the morning, 200 micrograms at night for 4 days; and then 300 micrograms twice daily for the remainder of the 12 weeks of treatment (64 days). See the table below for a summary of the study drug dosages. At the completion of the 12 weeks of dosing, all subjects will terminate study drug.

Table 2: 12-week Dosing Schedule - Titration Summary

Timeframe	Total Dose	Amount
4 Days	100 micrograms	AM: 100 Micrograms
4 Days	200 micrograms	AM: 100 Micrograms; PM: 100 Micrograms
4 Days	300 micrograms	AM: 200 Micrograms; PM: 100 Micrograms

4 Days	400 micrograms	AM: 200 Micrograms; PM: 200 Micrograms
4 Days	500 micrograms	AM: 300 Micrograms; PM: 200 Micrograms
Remainder treatment (64 days)	600 micrograms	AM: 300 Micrograms; PM: 300 Micrograms

Drug - Dose Intolerance:

In the case of a drug-dose intolerance, the dose of the study drug will be reduced by one dosage level. For example: from [AM: 300 mcg; PM: 300 mcg] to [AM: 300 mcg; PM: 200 mcg]. The subject will continue at the reduced dosage level for 7 days and then the dose will be escalated to the next dose level. Should the subject redevelop a drug-dose intolerance, the subject will stop the drug regimen.

Blinding Procedure:

Participants, family members, treating staff, physicians, RAs/data collectors, SRH pharmacists, site investigators and the statistician at the lead site will be blinded to group assignment until after the final analyses. The lead pharmacist who prepares the study drug kits will be blinded to group assignment. Johnson Compounding and Wellness Center, which is responsible for the randomization, will be unblinded. When Johnson Compounding and Wellness Center sends the medication to SRH for dispensing to the subject, a note indicating the contents, "Active" or "Placebo," will be with the medication. This note will be placed in a sealed envelope for storage should the subject's medication require unblinding to treat an adverse event.

Guidelines and Procedures for Breaking the Blind:

In rare cases, it may be necessary to break the blind to facilitate management of a serious adverse event (SAE). At each site, an assigned person (the PI or designee) will have access to unblinding. In the vast majority of cases where adverse events are noted, however, the decision about whether or not to continue the study drug, and what treatment to provide, if any, can be made without knowing whether the patient was receiving study drug or placebo. Since most SAEs might be due to something else even if the patient is in the active treatment group, the treating physician should assess multiple possible causes of the adverse event and should stop the study drug at least temporarily as long as there is some chance that it is responsible for the event. Just as in clinical practice, if an adverse event is thought to be due to a given drug, and that drug is stopped, clearance of the adverse event tends to support the causal connection. On the other hand, failure of the symptoms to resolve with stopping the drug requires the physician to search for alternative causes. Thus, in general, the treating physician should make a decision about whether or not an adverse event is likely to be connected to the study drug in a blinded fashion, and stop the drug where appropriate, at least temporarily. <u>If the adverse event does not resolve, the physician may identify another etiology and make a decision, in the future, to restart the study drug.</u>
In order to meet the criteria for unblinding the following scenario should be true. In uncommon instances, the treating physician may feel that it is critical to know which drug the subject is receiving, because:

1. The adverse event is serious; and
2. It will be potentially harmful or costly to stop the study drug *and* act simultaneously on

other possible causes of the adverse event; and
3. It will be dangerous to stop the study drug and wait for a few days to see whether the adverse event resolves before acting on other possible causes.

In all cases, the Treatment Unblinding form, which prompts the PI to address the need to unblind to ensure unblinding is indeed necessary, should be completed prior to unblinding the participant. Unmasking/unblinding should be considered a serious action. The treating physician may unblind the participant if unblinding is considered to be essential to clinical management.

The following questions should be answered on the Treatment Unblinding form by the treating physician whenever unblinding is being considered or has been implemented:

1. What is the adverse event that leads you to want to unblind the treatment condition?
2. What prevents you from addressing all possible causes of the adverse event simultaneously?
3. What prevents you from stopping the study drug blindly and waiting a few days to evaluate the course of the adverse event, and then make decisions about other interventions accordingly?

In all cases in which unblinding has occurred, the study physician treating the participant has been unblinded and will record and maintain these data in a confidential log so the case can be reviewed and the reasons for unblinding tracked. The treating physician should be reminded not to reveal the treatment assignment to any other staff members unless this information is essential to patient management, or to the patient or the patient's family.

