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Electrical Stimulation of the Midbrain to Promote Recovery from Traumatic Forebrain Injury

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14. ABSTRACT
This was a first attempt to improve recovery from traumatic brain injury by prolonged electrical stimulation of rat's midbrain. A fluid percussion injury was created over the right motor cortex. After 4-6 hours, we implanted a stimulating microelectrode protruding from a small, epoxy-encapsulated electronic stimulator, which was attached to the skull, and began delivering 30-microampere negative current pulses to one of two midbrain areas: the dorsal raphe or the median raphe. Stimulation was given 12 hours per day for 1 week, in 5-minute alternating on and off periods at 7-8 Hz. Comparisons were made with injured, non-stimulated rats and with uninjured rats (stimulated and non-stimulated). Behavioral testing at 6 weeks showed that learning in a hidden-platform water maze test was speeded by both dorsal and median raphe stimulation. Rearing movements in a transparent cylinder (sensorimotor performance) were normalized by the median but not the dorsal raphe. One adverse effect was seen: the dorsal but not the median raphe reduced working memory in the water maze. Initial histological inspection suggested that the dorsal raphe stimulation enlarged the hippocampus. We conclude that early median raphe stimulation with a temporary implant give permanent benefits in some types of traumatic brain injury.

15. SUBJECT TERMS
Traumatic brain injury; deep brain stimulation; rat

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INTRODUCTION

We began with the hypothesis that certain brainstem regions that release the monoamine neurotransmitter serotonin from extensively branching axonal systems can produce generalized repair of forebrain function. We further suggested that subjecting such a system to sustained electrical stimulation promoted general recovery following traumatic brain injury (TBI). Before the present project started, we had developed a small, cranially implantable, self-powered stimulator assembly for rats, consisting of a microprocessor-controlled generator of intermittent cathodal pulse trains provided with 2-way control and communication, and an integral protruding microelectrode. We had also shown that a few days of stimulation applied to the serotonergic system descending from the nucleus raphe magnus (NRM) of the medulla could enhance anatomical and behavioral recovery from spinal cord injury in rats if started within a few hours of the injury [3]. The concept proposed for the present grant was that one or both of the two main ascending serotonergic systems, originating the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN), following a parasagittal fluid percussion injury (PFPI), could improve recovery of hippocampus-based spatial learning and of cortically controlled sensorimotor performance motor. In addition, we proposed that improved performance would be correlated anatomically with the size of cortical (including hippocampal) white matter tracts and neuron numbers.

BODY

The timeline in the Statement of Work was brought forward 2 months, since funding was begun in April 2008, as opposed to the anticipated month of June 2008. We refer here to the earlier timeline. Otherwise, all items from the SOW are included as originally specified. Findings are detailed in the section on KEY RESEARCH ACCOMPLISHMENTS below.

Timeline.

a) Prior to grant start: January, 2008. Apply to Institutional Animal Care and Use Committee for approval of animal use.

Status: done.

b) Month 1: April, 2008 Fabricate 30 stimulator implants with attached electrodes for stimulating dorsal raphe nucleus (DRN).

Status: done.

c) Month 2: May, 2008 Begin Specific Aims 1 and 2, using groups A-D, entering 3-4 rats into study per week for 8 weeks.

Aim 1: to see if patterned electrical stimulation of the DRN for 4 weeks, started 4 hours after fluid percussion injury in rats, improves recovery of spatial learning.

Aim 2: to see if procedures of Aim 1 reduce gross lesion volume (shrinkage) of the forebrain and improve cell counts, axon pathology and 5-HT staining density in the dentate gyrus.

Group A (n=6) sham-operated rats with stimulator implants that are never activated

Group B (n=6) stimulated sham-operated rats

Group C (n=6) injured rats with stimulator implants that are not activated until 8 weeks after TBI.

Group D (n=6) DRN stimulation with 30-µA, 1-ms, 8-Hz cathodal pulses

Status: Work began on schedule. However, due to the multiple responsibilities of the technician responsible for creating the injuries, 2 rats per weeks were entered into the study.

d) Month 3: June, 2008. Begin behavioral testing for Specific Aim 1, 6 weeks after TBI. Begin turning on stimulators in group C, 8 weeks after TBI, for Specific Aim 3.
Aim 3: to reverse longer term (8-week) TBI, behaviorally and anatomically, using the same intervention as group D.

