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14. ABSTRACT
We have previously established that the pentapeptide CN-105 (Ac-VSRRR-COOH) improves functional and outcome and reduces histological injury in a murine model of traumatic brain injury (closed head injury caused by fluid percussion against the intact skull). The major goal of this project is to establish whether this neuroprotective pentapeptide would be effective in mitigating secondary neuronal injury and improving functional outcomes when administered prior to closed head injury, as this would be a potentially viable strategy to reduce morbidity of traumatic brain injury associated with combat. As described in the statement of work, during the initial year of this proposal, we have tested the efficacy of CN-105 when administered prophylactically in both a moderate single head injury, and paradigm of milder head injuries occurring on a weekly basis over 5 weeks. In addition, we performed the initial work comparing the bioavailability and pharmacokinetics between intravenous, intraperitoneal, intranasal, and oral modes of administration.

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1. INTRODUCTION:

We have previously established that the pentapeptide CN-105 (Ac-VSRRR-COOH) improves functional and outcome and reduces histological injury in a murine model of traumatic brain injury (closed head injury caused by fluid percussion against the intact skull). The major goal of this project is to establish whether this neuroprotective pentapeptide would be effective in mitigating secondary neuronal injury and improving functional outcomes when administered prior to closed head injury, as this would be a potentially viable strategy to reduce morbidity of traumatic brain injury associated with combat. As described in the statement of work, during the initial year of this proposal, we have tested the efficacy of CN-105 when administered prophylactically in both a moderate single head injury, and paradigm of milder head injuries occurring on a weekly basis over 5 weeks. In addition, we performed the initial work comparing the bioavailability and pharmacokinetics between intravenous, intraperitoneal, intranasal, and oral modes of administration. In the second year (no cost extension) we completed these objectives, including study replication for publication, and also completed ancillary work directly related to these objectives, including publication of a manuscript demonstrating efficacy of CN-105 in the setting of subarachnoid hemorrhage (Liu et al., *Stroke Vasc Neurology*, 2018), and presentation of our original research at the Military Health System Research Symposium in 2018, demonstrating efficacy of CN-105 in a model of blast injury to ferret gyrencephalic animals (MHSRS-18-1808). Since both subarachnoid bleeding and blast injury were related and facilitated by the work done in this research program, this grant was credited.

2. KEYWORDS:

Neuroprotection, closed head injury, murine models, neuroinflammation, functional recovery, peptide, therapeutic, glia

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

We have accomplished the major objectives, as defined in the original SOW. We have reproduce the short term (TBI) and long term studies (repetitive TBI) in preparation for manuscript submission and national presentation The major objectives, as stated in the approved SOW were:

Objective 1: Does prophylactic administration of CN-105 mitigate cellular injury and neurocognitive decline in a murine model of severe TBI? What is the optimal prophylactic therapeutic window of CN-105?

Objective 2: Does prophylactic administration of CN-105 mitigate cellular injury and neurocognitive decline in a murine model of mild, repetitive TBI?

Objective 3: What is the pharmacokinetic profile of CN-105 following intranasal and intraperitoneal administration?

Introduction: We have previously established that the pentapeptide CN-105 (Ac-VSRRR-COOH) improves functional and outcome and reduces histological injury in a murine model of traumatic brain injury (closed head injury). The major goal of this project was to establish whether this neuroprotective pentapeptide would be effective in mitigating secondary neuronal injury and improving functional outcomes when administered prior to closed head injury, as this would be a potentially viable strategy to reduce morbidity of traumatic brain injury associated with combat. As described in the statement of work, we tested the efficacy of CN-105 when administered prophylactically in both a moderate single head injury, and paradigm of repetitive milder head injuries occurring on a weekly basis over 5 weeks. We compared the bioavailability and pharmacokinetics between intravenous, intraperitoneal, intranasal, and oral mode of administration.

Objective 1: Does prophylactic administration of CN-105 mitigate cellular injury and neurocognitive decline in a murine model of severe TBI? What is the optimal prophylactic therapeutic window of CN-105?

Methods: To accomplish this objective, we utilized our model of closed head injury previously established in our lab. This murine model involves pneumatic impact against the closed skull with serial functional assessments of vestibulomotor (Rotorod latency) and cognitive (Morris water maze testing) performance.

Briefly, 12-14 week-old C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME) will be used for this model. The trachea is intubated after anesthesia induction with 4.6% isoflurane and the lungs are mechanically ventilated with 1.6% isoflurane in 30% O2/70% N2. Core body temperature is maintained at 37ºC through a rectal probe. The animal is secured in a stereotactic device, the scalp is incised and the skull exposed. A concave 3-mm metallic disc is adhered to the skull immediately caudal to bregma. A 2.0-mm diameter pneumatic impactor (Air-Power Inc., High Point, NC) is used to deliver a single midline impact to the center of the disc surface. The impactor is discharged at 6.8 ± 0.2 m/second with a head displacement of 3 mm. After impact, the animals are allowed to recover spontaneous ventilation and then the tracheas are extubated. Following recovery, mice are allowed free access to food and water.

In all studies, mice are randomized to treatment or vehicle, and both the surgeon perform in the TBI procedure and personnel performing behavioral assessments are blinded to treatment allocation. Animals are placed in a restrainer (Harvard Apparatus, Holliston, MA), and a single intravenous dose of drug will be administered by tail vein in a volume of 100 µL. Vehicle treated animals receive intravenous injection of 100 µL of normal saline at the same time points. Animals will be assigned to treatment group by a coded study identification number after injury using a paper randomization protocol. A block randomization scheme is used so that an equal number of animals are randomized to each of the treatment groups during concurrent experiments.

Clinically relevant behavioral assessments are performed on all animals. This includes tests of vestibulomotor function (Rotorod latency) and cognition (Morris water Maze). An automated Rotarod (Ugo Basile, Comerio, Italy) will be used to assess vestibulomotor function (Hamm et al., 1994). On the day prior to injury, mice (n=11-12 mice per group) will undergo one training trial at an accelerating rotational speed (4-40 rpm) for at least 200 seconds and then three additional test trials with the same accelerating rotational speed. The average time to fall from the rotating cylinder in the test trials is recorded as baseline latency. On days 1-7, 14, and 21 post-injury, the mice will have three consecutive daily trials with accelerating rotational speed (inter-trial interval = 15 minutes). The average latency to fall from the rod is recorded. Mice unable to grasp the rotating rod are given a latency value of 0 seconds. As described previously (Morris, et al. 1984), the Morris Water Maze assesses spatial learning and memory by testing the ability of mice to locate a submerged platform. The mice are placed in a pool (105 cm diameter) filled with liquid and allowed up to 90 seconds to locate the submerged platform. The mice will perform four trials/day for 4 consecutive days (inter-trial interval = 30 min). The mice are introduced in varying quadrants of the pool for each trial but the location of the platform never varies. The latency to locate the platform will be recorded, and the 4 trials per day will be averaged. Mice will be tested on days 28-31 post-injury (n=11-12 mice per group).

Based on the short half-life of CN-105, in the initial series of experiments, CN-105 compound was administered intravenously 10 and 20 minutes prior to injury, and this was compared to post-injury treatment. As demonstrated in **Figure 1A-C** prophylactic treatment improved outcome, although not to the extent of post-treatment**.** As defined in the grant proposal, once we established efficacy at 10 and 20 minutes prior to injury, we next tested the hypothesis that we could extend the therapeutic window of CN-105 prophylactic pre-administration to 30 and 60 minutes prior to closed head injury. As before, CN-105 was administered by intravenous administration at a dose of 0.05 mg/kg at 30 minutes (n=14 animals) or 60 minutes at a dose of 0.05 mg/k (n=15) and compared to vehicle treated TBI animals (n=14) and animals that underwent sham procedure (n=15). There was a statistically significant and durable improvement in functional performance when animals were treated with 0.05 mg/kg intravenously 30 minutes prior to the induction of head injury (p=0.0087 as compared to vehicle treated TBI animals; **Figure 2**). However, this neuroprotection was greatly diminished when drug was administered at the same dose and route 60 minutes after TBI. This may be due to the relatively short plasma half life of CN-105 (measured at 30 minutes in rodent models)

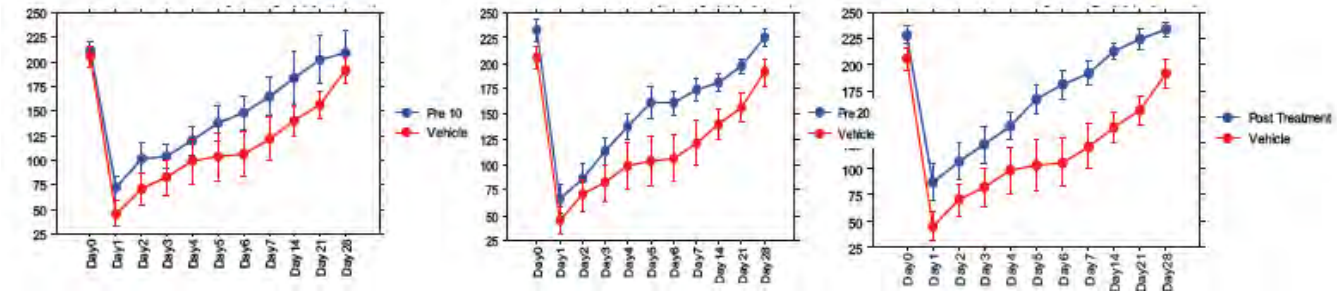
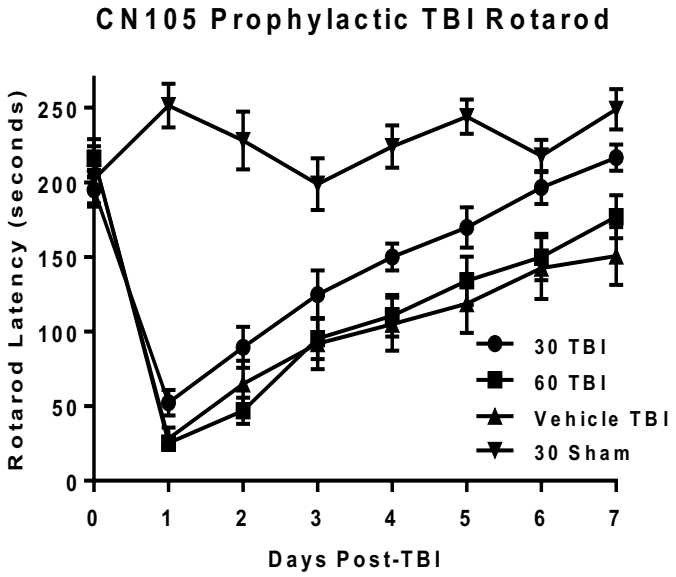


Figure 1A) 10-12 week old Male C57-BL6 were randomized to receive either saline vehicle (n=14) or 0.05 mg/kg CN-105 delivered intravenously by tail vein (n=11); there was a trend towards improved performance after administration of CN-105 (p=0.15 as assessed by two level ANOVA). **B**) Treatment at 20 minutes prior to TBI (n=13 animals randomized to 0.05 mg/kg CN-105) demonstrated statistically significant and durable improvement in Rotorod performance (p=0.2 as assessed by two level ANOVA) as compared to vehicle treated animals (n=14). **C)** Although pretreatment was associated with benefit, functional improvement was greatest when animals were treated with CN-105 0.05 mg/kg post-treatment (n= 14 animals post-treatment; as compared to vehicle, p=0.0054.

Figure 2: 10-12 week old male C57-BL6 were randomized to receive either sham procedure (inverted triangles; n=15); and closed head injury preceded by saline vehicle (n=14; filled upright triangles); and 0.05 mg/kg CN-105 by intravenous injection either 30 minutes prior to TBI (n=14, filled circles); or 60 minutes prior to TBI (n=15, filled squares). There was a statistically significant and durable improvement in Rotorod latency when animals were treated with CN-105 minutes 30 minutes after injury as compared to vehicle treated TBI animals (p.=0.0087 as assessed by two factor ANOVA). There was diminished efficacy, but still a trend for animals treated with CN-105 at 60 minutes prior to TBI to have improved rotorod performance as compared to vehicle treatment. Statistical assessments were made with 2 factor ANOVA with Sheffe post-hoc correction for multiple comparisons.



We next performed an follow-up series of experiments re-testing prophylactic efficacy at 3 and 6 hours prior to TBI. To test this, 10-12 week old male C57-BL/6 mice were randomized to receive vehicle (n=15); CN-105 at a dosage of 0.2 mg/kg delivered intravenously by tail vein injection in a volume of 100 ul at 3 hours prior to injury (n=15); CN-105 at a dosage of 0.2 mg/kg delivered intravenously by tail vein injection in a volume of 100 ul at 6 hours prior to closed head injury (n=15). As outlined in **Figure 3**, there was no statistically significant improvement at either 3 hour or 6 hour pre-incubation prior to injury

Thus, our data suggested that, although, as proof or principle, CN-105 was effective when administered prior to closed head injury, the peptide had to be present in sufficient concentrations at the time of injury to prevent pathological glial activation, and the relatively short half life (measured as 29 minutes) limited this therapeutic window to < 60 minutes. **To overcome this limitation,** we next performed an additional series of experiments, where higher dose of CN-105 (1 mg/kg) was administered intra-peritoneally to maximize drug availability. In this paradigm, we did demonstrate durable effect through 14 days tested, functional improvements most robust up to 14 days post-injury; (**Figure 4**)

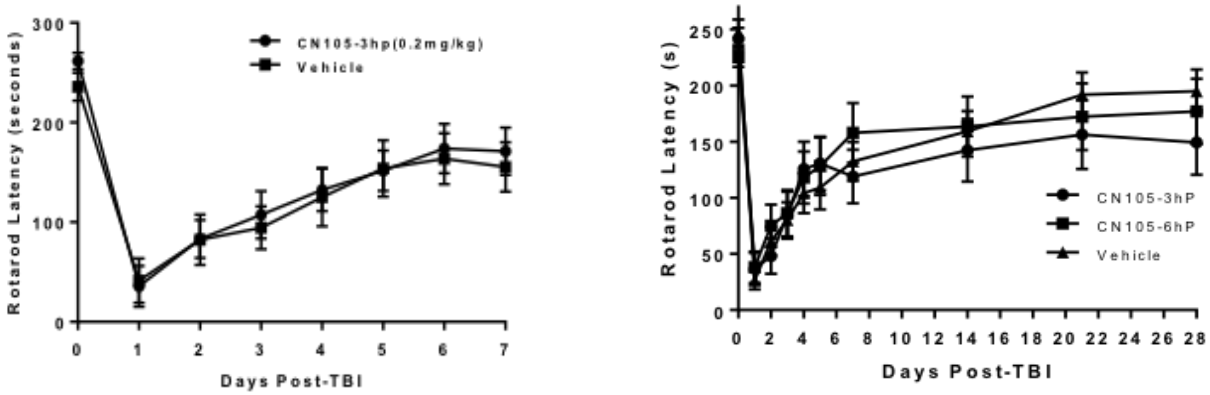


Figure 3: Pre-administration of intravenously administered CN-105 at a dose of 0.2 mg/kg did not affect functional outcome as assessed by serial Rotorod latency when administered at 3 hours or 6 hours (**B)** prior to TBI at a dose of 0.2 mg/kg (drug administered intravenously by tail vein in 100 ul sterile sterile isotonic saline)

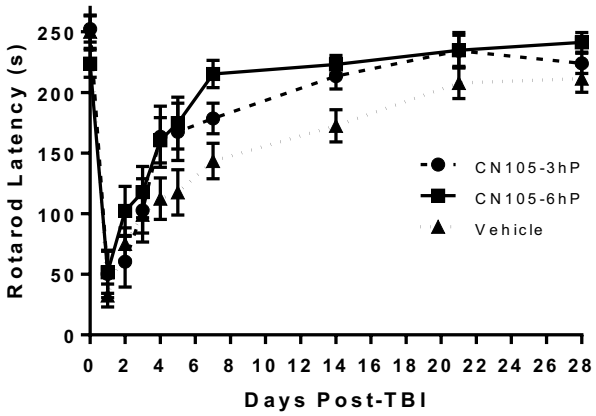


Figure 4: Pre-administration of CN-105 at a dose of 1.0 mg/kg improved functional outcome as assessed by serial at up to 14 days. Animals were randomized to receive 1 mg/kg CN-105 at 3 hours prior to injury (n-10); 6 hours prior to injury (n-11), or vehicle at 3 hours prior to injury n=12). Analysis was performed by 2 factor ANOVA (stratifying by treatment group and time), and conducted for the first 14 days) (* p<0.05)

In the no-cost extension period, based on the pharmacokinetic data (obtained in Objective 3) associated with different modes of administration of CN-105, we sought to replicate these results and optimize dosing by administration of concurrent intravenous (0.1 mg in 100 ul normal saline delivered by tail vein) and intraperitoneal (0.5 mg/kg) CN-105 at 3 hours (n=14) and 6 houra (n=14) prior to TBI and compared to vehicle treated control mice (n=14). As with our prior experiment, we found that prophylactic administration of CN-105 was associated with improved vestibulomotor functional outcomes. These effects were statistically significant, and durable throughout the 28 day testing period (**Figure 5**). Thus, our current study performed in the first quarter of the NCE period replicated our prior results, and demonstrated as proof of principle, that CN-105 has efficacy when used prophylactically prior to traumatic brain injury. The therapeutic window from drug administration is a direct function of maintaining sufficient concentrations of CN-105 at the time of injury. This is a function of the amount and mode of administration of drug. Therapeutic levels will be easier to accomplish after clinical administration, as the serum half-life in clinical phase 1 trail with normal health volunteers was approximately 3.5 hours, as compared to 0.5 hours in mouse and rat.

