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COMBINED CRISPR/CAS9 AND AAV FOR THE GENERATION OF CONDITIONAL ISOGENIC GENE KNOCK-INS

Prajwal Boddu, Abhishek Gupta, Todd Waldman and Manoj Pillai
Translational Science, Policy, & Health Outcomes Science

ABSTRACT IMPACT: We describe a novel methodology that combines CRISPR/Cas9-induced double stranded DNA breaks with homology dependent repair from an adeno associated virus (AAV) encoded template to generate single-allele edited isogenic cell line models of cancer-associated mutations with high efficiency. OBJECTIVES/GOALS: Conventional approaches to creating isogenic knock-ins, to model disease-associated mutations, are limited by poor efficiency and loss of the mutant allele on extended culture. We present an optimized editing approach combining CRISPR/Cas9 with Adeno-associated virus, using mutant SF3B1 as a prototype. METHODS/STUDY POPULATION: Left and right homology arms for SF3B1 were PCR amplified and cloned into pAAV-SEPT-Acceptor plasmid (containing a chimeric intron, neomycin resistance cassette and polyA tail). The disease-associated K700E mutation was introduced by site-directed mutagenesis. Single guide RNA (sgRNA) complexed with recombinant Cas9 along with the AAV donor were delivered into K562 cells, G418 resistant clones selected, and screened for integration by PCR. Confirmed clones were then transduced with a doxycycline-inducible Cre-recombinase containing lentiviral vector. Inducible expression of Cre-recombinase and expression of the mutant allele were confirmed by Western blot and Sanger sequencing respectively. RESULTS/ANTICIPATED RESULTS: Targeted-integration efficiencies among the Neo-resistant clones, generated by AAV-alone and AAV+CRISPR/Cas9, were 16% and 94%, respectively. Single cell cloning after Cre-mediated excision of loxp was unsuccessful presumably due to toxicity of the K700E mutation. To overcome this limitation, clones were transduced with doxycycline-inducible Cre-recombinase lentiviral vector. Doxycycline induction of Cre-recombinase resulted in reliable excision of the loxp cassette and expression of mutant allele at about 50% variable allele frequency (as determined by Sanger sequencing). The approach was validated in additional cell lines and for introduction of N-terminal FLAG tag for SF3B1. DISCUSSION/SIGNIFICANCE OF FINDINGS: Combining AAV and CRISPR/Cas9 can generate scalable single-dominant allele mutants with high precision and efficiency compared to AAV or CRISPR alone. Together with inducible Cre-recombinase, our approach can generate isogenic models where the mutation confers a growth disadvantage.

67553

Exoskeleton dynamics alter upper-limb coordination in a virtual reality reaching task

Alexander Brunfeldt[1], Alexander Dromerick[1], Barbara Bregman[1] and Peter Lum[2]
[1]Georgetown University and [2]The Catholic University of America

ABSTRACT IMPACT: This proof-of-concept study demonstrates that systematically altering limb dynamics with two exoskeleton devices alters ingrained, bilateral upper-limb coordination with the potential to rehabilitate functional reaching in chronic stroke survivors OBJECTIVES/GOALS: Advances in virtual reality and exoskeleton technologies have allowed researchers to alter upper limb coordination with more precision than ever before. The goal of this study was to systematically enhance the use of the nondominant limb during a bimanual reaching task, with an eye towards improving rehabilitative strategies post stroke. METHODS/STUDY POPULATION: Healthy, right-handed volunteers performed a bimanual reaching task in virtual reality (VR) space while simultaneously moving under the influence of two exoskeleton devices. The VR task had participants move a shared cursor, displayed at the midpoint between the hands, to targets arranged at shoulder and eye levels and located at 70% of full arm extension. Two exoskeleton devices applied either resistive torque to the dominant limb or assistive torque to the non-dominant limb. Three-dimensional hand position data were recorded at 50 Hz and analyzed offline. The primary outcome measure was relative contribution, calculated as the ratio of dominant/non-dominant displacement. RESULTS/ANTICIPATED RESULTS: Preliminary results from 3 participants showed that during baseline trials, when no torque was applied by the exoskeletons, relative contribution was 50.6% in favor of the dominant hand, with the dominant hand reaching on average 1.1cm farther than the left. When the exoskeletons resisted movement in the dominant limb while simultaneously assisting movement in the non-dominant limb, relative contribution was 49.7% indicating an increase in non-dominant limb usage. Further analysis showed that this effect was driven by one participant who reached 3.7cm farther with her non-dominant hand compared to baseline. DISCUSSION/SIGNIFICANCE OF FINDINGS: These pilot data suggest our testing platform is capable of altering normal coordination patterns and is likely the result of participants adopting an optimal control strategy imposed by the shared cursor. These findings will form the basis for a rehabilitation intervention to promote the use of the paretic limb in chronic stroke survivors.

