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

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14. ABSTRACT
We explored the novel concept that electrical stimulation of certain midbrain areas (the dorsal and median raphe nuclei) for one week improves recovery from acute traumatic brain injury. We designed and manufactured a wireless implantable stimulator for rats, with embedded stimulating electrode. A fluid percussion injury was created over the rat's right motor cortex. After 4-6 hours, we implanted the electrode in one of the target areas; the stimulator was attached to the skull. Stimulation was given 12 hours daily in 5-minute alternating periods at 8 Hz. Comparisons were made with injured, non-stimulated rats and with uninjured rats (stimulated and non-stimulated). Behavioral testing at 6 weeks, after either dorsal or median raphe stimulations, improved learning in a water maze test and normalized sensorimotor performance (movements in a transparent cylinder). Higher rates of stimulation (20 Hz) or one-week delay in starting treatment proved less efficacious. Histological inspection at 8 weeks showed an enlarged fiber tract (corpus callosum) after early 8-Hz median raphe stimulation, but no changes in cell counts or hippocampal or cortical volume with any treatment. Overall, the median raphe performed better. Its early stimulation with a temporary implant should be considered for enhancing recovery after certain traumatic brain injuries.

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

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INTRODUCTION

We proposed the hypothesis that certain brainstem regions, which release the monoamine neurotransmitter serotonin from extensively branching axonal systems, can enhance recovery of forebrain function after traumatic brain injury (TBI). We further suggested that subjecting such a system to sustained electrical stimulation would give additional recovery following TBI. We first 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 previously shown that a few days of stimulation applied to the serotonergic system descending from the nucleus raphe magnus 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), could improve recovery of hippocampus-based spatial learning and of cortically controlled sensorimotor performance motor after a parasagittal fluid percussion injury (FPI). 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 Statement of Work (SOW) proceeded basically according to plan, except that the dates of completion of certain milestones were different. A 6-month, no-cost extension to the 18-month Concept Award was granted. Delays were due to personnel problems and technical issues. Dr. Melissa Carballosa-Gonzalez, a key scientist on this project besides the PI, because she needed to finish her thesis work in Spain, did not start in Miami until August 2008. Lizbeth Manoah, BS, the project's main research technician, took time off for study from May to September 2008. Mr. Scott Burns, the student researcher who initially fabricated our electronic brain-stimulator implants, which were an essential part of all our work, left in June 2008 and we were unable to identify a satisfactory replacement engineer, Ms. Allison Irvine, until January 2009. Research output was slower than expected because a collaborating laboratory was unable to supply 4 operated rats with brain injury or sham surgery per week, as originally offered, and could only supply 2 per week. Our anatomical analysis was held back for 2 months by temporary problems with a microtome, preventing the cutting of brain sections. Finally, we spent a considerable effort performing quantitative histology on the cortex and hippocampus (volumes and cell counts) without finding an anatomical basis for the improved behavior. Recently, however, we have noted a significant increase in the volume of the corpus callosum and external (fiber tracts and have returned to processing and quantifying this tissue. Thus we have finally found a likely mechanism for the improved behavioral recovery in terms of improved intra-cortical conduction.

Tasks performed are outlined below with respect to the SOW Timeline. Findings are detailed in the following sections on KEY RESEARCH ACCOMPLISHMENTS.

SOW Timeline.
a) Prior to grant start. Apply to Institutional Animal Care and Use Committee for approval of animal use.
Status: done.

b) Month 1.
"Fabricate 30 stimulator implants with attached electrodes for stimulating dorsal raphe nucleus (DRN)."
Status: done, but with greater numbers of implants.

c) Month 2.
"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: Done. Final rat numbers are group A=8, group B=8, group C=16, group D=11
11.

d-e) Months 3-4.
"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 in the Morris water maze (hidden platform test for 3 days, probe trial on day 4, working memory test on days 4 and 5) and in the cylinder sensorimotor test has been completed for groups A-D.

f-h) Months 5-7.
"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 has been completed for all rats in groups A-D. Behavioral tests done at 5-6 weeks after traumatic brain injury (TBI) were not repeated at 14 weeks, because a major change in behavioral performance appeared unlikely in any group and personnel resources were limited.

i) Month 8. "Immunostaining of histological material from groups A-D. Analysis of swim-test data from groups A-D."

Status: Immunostaining with NeuN has been completed in all rats of these groups. We are currently staining material with the neuronal progenitor marker doublecortin (funded internally).

j) Month 9.
"Analysis of stained material and correlations with swim-test data. Start preparation of 1st report for publication and poster presentation."
Status: Abstracts were submitted to the Military Health Research Forum 2009, the National Neurotrauma Society 2009 annual meeting, and the Society for Neuroscience 2009 annual meeting.

k) Month 10.
"Fabricate 20 stimulator implants with attached electrodes, modified for Specific Aim 4."
Status: The implants were made as planned, but in greater numbers. We decided to modify the circuitry to provide a higher stimulus rate (24 Hz) rather than using higher stimulus amplitudes. This gives three times the release of serotonin [4] without undue current spread to tissue outside the target area [5].