Status: behavioral testing was begun, but Aim 3 was deferred and Aim 4 was started early (group G and additional group H). We added group H to provide appropriate control data for the effect of MRN stimulation on TBI. We deferred Aim 3 for several reasons. First, stimulators sometimes failed to turn on after 8 weeks of implantation. Second, we considered it more important to examine histological findings in group C with no further intervention. Third, we concluded that it would be better to prioritize the MRN study.
Aim 4: compare parametric alternatives of twice the stimulus amplitude, no nocturnal inactivation, and stimulating the serotonergic median raphe nucleus (MRN) on behavioral and anatomical outcomes from aims 1 and 2.
Group G (n=6) apply 30-µA pulses to the median raphe nucleus (MRN) in TBI.
Group H, same as group G but in sham-operated rats

e) Month 4: July, 2008. Testing continued from previous months.

Status: testing continued as planned.

f) Month 5: August, 2008. Begin repeat behavioral testing in group C, 14 weeks after TBI. Begin euthanasia and histological cutting and embedding in groups A-D, 15 weeks after TBI, for Specific Aim 2.

Status: histological processing started as planned.

g) Month 6: September, 2008. Testing continued from previous months.

Status: testing continued as planned.

h) Month 7: October, 2008. End of euthanasia and histological cutting and embedding for groups A-D.

Status: Because we added 2 rats per group, and because groups G and H were studied in parallel with A-D, these tasks were not completed until January 2009.

i) Month 8: November, 2008. Immunostaining of histological material from groups A-D. Analysis of swim-test data from groups A-D

Status: deferred to completion point of larger numbers of groups with more rats per group in February 2009.

j) Month 9: December, 2008. Analysis of stained material and correlations with swim-test data. Start preparation of 1st report for publication and poster presentation.

Status: deferred to completion point of larger numbers of groups in February 2009. Abstract was submitted on February 28, 2009 to Military Health Research Forum 2009 for meeting on August 31, 2009.

k) Month 10: January, 2009. Fabricate 20 stimulator implants with attached electrodes, modified for Specific Aim 4.

Status: the implants were made. However, we decided to modify the circuitry to provide a higher stimulus rate (x3, = 24 Hz) rather than a higher stimulus amplitude. This should give three times the release of serotonin [4] without undue current spread to tissue outside the target area [5].

l) Month 11: February, 2009. Begin Specific Aim 4, using groups E-G, entering 3-4 rats into study per week for 8 weeks.

Aim 4: compare parametric alternatives of twice the stimulus amplitude, no nocturnal inactivation, and stimulating the serotonergic median raphe nucleus (MRN) on behavioral and anatomical outcomes from aims 1 and 2.

Group E (n=6) apply 60-µA pulses to DRN (TBI).
Group F (n=6) apply 30-µA pulses to DRN without nocturnal (12-hour) hiatus (TBI)
Group G (n=6) apply 30-µA pulses to the median raphe nucleus (MRN) in TBI rats, or 60-µA pulses if group D's effects proved to be weak.

Status: Group E was modified as reported above for Month 10 to use of higher stimulation frequency instead of higher current. In addition, we chose the MRN rather than the DRN, due to superior outcome in initial behavioral findings (see KEY RESEARCH ACCOMPLISHMENTS below). Group F was converted to group H, as reported for month 3. In running group E, we added a contemporaneous control applying the higher frequencies to sham-operated rates (group I).

m) Month 12: March, 2009. Begin behavioral testing for Specific Aim 4, 6 weeks after TBI.

Status: testing has begun on schedule for groups E and I.

n) Month 13: April, 2009. 2nd budget year begins. Testing continued from previous months. Begin to analyze swim-test findings.

Status: to be done.

o) Month 14: May, 2009. Begin euthanasia and histological processing of groups E-G. Presentation of results at National Neurotrauma Society meeting.

Status: processing to be done. Meeting will be in September 2009. Abstract to be submitted on May 1. A similar abstract will be submitted to the Society for Neuroscience by May 14 for meeting in October 2009.

p) Month 15: June, 2009. End of euthanasia and histological processing in groups E-G. Staining of tissue from groups E-G

Status: to be done.

q) Month 16: July, 2009. Analysis of stained material from groups E-G and correlations with swim-test data

Status: to be done.

r) Month 17: August, 2009. Preparation of 2nd article and poster, comparing findings from groups E-G with A-D. Prepare possible 3rd article clinical translation feasibility and methodology.