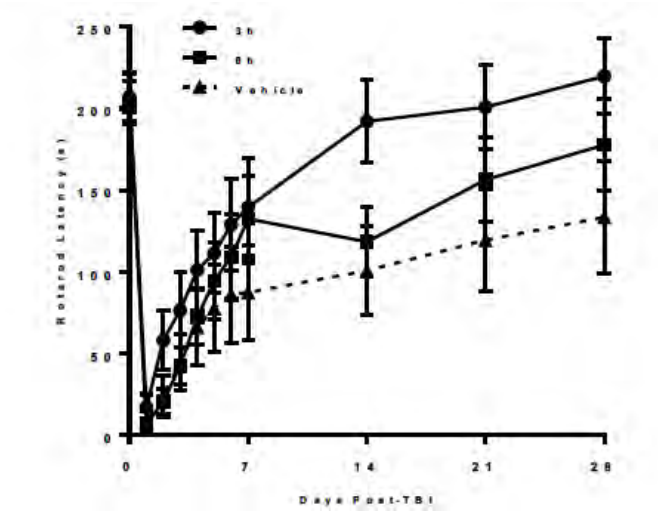


Figure 5: Male 10-12 week old C57-Bl/6 mice were randomly assigned to three groups (n=14/group): 3 h prophylactic administration of CN-105; 6 h prophyactic administration of CN-105, or 3 and 6 h administration of vehicle prior to closed head injury. CN-105 was administered as 0.1 mg/kg iv by tail vein + 0.5 mg/kg i.p in 100 ul NS; vehicle was delivered at the same time and volume. Serial rotorod performance was then assessed daily x 7 days and weekly until day 28. Both 3 h pre-administration and 6 h pre-administration of CN-105 was associated with durable functional improvement in Rotorod latency as compared to vehicle (* P<0.05 as assessed by 2 level ANOVA by time and treatment allocation) with Dunnet's post-hoc test to correct for multiple comparisons.

In summary, our data suggests as proof of principle, that CN-105 has efficacy when used prophylactically prior to traumatic brain injury. The therapeutic window from drug administration to injury is dependent on maintaining sufficient concentrations of CN-105 at the time of injury. This is a function of the amount and mode of administration of drug. Of note, the pharmacokinetics of CN-105 is a function of species; while rodent has a serum half life of 30 minutes, in phase 1 testing, serum half life in human subjects was approximately 3.5 hours (Guptill et al., 2017), which may make this more amenable to clinical use. However, to demonstrate proof of principle, in Objective 3, we define and compare pharmacokinetics associated with intranasal, oral, and intraperitoneal modes of administration with intravenous administration to assess alternative routes of delivery.

In the final quarter of the second year (NCE), we have replicated and compiled these results into a manuscript. Given the relevance to warfighters at risk for head injury, we have submitted this compiled these results and completed a manuscript for submission to *Military Medicine*, which is the official peer reviewed journal for the Association of Military Surgeons of the United States.

Our second research objective was to evaluate efficacy of CN-105 in a model of mild-moderate repeat TBI, occurring at weekly intervals, for a total of five injuries. As described in the original proposal, an additional aim is to evaluate whether prophylactic administration of CN-105 would mitigate cellular injury and neurocognitive decline in a murine model of mild, repetitive TBI. To investigate this, in Q3 we performed our initial experiment in repetitive mild TBI. Two groups of male 12 week old C57-BL/6 mice were randomized to receive either 0.5 mg/kg CN-105 delivered by intraperitoneal injection performed 30 minutes prior to and 72 hours following each mild TBI. Serial Rotarod assessment was performed one day prior to and following the mild TBI. Both placebo and CN-105-treated groups demonstrated acute decline after each injury. However, by day 38, the ApoE-treated group had significantly better motor outcomes (p= 0.015; two factor ANOVA) compared to the placebo group (**Figure 6A**). This trend continued through the final rotarod time point on day 90 (p=0.015; **Figure 6B**). A generalized linear model comparing the placebo and treatment groups over time demonstrated that the ApoE-group's motor performance was significantly different over time. We also conducted a MWM 1 week post the final TBI (TBI 4). Cognitive function as assessed by the Morris water maze did not show significant difference between placebo and treatment groups (**Figure 6C**).

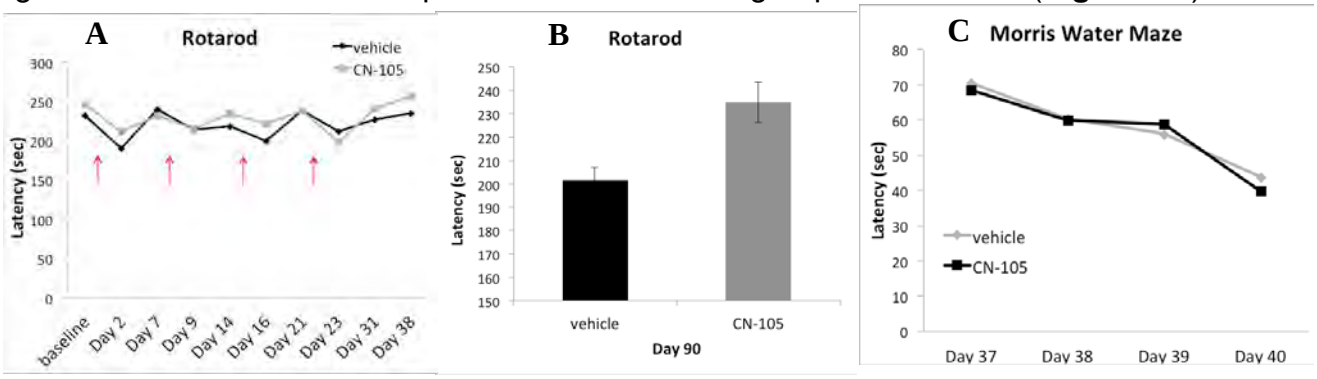


Figure 6A: As described in the protocol, serial mild TBI was performed by performing 4 consecutive mTBI (identified by red arrows), and allowing 1 week recovery intervals. There was significant decrement in Rotarod performance with near complete recovery immediately following each TBI. In the subacute phase phase (Day 38) there was improved performance at Day 38 in the CN-105 treated animals. **Figure 6B**: In the chronic phase (Day 90 following first injury), there was a significant improvement in motor performance off CN-105 treated animals as compared to vehicle treated animals. (*p=0.015) **Figure 6C**: In Morris Water Maze assessment of congnitive performance, there was no significant difference in latency between CN-105 and vehicle animals.

Thus, the results of our initial repetitive mild TBI experiment performed in Q3 demonstrated the late effects of mild repeat TBI, even though there was minimal clinical deficit after a one week recovery period between impacts. We found improvement in vestibulomotor (Rotorod) animals treated with prophylactic CN-105 at 90 days. During replication studies, we optimized and repeated the chronic TBI paradigm by slightly increasing the impact intensity (increasing the head displacement from impact from 2 mm to 2.5 mm). This reduced the ceiling effect whereby, in the short term, vehicle treated animals appeared to improve to near baseline (although at 90 days, they were left with significant deficits). Using the injury model, we randomized 10 week old C57-BL/5 (n=13/group) to receive 0.5 mg/kg CN-105 or vehicle by intraperitoneal injection 5 days/week. In this paradigm, vehicle treated aninals had progressive and cumulative injury after repeated TBI while they were still in a vulnerable period; animals treated with CN-105 had much less injury, and in fact were at baseline (pre-injury function) at the end of the series of closed head injuries (**Figure 7**). In Q4, we next performed histology from the initial animals that were tested in the prior quarter (animals described in Figure 5). We found that, consistent with their improved functional performance at 90 days, animals with repetive TBI protocol had a reduction in neuronal injury (**Figure 8**) and microgliosis (**Figure 9**) as compared to vehicle treated controls.

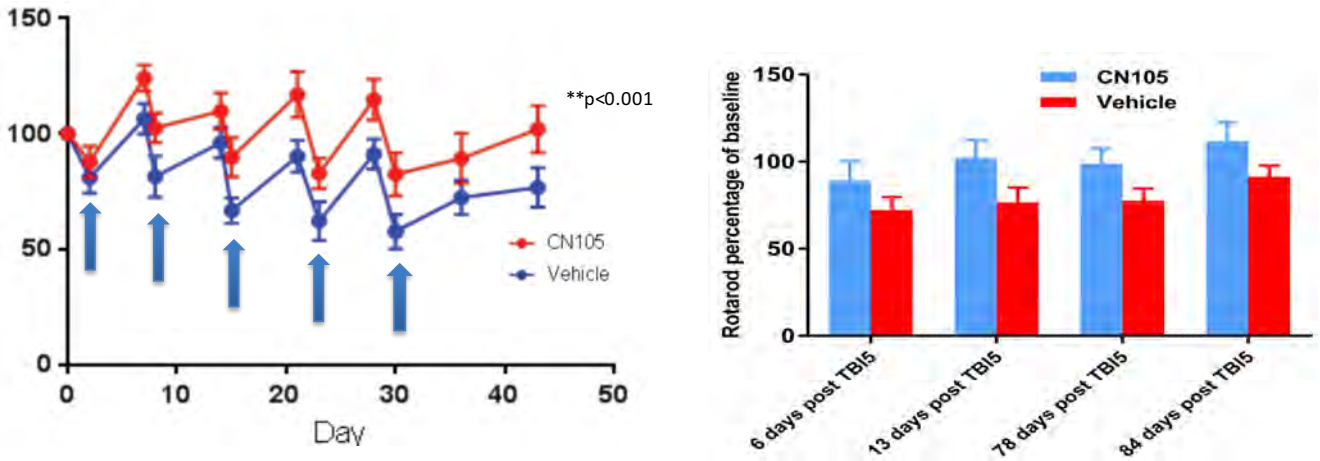


Figure 7A: 10 week old male C57-BL/6 animals were randomized to receive vehicle or 0.5 mg CN-105 by i.p injection 5/week. X 4 weeks (blue animals represent times of injury) Once/week animals receive a traumatic brain injury by pneumatic impactor with displacement of 2.5 mm, velocity of 6.8 m/sec. All animals tested serially to assess Rotorod displacement (initial value designated as 100%). CN-105 Treated animals had significantly better performance at all points tested ($p<0.001$, by two factor ANOVA) **Fig 7B**: **In Q5** we repeated long term vestibulomotor testing in this cohort and confirmed our prior finding of durable improvements in Rotorod latency ($p<0.01$ by ANOVA)

In the last quarter of NCE, next replicated these findings with an additional cohort of animals (Figure 9). This experiment was done to: 1) replicate and validate our prior findings; 2) to add an additional arm of sham treated animals (general anesthesia but no head injury); 3) to sacrifice at 11 days to evaluate short term histological changes. In particular, the sham group allows us to dissect the effects of learning (Rotorod performance improves over time with repeated testing), as well as test the effects of general anesthesia/stress associated with the TBI. In this cohort, animals were sacrificed earlier than the previous experiments (11 days following the fifth and final head injury). This experiment was carried out in similar fashion to the experiment described in Figure 7. 10 week old C57-BL/6 male mice were randomly assigned to one of three groups: 1) 0.5 mg/kg CN-105 i.p 5 days/week 2) identical volume and schedule of saline (vehicle) by intraperitoneal injection 5 days/week; 3) sham animal which

received identical treatment with the exception of no closed head injury. As described in Figure 7, TBI was performed with a displacement of 2.5 mm, and occurred weekly for 5 impacts.

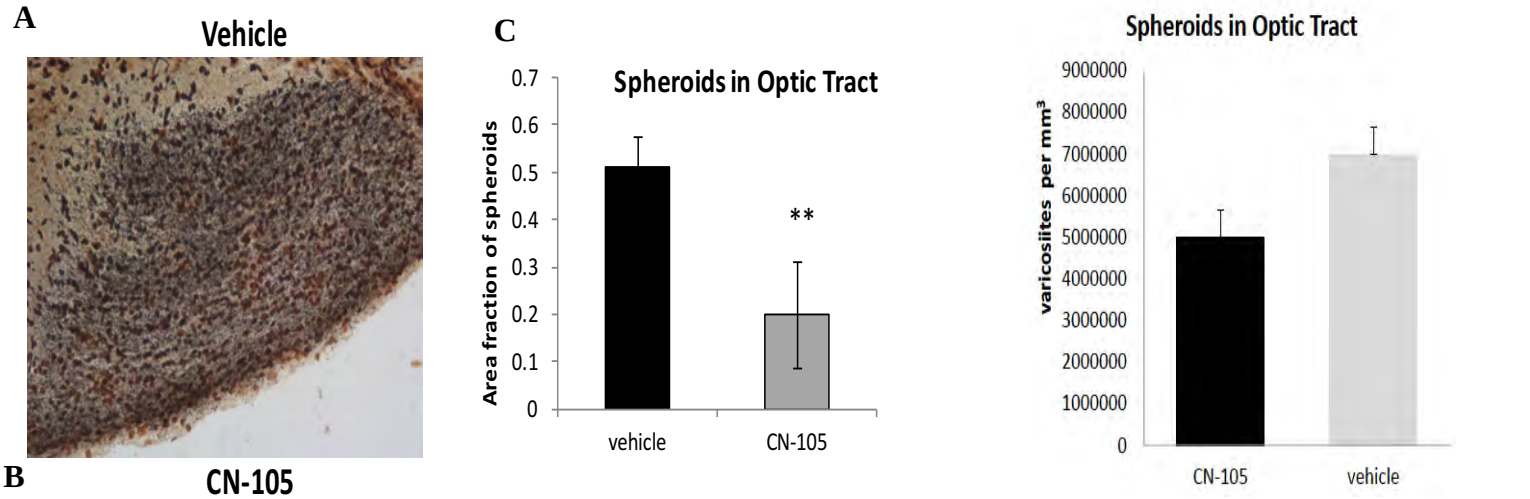


Figure 8: A characteristic finding in our model of repetitive TBI is the degeneration of white matter tracts visualized by Neurosilver staining. This is most evident is heavily myelinated tracts such as optic tract. Furthermore, it has been shown that in rodent models, vision is clearly affected. Using Neurosilver stain, Neurosilver positive spheroids were counted in the optic tract in saggital sections 1.3 mm lateral to midline 90 days after last impact. Reductions in spheroids were seen in treated animals (**A,B**), and confirmed using the area fraction fractionator method (**C**) of unbiased stereology (** < 0.001 by t-test). (**D**) **In the current quarter,** these histological findings were replicated in an independent cohort of rTBI animals. Again, CN-105 was associated with reduced axonal injury (p<0.02)

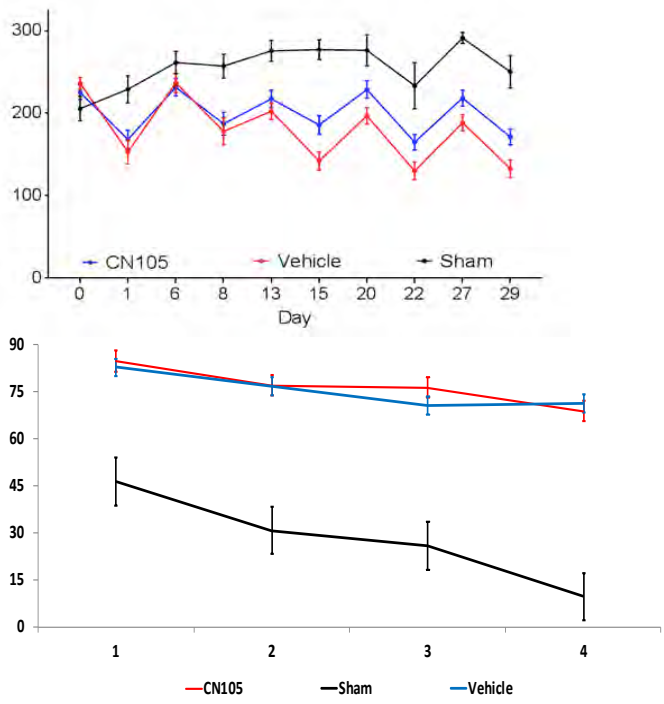
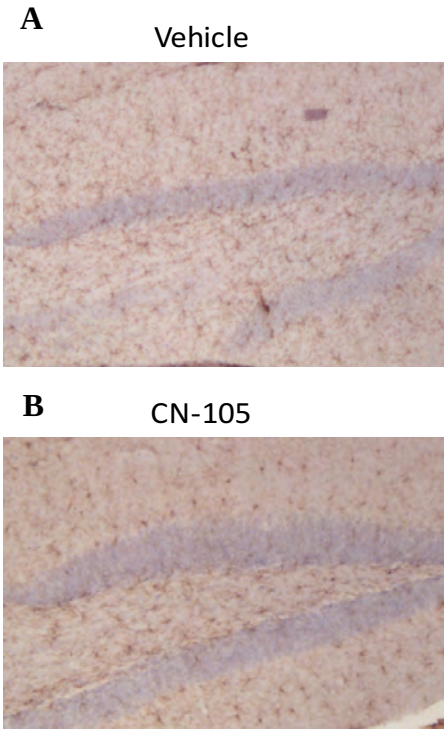