68415

Neural Impact of Neighborhood Disadvantage in Traumatically-Injured Adults: a Multi-Modal Investigation

E. Kate Webb[1], Carissa Weis[1], Ken Bennett[2], Ashley Huggins[1], Elizabeth Parisi[1], Jessica Krukowski[3], Terri deRoon-Cassini[4] and Christine Larson[1]
[1]University of Wisconsin-Milwaukee, [2]Montana VA Healthcare System, [3]Marquette University and [4]Medical College of Wisconsin

ABSTRACT IMPACT: Neighborhood disadvantage was significantly associated with brain structure and function in trauma-exposed adults, providing evidence that contextual factors should be assessed in mental health research, particularly in high-risk populations. OBJECTIVES/GOALS: Over 13 percent of Americans live in a socioeconomically disadvantaged neighborhood. Previous work has linked lower individual socioeconomic position to alterations in brain structure and function. However, the neural effects of area-level socioeconomic factors, such as neighborhood disadvantage, are unclear. METHODS/STUDY POPULATION: We recruited two-hundred and fifteen traumatically-injured participants from an Emergency Department in southeastern Wisconsin. An Area Deprivation Index (ADI) score, a national measure of neighborhood socioeconomic disadvantage, was derived from each participant's home address. Two-weeks post-trauma, participants underwent a battery of self-report measures and functional magnetic resonance imaging (fMRI) scans. Using a multi-modal approach, we investigated the impact of ADI on brain structure as well as neural activation during rest and during an emotional uncertainty task. We sought to disentangle the relationship between neighborhood and individual socioeconomic position and neural activity in the context

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of trauma. RESULTS/ANTICIPATED RESULTS: We demonstrated that neighborhood disadvantage is associated with decreased volume and alterations of resting state functional connectivity of structures implicated in affect processing, including the hippocampus, amygdala, and ventromedial prefrontal cortex. These results held even after controlling for relevant individual variables, including acute post-traumatic stress symptoms and years of education. Moreover, individuals from disadvantaged neighborhoods exhibited heighted activation of these same structures in response to aversive stimuli. Thus, brain regions critical for recognizing and processing negative stimuli are susceptible to the effects of area-level socioeconomic factors. DISCUSSION/SIGNIFICANCE OF FINDINGS: The results offer additional evidence that neurobiological mechanisms clarify how stress 'gets under the skin'. Changes to key brain regions may explain why those living in disadvantaged neighborhoods are at a heighted risk of PTSD. Broadly, these findings should inform future policies and community-driven interventions aimed at reducing poverty.