l) Month 11.
"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: We created slightly different groups, here listed with their final numbers (n)
Group G (n=9): apply 30-µA pulses to the median raphe nucleus (MRN) in TBI.
Group H (n=9): same as group G but in sham-operated rats. This necessary control was lacking in the original plan.
Group J (n=9): same as group G, but using 20 Hz stimulation rate
Group K (n=10): same as group G, but starting 1 week after injury.
We chose the MRN rather than the DRN for groups J and K due to superior outcome in initial behavioral findings (see KEY RESEARCH ACCOMPLISHMENTS below).
m-n).

m-n) Months 12-13.
"Behavioral testing for Specific Aim 4, 6 weeks after TBI."
Status: Behavioral testing done for all later groups.

o) Month 14.
"Histological processing of groups E-G. Presentation of results at National Neurotrauma Society meeting."
Status: processing done for all remaining groups. Abstract submitted on May 1.

p) Month 15.
"End of euthanasia and histological processing in groups E-G. Staining of tissue from groups E-G"
Status: Staining done for all remaining groups..

q) Month 16.
"Analysis of stained material from groups E-G and correlations with swim-test data."
Status: Continuing, almost complete.

r-s) Month 17-18.
"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: articles remain to be submitted, pending completion of analysis of the corpus callosum. We plan one article, combining behavioral and anatomical results with MRN and DRN stimulation. A second article will focus on work with cAMP (see below). A review on restorative brain regions is also planned.

s) Month 19-24 (no-cost extension, not in original SOW)
Status: In addition to completing histological analysis as explained in the extension request letter, we studied the molecular mechanism of the improvements seen. We focused on the molecule cyclic adenylate monophosphate (cAMP), whose intracellular levels can be raised by release of serotonin via activation of 5-HT7 subtype receptors. We used 4 groups, with or without TBI and with or without MRN stimulation (8 Hz). Three days after the injury or sham surgery, stimulation was given for 2 hours. Tissue was extracted immediately, and the amount of cAMP in the cortex, hippocampus, and thalamus was measured by immunoassay (ELISA). Histological analysis and the cAMP assay will be completed by the end of this month.

Milestones (from original proposal)
"1. November, 2008. Evidence with respect to the effect of DRN stimulation on behavioral recovery emerges."
Status: completed.

"2. December, 2008. Evidence with respect to the effect of DRN stimulation on anatomical recovery emerges. Reports submitted for publication and conference on DRN effects on recovery from TBI."
Status: Anatomical analysis was initially negative. This has recently changed due to further work on this topic. An abstract was presented at the Military Health Research Forum 2009 (August-September, 2009).

"3. May, 2009. Presentation of results at National Neurotrauma Society (NNS) meeting.
Status: An abstract was presented 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. "

Status: Experiments on all behavioral tests have been completed and analyzed. Abstracts were presented in September 2009 to the Society for Neuroscience annual meeting, in April 2010 to the American Society for Neural Transplantation and Repair annual meeting, and (pending) in June 2010 to the National Neurotrauma Society annual meeting.

Methods.
All methods were performed according to the original Statement of Work. Male, 250 gm Sprague-Dawley rats were used. However, group sizes were increased (to between 8 and 16) to allow for lower statistical power of effects and early problems with histological embedding (now solved). The TBI was created as originally planned. Stimulator implantation and the treatment protocols, including the use of 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.), instead of 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 blank, custom-designed printed circuit boards from an outside vendor. Finally, we added to our methods some assays of cAMP after acute MRN stimulation, partially funded by this grant.

Outcomes, products, and deliverables (from original proposal).
1. "2-3 published papers."
Status: Articles remain to be submitted, pending completion of analysis of the corpus callosum. We plan one article, combining behavioral and anatomical results with MRN and DRN stimulation for July 2010. A second article will focus on work with cAMP. A review on restorative brain regions is also planned for September 2010.

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

Status: The following 5 abstracts have been submitted
i) Military Health Research Forum 2009. Poster and talk.
Title: Prolonged midbrain stimulation early after traumatic brain injury aids behavioral recovery in rats.
Authors: Ian Hentall PhD, Melissa Carballosa-Gonzalez PhD, Lizbeth Manoah BS, Meghan O'Connell BS, Helen Bramlett PhD.

ii) National Neurotrauma Society 2009. Poster and talk. (Student Research Finalist and Travel Award.)
Title: Cognitive and sensorimotor recovery after fluid-percussion brain injury modified by early intermittent stimulation for one week in the rat's dorsal or median raphe.
Authors: Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Bramlett HM, Hentall ID

iii) Society for Neuroscience 2009. Poster.
Title: Improved recovery from acute traumatic brain injury (TBI) in rats after one week of electrical stimulation in midbrain raphe nuclei.
Authors: Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Bramlett HM, Hentall, ID.