Figure 9A: 10 week old male C57-BL/6 animals were randomized to 3 groups: vehicle (100 ul saline i.p 5x/week; n=8); or 0.5 mg CN-105 (i.p injection 5/week; n=12), sham (identical handling and anesrthesia without injury or injection, n=5) Animals received pneumatic TBI injury once/week animals receive a traumatic brain injury by pneumatic impactor with displacement of 2.5 mm, velocity of 6.8 m/sec. All animals tested serially to assess Rotorod displacement (initial value designated as 100%). CN-105 treated animals had significantly better performance (p<0.001, by two factor ANOVA) **Fig 9 B:** There was no improvement in cognitive function (assessed by daily latency to find platform in Morris Water Maze between vehicle and CN-105 treatment, although sham animals had significantly reduced latencies suggesting improved reference memory as compared to either of the TBI groups. (p<0.01 by ANOVA)

In summary, this repeat experiment performed in the replicated our prior findings, demonstrating benefit with CN-105 treatment and functional vestibulomotor performance as assessed by serial Rotorod latency, although no difference was observed in Morris Water Maze testing. We repeated the contextual fear response paradigm as described below, (Figure 11, and again found no differences as a function of CN-105 treatment. One of the goals of this repeat experiment was to sacrifice this cohort of animals for short term histology (day 11 after 5th TBI). These animals have been sacrificed, brain sections prepared and stained, and undewent analysis for microgliosis (F4/80 staining). Thus, CN-105 represents the first potential pharmacological intervention to improve long term functional outcomes (vestibulomotor function as defined by Rotorod latency) in a murine model of TBI. In the final quarter of the NCE period, we completed histological analysis, prepared and completed a publication to *Military Medicine.*

Another chronic finding that is observed in this model of repetitive TBI is diffuse microgliosis, which is often pronounced in the hippocampus. To assess whether CN-105 influenced the development of microgliosis, we performed unbiased formal stereology to examine F4/80 positive neurons (this stain does not differentiate intrinsic microglia from hematogenously recruited monocytes, but does reflect the CNS inflammatory response. We found that treatment with CN-105 significantly reduced microgliosis 90 days after injury (**Figure 10**).

These histological results suggest long term atrophy of heavily myelinated white matter tracts as well as dystrophic neurites and microgliosis (neuroinflammation) that are mitigated by treatment with CN-105, and would be consistent with the durable functional improvements associated with CN-105 treatment. In the next quarter (Q6; Q2 of NCE), we will attempt to replicate these histological findings in an independent cohort of mice exposed to our model of mild repetitive subconcussive closed head injury.



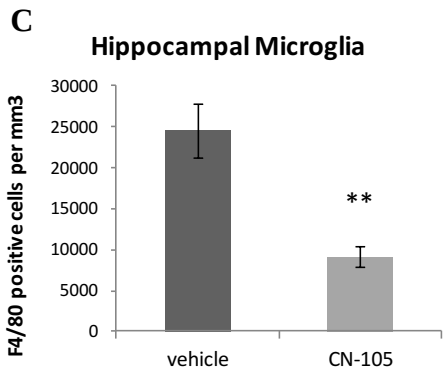


Figure 10: At 90 days following the final impact, the C57-BL/6 mice previously treated with CN-105 had significantly greater F4/80 positive microglia in the dorsal hippocampus(A,B). This is reflective of a dished neuroinflammatory response, and was confirmed by F4/80 staining using the optical fractionator method of unbiased stereology (**C**; $p<0.01$ by t-test)

One of the difficulties with repetitive subconcussive TBI models is difficulty demonstrated a reproducible and clinically relevant behavioural phenotype. To address this, in the NCE year,, we used a model of contextual fear conditioning, which assesses recall under stress.

Contextual Fear Conditioning Model: Animals were then singly transported into the behavioral room and placed in the fear conditioning chamber (12inch x 12inch x 12inch, L x W x H; steel beam floor; clear glass walls; acetic acid scent). After 120 seconds of habituation to the chamber, a tone of 2800Hz frequency and 80dB intensity was presented for 30 seconds, with the last two seconds co-terminating with a footshock (0.5mA). The animals were kept in the chamber for an additional 30s before being removed from the chamber. To test for recall of context, animals were placed in the original fear conditioning chamber 24 hours after conditioning for four minutes (no tone or foot shock was presented during this period). To test for recall of the cue (tone), animals were placed in a modified fear conditioning chamber
(felt floor; painted walls; vanilla scent). Animals were allowed to explore this chamber for 2 minutes, before the tone was presented for an additional 2 minutes. Animal movement was determined by photobeam interruption of a 16x16 array spaced 0.75 inches apart. Freezing was defined as failure to break 3 new beams, a metric that has been previously described to have strong correlation with both manual freezing determination as well as video capture. Normalized freezing was calculated by taking the ratio of the time spent freezing during the contextual recall and dividing by the time spent freezing during the contextual habituation.

When the contextual fear conditioning model was performed, there was a clear and reproducible behavioral phenotype associated with repetitive head injury; however, there was no long term improvement in performance as a function of CN-105 (**Figure 11**) in the paradigm described in our repetitive TBI model , in which 10 week old male C57-BL/6 animals were randomized to receive vehicle or 0.5 mg CN-105 by i.p injection 5/week. X 4 weeks, and once/week animals receive a traumatic brain injury by pneumatic impactor with displacement of 2.5 mm, velocity of 6.8 m/sec, as described in Figure 6

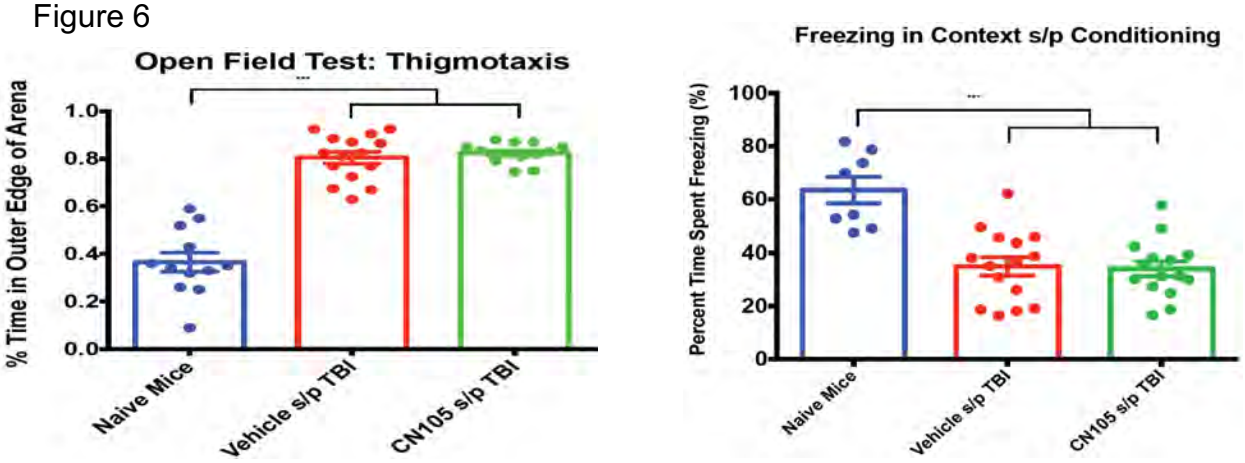


Figure 11: The repetitive TBI model resulted in a distinct phenotype associated with anxiety (Thigmotoaxis); **(A)** (***p<0.01 ANOVA, Tukey test for post-hoc comparisons; n=15 animals/group); and(**B)** memory deficits (contextual freezing after foot shock conditioning; n=15 animals/group). However, no significant differences were observed as a function of treatment with CN-105 vs. vehicle treatment for either of these measures.

During the NCE period, we also initiated several experiments evaluating the prophylactic use of CN-105 in preventing vasospasm and secondary ischemia after subarachnoid hemorrhage (SAH). Although this was not included in the original objectives, SAH represents an important component of traumatic brain injury and may result in vasospasm (delayed proliferative arteroiopathy) and secondary ischemia. Our laboratory has longstanding experience creating a repreoducible SAH. Briefly, 10 to 12 week old male mice are induced in a chamber with 4.6% isoflurane. The trachea was intubated and the lungs were mechanically ventilated with 1.6% isoflurane in 30% O_2/70% N_2. Rectal temperature is servoregulated to maintain a temperature of 37.0° ± 0.2°C. The right common carotid artery was exposed and right external carotid artery (RECA) was isolated and ligated. A blunted 5-0 monofilament nylon suture, is introduced to the bifurcation of the right anterior cerebral artery (RACA) and right middle cerebral artery (RMCA). After perforation of the ACA, the RECA stump is ligated and the skin was closed. free access to food and water. Functional testing (Rotorod latency) is performed daily until sacrifice and histological evidence of vasospasm (cerebrovascular diameter) is assessed at day 5. Treatment with CN105 significantly improved functional outcomes (Rotorod latency days 1-5) and reduced RMCA vasospasm at 5 days after SAH compared to vehicle group (vehicle 81.39 ± 31.52µm; CN-105 109.30 ± 28.95µm; p = 0.044), **Figure 12.** Although this data represents additional experiments that go beyond the original scope of work, they were discussed at the JPC-6-CCCRP Neurotrauma IPR Presentation in 2017. SAH represents an important facet of TBI, and demonstration that CN-105 reduced the deficit associated with SAH and prevents the development of vasospasm represents will facilitate the possibility of clinical translation. **In the final quarter of the NCE**, we completed these studies, we submitted and revised a manuscript demonstrating efficacy of CN-105 in SAH which has been accepted for publication in *Stroke and Vascular Neurology (the current funding is credited).*This manuscript is included in the Appendix.

During the NCE, we also completed work demonstrating that CN-105 improves functional outcome in a gyrencephalic (ferret) model of blast injury. This work was presented at the Military Health System Research Symposium Military Health System Research Symposium in 2018, (MHSRS-18-1808). As with the SAH data, although not a specific objective of this grant, this work was directly related to the translatability of CN-105 to benefit war fighter following brain injury, and was facilitated by this award, so the current funding was credited. The presentation describing efficacy in blast injury is included as an Appendix.

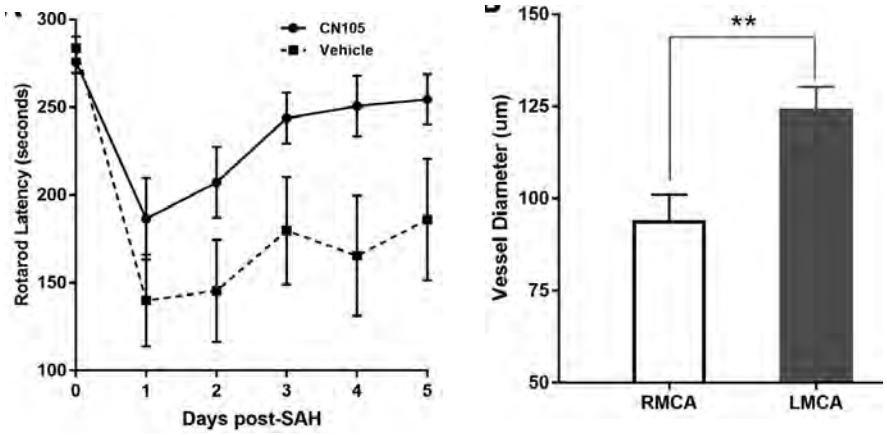


Figure 12: Treatment with CN105 significantly improved functional outcomes (Rotorod latency days 1-5; p<0.01 by ANOVA) and reduced MCA vasospasm at 5 days after SAH compared to vehicle group (vehicle 81.4 ± 31.52µm; CN-105 109.3 ± 28.95µm; p = 0.04).

The research objective outlined in this proposal was to compare the feasibility of different routes of administration of CN-105. Briefly, 1 mg/kg was administered as a single dose by intranasal (IN; dose volume 40 microliter/nares)), intraperitoneal (IP), and oral (PO) dosing in cannulated 13-16 week old male Sprague-Dawley rats (n=3/group). Pharmacokinetic (PK) samples were collected by sublingual vein at approximately 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dose. Aside from the IV formulation, CN-105 was only quantifiable at 0.083 (5 min) hours postdose for animals in Group 2 IN administration of CN-105 and all CN-105 plasma concentration were BLQ (< 25 ng/mL) for animals after PO administration of CN-105; therefore, exposure ratios between the different routes of administration could not be calculated (no AUC determined after IN or PO administration. Following a single IP administration of 1 mg/kg CN-105, mean C_{max} and $AUC_{0\text{-}24hr}$ values for CN-105 were 725 ng/mL and 830 hr*ng/mL, respectively Figure 12). These results are summarized in Table 1 and Figure 13.

Table 1: CN-105 Pharmacokinetics following IP, IN, and PO Delivery

Group	Dose (mg/kg)	Route	Subject	C_{max} (ng/mL)	C_{max}/Dose (kg*ng/mL/mg)	T_{max} (hr)	T_{last} (hr)	AUC_{Tlast} (hr*ng/mL)	$AUC_{0\text{-}24hr}$ (hr*ng/mL)	$AUC_{0\text{-}24hr}$/Dose (hr*kg*ng/mL/mg)	$T_{1/2}$ (hr)	AUC_{INF} (hr*ng/mL)	AUC_{INF}/Dose (hr*kg*ng/mL/mg)
1	1	Intraperitoneal	101	1040	1040	0.5	2	1180	1370	1370	NA[c]	NA[c]	NA[c]
			102	507	507	0.083	2	515	629	629	0.739	632	632
			103	628	628	0.083	2	386	495	495	NA[d]	NA[d]	NA[d]
2	1	Intranasal	104	2790	2790	0.083	0.083	NA[b]	NA[b]	NA[b]	NA[c]	NA[c]	NA[c]
			105	145	145	0.083	0.083	NA[b]	NA[b]	NA[b]	NA[c]	NA[c]	NA[c]
			106	307	307	0.083	0.083	NA[b]	NA[b]	NA[b]	NA[c]	NA[c]	NA[c]
3	1	PO	107[a]	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA
			108[a]	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA
			109[a]	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA - Not applicable
[a] All CN-105 plasma concentrations were BLQ (< 25 ng/mL)
[b] AUC not reported due to less than three consecutive quantifiable concentrations
[c] Secondary parameters ($T_{1/2}$ and AUC_{INF}) not reported due to insufficient plasma concentration-time data
[d] Secondary parameters ($T_{1/2}$ and AUC_{INF}) not reported due to an adjusted R^2 less than 0.9

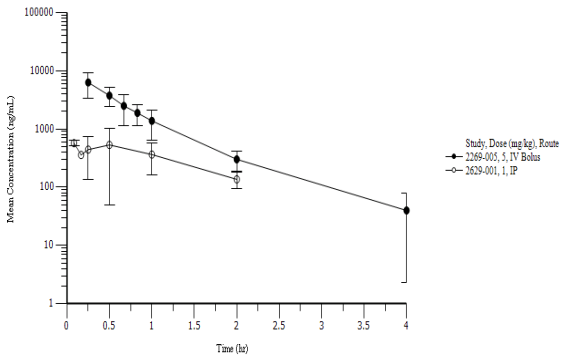


Figure 13: Mean +/- SD plasma concentration-time profile following a single intraperitoneal administration of CN-105 at a dosage of 1 mg/kg in male Sprague-Dawley rats

Based on this pharmacokinetic information demonstrating a more prolonged half life associated with i.p administration as opposed to i.v. route of administration (intraperitoneal $T_{1/2}$ 0.74 hours vs. intravenous $T_{1/2}$ 0.49 hours), we next optimized the prophylactic administration dosing paradigm described in Objective 1 by administering intravenous and intraperitoneal administration simultaneously at 3 and 6 hours prior to subjecting mice to moderate-severe closed head injury paradigm. Male 10-12 week old C57-BL6 mice were randomized into one of three groups: animals pretreated 3 hours prior to TBI with a combination of CN-105 0.1 mg/kg i.v. plus 0.5 mg/kg i.p (n=14); animals pretreated 6 hours prior to TBI with a combination of CN-105 0.1 mg/kg i.v. plus 0.5 mg/kg i.p (n=14); animals pretreated 3 and 6 hours prior to TBI with a combination of 100 microliters vehicle i.v. plus 0.5 mg/kg i.p (n=14). Serial Rotorod was performed daily for days 1-7

post-injury , and weekly until Day 28. With this optimized treatment regimen, we were able to observe a significant improvement in animals pretreated at 3 hours prior to injury ($p<0.01$ at day 28; Figure 14).