Status: to be done.

s) Month 18: September, 2009. Continue preparation of articles and other reports until end of grant period (October 5, 2009).

Status: to be done.

Milestones

1. November, 2008. Evidence with respect to effect of DRN stimulation on behavioral recovery emerges.

Status: done.

2. December, 2008. Evidence with respect to effect of DRN stimulation on anatomical recovery emerges. Reports submitted for publication and conference on DRN effects on recovery from TBI.

Status: anatomical analysis and preparation of publication in progress. Abstract was submitted on February 28, 2009 to Military Health Research Forum 2009 (for meeting on August 31, 2009).

3. May, 2009. Presentation of results at National Neurotrauma Society (NNS) meeting.
Status: abstract to be submitted to NNS by May 1, meeting takes place in September, 2009.

4. August, 2009. Effect of stronger DRN stimulation, no nighttime pause in this stimulation and MRN stimulation on anatomical and behavioral recovery from TBI emerges. Reports on these comparative aspects are sent for publication and presentation at meetings. Presentation of early results at Society for Neuroscience meeting.

Status: to be done. Abstract will be submitted to the Society for Neuroscience by May 14 for meeting in October 2009.

Methods.
All methods were performed according to the original Statement of Work. Male, 250 gm Sprague-Dawley rats were used. However, 8 or 9 rats per group, as opposed to 6, became the target number, due to weaker than expected effects and the need for secure statistical validation. The TBI was created as originally planned. Stimulator implantation and the treatment protocols, including the platinum-iridium microelectrodes, were followed exactly. Behavioral testing and histological analysis was carried out as first proposed. A minor but significant improvement in stimulator construction was to use epoxy embedding (DP420, 3M Corp.), not silicone, which gave a mechanically more secure and watertight device, with no chemical degradation. We also did all stimulator fabrication in-house, other than obtaining the unfilled, custom-designed printed circuit boards from an outside vendor.

Outcomes, products, and deliverables.
1. 2-3 published papers.
Status: a larger combined paper will be submitted by August 2009.

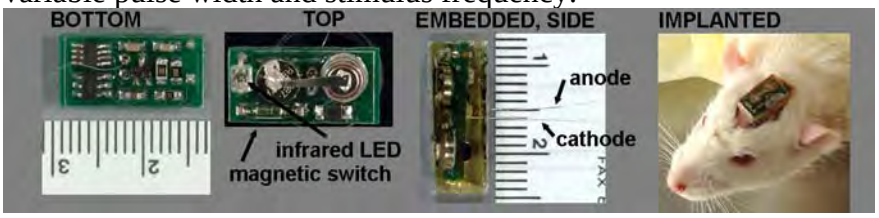
2. 2-3 posters at 2009 meetings of the Society for Neuroscience and the National Neurotrauma Society.

Status: abstracts will be submitted this month. One abstract was submitted to on February 28, 2009 to Military Health Research Forum 2009.

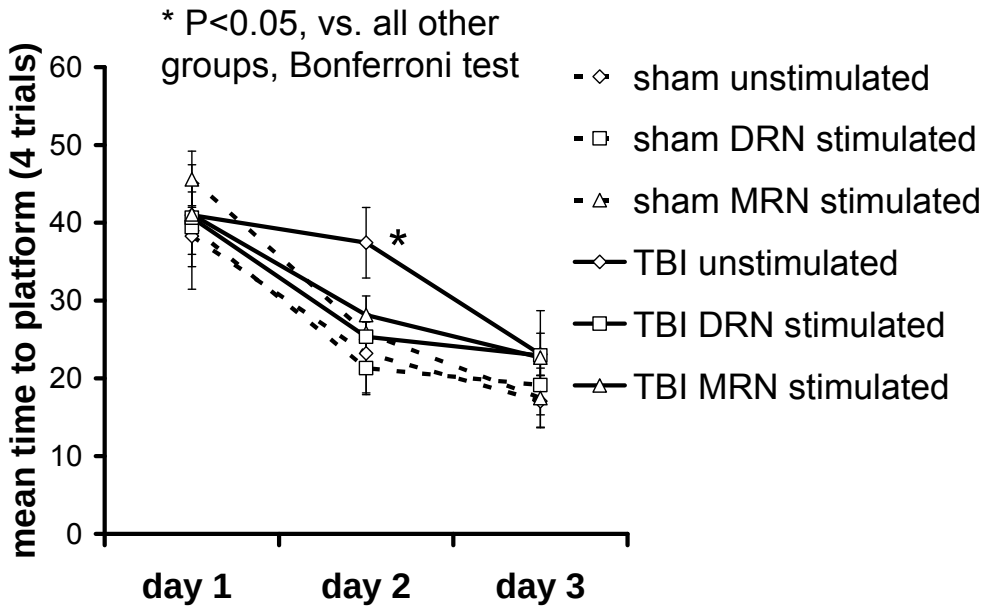
3. A method to be refined in collaboration with neurosurgeons and biomedical engineers for reducing behavioral deficits after severe TBI in humans.