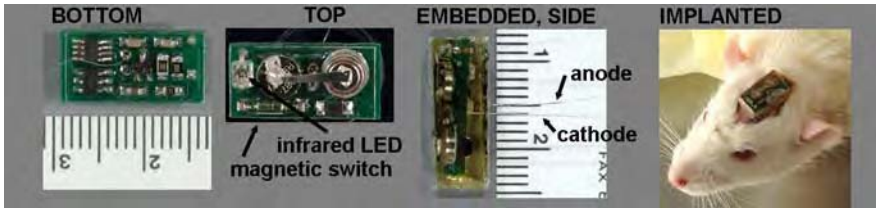
iv) American Society for Neural Transplantation and Repair 2010. Poster. (Student Travel Award.)
Title: Cognitive and sensorimotor recovery after fluid-percussion brain injury modified by early intermittent stimulation for one week in the rat's dorsal or median raphe.
Authors: Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Furones-Alonso O, Bramlett HM, Hentall ID.

v) National Neurotrauma Society 2010. Poster.
Title: Serotonergic brainstem areas controlling neural repair: inputs, effects, mechanisms.
Authors: Hentall ID, Carballosa-Gonzalez MM, Furones-Alonso O, Bramlett HM.

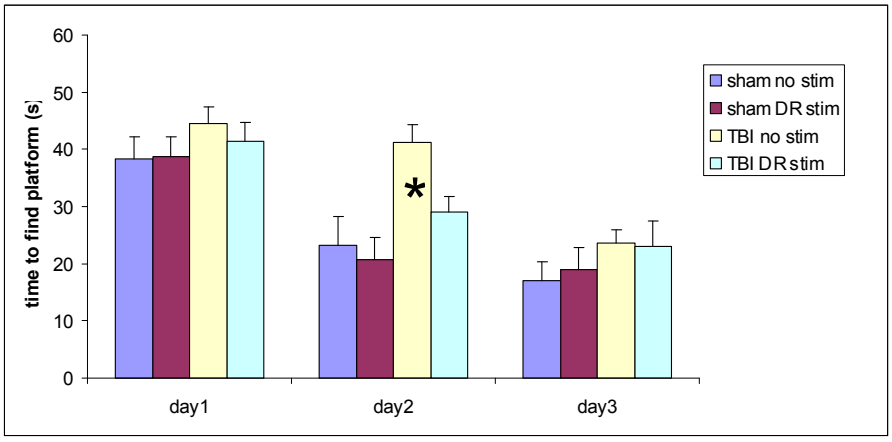
3. "A method to be refined in collaboration with neurosurgeons and biomedical engineers for reducing behavioral deficits after severe TBI in humans."
Status: A suitable method seems to have been devised for reducing behavioral deficits after TBI that is testable in humans. Collaborations are being developed with neurosurgeons and biomedical engineers. A corresponding grant pre-proposal was sent to the CDMRP, and more such collaborative grant proposals are being formulated.

KEY RESEARCH ACCOMPLISHMENTS:

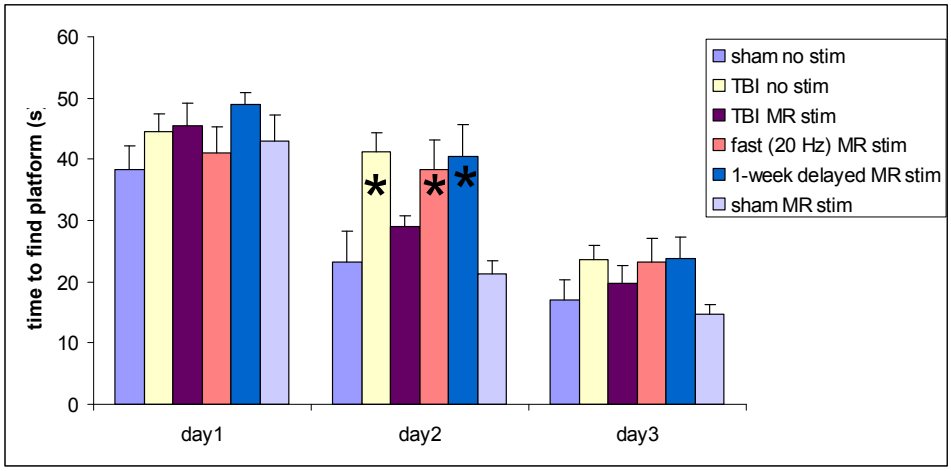
- We designed and manufactured an epoxy-embedded wireless stimulator for freely moving rats. Its latest implementation (below) is very reliable and offers variable pulse width and stimulus frequency.



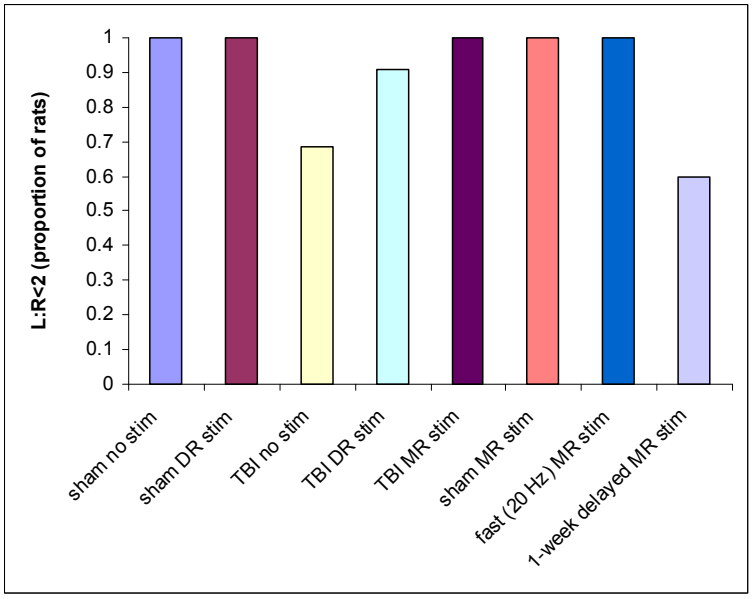
- We studied the effects of deep brain stimulation in the dorsal raphe nucleus (DRN) on recovery from parasagittal fluid percussion injury as a model of traumatic brain injury (TBI). We found that the rate of learning in the hidden platform test in a Morris water maze was restored by 12-hours daily intermittent DRN stimulation for 1 week. This was seen on the 2nd day of 3 days of testing, when rats with untreated TBI found the platform less rapidly than other groups (asterisk in figure below).