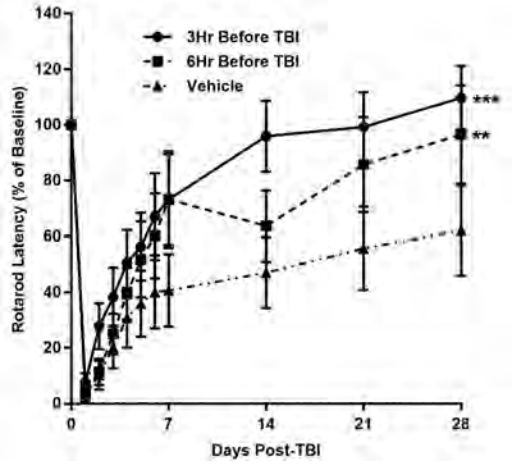


Figure 14: C57-BL/6 male 12 week old mice were treated with a combination of 0.5 mg/kg i.p. and 0.1 mg/kg i.v CN-105 or i.p and i.v vehicle at 3 hours and 6 hours prior to moderate to severe closed head injury. In the the 3 hour pre-treatment group, there was functional benefit, as defined by improved vestiubulomotor function (Rotorod latency) for animals pre-treated 3 hours prior to injury ($p<0.01$ by ANOVA).

These results demonstrate that, in theory, pretreatment with CN-105 could reduce functional deficits associated with subsequent head injury as long as dosing can be optimized so that there are adequate drug concentrations at the time of injury. It is important to note that, although the serum half-life of CN-105 is 29 minutes in murine model, the half life is 3.5 hours in humans. This would suggest that chronic pretreatment may be a viable strategy for military at risk for TBI. To complete our dataset for publication, in the final quarter NCE, we repeated the PK experiments in the same dose that was efficacious in mouse models (0.1-1.0 mg/kg; Figure 15).

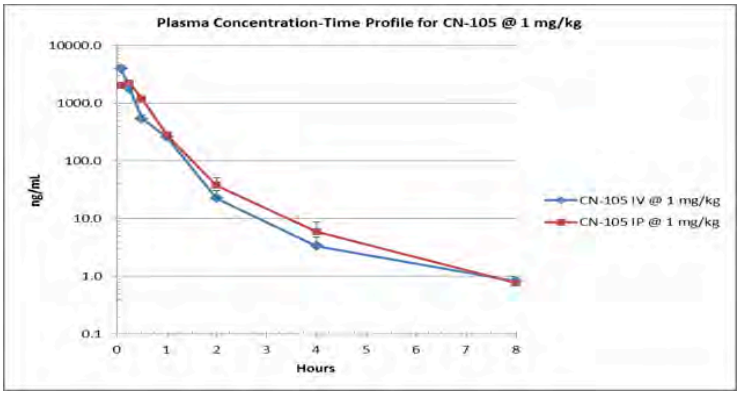


Figure 2: Pharmacokinetic parameters were assessed following 1 mg/kg administration of CN-105 in intraperitoneal vs intravenous formulation in Faster t(max) associated with i.v administration was 0.083/h as opposed to 0.25/h with i.p administration. C(max) was increased with IV as compared to IP administration (3997 vs 2230 ng/ml); as was AUC (0-inf) 1568 vs 1455 ng.hr/ml. Half life was comparable in IV vs IP administration (1.33 vs 1.1/h in IV vs IP)

What opportunities for training and professional development has the project provided?

Nothing to report.

This project was not intended to provide training and professional development opportunities.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

As noted below, in the body of the grant during the period of no-cost extension, we replicated our experiments for presentation and publication. The data regarding the efficacy of prophylaxis and pharmacokinetics is being prepared for submission to *Military Medicine.* The data regarding efficacy in subarachnoid hemorrhage was published in *Stroke Vascular Neurology*. The data demonstrating efficacy in a gyrencephalic blast injury was presented recently at Military Health System Research Symposium Military Health System Research Symposium in 2018, (MHSRS-18-1808). It is our intent ti complete manuscript preparation for the data demonstrating efficacy on the repetitive subconcussive head injury model (rTBI) for submission to *J Neurotrauma* in this calendar year.

What do you plan to do during the next reporting period to accomplish the goals?
This is the final report.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

This is the final report .

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

CN-105 was designed from the receptor binding face of apolipoprotein E, which is an endogenous protein that has been demonstrated to modify injury responses and outcomes after acute brain injury. In particular, we have demonstrated that CN-105 mimics the neuroprotective and anti-inflammatory actions of the native holoprotein. As there are no pharmacological interventions that have ever been demonstrated to improve functional outcomes after acute brain injury, this would represent the first-in class and first-in-disease-state therapeutic to improve outcomes following for acute brain injury. We are particularly excited by the fact that CN-105 improves long term functional outcomes and reduces chronic histological and structural changes associated with mild repetitive head injury.

Since CN-105 is currently in clinical trials, where it is has demonstrated an excellent safety profile in patients with acute brain injury (intracranial hemorrhage), we feel that is has high potential to translate to the military TBI population. Our intent is to work with the MTEC consortium (which we have joined) to facilitate translation.

What was the impact on other disciplines?

We have initiated a multi-center Phase 2 open label study evaluating the safety and feasibility of administering CN-105 in patients with spontaneous intracranial hemorrhage. Although this is a different patient population than soldiers with traumatic brain injury, many of the mechanisms of neuroinflammation, secondary neuronal injury, and development of cerebral edema are similar. In fact, parenchymal hemorrhage is a common pathological feature of brain injury associated with TBI. We feel that this study may facilitate an early clinical study in patients with traumatic brain injury.

What was the impact on technology transfer?

There is no new intellectual property that was developed during this reporting period.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report (we have initiated a phase 2 multicenter trial in spontaneous intracranial hemorrhage, as noted above)

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report. We have utilized the budget in the no cost extension period in a manner consistent completing the stated objectives of this proposal.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

There are no human subjects associated with any of the research activities included in this grant.

Significant changes in use or care of vertebrate animals

As described in the initial SOW, Institutional Animal Care and Use Committee approval and ACURO approval were obtained in the first month of this grant. All procedures described have remained compliant with these approvals.

Significant changes in use of biohazards and/or select agents

Nothing to report. There were no biohazards associated with this proposal.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications.

We will be submitting a peer review publication describing the effects of CN-105 when used as a prophylactic neuroprotectant therapy. Our intent is to submit to *Military Medicine*.

Included as an Appendix are two manuscripts directly relevant to this award. The first is pursuant to the suggestion that we define the neuroprotective efficacy of CN-105 when administered after injury (this award is cited); the second demonstrates safety pharmacokinetic profile of CN-105 in a phase 1 study, which is important to translation to a clinical study (this work was not performed within the scope of the current award, which is not cited).

Laskowitz DT, Wang H, Chen T, Lubkin DT, Cantillana V, Tian-Ming T, Kernagis D, Zhou G, Macy G, Kolls BJ, Dawson HN" Neuroprotective pentapeptide CN-105 is associated with reduced sterile inflammation and improved functional outcome is a traumatic brain injury murine model" Scientific Reports, 2017:46461PMID: 28429734 (*this award was cited*)]

Guptill JT, Raja S, Ramey S, Boakye-Agyeman F, Noveck R, Tu T-M, **Laskowitz DT**. Phase I, randomized, double-blind, placebo controlled study to determine the safety, tolerability, and pharmacokinetics of a single escalating dose and repeated doses of CN-105 in healthy adult subjects. J Clinical Pharmacology, 2016. PMID: 27990643

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

We have presented our data from the current research project demonstrating that CN-105 exerts neuroprotection in a gyrencephalic blast model of ferret at the 2018 Annual Military Health System Research Symposium.

In addition, there are several other recent publications demonstrating that CN-105 exerts neuroprotective effect after different components of traumatic brain injury (i.e. ischemia, parenchymal, contusion, and subarachnoid hemorrhage)

Tu TM, Wang H, Dawson H, Kolls B, **Laskowitz DT**. "Apolipoprotein E mimetic peptide, CN-105, improves outcomes in ischemic stroke" Annals of Clinical and Translational Neurology 4(4):246-265, 2017 PMID: 28382306

Lei B, James ML, Liu J, Zhou G, Venkatramen TN, Lascola CD, Acheson S, Dubois LG, **Laskowitz DT**, Wang H Neuroprotective pentapeptide CN-105 improves functional and histological outcomes in a murine model of intracerebral hemorrhage Scientific Reports 6:34834, 2016 27713572

Laskowitz DT, Wang H, Chen T, Lubkin DT, Cantillana V, Tian-Ming T, Kernagis D, Zhou G, Macy G, Kolls BJ, Dawson HN" Neuroprotective pentapeptide CN-105 is associated with reduced sterile inflammation and improved functional outcome is a traumatic brain injury murine model" Scientific Reports, 7(46461), 2017 PMID: 28429734

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

There was no new technologies or techniques.

- **Inventions, patent applications, and/or licenses**

There was no new IP filed during the course of this grant.

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	**Daniel Laskowitz, MD, MHS:**
Project role:	PI
Nearest person month worked:	2.0
Contribution to project:	Dr. Laskowitz serves as the PI, and is responsible for study design and conduct, reporting. (unchanged)
Funding Support:	Cure Alzheimer's Foundation (0.12 calendar month) (described in next section)

Name:	**Haichen Wang, MD**
Project Role:	Co-investigator
Nearest person month worked:	4
Contribution to Project:	Responsible for animal surgical procedures; preclinical models of neurotrauma (unchanged)
Funding Support	Yunnan Valley Pharma contract to test new pharmacological therapies in stroke (3 calendar months)

Name:	**Viviana Cantillina-Riquelme**
Project Role:	Research analyst
Contribution to Project:	Animal behavior and functional testing, histology and unbiased formal stereology, data analysis
Nearest person month worked:	10
Funding support:	No other Funding support

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Since this proposal has initiated, the PI has had funded one additional grant, entitled "*A novel apoE mimetic therapeutic peptide CN-105 attenuates AD pathology and improves functional outcomes in a murine model of Alzheimer's disease".* In this current study, we will test the hypothesis that CN-105, delivered as a continuous infusion over 6 weeks, will reduce AD pathology and improve behavioural outcomes. There is no scientific or budgetary overlap. He contributes 0.12 calendar months/year towards this grant which runs from 2/1/17 through 1/31/2018.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Attached are two appendices; both are manuscripts that were published recently:

Laskowitz DT, Wang H, Chen T, Lubkin DT, Cantillana V, Tian-Ming T, Kernagis D, Zhou G, Macy G, Kolls BJ, Dawson HN" Neuroprotective pentapeptide CN-105 is associated with reduced sterile inflammation and improved functional outcome is a traumatic brain injury murine model" Scientific Reports, 2017:46461PMID: 28429734 *(this grant is cited)*

CN-105 is neuroprotective in a gyrencephalic blast model of ferret at the 2018 Annual Military Health System Research Symposium

Appendix 1:

Presentation at the Military Health System Research Symposium (Aug 20-23, 2018; Orlando, FL)

Duke
DEPARTMENT OF
Biomedical Engineering

Neuroprotective Effect of an ApoE Mimetic Peptide in a Gyrencephalic Blast Animal Model

Allen W. Yu, BS[1], Hattie C. Cutcliffe, MS[1] Daniel T. Laskowitz, MD[2,3], Haichen Wang, MD[2], Brad J. Kolls, MD PhD MMCi[2], Jason Kait, MS[1], Cameron R. Bass, PhD[1]

[1] Duke University, Durham, NC
[2] Duke University School of Medicine, Durham, NC
[3] Aegis, LLC, Durham, NC

MHSRS 2018
Orlando, FL USA
Aug 20 – 23, 2018

Disclosures

- Daniel T. Laskowitz
  – Officer of Aegis LLC
  -Supported by CDMRP Contract: W81XWH-16-C-0142
- Brad J. Kolls
  – Co-inventor on Duke held patent

No Disclosures

- Allen W. Yu, Cameron R. Bass, and the Injury Biomechanics Laboratory at Duke University

→Affiliations do not influence the statements presented
→Views here reflect those of authors only

Neurotrauma: Trouble?

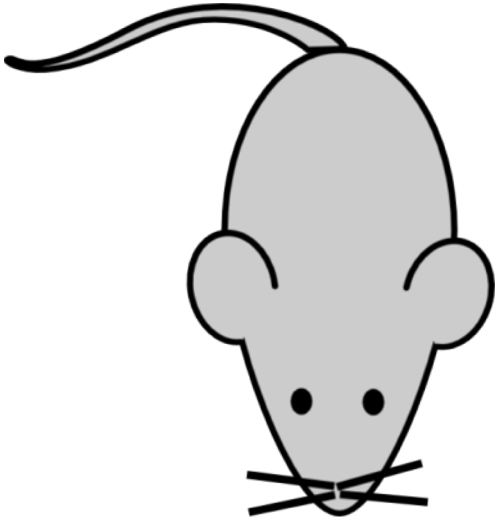
FDA Approved Neuroprotective Agents (~30 years)

Zero

DUKE BME

What are the roadblocks to clinical translation?

Of Mice And Men



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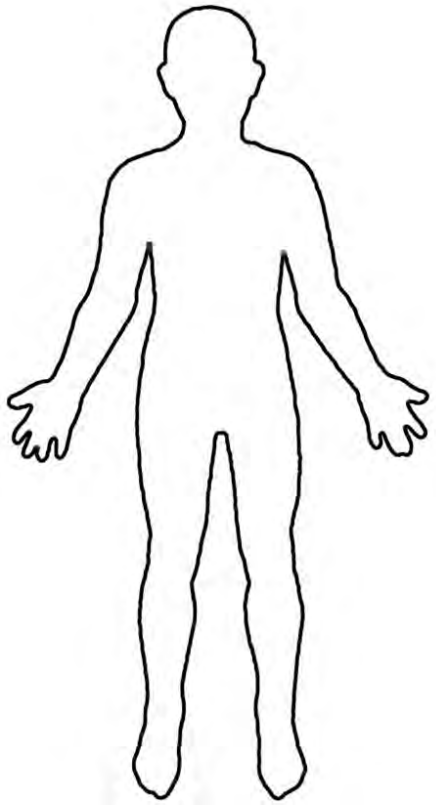
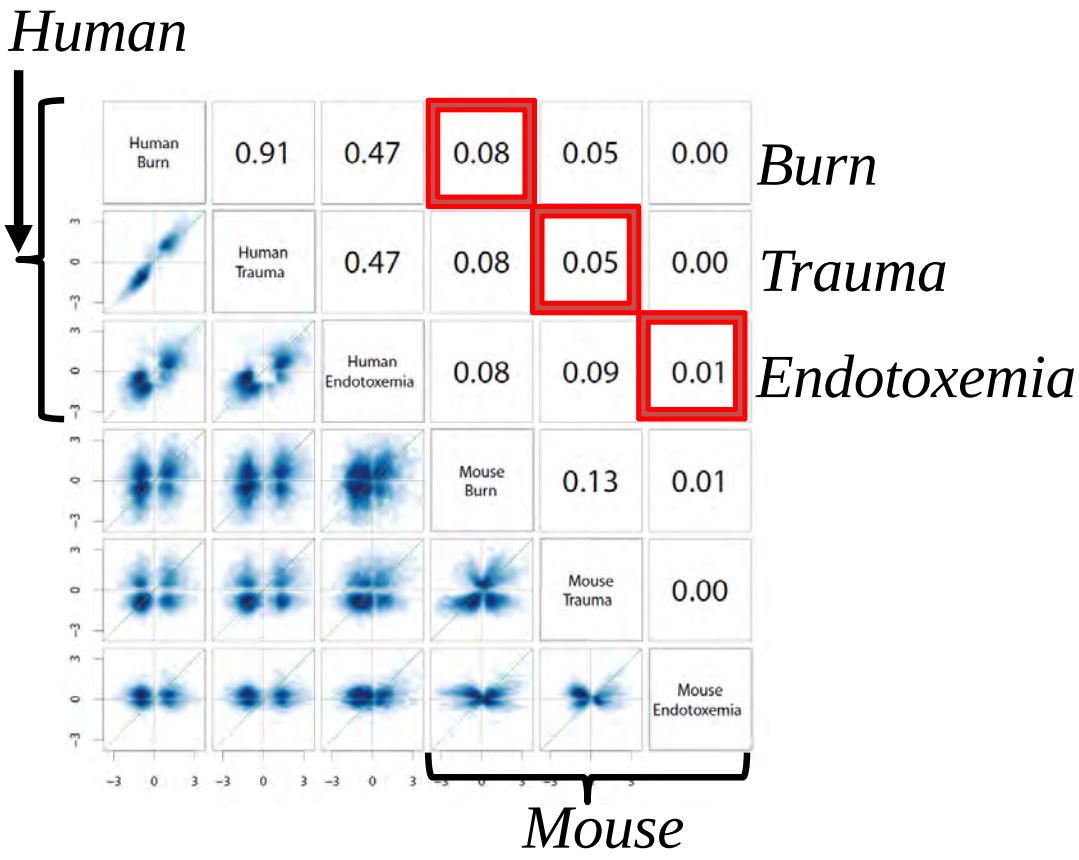