KEY RESEARCH ACCOMPLISHMENTS:
Bulleted list of key research accomplishments emanating from this research.

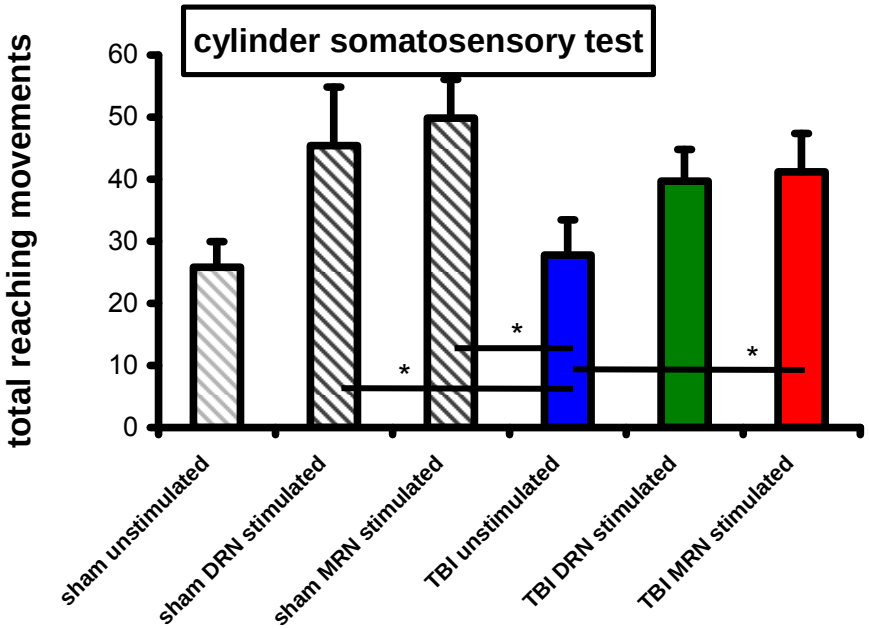
- We studied the effects of deep brain stimulation in the median raphe nucleus (MRN) and dorsal raphe nucleus (DRN) on recovery from parasagittal fluid percussion injury as a model of traumatic brain injury (TBI) in 60 rats. Created epoxy-embedded wireless stimulator for freely moving rats. Latest implementation is very reliable and offers variable pulse width and stimulus frequency.



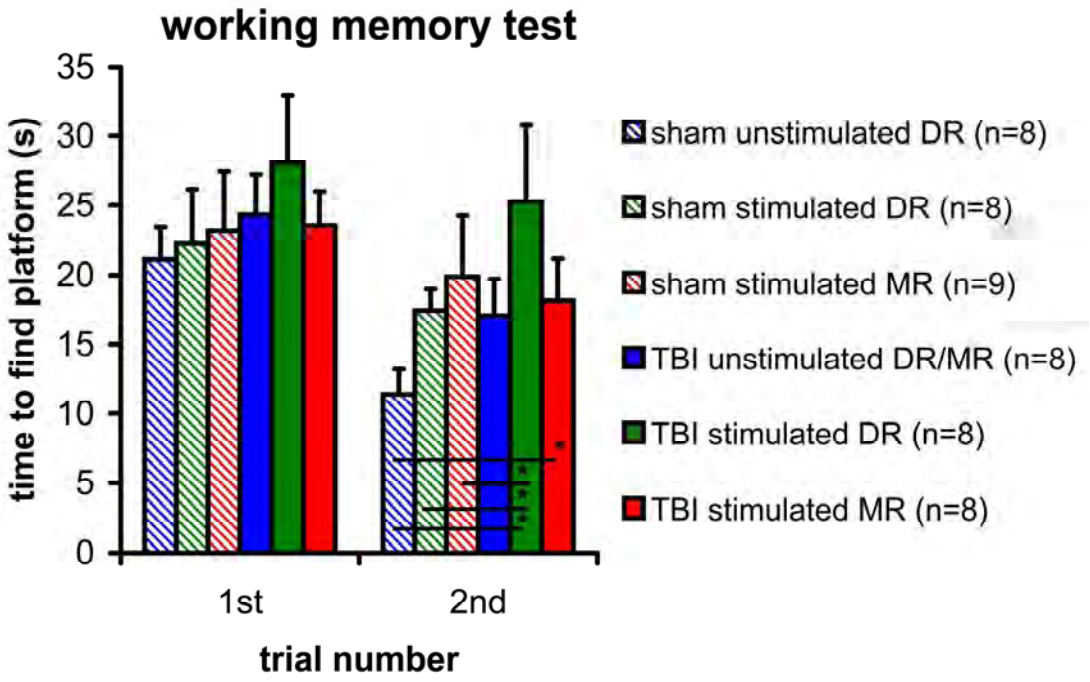
- We found that the rate of learning in the hidden platform test in a Morris water maze was restored by 12-hour daily intermittent DRN or MRN stimulation for 1 week. This was seen on the 2nd day of 3 days of testing, when rats with untreated TBI discovered the platform less rapidly than other groups (see figure below).