Note: To avoid inadvertent or non-essential episodes of unblinding, the PI (or designee) should assure that the covering physician staff, residents, physician's assistants and nursing staff is informed that stringent guidelines must be followed when starting and stopping all medications. Unblinding cannot occur unless the Guidelines for Unblinding have been reviewed and completed by the PI.

A record of each subjects' assignment from Johnson Compounding and Wellness Center, "Active" or "Placebo," is placed in a sealed envelope labeled with the Study Name and ID along with the subject's name and Study ID Number on the outside. This envelope is placed in a larger envelope labeled with the Study Name and ID in the SRH Night Pharmacy Omnicell automated dispensing machine. Only SRH Nursing Managers and the SRH IND Pharmacist, using the Omnicell machine can access the study envelope using the product identifier "unblinding key."

Follow-up Assessments:

Subjects/surrogates will be contacted by the study coordinator two weeks before each scheduled follow-up visit to arrange an in-person visit at Spaulding. Follow-up visits will be conducted at 6, 12, 13, 24, and 52 weeks. There will be a **+/- 2-week** window to complete these milestone visits to accommodate for scheduling issues. Actual dates for each visit will be recorded to ensure that the follow up visits are within the appropriate time window. In addition, we will conduct a telephone call to the subject at week 13 (one week after drug has stopped) as a safety assessment. If it is not possible to complete an in-person assessment, follow-up activities involving symptom

reporting, adverse events and medication changes will be conducted by telephone.

Inpatient-Outpatient and Acute Care Transition Procedures:

As part of this study focuses on Traumatic Brain Injury in an inpatient population, there may be subjects who are transferred from Spaulding Rehabilitation Network (SRN) inpatient to other outpatient sites (either inside or outside SRN), to acute care based on clinical treatment needs, or home. In these cases, we will follow our subjects with an intent-to-treat procedure as outlined below.

1. In all cases of discharge or transfer (prior to 12 weeks) we will attempt to continue the subject's dosage schedule of the investigational drug. For all subjects, regardless of whether dosing continued for the full 12 weeks, we will attempt to follow up with these subjects for follow-up assessments. Subjects who do not return for any follow-up assessments will be considered lost to follow up.
    a. Upon discharge, instructions will be given to the subject and/or caretaker on how to continue the dosage schedule. Study staff will follow up with all monitoring procedures (as outlined in the study visit table) via weekly phone calls.
2. In addition, if the subject transfers to acute care:
    a. We will attempt to continue the subject's dosage schedule as outlined above.
    b. If this is not possible, there will be a **1 week** window during which the treatment schedule will be deferred to accommodate for appropriate acute clinical care during this time; that is, study drug may be stopped for up to 1 week, and then restarted. This restart may only happen once per patient during the trial.
    c. If the subject returns to the study within **1 week**, he/she will continue the dosage schedule at the last administered dose. If the subject is unable to return within 1 week, he/she will be analyzed as a non-compliant patient.

IX. DESCRIPTION OF ASSESSMENT AND OUTCOME MEASURES

Outcome measures:

The outcome measures, assessment schedule, and change indices we selected are designed to optimize capture of the primary treatment effect (CVLT-II) while considering normal variation in test performance as well as practice effects which may occur with repeated assessments. The proposed test battery consists of measures selected from the NIH NCMRR TBI study group for the COBRIT study [23].

All outcome measures will be assessed in person, with the following schedule: baseline (pretreatment), 6, 12, 24, and 52 weeks post-intervention.

The primary outcome measure, the California Verbal Learning Test-II (CVLT-II), is sensitive to attention, memory and aspects of executive functions, all of which may be altered by Huperzine A because of their dependence on dopaminergic and cholinergic activity. The CVLT-II will be administered at baseline, 6 weeks, 12 weeks, and 52 weeks. With the exception of the 24-week timepoint, these assessment points coincide with the neurophysiologic assessment schedule

(described below) and will allow us to explore the correspondence between temporal changes in biologic and cognitive markers associated with the intervention.
Variability in cognitive performance within and across assessments is common in both healthy and neurologically-impaired populations and may exceed two standard deviations in some cases. Practice effects are also common and generally attributable to increased familiarity with test materials, content, and process. To help mitigate these potential influences on test scores, Reliable Change Indices (RCI) [26] will be calculated at the 3 and 12 month reassessments. RCI's are determined by first subtracting the mean T2 - T1 change from the difference between the two testings for each individual, and then comparing it to 1.64 times the standard deviation of the difference. A difference of this magnitude is exceeded only 10% of the time (in either the positive or negative direction) if there is no real change. Applying a correction to change scores is particularly important in samples that include patients with severe TBI, as practice effects are magnified at higher levels of impairment and with shorter test-retest intervals [27].

