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Pre-Clinical Models of Penetrating Brain Injury: Study Protocol for a Systematic Review

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OBJECTIVES/GOALS: Penetrating brain injury (PBI) differs both physiologically and in clinical outcomes when compared to blunt-force traumatic brain injury (TBI). Despite this, there are few pre-clinical models of PBI described in the literature. To address this gap, we will develop a study protocol for a systematic review. METHODS/STUDY POPULATION: Three electronic databases (PubMed, Embase, Web of Science) will be searched using keywords and controlled vocabulary related to animal models, computational models, simulations, and disease key words including traumatic brain injury and penetrating brain injury. The primary outcome will be the method of PBI modeling. Secondary outcomes will be related to bibliographic information, computational analysis, and histochemical, radiographic, behavioral, and human clinical biomarkers and outcome measures used in PBI models. A panel of independent investigators will review publications resulting from this search strategy to identify relevant studies. The protocol will adhere to PRISMA-P guidelines. RESULTS/ANTICIPATED RESULTS: Eligible studies will include both exploratory and descriptive research, and both quantitative and qualitative data. A summary of selected studies will be presented, and the synthesis will follow a narrative framework. DISCUSSION/SIGNIFICANCE: This protocol provides a framework for comprehensively evaluating pre-clinical PBI models with focus on methodology. PBI is a phenotypically unique disease and is under studied. This protocol will be of great benefit to clinicians and scientists in this emerging field and can help monitor future progress in translational research.

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The brain under stress: How a history of unpredictable shock affects neural processing of future stressors

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OBJECTIVES/GOALS: Determine how a history of unpredictable foot shock in mice affects brain wide patterns of neural activation to future stressors. Additionally, we aimed to characterize how the paraventricular nucleus of the thalamus (PVT) is involved in the fear sensitization process. METHODS/STUDY POPULATION: We used a mouse model of stress enhanced fear learning, where stressed mice are first subjected to a series of unpredictable foot shocks in a novel context while control mice undergo exposure to the novel context without experiencing foot shock. Mice are then left undisturbed for 28 days, following which they are exposed to a single foot shock in a novel context. Mice are tested in the second context 24 hours after single shock, and the amount of time spent frozen in the context provides a measure of fear sensitization. Whole brain patterns of activation during the second context test will be assessed via whole brain optical clearing with antibody staining of immediate early genes. The role of the PVT in fear sensitization will be characterized using chemogenetic approaches. RESULTS/ANTICIPATED RESULTS: Our preliminary results demonstrate that mice display enhanced fear acquisition long after the initial experience of unpredictable shocks. We anticipate to identify regions previously implicated in fear learning and novel regions not previously described through our brain clearing approach. In addition, we anticipate chemogenetic inhibition of the PVT will reduce freezing to an auditory cue associated with the shock in the second context but not to the context itself. DISCUSSION/SIGNIFICANCE: Our findings will provide a comprehensive view of how a history of unpredictable stress affects whole brain processing of subsequent stressful experiences, and describe the role of the PVT in cued fear sensitization.

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Operationalization of a Translational Ethics Program

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OBJECTIVES/GOALS: Within our CTSA hub, greater emphasis is on understanding innovation and human health impact of translational science writ large rather than focus on translational research projects. Our program is restructured to reflect distinguishing ethical features of translational science which are complimentary to traditional research ethics issues. METHODS/STUDY POPULATION: This descriptive analysis depicts the development of our ethics program as an exemplar of how to integrate into the research enterprise of an academic health science center that engages in translational research. Our relational approach is predicated on the embodiment of ethical values by all who are involved in research committed to proactive dialog, team building, and collaboration. This translational research culture is facilitated by a multidisciplinary ethics team who are embedded throughout the translational research enterprise. RESULTS/ANTICIPATED RESULTS: Our program is integrated into a translational science enterprise within a CTSA hub in four areas: relational structure (from leadership team to community engagement), education (from trainees to the research community to the public), support (through ethics consultation with multiple touchpoints in the translational science pathway), and team science (from team on-boarding and communication to D&I research of team science interventions). We have developed a research agenda examining research ethics topics that increase quality, applicability and downstream social impact of research; understanding translational science through historical and science & technologies studies lenses, and ethnographic and mixed-method approaches to understanding team science and the science of team science. DISCUSSION/SIGNIFICANCE: The integration of a translational ethics program provides attention to traditional research ethics issues regarding study conduct and integrity but also transcends those concerns to focus on the translational science enterprise itself through relationships, cultivating trust, team science, DEIA, and social responsibility.

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Pathophysiology of voluntary motor commands in patients with multiple sclerosis identified using reverse engineering of motor unit population discharge.