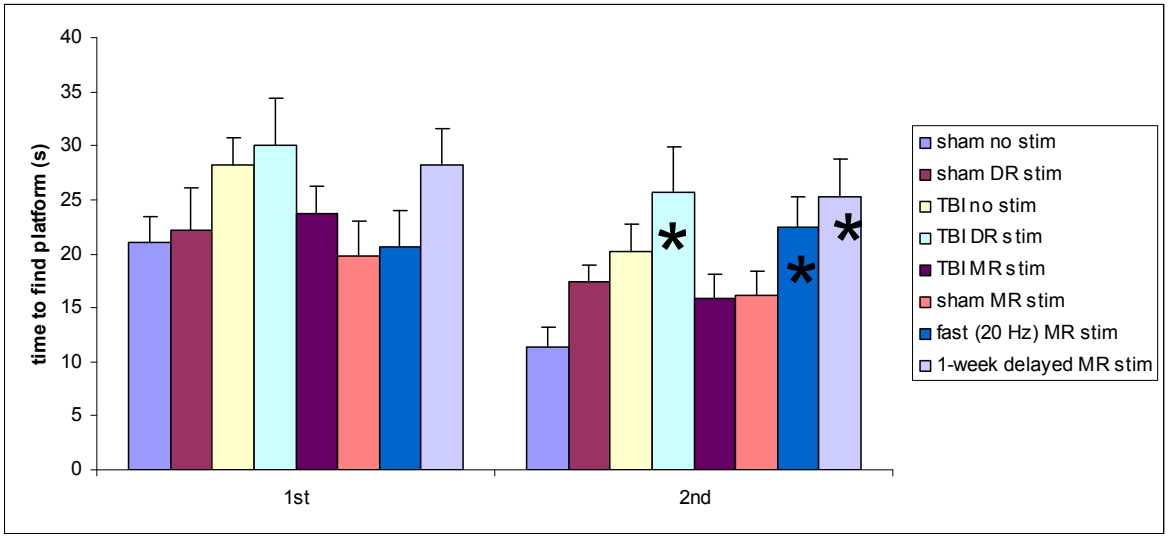
- We repeated the above study in the median raphe nucleus (MRN). Again, the rate of learning in the hidden platform test in a Morris water maze was restored by 12-hour daily intermittent MRN stimulation for 1 week, as seen on the 2nd day of 3 days of testing, when rats with untreated TBI found the platform less rapidly than other groups. Delaying the onset of RN stimulation by 1 week after TBI, or giving a higher rate of stimulation were not effective in enhancing recovery compared with untreated injured rats (see figure below). Asterisks indicate difference from sham/untreated in Bonferroni post-hoc analysis on 1-way ANOVA.



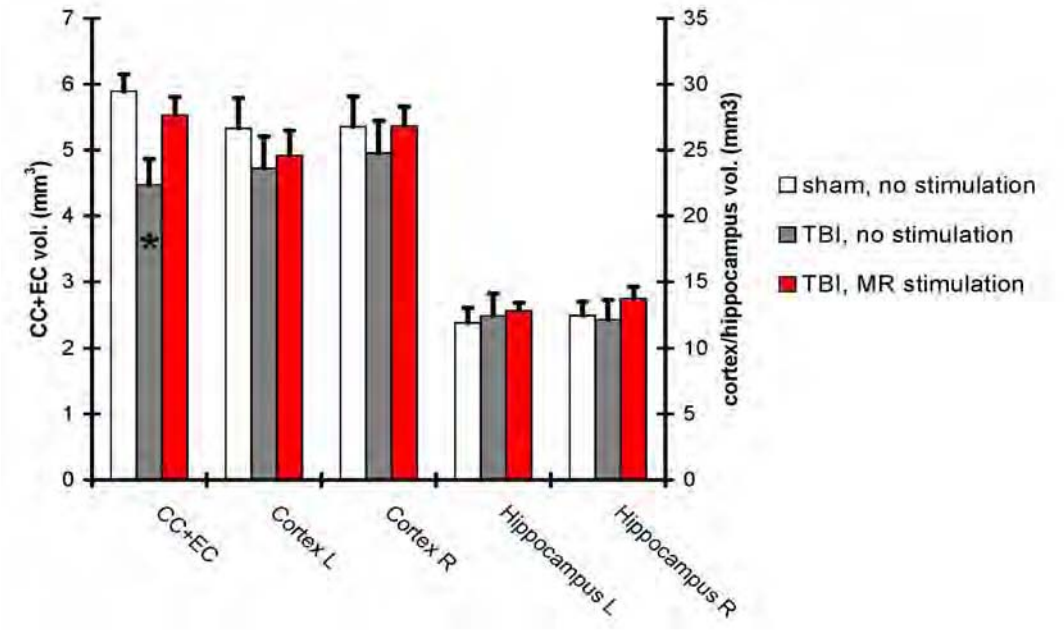
- We found that sensorimotor ability 5 weeks after injury, as measured by spontaneous rearing movements involving one or both forelimbs in a transparent cylinder, was increased by 1 week of MRN stimulation or DRN stimulation started at week 0 (see figure below). Unlike the cognitive performance in the swim test, performance was also improved by faster (20 Hz) or delayed (1 week) stimulation. The graph below shows the proportion of rats in each group that did not have severe left:right asymmetry in reaching movements (favoring the side ipsilateral to the injury >200%). The chi-square test showed unequal frequency distribution ($p<0.05$).