Of Mice And Men

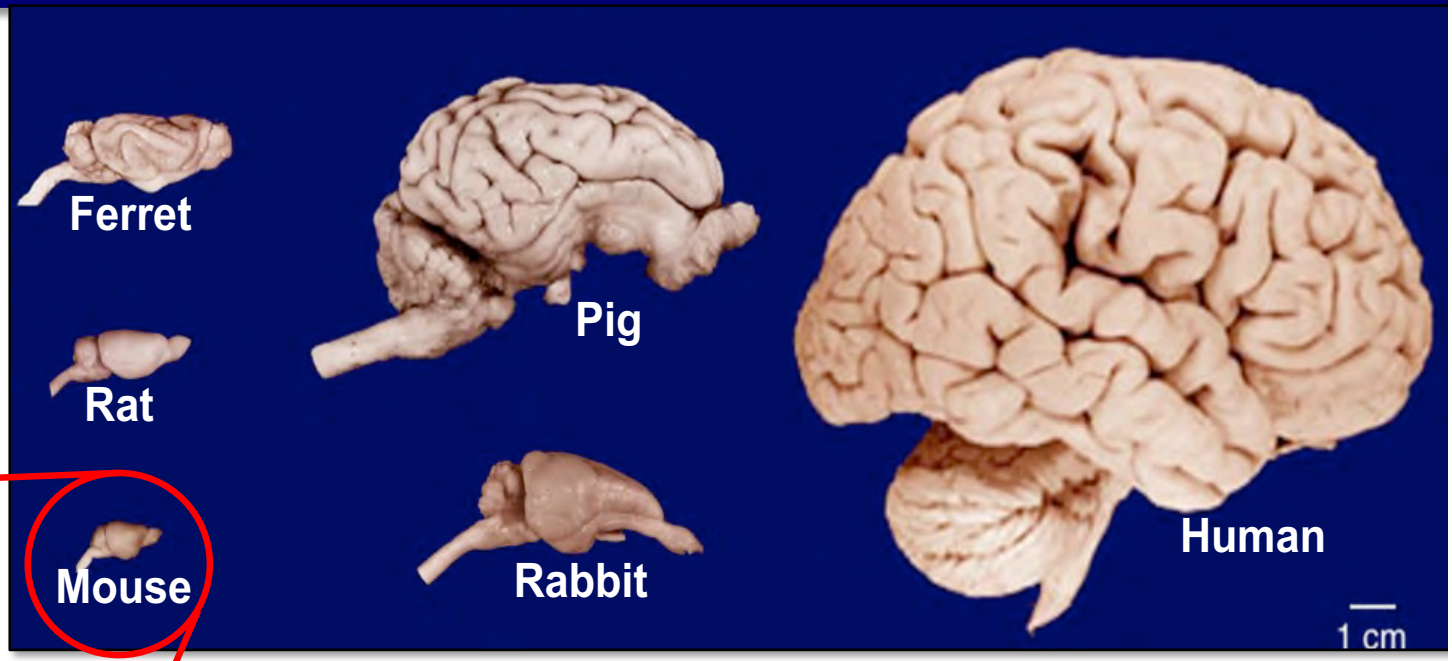
Seok et al. 2013: systematic comparison of murine & human inflammatory response

- Different genomic response
  - Correlations of gene changes in human response to disease and mouse models are quite low
- Different temporal response
  - Mice tend recover much more quickly than humans; gene expression levels return to normal faster too
- Different signaling pathways involved in inflammatory response

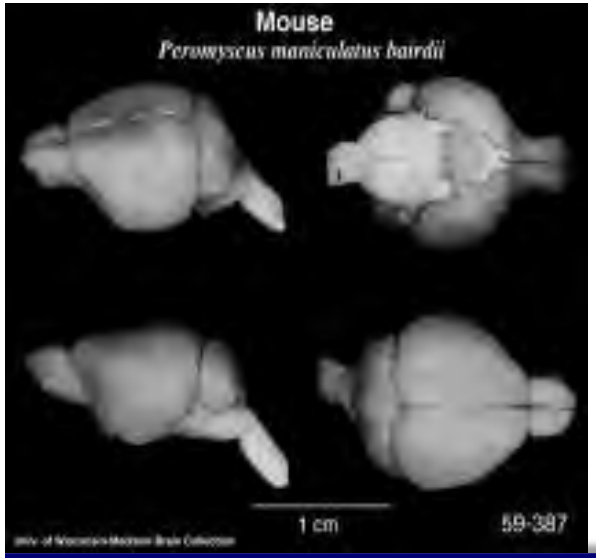


Of Mice And Men

Comparative neuroanatomy?



(Adapted from the Wisconsin Brain Collection)



- Gyrencephalic vs. Lissencephalic
- White/gray matter

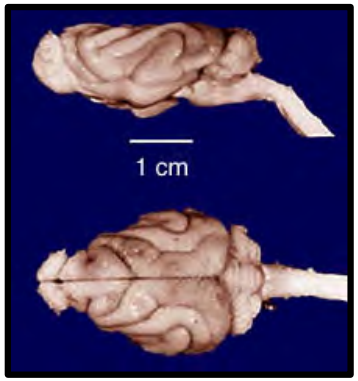
So?

- Rodent models are likely one of the problems
  - Not well correlated with human injury response, pathophysiologically.
  - Mostly employ biofidelically irrelevant inputs to the model for TBI.
  - The translational relevance of simple behavioral tasks is limited as rodents do not provide the full clinical spectrum of TBI symptomology

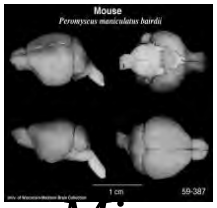
Why Ferrets?

- Gyrencephalic/Small/Blast Injury Model
  - Gray-white boundaries, wave transmission problem
  - Well-characterized neuroanatomy (e.g. Hein, 1990)

Ferret



Rabbit



Mice

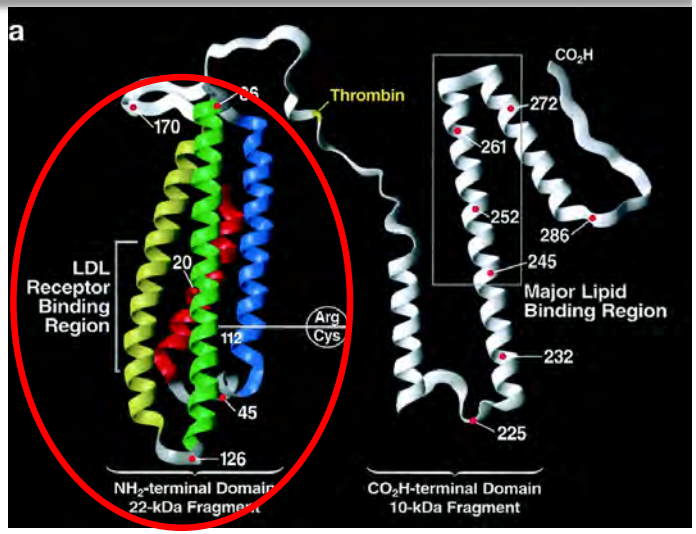
- Behavior
  - Predator -> Not naturally fearful (PTSD later…)
  - Clinical Correlates with VA Blast Neurotrauma Patients (Oculomotor/vestibular response)

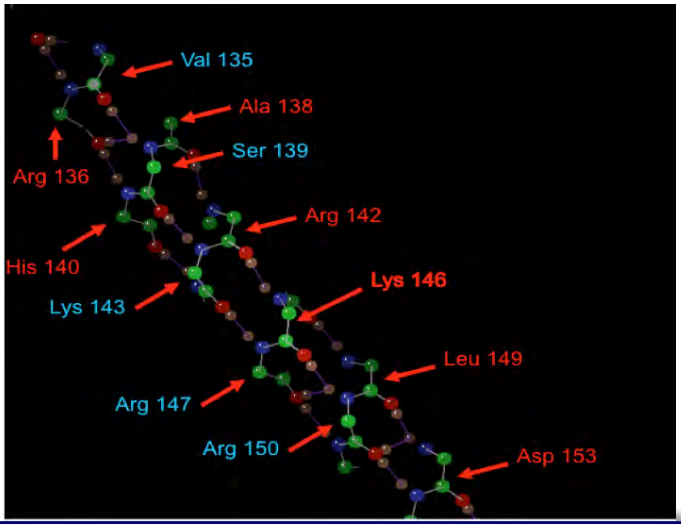
Apolipoprotein E (ApoE) Mimetic Peptide

- ApoE polymorphism in neurological disease
  - E4: Alzheimers Disease, recovery from brain injury
- ApoE induces signaling cascade resulting in reduced neuroinflammatory responses (Laskowitz et al., 1997)

In collaboration with the Multidisciplinary Neuroprotection Laboratory at Duke Med Center:

- Linearized pentapeptide models the receptor binding face of endogenous apoE (Laskowitz et al., 2001)
- ApoE mimetic peptide protects against NMDA mediated neuronal excitotoxicity and modulates glial responses (Lei et al., 2016)



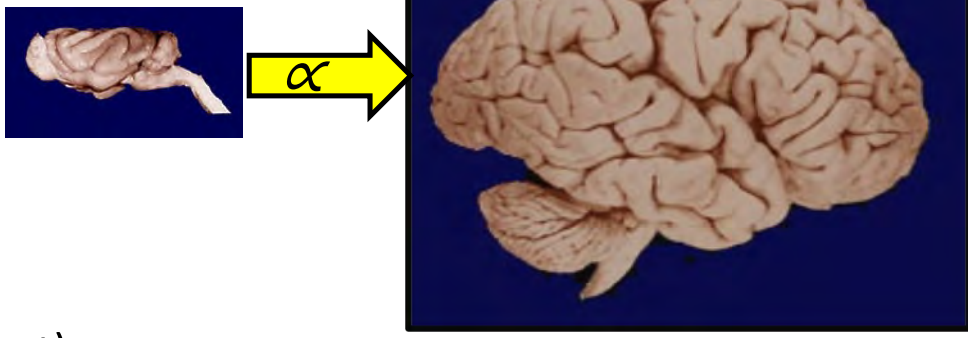


ApoE Mimetic Peptide in Acute Brain Injury

- Administration of peptide is well tolerated, associated with improved histological and functional outcomes in:
  - Intracranial Hemorrhage
    - (Lei et al., Sci Rep, 2016)
  - Focal Stroke
    - (Tu et al., *Ann Clin Trans Neurol*, 2017)
  - Subarachnoid hemorrhage
    - (Wang et al*., Stroke Vasc Neurol,* in press)
  - Traumatic Brain Injury
    - (Laskowitz et al., *Sci Rep*, 2018)
  - Blast Injury?

Study Objectives

- Create a repeatable musteline model of blast brain injury reflecting conditions seen in theater:
  - Thoracic Protection (hard body armor)
  - Scaling (representative of realistic exposures from high explosives)
- Assess behavioral paradigms based on clinical tests and observed deficits in humans
  - Motion coherence (visual choice experiment)
  - Executive function (prey tracking)
- Treat blast-exposed ferrets with candidate peptide and assess functional recovery

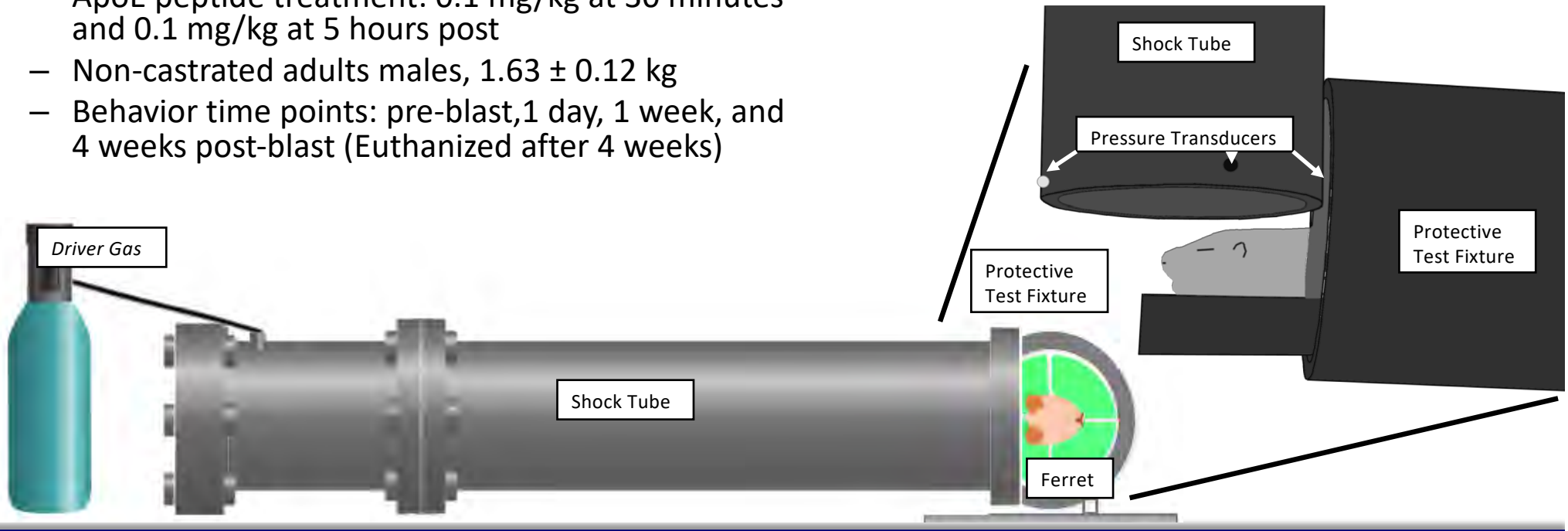


(Adapted from the Wisconsin Brain Collection)

Ferret Blast Model

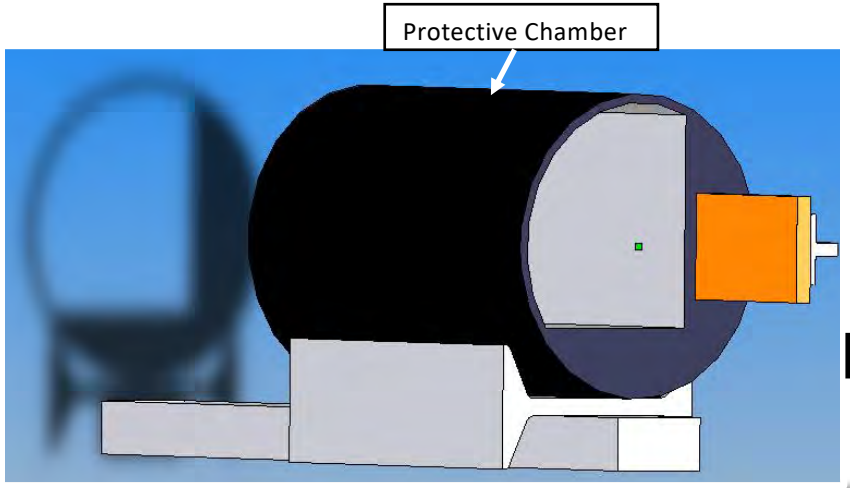
- Ferrets (*Mustela putorius furo*) were anesthetized and subjected to blast waves according to protocols approved by the Duke IACUC
  - N = 20 (12 blasted non-treated, 5 blasted peptide-treated, 3 sham controls)
  - ApoE peptide treatment: 0.1 mg/kg at 30 minutes and 0.1 mg/kg at 5 hours post
  - Non-castrated adults males, 1.63 ± 0.12 kg
  - Behavior time points: pre-blast,1 day, 1 week, and 4 weeks post-blast (Euthanized after 4 weeks)

- Shock tube (Rafaels et al., 2012)
  - 8 in. diameter, horizontally mounted, aluminum shock tube, helium driver gas, PET bursting diaphragms
  - Peak incident overpressure of 265±12 kPa and positive phase duration of 2.7±0.2 ms



Blast Exposure

- Isolate blast exposure to the head
  - Thorax and abdomen placed inside steel tubing
  - Vinyl nitrile closed cell foam used to close the open end of the cylinder
  - Protect air-filled organs and avoid potential downstream effects