All subjects will also undergo a neurophysiologic examination at the time of their baseline cognitive testing, and at 6 weeks, 12 weeks, 24 weeks and 52 weeks.

To control for the potential confounding effects of mood, pain and subjective distress on treatment, we will administer self-report measures in these areas at the time of enrollment. Scores on the Beck Depression Inventory, modified Brief Pain Inventory and British Columbia Post-Concussion Scale, obtained at baseline, will be used as covariates in our primary analysis. We will also use time post-injury as a covariate to control for the possible influence of length of time since injury on treatment outcome as spontaneous recovery will be more rapid in subjects enrolled early versus late, and because those enrolled later will be higher functioning at baseline.

Screening/Follow-up measures:

- Galveston Orientation Amnesia Test (GOAT): A series of 10 questions asked to a patient to help evaluate posttraumatic amnesia. The test is repeated at each visit, and is scored on a scale of 0 to 100. A patient is determined to be out of the amnesic state when the score exceeds 75 on three consecutive examinations.

- Trail Making Test B: Part B of this test requires the patient to alternate between numbers and letters (i.e., 1-A-2-B, etc.) distributed in a spatial array [28, 29]. The task alternation aspect of Trail Making B increases its sensitivity to aspects of executive/frontal lobe function that are frequently altered in patients after TBI.

- Traumatic Brain Injury Quality of Life (TBI-QOL): The TBI-QOL [28] was developed as a comprehensive patient-reported outcomes (PRO) measurement system specifically for individuals with traumatic brain injury (TBI). It consists of 20 independent calibrated item banks and 2 uncalibrated scales that measure physical, emotional, cognitive, and social aspects of health-related quality of life. We will administer only the short form (6 questions) of the TBI-QOL Learning and Memory subscale.

- Ruff Neurobehavioral Inventory (RNBI): The RNBI [29] is a self-report instrument for assessment of a wide range of symptoms (cognitive, emotional, and physical), as well as

quality of life and daily functioning. It was designed to assess these areas in individuals who have recently been affected by an injury, illness, or other stressor. We will be administering the 24 question Postmorbid Cognitive Domain form of the RNBI.

Primary measure:

- CVLT-II: The California Verbal Learning Test provides a reliable measure of memory encoding and retrieval, which is often affected in patients with TBI. The California Verbal Learning Test second edition [30, 31] measures verbal learning and memory using a multiple-trial list-learning task. Sixteen words from 4 semantic categories are repeated for each of the five learning trials for list A. A distractor list (B) of 16 words from 2 of the same and 2 different semantic categories is then given. Short and long free and cued recall measures of list A are presented. There is also a recognition paradigm. Z-scores are computed for total learning trials 1-5 and all recall and recognition trials.

Covariates:

- Beck Depression Inventory (BDI): The Beck Depression Inventory (BDI) is a 21-item test presented in multiple-choice format that measures the presence of and the degree of depression in adults [32].The BDI will be used to evaluate if mood is a confounder in this study.
- The modified Brief Pain Inventory: The BPI is a short self-assessment questionnaire that provides information on various dimensions of pain including how pain developed, the types of pain a patient experiences, and time of day pain is experienced, as well as current ways of alleviating pain[33].
- The British Columbia Post-concussion Scale: The scale evaluates several clinical symptoms (13 categories) of concussion (i.e. headache fatigue, dizziness, sleep disturbance, etc) and asks individuals to endorse the level of severity [34]. Symptom frequency ratings over the prior two weeks are also evaluated. The scale has been normed to investigate the expected rate of symptomatology in the non TBI population, making it an attractive metric for TBI.
- Time since injury: Time since injury will be used as a covariate to control for the possible influence of injury chronicity on treatment outcome.

Secondary measures (neurophysiological assessments):

In addition to the neurocognitive assessment, we will also conduct several neurophysiologic assessments in line with our secondary aim of investigating the neurophysiologic effects of Huperzine A. These assessments will provide insights into the mechanisms of Huperzine A in modulating mood and cognition.