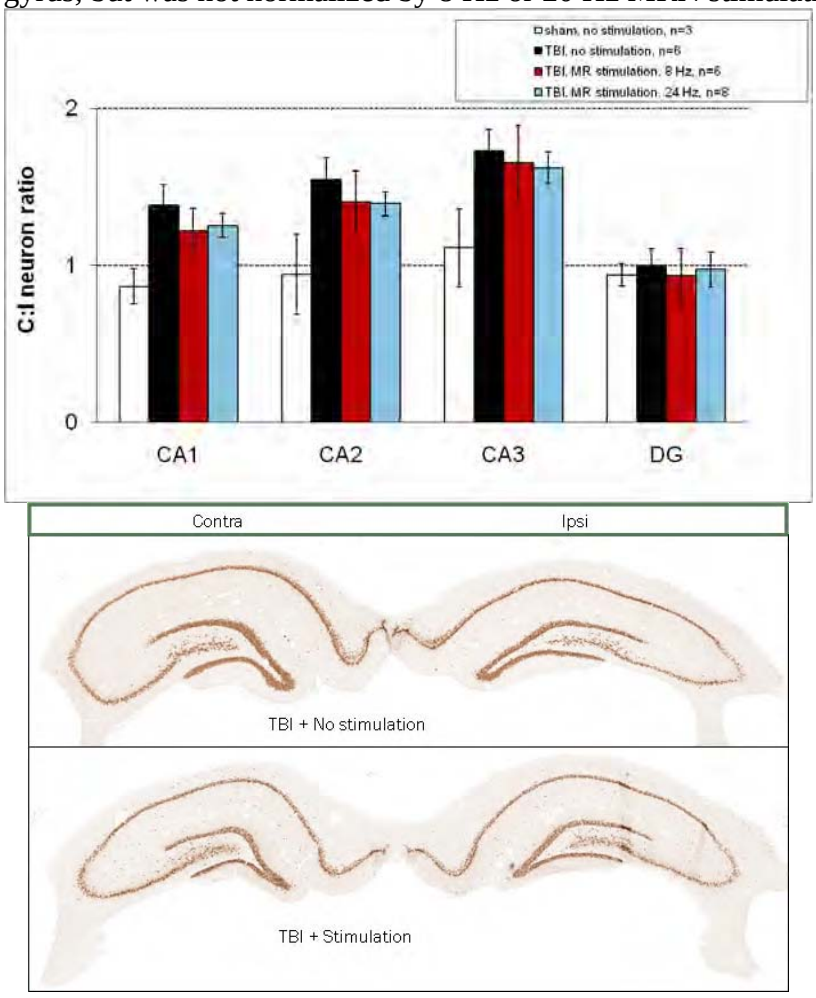
- We found that working memory was worsened by DRN stimulation and not significantly improved by MRN stimulation. In this test, on two consecutive days, the rat had to swim to a hidden platform which it hds recently been shown. Improved performance (lower latency) in the 2nd trial was used to indicate that the memory of the first trial had been retained. Statistical analysis was done as in hidden-platform test.



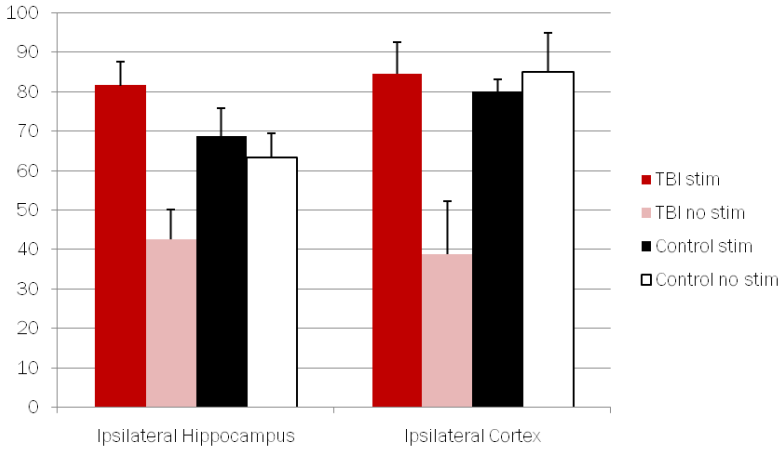
- The volume of the hippocampus and cortex, on the injured or uninjured sides, was not by changed by MRN stimulation (see figure below). However, changes were seen in the volume of the corpus callosum. In the previous annual report, we had stated that DRN stimulation did seem to increase hippocampus volume. We now withdraw this conclusion. DRN stimulation did not alter any measured forebrain volumes.