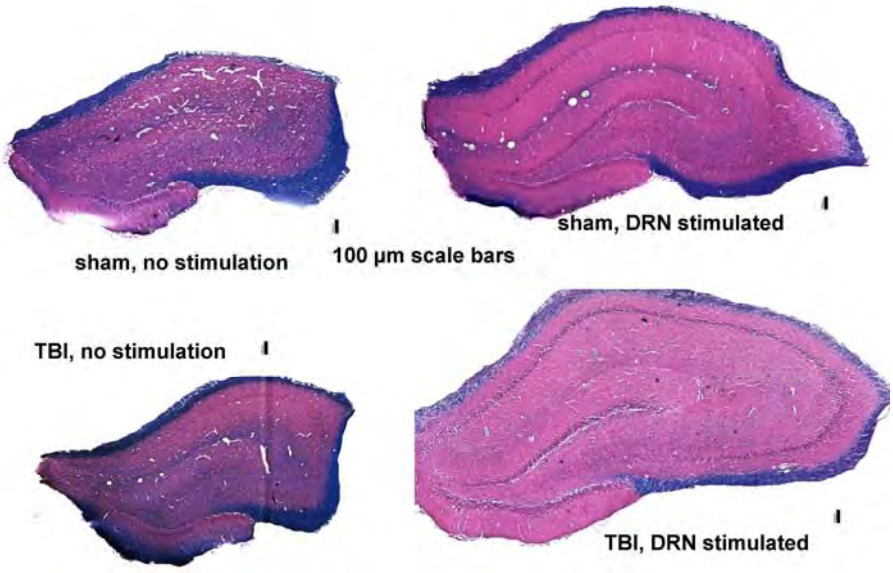
- We found that sensorimotor ability at 5 weeks, as measured by spontaneous rearing movements involving one or both forelimbs in a transparent cylinder, was increased by 1 week of MRN stimulation or DRN stimulation started at week 0 (see figure below). The bars with asterisks indicate significant at P<0.05 in the Fisher LSD test after 1-way ANOVA.



- We found that working memory was worsened by MRN (MR) and DRN (DR) stimulation, when in two consecutive days a rat had to swim to a hidden platform which it has recently been shown (see figure below). Statistical analysis was done as in the above graphs.



- We found that the volume of the hippocampus was increased by DRN stimulation (see figure below). We have analyzed 8 rats from the first 4 groups (exclude MRN studies). After either sham surgery or TBI, the hippocampal volume appeared to be larger by about 40%, as quantified by contour tracing using image-processing software (Stereo Investigator). This analysis continues.



REPORTABLE OUTCOMES:

1. An abstract was submitted on February 28, 2009 to Military Health Research Forum 2009 (Appendix).
2. A grant was submitted to the National Institutes of Health: 1R21NS067268-01, Repair Pathways in Traumatic Brain Injury. The proposal focuses on mechanisms of effects of DBS in the DRN and MRN on TBI (in contrast to the present grant, which has a translational focus).

CONCLUSION:

We have demonstrated some encouraging but mixed behavioral effects on hippocampal and cortical based behaviors of prolonged DRN and MRN stimulation following TBI. Long-lasting effects in sham-operated rats also emerged. Initial anatomical explorations, now being followed by extensive investigation and analysis, showed a considerable enlargement of the hippocampus.