- Scaling
  - Shock positive phase duration was scaled to a 70 kg human according to cube-root of mass scaling rules commonly used in blast studies (eg. Bowen et al. 1968)
  - Scaled duration of 9.4±0.6 ms is representative of shorter duration blasts found in the military theater

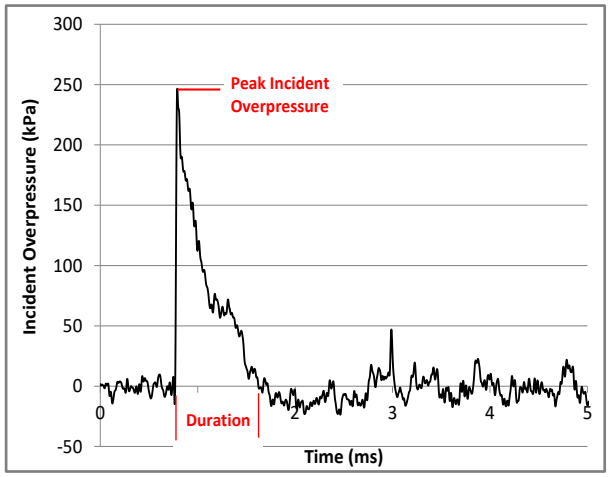
$$\Delta t_{scaled} = \lambda \Delta t \qquad \lambda = \left(\frac{70kg}{m}\right)^{1/3}$$

Interceptor Body Armors *(Adapted from U.S. Army)*

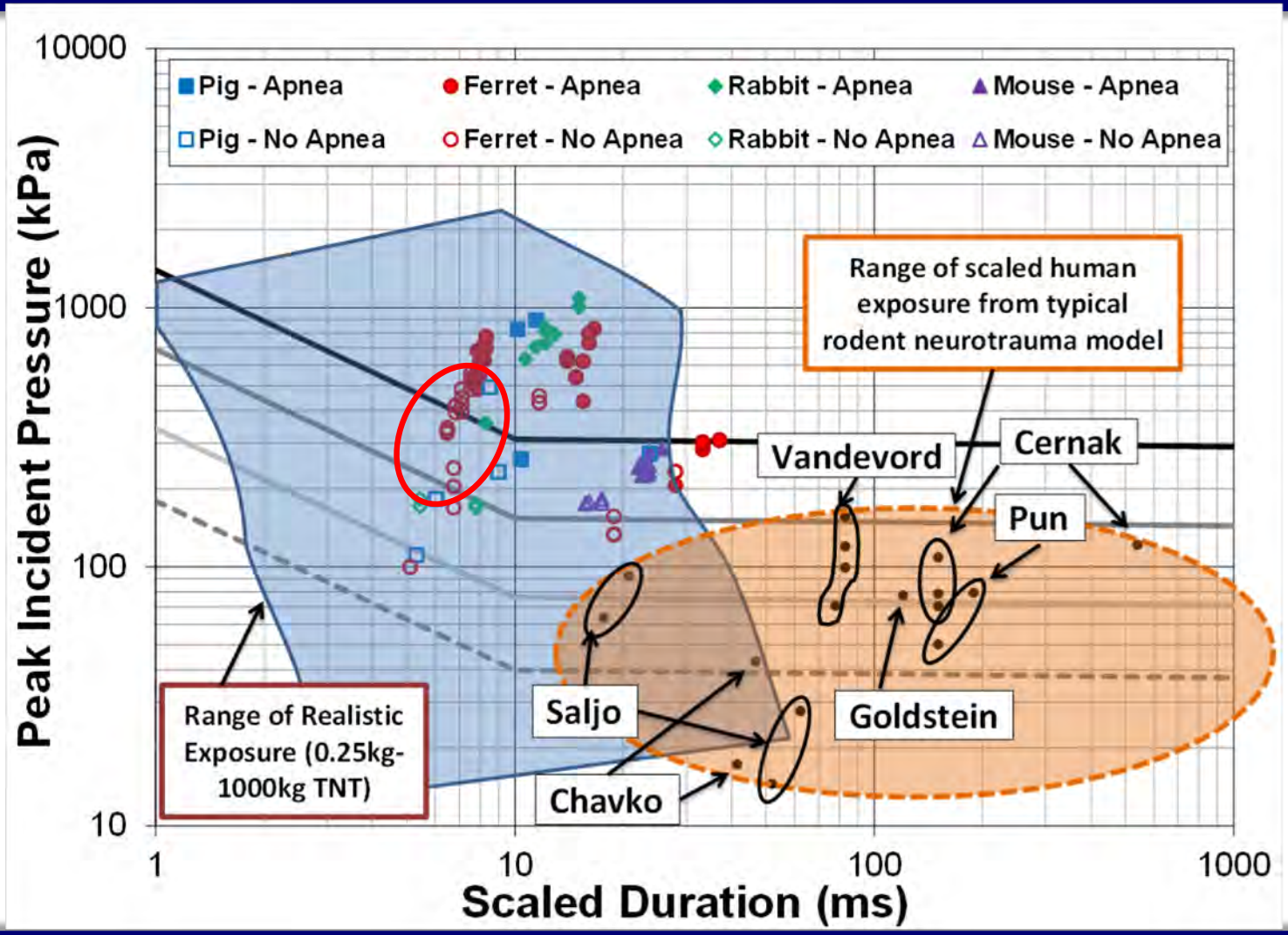
Blast Exposure

Blast-induced Injury

- All blast-tested ferrets survived the injury
- Post-blast, little to no apneic responses were observed. No serious pulmonary or gastrointestinal injuries were observed during necropsy, indicating suitable protection from steel thoracic chamber.
- Peak incident overpressure of 265±12 kPa and positive phase duration of 2.7±0.2 ms (unscaled) represent a 30% risk of apnea from established risk curves (Rafaels et al., 2012)



Ferret blasts simulate high explosives seen in combat



Rodents!

- Most existing rodent blast experiments…
  - … have biomechanical scaled durations typical of large nuclear blasts!

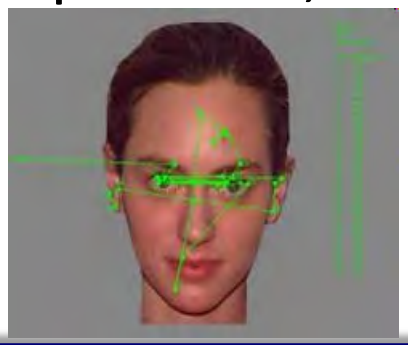
- The nature of the ‘Whack’ matters!

Human Oculomotor Response in Blast
(Capehart, Bass, 2011)

- Blast without Blunt Trauma, Abnormal:

1. Saccades – Rapid eye motions – scene processing into fovea
2. Smooth Pursuit – Visual stimulus – smooth trajectory
3. Optokinetic Nystagmus – Involuntary eye movement when processing a field

- Depend on variety of cortical, subcortical, cerebellar, and brainstem structures
- Unique to primates, some carnivores (like ferrets)



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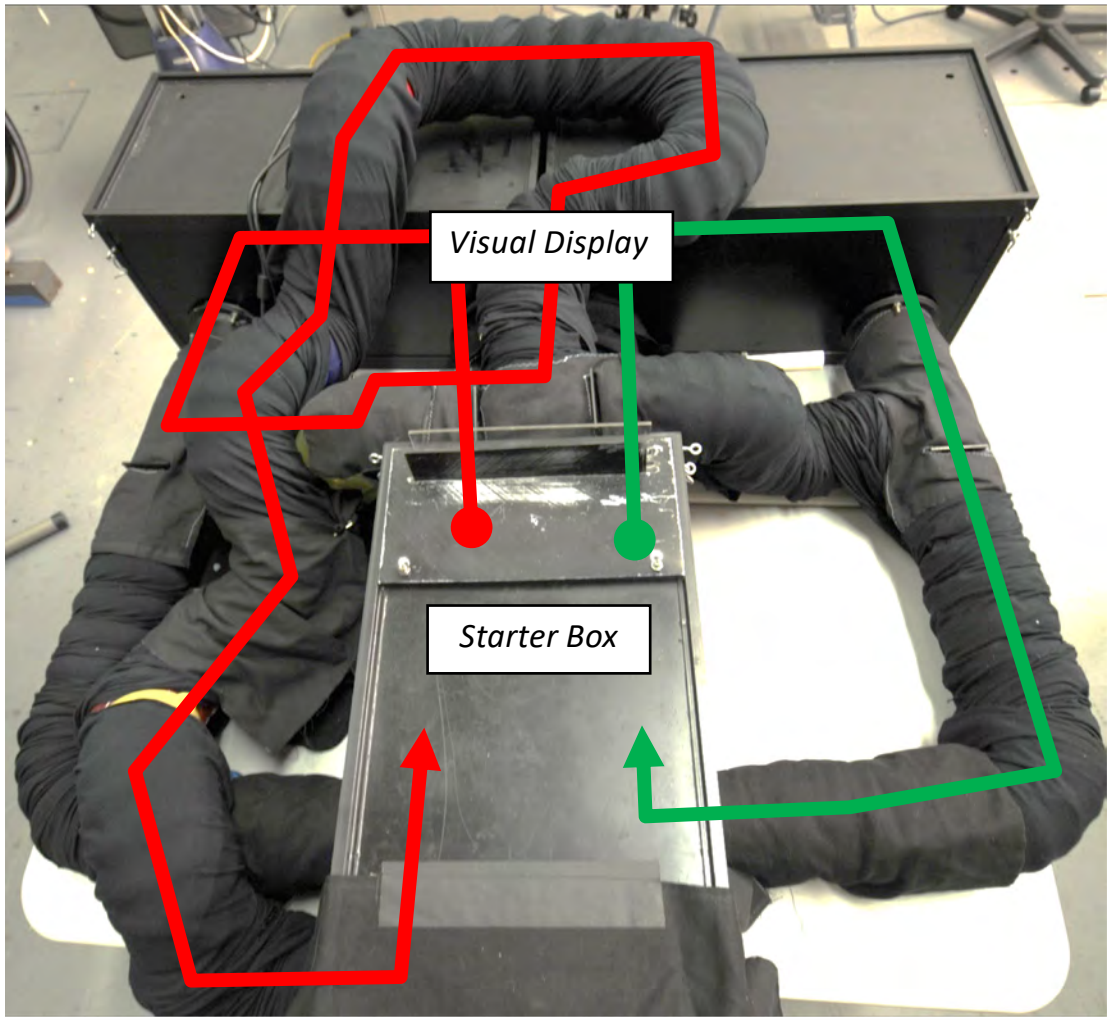
www.opt.uab.edu

Motion Coherence (MC) Forced Choice Paradigm



Based on Hupfeld et al., 1997

– Assessment of blast-induced reduced visual motion perception (akinetopsia) and discrimination, implicates cortical, subcortical, cerebellar, and brainstem structures

• Visual Discrimination box

– Ferrets placed into starter box and two paths presented simultaneously

(1) Coherent dot display: Food reward, short return

(2) Noncoherent dot display: No food reward, long return

Motion Coherence Visual Display

Left Display:
100% Coherent Motion
(correct choice)

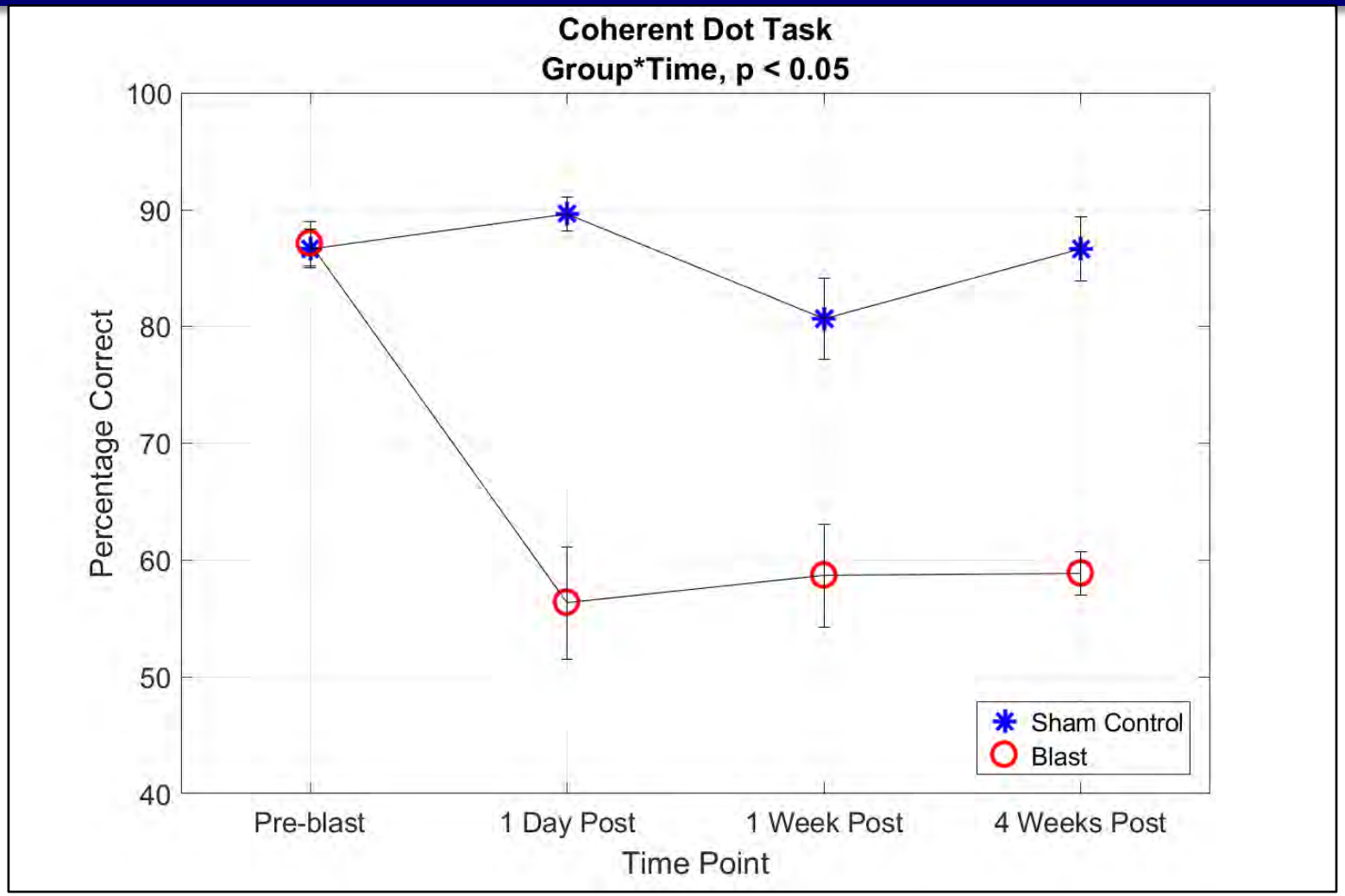
Right Display:
0% Coherent Motion
(incorrect choice)

Food reward
Shorter return path

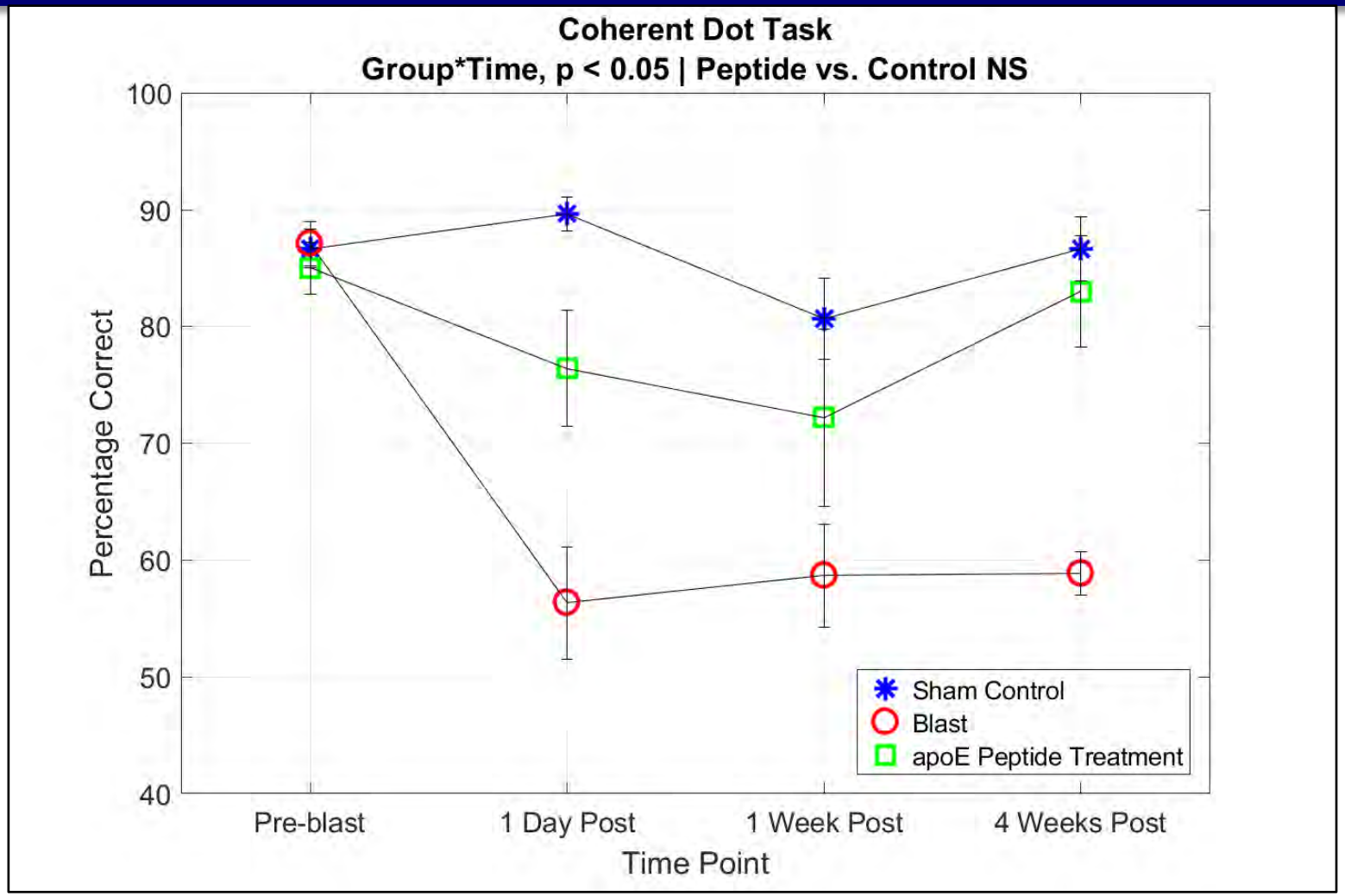
No food reward
Longer return path

- Ferrets trained 20 trials per time point (pre-blast, 1 day, 7 days, 28 days post injury). Time and percentage correct logged.