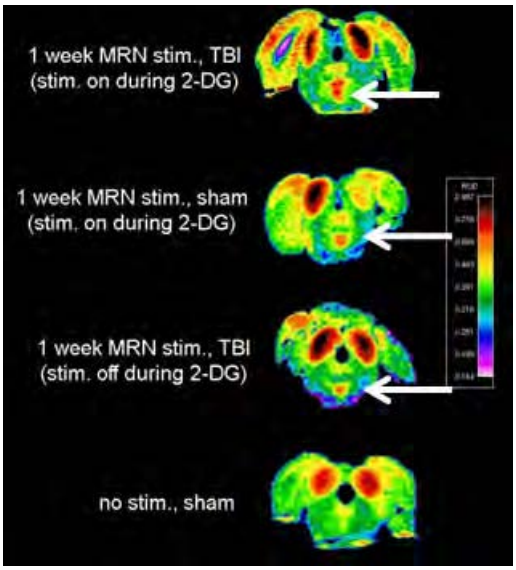
- The number of neurons stained with the neuronal marker NeuN was very variable. We saw no significant effects of stimulation on these numbers. As in the graph below, the left:right (control:injured) ratio clearly was affected by TBI in the hippocampus, except the dentate gyrus, but was not normalized by 8 Hz or 20 Hz MRN stimulation.



- We showed that stimulation for 2 hours, 3 days after TBI, restored cAMP levels in various forebrain regions. cAMP in pmol/ml, measured by ELISA, is shown below.



- To confirm that the stimulator was still working 1 week after implantation, we measured uptake of radiolabeled 2-deoxyglucose in the midbrain and forebrain. In the color-coded autoradiograms shown below, arrows indicate the area or relative hyperactivity at site of the microelectrode.



REPORTABLE OUTCOMES:
1. Five abstracts were submitted (attached in appendix).
i) Military Health Research Forum 2009. Poster and talk.
ii) National Neurotrauma Society 2009. Poster and talk.
iii) Society for Neuroscience 2009. Poster.
iv) American Society for Neural Transplantation and Repair 2010.
v) National Neurotrauma Society 2010.

2. Two grants and one additional accepted pre-proposal were submitted. One pre-proposal was rejected. All proposals the present PI (Hentall) was named as PI.

i) 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.

ii) CDMRP. PT090294. Individual Investigator Award. "Forebrain repair mechanisms under neural control". This proposal also focuses on mechanisms. It is pending approval as of May 5 2010.

iii) CDMRP. PT090617. Advanced Technology/Clinical Trials Award. "Midbrain stimulation to repair recent brain trauma in man". An off-label test of an FDA-approved device to stimulate the MRN for 1-2 weeks early after a moderately severe TBI in human patients was proposed. The pre-proposal was accepted. Neurosurgeons at the University of Miami and the president of a bioengineering company (Triangle Biosystems, Inc., North Carolina) were named as Co-PIs.

After discussion with neurosurgeons, it was decided to pursue further safety studies in large animals before submission.

iv) DMRDP. DM102397. “Deep Brain Stimulation for Acute Neurotrauma”. This was sent as pre-proposal, but an invitation to send a full proposal was not obtained. The proposal was to use pigs as a large animal preclinical model for safety and efficacy studies of MRN stimulation.

CONCLUSION:

We have demonstrated some encouraging behavioral effects of prolonged DRN and MRN stimulation on hippocampal and cortical based behaviors following TBI. Anatomical exploration showed enlargement of the corpus callosum. One behavioral outcome with clear therapeutic value was recovery in the rate of learning of a navigation task (swim test) produced by either DRN or MRN stimulation. No effect of the stimulation on performance was seen in uninjured animals. Also, stimulation restored bilateral reaching in a behavioral test (cylinder test) of sensorimotor performance. In contrast, the swim test for working memory showed performance to be hindered by the DRN stimulation. The MRN gave somewhat better results than the DRN, as in the working memory and cylinder tests and the anatomical recovery. An acute, MRN-evoked rise in cyclic adenylyl monophosphate (cAMP) was also measured experimentally. Several 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 terminals activates 5-HT7 receptors to increase cAMP, which we propose to be the primary mechanism for the beneficial trophic effects.

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 provide the first evidence that DBS in the midbrain, near sites that have been safely targeted already in many hundreds of patients for chronic pain [2, 7], can reverse some of these deficits. 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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Appendix 1.
Abstract. Military Health Research Forum 2009.

Prolonged midbrain stimulation early after traumatic brain injury aids behavioral recovery in rats. Ian Hentall PhD, Melissa Carballosa-Gonzalez PhD, Lizbeth Manoah BS, Meghan O'Connell BS, Helen Bramlett PhD.