The behavioral outcome with clearest positive therapeutic value was recovery in the rate of learning of a navigation task (swim test) produced by either DRN or MRN stimulation. This entailed no effect of the stimulation on performance in uninjured animals. In contrast, the swim test for working memory showed performance to be hindered by the MRN and DRN stimulation. It is interesting that at least seven other published studies have demonstrated that elevating cyclic adenylyl monophosphate cAMP in aged animals improves hippocampal-dependent learning but worsens working memory [1, 6, 8-12]. This is consistent with our hypothesis that serotonin released by raphe terminal activates 5-HT7 receptors to increase cAMP, which we propose to be the primary mechanism for the beneficial trophic effects. The cylinder test, taken to indicate somatosensory function, gave highly variable results. We were unable to see a statistically significant effect of the injury of the treatment on laterality, but it did appear that injury slightly reduces total movement and that MRN or DRN stimulation reverses this.

Our future plans, as outlined in the Statement of Work for the next 6 months, include completion of the anatomical analysis, study of the effect of MRN stimulation at higher frequency (24 Hz as opposed to 8 Hz) and examination of the treatment for 8-week old injuries. The MRN is our focus because it appears to have characteristics somewhat superior to the DRN, as in the graphs for working memory and the cylinder test above.

There is currently no adequate internal treatment for the chronic behavioral deficits that follow TBI. The military and public health problem is very serious. The present findings present the first evidence that DBS in the midbrain, near central gray sites that have been safely targeted already in many hundreds of patients for chronic pain [2, 7], can reverse some of these deficits. Clearly some of the detailed results raise caveats, especially those showing long-term behavioral sequelae of stimulation in sham-operated rats. However, most medical treatments for severe ailments come with inconvenient or adverse side effects, so this aspect does not rule out clinical translation. Thus we strongly advocate further animal research that can lead promptly to early clinical trials of midbrain DBS for partially restoring the deficits of moderate TBI.

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Abstract

(a) Background and Objectives. Traumatic brain injury (TBI) has large costs to military and civilian organizations and individuals, but few effective treatment options. We explored the new concept that certain brainstem neurons, whose terminations release serotonin in widespread forebrain areas, are restorative after TBI. Specifically, we tested whether one week of intermittent electrical stimulation in either the dorsal raphe nucleus (DRN) or the median raphe nucleus (MRN), started within hours of a moderate TBI, would enhance sensorimotor and cognitive recovery.

(b) Methodologies. Clinically realistic TBI was modeled in adult male Sprague-Dawley rats (now n=42) under isoflurane anesthesia by applying a brief (18 ms) epidural pressure pulse (1.8-2.2 atm) through a fluid-coupling over the lateral forebrain. A self-contained, battery-powered electronic stimulator (about 2 g) with 2-way remote readout and control was cranially implanted 4-6 hours later in rats with sham-injury or TBI. A platinum-iridium microelectrode, placed stereotaxically in the DRN or MRN, delivered 5-minute alternating periods of stimulus trains (-30 μA, 1 ms, 8 Hz) and rest. Some rats had inactive control stimulators. The stimulus was off at night (1800-0600 hr). At 6 weeks, hidden-platform spatial learning and working memory were measured in a Morris water maze, and forelimb-use symmetry was quantified in a transparent cylinder. At 14 weeks, brains were examined histologically.

(c) Results. Spatial learning was faster in TBI rats if the DRN or MRN was stimulated ($P<0.05$, in preliminary post-hoc comparisons after ANOVA). Forelimb-use symmetry also recovered more after stimulation. The working memory test was inconclusive, showing high intra-group variability. Anatomically, in the 7 rats analyzed so far, DRN stimulation increased bilateral hippocampal volume relative to cortex by about 30% in TBI.

(d) Conclusions. Preliminary results point to a restorative effect of sustained MRN or DRN activity on motor and cognitive behavior, possibly reflected in forebrain tissue changes. We will next study stronger stimulation amplitudes, which may show a clearer effect, and older injuries.

(e) Deep brain stimulation (DBS) is used extensively for Parkinson's disease and related disorders. Technically, its translation to early TBI would seem easy. However, preclinical research first must determine which DBS protocol offers best outcomes and fewest risks in TBI. Ahead, early post-injury patient selection and consent could be difficulty, but may be justifiable compared to alternatives.

Agencies	DOD (CDMRP)
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