- P50 and P300 (via EEG) – event related potentials:
  We will measure P50 and P300 using auditory stimuli. These neurophysiological measurements index cortical electrical activity associated with a given stimulus and therefore our hypothesis is that if there is an improvement in activation of cognitive-related

neural networks, then the signal as indexed by P50 and P300 will increase. Specifically, P50 represents an index of activity in the cholinergic system and has been used to characterize presynaptic cholinergic deficit [35] and P300 is a measure of general cognitive processing elicited during attention, memory, and executive tasks. A decrease in P300 latency indicates faster processing and can serve as an index of cognitive improvement.

- Short Latency Afferent Inhibition (SAI) (via TMS):
  TMS can index cortical activity as it is possible to elicit a cortical motor evoked potential. Before undergoing the procedure, we will administer a TMS screening checklist to the subject so as to assess whether the subject is eligible to receive TMS. Contraindications to TMS include (but are not limited to): metal in the head and/or implanted brain medical devices. If the patient has recently undergone a craniectomy TMS will be administered to the contralateral hemisphere. If it is determined that subject is not eligible for this procedure, he/she will continue the study without collecting the TMS data, as this measure is a secondary outcome.
  Responses to stimuli applied to the motor cortex will be recorded from surface electromyography (EMG). Silver/silver chloride electrodes will be placed over the muscle belly (active electrode) and joint or tendon of the muscle (reference electrode) to record MEPs. Conditioning stimuli will be done via single pulses of electrical stimulation applied to the median nerve at the wrist. The intensity of the conditioning stimulus will be adjusted over motor threshold for evoking a visible movement of the thenar muscles. The conditioning stimulus to the peripheral nerve will be applied before the magnetic test stimulus. Furthermore, according to the parameters used it is possible to assess the activity of certain neurotransmitters. In this application, we will measure SAI that can also be considered a measure of cholinergic activity. Our hypothesis is that cholinergic activity will increase in subjects randomized to Huperzine A compared to those randomized to placebo, and therefore a decrease in SAI will be seen in the Huperzine A arm. We will monitor for adverse effects using an adverse effect questionnaire designed for TMS stimulation.

- Task-evoked pupillary response (TEPR):
  Changes in pupil diameter due a cognitive task are considered a reliable and sensitive index of the degree of cognitive load and resource demand to the task. We will record pupillary response, indexed as changes in pupil diameter, to two sets of tasks measuring cognitive performance: a low cognitive-load task vs. a high-cognitive load task. The first will consist of a set of simple stimuli presented to the patient in an organized and timely manner, which will then be recognized and named by the subject. The high-cognitive load task includes the presentation of a larger and more complex array of information for the subject to recall. Both sets of stimuli will be shown in a computer screen with a fixed interval between stimuli. Measurement of pupil diameter and reactivity will be conducted with a remote eye-tracker device. It consists of a high-resolution 17" computer screen placed in front of the subject's face that allows both stimuli display and pupil tracking through infrared diodes that generate reflection patterns of the user's cornea.

- Smooth pursuit eye movement (SPEM):

Eye movements will be recorded by an infrared-based eye-tracking device consisting of a high-resolution computer screen that will be placed in front of the subject's face at a 40cm distance. Subjects will be presented with a target stimulus that will move in a predetermined and consistent trajectory in the fixed background screen. We will measure different parameters based on this recording both at baseline and treatment completion, including eye and target velocity, index of target prediction, eye position error and intra-individual variability of eye error. Comparison of baseline to after-treatment measurements will be considered a reflection of cognitive changes.

X. STATISTICAL ANALYSIS

This is an exploratory phase II study with the intent of generating hypotheses for a confirmatory phase II/III trial, as well as in obtaining effect sizes and variances for sample size calculation for further confirmatory studies. The study is also designed to address the tolerability of Huperzine A in the moderate and severe TBI populations.