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OBJECTIVES/GOALS: Our objective is to characterize excitatory, inhibitory, and neuromodulatory components of the voluntary

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motor command at the level of the spinal motoneuron in people with multiple sclerosis (MS). This information will provide insight into neural mechanisms of motor dysfunction and their heterogeneity among patients with MS. METHODS/STUDY POPULATION: Due to advances in high-density surface EMG (HDsEMG) decomposition and the recent development of a paradigm for reverse engineering of motor unit population discharge, we can feasibly estimate aspects of excitatory, inhibitory, and neuromodulatory components of the voluntary motor command in humans on a person-specific basis. We tested 11 ambulatory patients with MS and mild-moderate disability. We recorded HDsEMG from tibialis anterior (TA) and soleus (SOL) during isometric plantarflexion and dorsiflexion, performed as slow triangle contractions. EMG was decomposed into motor unit spike trains using blind source separation. We calculated a number of motor unit variables, most notably delta-F, which estimates motoneuron excitability and the balance of neuromodulatory and inhibitory inputs. RESULTS/ANTICIPATED RESULTS: There were consistent differences in MS patients vs. controls. For TA, values were decreased for delta-F (3.9 vs. 5.9 pps), initial firing rate acceleration (5.8 vs. 7.1 pps), firing rate range (9.3 vs. 11.9 pps), and max firing rate (12.3 vs. 15.0 pps). SOL had more modest decreases in delta-F (3.0 vs. 3.8 pps) and firing rate range (4.8 vs. 5.6 pps). Self-sustained firing was longer for MS patients. Within a patient, abnormalities in motor unit variables were not consistent across muscles and legs. Interestingly, there were several abnormalities in the patients with a normal clinical motor exam, indicating that perhaps our measures are sensitive to subclinical changes in processing of voluntary motor commands. DISCUSSION/SIGNIFICANCE: Excitatory, inhibitory, and neuromodulatory components of the voluntary motor command must be appropriately balanced for skilled motor output. This study is the first to characterize how they are disrupted in MS, providing foundational information to inform the development of mechanistically-based rehabilitation interventions.

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Hydroxypropyl beta cyclodextrin barrier prevents respiratory and eye viral infections

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OBJECTIVES/GOALS: Susceptible mucocutaneous membranes of the eye and nasal cavity are easily infected by viruses leading to pink eye or respiratory infections whose direct cost has been estimated as $16 billion annually in the United States. We have developed a novel and effective barrier that will be agnostic to variants enveloped viruses like coronaviruses. METHODS/STUDY POPULATION: We evaluated the efficacy of hydroxypropyl cyclodextrin barrier in preventing respiratory coronavirus infections using 25 humanized angiotensin converting enzyme-2 receptor (hACE-2) mice under a BSL3 laboratory setting. We have shown the barrier is safe and efficacious in preventing coronavirus infections in in vitro respiratory cell lines. We instilled 10 uL aliquot of the barrier into the nostril of the mouse 30 minutes before exposing them to a 10uL titer containing 10,000 plaque forming units of the SARS-CoV-2 delta variant. The control mice received the SARS-CoV-2 infection but not the barrier. The mice were observed for 5 days after which they were sacrificed. We analyzed the lungs and nasal palates for viral load using reverse transcription-polymerase chain reaction. RESULTS/ANTICIPATED RESULTS: We observed our barrier to be effective in preventing SARS-CoV-2 delta variant infection in hACE2 mice models. The lungs and nasal secretions of treated mice were less infectious with lower viral load than the control mice. The lungs of treated mice showed decrease in IFN gene expression and many cytokines and chemokines that regulate virally induced inflammatory responses such as IL-1b, IL-8, CXCL9, CXCL10, and the CCLs. We observed the plasma Angiotensin I and Angiotensin II decreased with barrier treatment, correlating with the viral load observed in the lungs. These peptides may be useful biomarkers for monitoring viral load within the lungs of virally infected individuals. DISCUSSION/SIGNIFICANCE: This supports the barrier's efficacy to reduce transmission and prevent infections of SARS-CoV-2. This easy to use barrier can augment the mucocutaneous layers of the eye and nasal cavity. Our agnostic barrier will reduce the economic and public health burden of seasonal respiratory and eye viral infections and their related deaths amongst the public.

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Characterizing the single-cell transcriptomes of fetal natural killer cells isolated from the umbilical cord of fetuses exposed to human cytomegalovirus during gestation†

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OBJECTIVES/GOALS: Congenital cytomegalovirus (cCMV) remains to be the leading infectious cause of fetal anomalies. The role of fetal natural killer (NK) cells during cCMV remains largely unknown. The objective of this study is to define the transcriptomes of fetal NK cells exposed to human cytomegalovirus (HCMV) infection during gestation. METHODS/STUDY POPULATION: Four sets of umbilical cord blood and matching umbilical cord tissues were collected from two HCMV seropositive (HCMV+) and two HCMV seronegative (HCMV-) fetuses that did not experience any complications during gestation. These samples were provided by the Medical College of Wisconsin Tissue Bank and were processed within 24 hours following live birth. CD7+ CD3e-CD14-CD19-CD20- fetal NK cells were isolated, using the BD FACSAria sorter. Following cell sorting, single-cell RNA sequencing (scRNA-seq) was performed, and cDNA libraries were constructed and sequenced via NextSeq 550. Cell Ranger was then used to algin the cDNA reads and the Seurat R package was used to analyze the transcriptional data. Cells were filtered and clustered based on the number of uniquely expressed genes. RESULTS/ANTICIPATED RESULTS: Four sets of umbilical cord blood and matching umbilical cord tissues were collected from two HCMV+ and two HCMV- fetuses. We were able to successfully sort and capture fetal NK cells and perform

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