(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.

Appendix 2.
Abstract. National Neurotrauma Society 2009.

Cognitive and sensorimotor recovery after fluid-percussion brain injury modified by early intermittent stimulation for one week in the rat's dorsal or median raphe. Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Bramlett HM, Hentall ID

Our laboratory has previously shown that the medulla's raphe magnus, which has a highly branched descending serotonergic projection, improves anatomical and behavioral recovery from an incomplete thoracic spinal contusion if stimulated for several days, starting within hours to a few days of the injury. We proposed that stimulation of divergent ascending serotonergic systems in the midbrain could have similar benefits in traumatic brain injury (TBI). Hence we tested the effect of one week of intermittent electrical stimulation in the dorsal raphe nucleus (DRN) or the median raphe nucleus (MRN) on sensorimotor and cognitive recovery after a moderate TBI. Adult male Sprague-Dawley rats (n=50) received under isoflurane anesthesia a brief (18 ms) epidural pressure pulse (1.8-2.2 atm) applied through a fluid-coupling device over the lateral forebrain, or else received a sham operation. An epoxy-embedded battery-powered stimulator (about 2 g) with 2-way remote readout and control was cranially implanted 4-6 hours later. This delivered 5-minute periods of alternating rest and stimulation (-30 μA, 1 ms pulses at 8 Hz) for 12 daylight hours to a Pt-Ir microelectrode in the midline DRN or MRN. Some rats had inactive stimulators as controls. At 6 weeks, hidden-platform spatial learning and working memory were measured in a Morris water maze, and forelimb-use asymmetry was quantified during rearing movement in a transparent cylinder, all assessed by ANOVA ($P<0.05$) with Bonferroni post-hoc comparison. Spatial learning in rats with TBI was superior on the 2nd trial day when the DRN or MRN was stimulated. Forelimb-use symmetry also recovered more in stimulated groups. Working memory was worsened by DRN but not MRN stimulation in both injured and sham groups. At 14 weeks, brains were examined histologically. Anatomical analysis, presently confined to DRN, showed that stimulation bilaterally increased hippocampal volume relative to cortex (30%). In conclusion, sustained MRN or DRN activity can restore some kinds of motor and cognitive performance after TBI but may also have adverse effects. Surgery and hardware for deep brain stimulation (DBS) are readily adaptable to midbrain sites for early TBI, but benefits and drawbacks in relation to outcome predictors (injury status, stimulation modality) need further assessment. Supported by USAMRMC W81XWH0810288

Appendix 3.
Abstract. Society for Neuroscience 2009.

Improved recovery from acute traumatic brain injury (TBI) in rats after one week of electrical stimulation in midbrain raphe nuclei.Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Bramlett HM, Hentall, ID.

Abstract: Based on accumulating evidence for neurotrophic effects initiated by serotonin, we suggested that stimulation of divergent ascending serotonergic systems in the midbrain could improve behavioral and anatomical recovery from TBI. To test this idea, the rat's DR or MR was stimulated intermittently for 1 week, beginning early after a moderate parasagittal fluid-percussion injury over the cortex. Recovery of sensorimotor and cognitive behavior was assessed 6 weeks after the TBI, and post-mortem quantitative histology was performed at 8 weeks. Adult male Sprague-Dawley rats (n=50) received under isoflurane anesthesia a brief (18 ms) epidural pressure pulse (1.8-2.2 atm) applied through a fluid-coupling device over the lateral forebrain, or else received a sham operation. An epoxy-embedded battery-powered stimulator (about 2 g) with 2-way remote readout and control was cranially implanted 4-6 hours later. This delivered 5-minute periods of alternating rest and stimulation (-30 μA, 1 ms pulses at 8 Hz) for 12 daylight hours to a Pt-Ir microelectrode in the midline DR or MR. Some rats had inactive stimulators as controls. At 6 weeks, hidden-platform spatial learning and working memory were measured in a Morris water maze, and forelimb-use asymmetry was quantified during rearing movement in a transparent cylinder, all assessed by ANOVA ($P<0.05$) with Bonferroni post-hoc comparison. Spatial learning in rats with TBI was superior on the 2nd trial day if the DR or MR had been stimulated 5-6 weeks earlier. Forelimb-use symmetry also recovered more in stimulated groups. Working memory was worsened by DR but not MR stimulation in both injured and sham groups. Anatomical analysis has revealed increases in hippocampal volume after DR or MR stimulation. In conclusion, sustained MR or DR activity can restore some types of motor and cognitive performance after TBI but the DR may also have adverse behavioral effects. These findings provide a basis for prospective treatment of human TBI by deep brain stimulation (DBS), and are consistent with a role for serotonin in long-lasting repair of the central nervous system.

Supported by USAMRMC grant W81XWH0810288

Appendix 4.

Abstract. American Society for Neural Transplantation and Repair 2010.

Cognitive and sensorimotor recovery after fluid-percussion brain injury modified by early intermittent stimulation for one week in the rat's dorsal or median raphe. Carballosa-Gonzalez MM, Manoah L, O'Connell MK, Furones-Alonso O, Bramlett HM, Hentall ID.