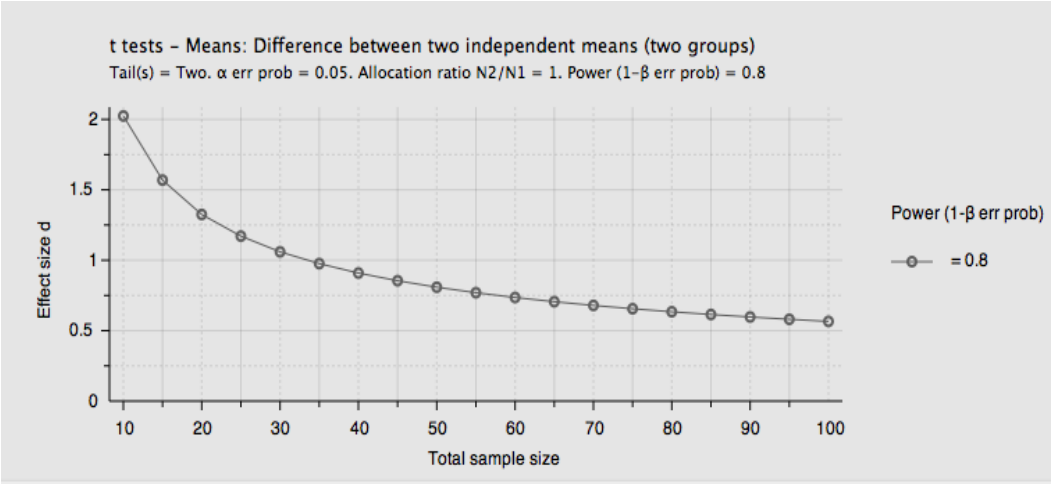

Figure 2: Effect size to total sample size ratio necessary for a powerful study

In designing this exploratory study, we conducted a sensitivity analysis to obtain a sample size that would provide a reasonable effect size when comparing Huperzine A to placebo (Figure 2). This allows for detection of differences between the two treatments, to plan for further studies. Assuming a power of 80% and an alpha of 5%, we originally projected that a sample of 60 patients (30 patients in each group) would allow us to detect an effect size of 0.75 between the two groups (placebo and active). Given our prior studies in TBI and cognition, an effect size of 0.75 for the primary outcome measure (CVLT-II) is clinically meaningful, to compare two treatments.

The authorized addition of two self-report measures to our outcome assessment battery (i.e., Cognitive Function subscale from the Neurological Quality of Life Questionnaire and the Ruff Neurobehavioral Inventory) was expected to influence our original power estimate and required sample size. We calculated our effect size by estimating the upper and lower limits of the change score that would be demonstrated by the control group on our primary outcome measure (i.e., California Verbal Learning Test-II). This analysis showed that, to achieve an effect size of 0.75, the final sample size could vary between 15 and 39 subjects per group, given a mean difference

in performance between groups of 3.59 with a standard deviation of 3.30 to 5.13. In view of the variability in this estimate, we proposed that we would run 10 subjects, determine the degree of variability in performance and then adjust the sample size accordingly.

After enrolling 10 subjects, we assessed the data for an estimate of the average difference between groups (difference to baseline) on a ratio of 3:6. There was an estimated effect size of 1.19 between group A (M=4.67, SD=4.51) and group B (M=0.33, SD=2.50). Using a two sample t-test to detect the difference between groups, a minimum sample of 26 subjects will be required (13 per group).

The anticipated loss to follow-up rate in this study is around 15% - like our other drug studies. To account for this loss-to-follow-up rate**, a total of 15 patients per group (30 total) will be enrolled in the study**. The primary analysis will be an intent-to-treat analysis that will include all randomized participants regardless of their compliance with the study treatment or follow-up schedule. Study subjects should have received at least 1 dose of study drug/placebo. All hypothesis testing will be conducted using $\alpha = 0.05$, two-sided.

To analyze the data, we will compare means using t-test and modeling the data with ANOVA for multivariate analysis controlling for baseline values and other important covariates. If necessary, non-parametric approaches including Mann-Whitney and Friedman's test for multivariate analysis will be used with data that are not normally distributed. We will also use Fisher's exact test for comparison of categorical variables such as proportion of adverse effects. In summary, we will use the following statistical tests according to our primary aims:

- Primary Aim: To determine whether Huperzine A, as compared with placebo, has a differential effect on learning and memory functions after moderate to severe TBI.

For this aim we will compare means using t-test (comparing differences (Week 12 – baseline) between the two groups) and modeling the data with ANOVA for multivariate analysis controlling for baseline values and other important covariates (for secondary analysis). If necessary, non-parametric approaches including Mann-Whitney and Friedman's test for multivariate analysis will be used with data that are not normally distributed.

- Secondary aim 1: To determine whether administration of Huperzine A produces significant differences in neurophysiologic markers (as indexed by EEG event related potentials (P50 and P300) and TMS-indexed cortical excitability (cholinergic activity)) associated with cognition, relative to a placebo.

For this aim we will also build models in which the main dependent outcome will be amplitude or latency measures (from P50, P300 and SAI) and the independent variables will be group, time and the interaction group*time.