Mild Blast Causes MC Impairment



ApoE mimetic peptide restores MC performance



Executive Function – Simulated Prey Interaction

Paradigm Summary

- Presented ferret animal model serves as a clinically relevant and repeatable laboratory model of blast-induced TBI
- Behavior testing sensitive to very mild blasts, allows assessment of more subtle functional and cognitive endpoints. Analysis still ongoing.
- Behavior is persistent and has been assessed at up to 2 years (equivalent to ~5-10 years in humans)
- Gyrencephalic model amenable to therapeutic intervention
  - Early pilot cohort treated with apoE mimetic peptide restores behavior by 4 weeks in motion coherence paradigm
  - Given the lack of approved neuroprotectants, apoE based therapeutics are a promising strategy

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Questions?

Duke
DEPARTMENT OF
Biomedical Engineering

Neuroprotective Effect of an ApoE Mimetic Peptide in a Gyrencephalic Blast Animal Model

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MHSRS 2018
Orlando, FL USA
Aug 20 – 23, 2018

Appendix 2:

Submission of data demonstrating efficacy of CN-105 when administered prophylactically in murine TBI model to *Military Medicine* (AMSUS' official peer reviewed journal)

Military Medicine

Prophylactic Treatment with CN-105 Improves Functional Outcomes in a Murine Model of Closed Head Injury

--Manuscript Draft--

Manuscript Number:	
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Abstract:	Structured Summary Introduction: Traumatic brain injury (TBI) has become the signature wound of recent military operations and training over the past two decades. Throughout the Department of Defense (DoD) from 2000 through the first quarter of 2018, there have been 383, 947 reported TBIs of all severities resulting in significant morbidity and high healthcare costs. A treatment that would protect against secondary brain injury in TBI and improve the long-term functional outcomes of traumatic brain injury on service personnel is therefore of great interest. One agent that has shown promise in modifying the post-traumatic neuroinflammatory response in murine closed-head injury models of TBI is a novel, small 5 amino acid apolipoprotein E (apoE) mimetic peptide, CN-105. The goal of this study was to determine whether CN-105 would maintain its neuroprotective effects if administered prior to closed-head injury. Materials and Methods: CN-105 was synthesized by Polypeptide Inc. (San Diego, CA) to > 99% purity. We examined the efficacy of prophylactic administration of CN-105 in a well-established closed head injury model associated with reproducible vestibulomotor deficits. CN-105 was dissolved in sterile 0.9% saline and administered intravenously (IV) through a tail vein and/or by intraperitoneal (IP) injection in a volume of 100 microliters at various time points prior to injury. Vehicle treated animals

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Opposed Reviewers:	

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Prophylactic Treatment with CN-105 Improves Functional Outcomes in a Murine Model of Closed Head Injury

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Disclaimer: The views expressed are solely those of the authors and do not reflect the official policy or position of the US Army, US Navy, US Air Force, the Department of Defense, or the US Government. DTL and RTF are officers of Aegis CN, LLC, which supplied study drug. Aegis had no editorial control over study design, execution, or the writing of this manuscript. DTL and BJK are co-inventors of the Duke-held patent for CN-105. This work was funded by DOD contract CDMRP#W81XWH-16-C-0142

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Results: IV administration of CN-105 up to thirty minutes prior to closed head injury significantly improved durable vestibulomotor functions compared to vehicle control-treated animals. In pharmacokinetic studies in uninjured CD-1 mice, IP administration of CN-105 resulted in an ~ 3-fold increase in the time to reach maximal plasma concentration (Tmax) and an ~1.5-fold increase in mean plasma residence time (MRT) compared to IV administration although the terminal elimination half-lives ($T_{1/2}$) were similar. When CN-105 was co-administered by IP and IV dosing 6 hours prior to injury, a durable improvement in vestibulomotor function was observed up to 28 days following injury.

Conclusion: CN-105 improves functional outcomes when administered prior to injury. This may be adaptable in the future as a means of prophylaxis against TBI-related secondary brain injury that improve the outcomes of affected service members.

Introduction

Traumatic brain injury (TBI) has come to be known as the “signature injury” of the recent conflicts in Afghanistan, Iraq and other theaters comprising the Global War on Terror.[1,2] Furthermore, TBI has become increasingly recognized as a complication of military training exercises such as combatives, obstacle courses, airborne operations and heavy-weapons training.[2,3,4,5,6] According to the most recent data there have been 383,947 total TBIs of all severities reported across the Department of Defense (DoD) between 2000 and the first quarter of 2018[7] with projected health care costs related to the care of TBI of approximately $14 billion over the next 20 years.[8] Unfortunately, there are no neuroprotective pharmacological therapies that have been demonstrated to improve long term functional outcome following TBI, and the treatment options for patients with persistent TBI symptoms remains primarily supportive.[9] Considering the detrimental impact of TBI on the health and safety of individual service members and the resulting strain on individual and unit health and readiness, filling this therapeutic vacancy has become a top priority for the DoD.

Primary brain injury, or the injury to the brain that occurs as a direct result of head trauma, is frequently exacerbated by indirect, secondary brain injury that consists of cellular energy depletion, excitotoxicity and ischemia in the acute phase, followed by neuroinflammation in the intermediate phase. The corresponding secondary injury typically leads to cerebral edema, oxidative damage, and intracranial hypertension.[3,10,11,12] Thus, for optimal benefit, a pharmacological intervention that targets these processes should be administered as quickly as possible post-injury. Alternatively, prophylactic administration to soldiers in high-risk environments might also be of benefit provided that adequate tissue concentrations of the drug are achieved by the time of injury and are able to effectively slow the progression of secondary injury. Although no proposed neuroprotectants improve long-term functional outcomes, several investigational agents have shown great promise in preclinical studies.[9] Additionally, for military applications, a TBI intervention should not only be effective at limiting secondary injury, but should also be safe, available and feasible for administration at or near the point of injury, stable throughout a range of climate extremes, easy to administer and safe.

Increasing evidence suggests that functional outcomes after acute brain injury are modified by genetic factors, which may influence neuroinflammation and secondary neuronal injury as well as plasticity and recovery.[13,14] One of the most robust genetic associations with outcome after TBI is the apolipoprotein E (APOE) polymorphism. [15,16] ApoE is the primary apolipoprotein produced in the brain, where its synthesis is upregulated after injury, and has been demonstrated to reduce glial activation and inflammatory cytokine release in vitro and in vivo models of closed head injury.[16] Although apoE does not readily cross the blood brain barrier, we recently developed CN-105, a 5 amino acid peptide (Ac-VSRRR-COOH) derived from the polar face of the apoE receptor binding region. This peptide readily crosses into the CNS compartment, is well tolerated, and improves long-term histological and functional outcome in multiple preclinical models of acute brain injury, including subarachnoid hemorrhage, intracranial hemorrhage, stroke, blast injury, and closed head injury.[17,18,19] Moreover, CN-105 is an

excellent candidate for clinical translation, and has demonstrated an excellent safety profile, as well as a linear and predictable pharmokinetic profile in phase 1 escalating dose studies involving healthy volunteers.[20] We now test the hypothesis that CN-105 retains its neuroprotective and anti-inflammatory effects when administered prior to injury.

Methods

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Duke University Institutional Animal Care and Use Committee, Durham North Carolina.

Closed Head Injury Model: The murine closed head injury model used in this study has been previously described. [17] The closed head impact results in injury to selectively vulnerable neurons in the cortex and hippocampus and is associated with vestibulomotor deficits and long-term neurocognitive deficits. Briefly,10–12-week-old C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME) were used. The trachea was intubated after anesthesia induction with 4.6% isoflurane and the lungs were mechanically ventilated with 1.6% isoflurane in 30% O2/70% N2. Core body temperature was servo regulated at 37° C via rectal probe. To avoid basilar skull fracture, ear bars were not used. The animal was secured in a stereotactic device, mechanically ventilated, and the head shaved to identify anatomical landmarks. A concave 3-mm metallic disc was adhered to the skull immediately caudal to the bregma. A 2.0-mm diameter pneumatic impactor (Air-Power Inc., High Point, NC) was used to deliver a single midline impact to the disc surface. The impactor was discharged at 6.8 +/- 0.2 m/second with a head displacement of 3 mm. After impact, the animals were allowed to recover. Once spontaneous ventilation resumed the mice were extubated. Mice were allowed free access to food and water. Control mice were treated identically but had no induced head injury. All mice were housed in the same facility.

Drug Administration: CN-105 (Ac-VSRRR-amide) was synthesized by Polypeptide Inc. (San Diego, CA) to a purity of > 99%. It was dissolved in sterile, 0.9% saline and administered IV through a tail vein and/or by IP injection in a volume of 100 µL. Vehicle treated animals received IV and/or IP injection of 100 µL of normal saline at the same time points. Animals were randomly assigned to treatment groups by a coded study identification number after injury using Graphpad online randomizer.

Testing of Functional Deficits. Mice were randomly assigned to treatment groups immediately following injury and all behavioral evaluations were performed by investigators blinded to treatment. An automated Rotarod (Ugo Basile, Comerio, Italy) was used to assess vestibulomotor function. On the day prior to injury, mice (n = 10–15 mice per group) underwent one training trial at an accelerating rotational speed (4–40 rpm) for at least 200 seconds and then three additional test trials with the same accelerating rotational speed. The average time to fall from the rotating cylinder in the test trials was recorded as baseline latency. Mice were tested on consecutive days post-injury and received three consecutive daily trials with accelerating rotational speed

(inter-trial interval = 15 minutes; n = 10–15 mice). The average latency to fall from the rod was recorded. Mice unable to grasp the rotating rod were given a latency value of 0 seconds.

Pharmacokinetic methods: An *in vivo* assessment of the pharmacokinetics of CN-105 following IV or intraperitoneal IP administration in healthy fed adult male CD-1 mice was conducted by Charles River (Worcester, MA). Briefly, CN-105 was aseptically resuspended in 0.9% sodium chloride and filtered through a 0.22-micron PVDF syringe filter prior to IV or IP dosing. Terminal blood was collected at 0.083, 0.25, 0.5, 1, 2, 4, and 8 hours following IV or IP administration (n=3 per dose group) in K_2EDTA tubes and stored on wet ice until processed to plasma by centrifugation at 3500 rpm for 10 min at 5^0C within 30 min of collection. Plasma was transferred to fresh tubes containing HALT protease inhibitor cocktail (ThermoFisher Scientific) at a final concentration of 1%, vortexed, frozen and stored at -80^0C. Plasma CN-105 levels were determined by liquid chromatography (Ajilent 1290)-tandem mass spectrometry (API6500+). The analytical range was 5 ng/ml to 5000 ng/ml (R2 = 0.995). Pharmacokinetic parameters were determined by non-linear compartmental analysis using WinNonlin (Certara, Princeton, NJ).

Results:

We first designed a series of experiments to assess whether CN-105 could reduce functional deficits as defined by Rotorod latency when administered intravenously prior to injury. Previously we demonstrated neuroprotection when CN-105 was administered post-treatment, and this was used as a positive control.[17] At 10 and 20 minutes prior to injury, IV administration of 0.05 mg/kg CN-105 was associated with durable motor improvements in TBI-associated functional deficits as compared to administration of vehicle control (Fig. 1A). In an independent study, functional benefit was also observed when CN-105 was administered 30 minutes prior to injury, although there was no benefit when administration occurred 60 minutes prior to injury (Fig. 1B). These data indicate that prophylactic dosing of CN-105 may be effective in improving functional outcomes when administered prior to traumatic brain injury. However, the short half-life associated with intravenous dosing in a murine model may limit the prophylactic window required to achieve therapeutic tissue concentrations at the time of injury.

To address this limitation, we conducted a series of pharmacokinetic experiments to explore the possibility that intraperitoneal injection of drug would result in higher sustained tissue concentrations. **Figure 2** shows the mean CN-105 plasma concentrations as a function of time in uninjured CD-1 mice following IV or IP administration of 1 mg/kg CN-105 in 0.9% sterile sodium chloride. Following IV infusion, CN-105 plasma concentration declined in a polyphasic manner. Following IP administration, a short distribution phase was seen followed by a polyphasic reduction in CN-105 plasma concentration that closely mirrored that seen following IV infusion. Individual plasma concentration versus time profiles of CN-105 following IV versus IP administration were analyzed by non-compartmental analysis to determine the PK parameters (Table 1). The bioavailability of CN-105 after IP dosing was 93%. Although the CN-105 terminal elimination half-life ($T_{1/2}$) was similar following IP versus IV

administration (T1,2's ~ 1.1 – 1.3 hours), the Tmax was ~ 3-fold longer, and the mean residence time was ~ 1.5-fold longer following IP administration.

Based on these results, we modified the dosing paradigm to include co-administration of CN-105 by IP (0.5 mg/kg) as well as IV (0.1 mg/kg) dosing at 3 or 6 hours prior to injury. As shown in **Figure 3**, this dosing regimen was associated with an improved trajectory of vestibulomotor recovery that was durable when longitudinal testing was performed out to 28 days following injury.

Discussion:

Traumatic brain injury is a heterogenous disorder that is the result of both primary and secondary injury mechanisms. It can vary in severity and is frequently associated with high morbidity in all forms that negatively impacts the health and readiness of individual soldiers. As a result, considerable attention has been given to understanding the complex array of secondary injury mechanisms in order to develop neuroprotective therapies. Although many interventions have been evaluated for this purpose, over 30 phase III prospective clinical trials evaluating various therapies have failed to reach their primary endpoints.[21,22,23] Furthermore, many of these therapies have been evaluated as post-injury interventions. While this may serve civilian TBI patients, where approximately 60-75% of patients present for medical evaluation shortly after injury[24,25] evaluation and documentation rates following acute TBI, particularly mild TBI, has historically been low.[26] A number of factors unique to military service have been identified as barriers to acute evaluation and care, including various in-theater assessment barriers, exposure to other, concurrent injuries that are often more severe, symptoms that are common to various other diagnoses or attributed to the stresses of military service, and co-existing mental health comorbidities. This is further complicated by a history of underreporting amongst military personal for a disease process that continues to rely on accurate reporting of history and clinical symptoms to make a diagnosis. Suggested hypotheses to explain this underreporting have included concerns amongst affected soldiers that reporting symptoms may result in removal from their units, colleagues and responsibilities, fear of delays return to home and family following deployment, beliefs that existing symptoms are minor and will resolve on their own, fear of stigmatization and a reduced ability to recognize TBI symptoms until return to less structured garrison and civilian lifestyles (Milliken, Brenner, French).[27,28,29] In the current study, we demonstrate that CN-105 improves functional outcomes following TBI when administered prior to injury. These findings, along with its ease of administration and safety profile, make it an attractive prospect for providing a degree of neuroprotection to soldiers who are deemed by military leadership and military healthcare providers to be at high risk for TBI.