Our laboratory has previously shown that the medulla's raphe magnus, which has a highly branched descending serotonergic projection, improves anatomical and behavioral recovery from an incomplete thoracic spinal contusion if stimulated for several days, starting within hours to a few days of the injury. We proposed that stimulation of divergent ascending serotonergic systems in the midbrain could have similar benefits in traumatic brain injury (TBI). Hence we tested the effect of one week of intermittent electrical stimulation in the dorsal raphe nucleus (DRN) or the median raphe nucleus (MRN) on sensorimotor and cognitive recovery after a moderate TBI. Adult male Sprague-Dawley rats (n=50) received under isoflurane anesthesia a brief (18 ms) epidural pressure pulse (1.8-2.2 atm) applied through a fluid-coupling device over the lateral forebrain, or else received a sham operation. An epoxy-embedded battery-powered stimulator (about 2 g) with 2-way remote readout and control was cranially implanted 4-6 hours later. This delivered 5-minute periods of alternating rest and stimulation (-30 μA, 1 ms pulses at 8 Hz) for 12 daylight hours to a Pt-Ir microelectrode in the midline DRN or MRN. Some rats had inactive stimulators as controls. At 6 weeks, hidden-platform spatial learning and working memory were measured in a Morris water maze, and forelimb-use asymmetry was quantified during rearing movement in a transparent cylinder, all assessed by ANOVA ($P<0.05$) with Bonferroni post-hoc comparison. Spatial learning in rats with TBI was superior on the 2nd trial day when the DRN or MRN was stimulated. Forelimb-use symmetry also recovered more in stimulated groups. At 14 weeks, brains were examined histologically. Anatomical analysis, presently confined to DRN, showed that stimulation bilaterally increased hippocampal volume relative to cortex (30%). Furthermore, preliminary data has demonstrated an increase in cAMP following stimulation of the MR. We propose that the enhanced sensorimotor and anatomical recovery is due to a widespread release of serotonin, leading to the increase in cAMP and the subsequent expression of neurotrophic and neuroprotective genes. In conclusion, sustained MRN or DRN activity can restore some kinds of motor and cognitive performance after TBI but may also have adverse effects. Surgery and hardware for deep brain stimulation (DBS) are readily adaptable to midbrain sites for early TBI, but benefits and drawbacks in relation to outcome predictors (injury status, stimulation modality) need further assessment. Supported by USAMRMC W81XWH0810288

Appendix 5.
Abstract. National Neurotrauma Society 2010.

Serotonergic brainstem areas controlling neural repair: inputs, effects, mechanisms. Hentall ID, Carballosa-Gonzalez MM, Furones-Alonso O, Bramlett HM.
Endogenous repair of neural tissue, for example after traumatic brain injury (TBI) or spinal cord injury (SCI), involves numerous molecular pathways. Understandably, given this multiplicity, drug monotherapy for enhancing recovery from TBI or SCI has proved relatively unsatisfactory, prompting research into combination therapies. These present a problem for the researcher similar to that which evolution is continuously solving through natural selection, namely how to coordinate many molecular processes in order to optimize possible repair. Such coordination, we propose, is carried out by centralized neural control from brainstem raphe nuclei, whose widespread axonal projection release serotonin (5-HT) and co-release certain neuropeptides such as TRH. The concept has three main parts: (1) neurons respond to signs of injury; (2) their axons release substances with protective and trophic effects; (3) their activation enhances behavioral and anatomical restoration. We focused initially on the 3rd part, applying intermittent electrical stimulation to rats through wireless implants, beginning within a few hours of moderate neurotrauma and ending typically about 1 week later. When applied among the ascending neurons of the midbrain's dorsal raphe (DR) or median raphe (MR) following a fluid-percussion injury (FPI) over the right cortex, the stimulation enhanced sensorimotor and cognitive recovery measured 5-6 weeks later. When applied among descending neurons of the hindbrain's nucleus raphe magus (NRM) after weight-drop contusion in the T8 spinal cord, various sensorimotor variables showed strong long-term improvement; greater myelination and serotonergic terminal density near the injury zone were measured at 14 weeks. We have replicated many of the findings with the NRM by stimulating one of its major input regions, the midbrain's periaqueductal gray (PAG), a neurosurgical target that has proven safe in deep brain stimulation (DBS). More recently we have focused on mechanisms and on detection of injury by neurons, the 2nd and 1st main parts respectively. We have found increases in cyclic AMP (cAMP) measured by ELISA in the lumbar and thoracic spinal cord after NRM stimulation. Similarly, in the hippocampus after MR stimulation, cAMP was elevated, the difference between stimulated and non-stimulated levels being accentuated by FPI. cAMP is known to increase expression of several important neurotrophic genes and to block inhibition of myelination. Its elevation via activation of 5-HT7 receptors could account for some of the benefits of raphe stimulation after neurotrauma. With regard to the 1st main part of the general concept, we have recorded changes in neuronal activity in the NRM through 16-electrode probes during a T8 weight-drop contusion. As a whole, our findings confirm the general concept of serotonergic repair centers, which may be effective only for early, mild injury unless boosted artificially, and suggest the possibility of treating early neurotrauma by DBS. Supported by USAMRMC W81XWH0810288