- Secondary aim 2: To determine whether Huperzine A reduces the prevalence of post-traumatic seizures after moderate and severe TBI as compared to placebo

For this aim, we will compare proportion of seizures within the first 6 weeks between Huperzine vs. placebo group using a Fisher's exact test (as we expect small numbers of expected frequencies in the cells).

- Secondary aim 3: To evaluate the safety and tolerability of Huperzine A in this patient population as compared to placebo. Safety and tolerability will be assessed by frequency of adverse effects, including laboratory abnormalities.

XI. RISKS AND DISCOMFORTS

Transcranial Magnetic Stimulation:

Note that we will be using single and paired-pulse TMS as a diagnostic tool. This type of TMS stimulation does not change cortical excitability or brain activity. There is a small chance of single pulse TMS inducing a mild, transient headache, or neck pain. TMS equipment produces a clicking sound when a current is passed through the stimulation coil. This click can result in ringing in the ear and temporary auditory threshold shifts if no protection is used. In order to prevent this potential adverse effect subjects will wear earplugs during TMS.

Electroencephalography (EEG):

The EEG test is performed to measure the electrical activity in the brain and to examine the dynamic changes. It also allows for better understanding of the effects of electrical activity generated in different areas of the brain. EEG only measures brain activity and does not induce electrical current. It is non-invasive and has been used extensively in clinical practice for diagnosis or neurological conditions such as epilepsy. There are no risks associated with EEG other than a mild discomfort caused by the tightness of the EEG net. The investigator will adjust the net to allow for the comfort of the subject.

Eyetracking and pupil dilation measurement:

The eyetracking tasks that will be performed help us to measure cognitive load capabilities of the subjects. Subjects will complete both task-evoked pupillary response and smooth-pursuit eye movements by watching a high-resolution computer screen while resting their head on a headrest. There are therefore no added risks associated with this device. During the procedure, participants may experience minor discomfort resulting from sitting still and minimizing movements during the experiment. Rarely, some eye dryness or watering of the eyes may result from the test. If the subject's eyes become uncomfortable, the person administering the test will stop the procedure so the subject may rest their eyes.

Huperzine-A:

Potential side effects of Huperzine A include: nausea, vomiting, diarrhea, sweating, blurred vision, slurred speech, loss of appetite, contraction and twitching of muscle fibers, cramping, increased saliva and urine, inability to control urination (incontinence), hypertension, and slowed heart rate (bradycardia) [36]. As with any investigational agent, there may be side effects that are unknown that may occur.

Neuropsychological assessments/questionnaires:

There are no risks involved with the neuropsychological testing batteries used in this study. If the subject feels uncomfortable answering any of the questions or performing any of the neuropsychological assessments, he/she may stop at any time. If the subject becomes bored or fatigued while completing the battery, he/she may be able to take a break and rest before completion.

Expected symptoms of Traumatic Brain Injury:

Based on the severity of the subject's TBI, there are many different symptoms expected that are related to the injury. Thus, these symptoms can change as the patient's injury status progresses from the acute to chronic phase. These expected symptoms may include medical (e.g., hemodynamic changes, fever, dysautonomia), cognitive (e.g., confusion, distractibility, memory impairment) and behavioral (e.g., agitation) problems. Please see Appendix A for the list of adverse events frequently seen in the TBI population.

In order to determine whether side effects seen during the study are related to TBI or Huperzine A, we will review baseline symptoms of TBI for the patient prior to starting Huperzine A, monitor for exacerbation of existing symptoms and assess for the onset of new symptoms through systematic medical record review, medication/seizure/side effects checklists and also through contact with the subject and treating physician/nursing team.

Data Safety Monitoring Board and stopping rules:

Adverse events will be collected from the start of Huperzine A treatment through the end of study participation. During the 12 weeks of study drug, all adverse events will be collected. However, after study drug dosing is complete, we will only collect information on serious and/or unresolved/unstable adverse events that are clinically meaningful. All adverse events regardless of attribution to Huperzine A will be collected and recorded, using standard adverse event forms. All applicable local regulatory requirements related to the reporting of serious adverse events to regulatory authorities and the IRB will be followed during this study. Serious adverse events will be promptly reported to the IRB. The issue of placing the study on hold will be raised by the investigators with our local IRB if any serious adverse events occur.