The development of apoE mimetic neuroprotective therapies was based on initial observations demonstrating that endogenous apolipoprotein E (apoE) played an adaptive role following brain injury by reducing neuroinflammation and secondary neuronal injury.[30,31,32] Although the intact apoE lipoprotein is too large to cross the blood brain barrier and thus cannot serve as a viable therapeutic[33], apoE peptides can be created from the apoE receptor binding region, which is believed to mediate its anti-

inflammatory and neuroprotective effects via interactions with the glial LRP-1 receptor.[34,35] Importantly, there is convergent evidence that apoE-based neuroprotectants improve outcomes in a variety of preclinical animal models that recreate the different features of TBI pathology, including closed head injury, parenchymal and subarachnoid hemorrhage, and ischemia (Table 2).

CN-105 was developed to optimize potency and CNS penetration by linearizing the polar surface of the helical receptor binding region of apoE. CN-105 has been demonstrated to reduce glial activation in vitro, and in vivo and to improve histological and functional outcomes in a number of preclinical models of brain injury. Moreover, CN-105 is stable and can be stored in lyophilized form or in solution. Importantly, phase 1 single and multiple dose escalation studies have demonstrated linear and predictable pharmacokinetic profile, and a favorable toxicity profile both in the Phase 1 and ongoing Phase 2 trials.[20] Our current observations demonstrating prophylactic efficacy of CN-105 reducing vestibulomotor deficit was likely a function of adequate blood levels at the time of injury. Of note, the measured half-life in humans ~3.5 hours, is considerably longer than in rodent models (<1.5 hours) (Table 1 and Ref. 20) and increases the feasibility of prophylactic administration in the military setting.

Although CN-105 represents an excellent candidate for clinical translation in the setting of traumatic brain injury, there are several potential limitations, which should be addressed. As a peptide, CN-105 has minimal oral bioavailability, and current clinical trials utilize intravenous administration.[16,17,18,20] Although this does not represent a challenge for administration following injury, it would not be optimal for repeated prophylactic administration. To this end, we are exploring minimally or noninvasive routes of delivery, such as intranasal, subcutaneous, or transdermal administration. The mechanism(s) by which CN-105 exerts its neuroprotective effects remains incompletely defined, although convergent data suggests that both apoE and the apoE mimetic peptides exert direct neuroprotective and anti-inflammatory effects via interaction with the LRP-1 receptor, which is present on neurons and glial.[34,35] A better understanding of the physicochemical nature of this interaction may allow the rational development of small molecule therapies. Finally, although we demonstrate as proof of principle that prophylactic administration of CN-105 improves recovery and functional outcome after TBI, as long as adequate blood/tissue concentrations are achieved, intraperitoneal administration is not feasible in the clinical setting. Moreover, rodent models are not ideal for studies of human clinical pharmacokinetics or disease intervention.

Conclusions:

Our results demonstrate that administration of CN-105 prior to an induced murine closed head injury produced a durable improvement in vestibulomotor function. These findings suggest that based on the longer half-life of the drug observed in humans, CN-105 may be an effective prophylactic strategy for improving functional outcomes in soldiers at high risk for head injury in both training and combat environments.

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Figure Legends

Figure 1. (A) A single intravenous administration of CN-105 (0.1 mg/kg) improved vestibulomotor function as assessed by Rotarod when administered 10 min (A), 20 min (A) or 30 min (B), but not 60 min (B) before the induction of TBI.

Figure 2. Mean CN-105 plasma concentration as a function of time following an intravenous (IV) or intraperitoneal (IP) injection of 1 mg/ml CN-105 in sterile saline. The data represent the mean ± the standard error of 3 animals per time point per dose group.

Figure 3. Co-administration of CN-105 by intravenous (0.1 mg/kg) and intraperitoneal injection (0.5 mg/kg) for 3 or 6 hours before the induction of TBI resulted in a durable improvement of vestibulomotor function up to 28 days after injury as assessed by Rotarod.

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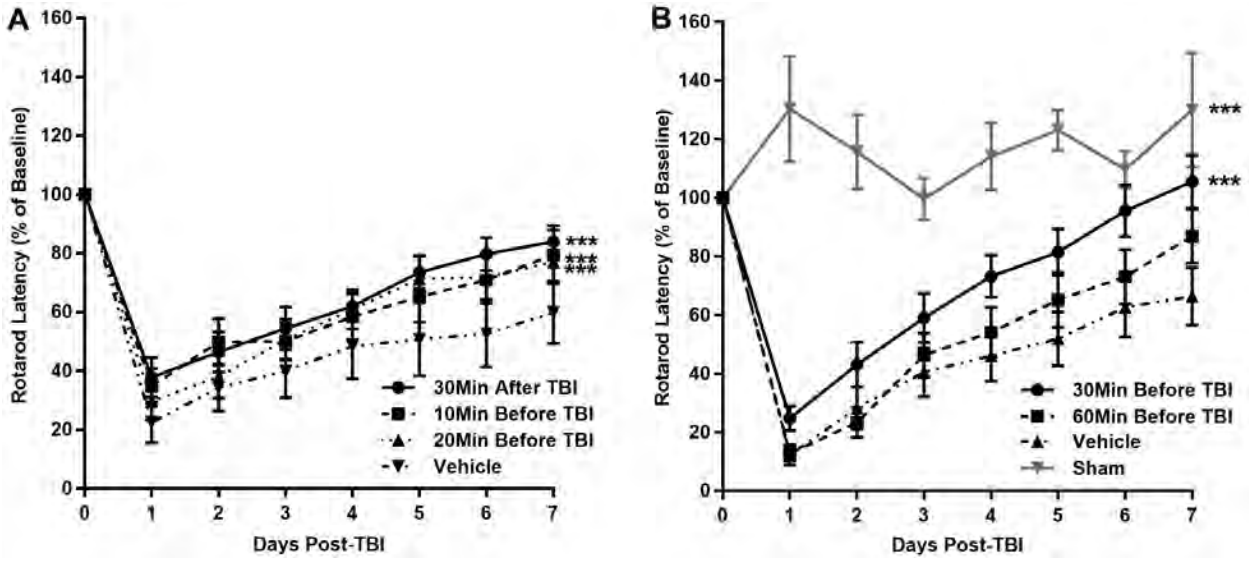

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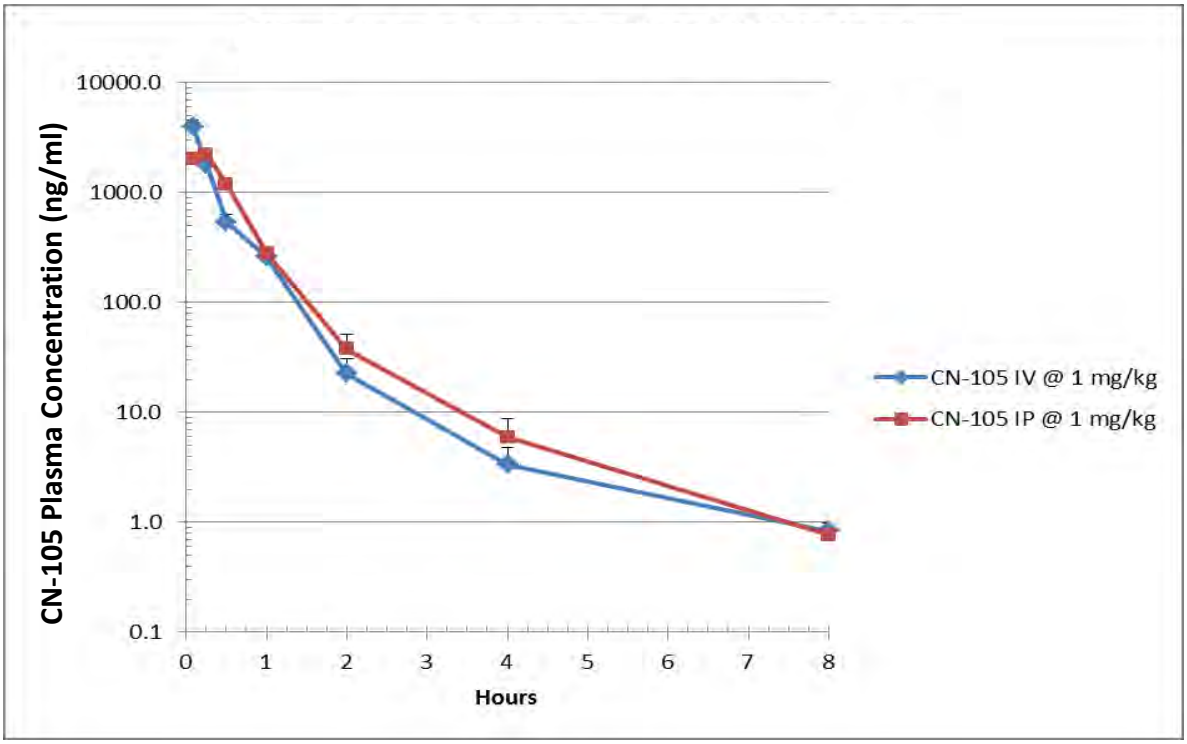

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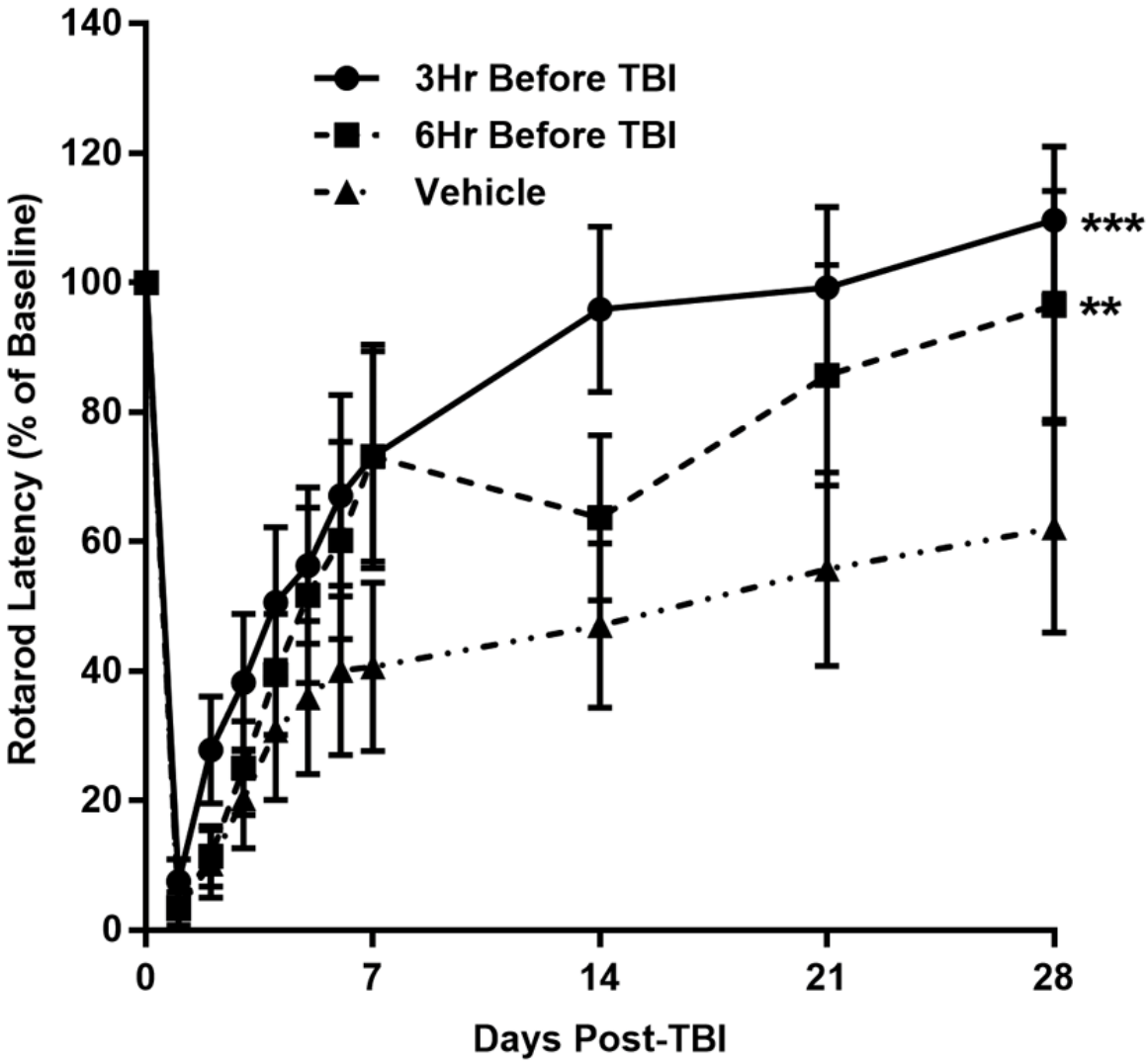

Table 1. CN-105 Pharmacokinetic Parameters Following Intravenous (IP) Versus Intraperitoneal (IP) Administration in CD-1 Mice

PK Parameter	IV	IP	Units
T_{max}	0.083	0.25	Hr
C_{max}	3997	2230	ng/ml
$AUC_{0\text{-}inf}$	1568	1455	ng.hr/ml
$T_{1/2}$	1.33	1.10	Hr
CL	662	830	ml/hr.kg
MRT	0.35	0.53	Hr
$V_{z,obs}$	1270	1164	ml/kg
F	100	93	%

Table 2. Therapeutic effects of apoE-mimetic peptides in a variety of preclinical CNS disease models.

Injury	Species	Histological/biochemical outcome measures	Functional outcome measures	References
Stroke-MCAO	Rat	Reduction in infact volume (35 days)	Improved vestibulomotor function, locomotor function	41
Stroke-MCAO	Mouse	Reduced microglial activation, infarct volume, improved survival	Improved vestibulomotor function	18
Hypoxia-Ischemia	Rat	Less tissue loss	Reduced mortality	42
Hypoxia-Ischemia	Mouse	Reduced infarct volume and radiographic progression of infarct	Improved vestibulomotor function	43
ICH (collagenase injection)	Mouse	Reduction in inflammatory cytokines, cerebral edema	Improved vestibulomotor function	44
ICH (collagenase injection)	Mouse	Reduction in microgliosis, edema, neuronal injury, inflammatory signaling (p38; NFKB)	Improved vestibulomotor function and memory	45
ICH	Mouse	Brain water content reduced, neuroinflammation was decreased, neuronal survival was increased, hemorrhage volume was not affected	Durable improvement in vestibulomotor and cognitive function	19
SAH	Mouse	Reduction in vasospasm, edema, mortality	Improved functional exam, vestibulomotor function	36
SAH	Mouse	Reduction in vasospasm	Improved vestibulomotor function	46
SAH	Mouse	Reduced microgliosis and apoptosis, enhanced Akt activation and suppressed caspase-3 cleavage, attenuated cytokine	Alleviated neurological deficits	47

		production (IL-1β, IL-6, TNFα)		
SAH	Mouse	Reversed blood-brain barrier disruption, reduced brain edema and neuron apoptosis, increased cerebral glucose uptake, inhibited activation of MMP-9	Improved neurological functions	48
SAH	Mouse	Suppressed JAK/STAT3 signaling, reduced M1 microglia activation	Attenuation of oxidative stress and inflammation	49
TBI (closed head injury)	Mouse	Reduction in oxidative stress (aconitase), neuronal degeneration, TNFα mRNA	Improved vestibulomotor function and memory	50
TBI (closed head injury)	Mouse	Reduction in degenerating neurons, microgliosis	Improved vestibulomotor function and memory	51
TBI (cortical contusion)	Rat	Smaller lesion volume, reduction in astrocytosis	Improved motor outcomes	52
TBI (closed head injury)	Mouse	Reduction in degenerating neurons, microgliosis, TNFα, Aβ	Improved vestibulomotor function	53
TBI (cortical contusion)	Rat	Reduction in degenerating neuons	Improved sensorimotor function, reference and working memory	54
TBI (closed head injury)	Mouse	Suppressed activation of MMP-9, reduced breakdown of blood-brain barrier, reduced TBI lesion volume and vasogenic edema	Decreased functional deficits compared with saline-treated TBI animals	55
TBI (closed head injury)	Mouse	Reduction in neuronal degeneration, microgliosis, reduced expression of a subset of inflammatory and immune-related genes	Improved vestibulomotor function, improved memory	17

TBI (closed head injury)	Mouse	Improved glucose uptake, reduced VEGF expression, edema, and neuronal degeneration	Improved vestibulomotor function	56
Spinal cord injury	Mouse	Reduced spinal cord demyelination induced by focal lysolecithin injection, increased axon survival, increased evoked potentials	Improved locomotor function	57
Spinal cord injury	Rat	Reduced microgliosis, reduced lesion size, reduced neuron degeneration	Ameliorated motor deficit 14 days post-injury	58
Spinal cord injury	Mouse	Partially restored spinal cord blood-brain barrier function, increased white matter spared from the injury, reduced inflammatory influx of leukocytes to site of injury	Improved locomotor function	59