Importantly, a data safety monitoring board (DSMB) will be established for the study, and will be comprised of a physiatrist (rehabilitation physician) with expertise in traumatic brain injury rehabilitation, a neuropsychologist with expertise in cognitive rehabilitation, and a biostatistician with expertise in clinical trials. This DSMB will meet after 50% of the subjects complete the study, and will meet again after 20 subjects have completed the study. The DSMB will be un-blinded in their assessment of AEs and we will adopt the following stopping rules:

- We will stop the study early if any patient dies, if any patient requires emergency surgery, or if any patient suffers a permanent and irreversible disability, only if the DSMB determines that this event was definitely or probably related to study drug.
- We will also stop the study if there is disproportionate incidence of serious adverse events in the active treatment arm vs. the placebo arm. Serious adverse effects will be defined as any adverse event, including a significant change in lab values, that is: (i) fatal, (ii) life-

threatening, (iii) requires or prolongs inpatient hospitalization, (iv) result in persistent or significant disability or incapacity, v) a congenital anomaly/birth defect, or vi) an important medical event. Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

Because this clinical trial is largely exploratory, we will not employ a stopping rule for efficacy or for futility.

The DSMB will review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol through oversight of study interventions and interactions and will provide an unbiased written report of the event. At a minimum, the DSMB must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The DSMB must also indicate whether it concurs with the details of the report provided by the Principal Investigator. Members of the DSMB may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research with respect to safety and well-being of participants. The DSMB will protect the safety and well-being of human subjects through its review and bring findings promptly to the Principal Investigator and then to the IRB/HRPO. The DSMB shall have authority to stop the protocol or remove subjects from the study in order to protect the well-being of the subjects enrolled, should this be necessary.

XII. MONITORING AND QUALITY ASSURANCE

Safety monitoring and subject compliance:

- Medication Log: Prior to randomization, study staff will review the results of screening tests to identify potential drop-outs; subjects identified as being at high risk for noncompliance with study drug will not be randomized. For Spaulding inpatients, nursing staff will maintain a treatment record which will be checked weekly by study staff, and transcribed onto a medication log which will include the time, date, and each dosage of Huperzine A taken. Finally, any deviation from adherence to the medicine regime will be documented. Study data will be monitored for irregularities. Any irregularities in the data will be investigated for possible deviations in adherence. We will also track the patient's other medications (not involving Huperzine) on this Log – including dosing amounts and times. For community-dwelling outpatients, subjects and/or caretakers will be given a medication diary to fill out daily.

- Laboratory Data: Serum measures of renal and liver function will be obtained at baseline, 6 weeks, 12 and 13 weeks to evaluate for eligibility to participate in the study as well as any potential unexpected side effects. Hematologic data will also be obtained at baseline, 6 weeks, 12 and 13 weeks to observe for any side effects. A general chemistry panel will be completed at baseline. Clinical blood results obtained within 48 hours of the study time

points will be able to be used for evaluation thereby, reducing the number of venipunctures for the subject.

- Biomarkers: Blood samples for UCH-L1, GFAP, NF-l and Tau will be obtained at the time of enrollment, at 24 hours, 23 days, 30 days post injury and 90 days post injury. De-identified samples will be shipped to Quanterix Corporation for analysis after processing by the SRH clinical lab. After analysis Quanterix Corporation will return the samples to the Spaulding Rehabilitation Network Research Institute.

- Study Drug Blood Levels: Blood samples will be drawn from 10% of subjects to assess efficacy vs. steady- state HupA concentrations and tolerability during the maintenance phase. Samples will be drawn pre-dose and 1 hour post dose on Day 21 or Day <21 (at the end of dose escalation for each patient in the 10%, whatever dose they escalate to). During the maintenance phase a weekly pre-dose sample will be drawn. Week 12, on the last day of dosing, a pre-dose and 1 hour post-dose sample will be drawn. These samples will be processed in the SRH clinical labs, stored at -80, and then forwarded to the Illinois Institute of Technology Research Institute (IITRI) in Chicago. De-identified samples will be shipped to IITRI and the link to the code will remain at SRH and not be provided to IITRI.

- Baseline Symptom Checklist: This will be completed before study drug is started. After treatment is initiated, the study staff should use the baseline symptom checklist list as a reference to identify and code new symptoms that arise after treatment is initiated, or to detect an exacerbation of a previously-noted symptom. For Spaulding inpatients, these data will be collected from systematic medical record review and also from speaking with the treating medical/nursing staff on a weekly basis. For outpatient, changes in baseline symptoms will be monitored through weekly phone calls.

- Drug Side Effects Checklist: For Spaulding inpatients, the study staff will evaluate potential side effects of Huperzine A using the drug side effect checklist during weekly systematic review of the medical record. This scale covers all known possible side effects of Huperzine A. In addition to speaking with medical/nursing staff, the subjects/caregivers may be asked to report whether they have experienced any side effects in an open-ended manner, and any side effects experienced by patients will be marked on a scale of 1 through 3 to measure severity (1- Mild, 2- Moderate, 3- Severe). If any side effects are reported, the degree of relatedness to the Huperzine A intervention will be assessed. For outpatient, side effects from Huperzine-A will be monitored through weekly phone calls.

- Seizure Log: Although it is not possible within the scope of this study to obtain definitive information regarding Huperzine A's anticonvulsant properties, a detailed seizure log will be utilized to evaluate for seizure events or signs associated with seizures in Spaulding inpatients. This log will be maintained weekly by the study staff collecting data through the systematic review of the medical record and speaking to the treating medical/nursing staff. Study staff will review documented chart evidence of seizure including any EEG that is performed as part of the routine medical care. For outpatient subjects, episodes of seizure activity will be monitored through weekly phone calls.

- Suicidality Scale – We will monitor for suicidality using the Columbia-Suicide Severity Rating Scale (C-SSRS) at baseline, 6 weeks and 13 weeks – as requested by the FDA Division of Neurology Products per our IND.

- Adverse event monitoring: In addition to the above, we will continually monitor for changes in patient health status. The baseline symptom checklist, side effect checklist and seizure log will be used to help monitor for changes in symptoms or side effects in Spaulding inpatients, and community-dwelling outpatients. For Spaulding inpatients, we will also monitor the patient's chemistry by the scheduled laboratory exams, and monitor compliance through the medication log. By using this multimodal approach, we will be able to appropriately rate events as they occur, and to accurately associate such events to the appropriate changes in subject status (ex. whether from TBI or Huperzine, expected or unexpected, etc). In addition, approximately every three months after the 12-week active treatment phase (i.e., week 24, week 36, week 52), subjects will be monitored for the occurrence of any new *serious* adverse events as well as any unresolved/unstable AEs that may have occurred during the 12-week treatment period. Subjects will be instructed to call the Study Coordinator if a serious adverse event occurs between scheduled follow-ups. Serious adverse events will be reported immediately, in accordance with DSMB and IRB policies.

Treatment Compliance:

Because effects will be dependent on patients taking Huperzine, treatment compliance will be essential in our study. We will measure compliance by asking the subject and/or caretaker to return the medication bottles at the end of the titration and maintenance periods. Any remaining pills will be counted, returned to the pharmacy and documented in the subject's folder, with specific forms. If the subject is still inpatient, we will monitor compliance through the medication administration system. Finally, we do not expect significant adverse effects associated with the use of Huperzine that would lead patients receiving this drug to become non-adherent.

Dropout in the placebo group:

Participants in the placebo group may disproportionately discontinue the study treatment since there will be little or no improvements in cognitive function. Although, this fact could bias the results, we expect that the placebo group will also have a small improvement (due to natural recovery, though smaller than in the active group) and we will conduct an analysis of the data using an intent-to treat population, to protect against disproportionate dropout.

XIII. POTENTIAL BENEFITS

It is possible that subjects participating in this research may benefit from the Huperzine-A given in the trial. Additionally, subjects may benefit from the use of TMS as the data could suggest plasticity changes and encourage clinicians to continue therapy or other interventions. Lastly

subjects may benefit from the close follow-up and enhanced observation of the clinical research team.

XIV. STIPEND

Each subject will receive a total of $450 paid throughout their study participation. The stipend payment distribution is as follows: Screening $25, Baseline $25, Week 6 $50, Week 12 $100, Week 13 $50, Week 24 $100 and Week 52 $100.

In addition, travel, parking and/or hotel costs will be reimbursed up to $200/ subject. Proof of payment of costs will be required for reimbursement.

Plan for Future Studies:

A major goal of this study is to collect data that may be useful to plan future phase II/III trials. In fact, this is one of the major goals of phase II studies that usually test several outcomes as there are no strong data to support a large trial. Therefore, with this study we expect to:

(1) Determine parameters for sample size calculation such as effect sizes for different endpoints.
(2) Based on the extensive assessments we are conducting, we will be able to determine in a preliminary manner the predictors associated with response. Therefore, further trials may optimize response.
(3) Determine duration of treatment as we may conclude that treatment duration is excessively long or short.
(4) Learn the neural mechanisms associated with Huperzine induced cognitive improvement; and thus, be able to optimize response.

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