77822

PSD95-nNOS interaction alters the basolateral amygdala transcriptome following fear conditioning: implications for molecular mechanisms underlying PTSD

Jheel Patel, Melissa Haulcomb, Liangping Li, Guanglong Jiang, Erik Dustrude, Yunlong Liu, Yvonne Lai, Andrei Molosh and Anantha Shekhar
Indiana University School of Medicine

ABSTRACT IMPACT: This research takes a transcriptomic approach to parse genes and molecular pathways that underlie the fear memory circuitry and, in doing so, identifies therapeutic targets that can further be developed into treatments for fear disorders, such as post-traumatic stress disorder. OBJECTIVES/GOALS: Normal fear learning produces avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Our goal is to understand the mechanism and identify novel genetic targets underlying fear responses. METHODS/STUDY POPULATION: Involvement of the basolateral amygdala (BLA) in fear acquisition is well established and requires activation of N-methyl-D-aspartic acid receptors (NMDARs). At a cellular level, NMDAR activation leads to production of nitric oxide (NO) by a process mediated by interaction between postsynaptic density protein 95 (PSD95) and neuronal nitric oxide synthase (nNOS). To elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, here we use rodent behavioral paradigms, pharmacological treatment with a small molecule PSD95-nNOS inhibitor, RNA-sequencing, and an AAV-mediated knockdown of the nNOS gene in the BLA. RESULTS/ANTICIPATED RESULTS: We show that treatment with ZL006 attenuates rodent cued-fear consolidation. Additionally, with RNA-sequencing, expression of 516 genes was altered in the BLA following fear expression; of these genes, 83 were restored with systemic ZL006 treatment. Network data and gene ontology enrichment analyses further revealed that cGMP effects, insulin-like growth factor binding, and cognition-related pathways were significantly altered. Finally, we show that a BLA-specific knockdown of nNOS attenuates cued fear consolidation, without adverse effects on other memory and motor behaviors. DISCUSSION/SIGNIFICANCE OF FINDINGS: Via a model of NMDAR-mediated fear consolidation, our results reveal novel pathways and genetic targets that underlie plasticity of fear memory circuitry. Importantly, these results will inform future therapeutic strategies for targeting fear related disorders like PTSD.

96101

Temporal Evolution of Neural Activity in Human Brain Organoids

Kobina G. Mensah-Brown, James Lim, Dennis Jgamadze, Guo-li Ming, Hongjun Song, John A. Wolf and Han-Chiao I. Chen
University of Pennsylvania

ABSTRACT IMPACT: This study will provide the essential characterization of intrinsic neural activity in human brain organoids, both at the single cell and network levels, to harness for translational purposes. OBJECTIVES/GOALS: Brain organoids are 3D, stem cell-derived neural tissues that recapitulate neurodevelopment. However, to levy their full translational potential, a deeper understanding of their intrinsic neural activity is essential. Here, we present our preliminary analysis of maturing neural activity in human forebrain organoids. METHODS/STUDY POPULATION: Forebrain organoids were generated from human iPSC lines derived from healthy volunteers. Linear microelectrode probes were employed to record spontaneous electrical activity from day 77, 100, and 130 organoids. Single unit recordings were collected during hour-long recordings, involving baseline recordings followed by glutamatergic blockade. Subsequently, tetrodotoxin, was used to abolish action potential firing. Single units were identified via spike sorting, and the spatiotemporal evolution of baseline neural properties and network dynamics was characterized. RESULTS/ANTICIPATED RESULTS: Nine organoids were recorded successfully (n=3 per timepoint). A significant difference in number of units was seen across age groups ($F(2,6) = 6.4178$, $p = 0.0323$). Post hoc comparisons by the Tukey HSD test showed significantly more units in day 130 (51.67 ±14.15) than day 77 (16.33 ±14.98) organoids. Mean firing rates were significantly different in organoids based on age, with drug condition also trending toward significance ($F(6,12) = 9.97$; $p = 0.0028$ and $p = 0.08$ respectively). Post hoc comparisons showed a higher baseline firing rate in day 130 (0.99Hz ±0.30) organoids than their day 77 counterparts at baseline (0.31Hz ±0.066) and glutamate blockade (0.31Hz ±0.045). Preliminary network analysis showed no modularity or small-world features; however, these features are expected to emerge as organoids mature. DISCUSSION/SIGNIFICANCE OF FINDINGS: Initial analysis of brain organoid activity demonstrates changes in single unit properties as they mature. Additional work in this area, as well as further network analyses, will confer better sense of how to rationally utilize brain organoids for translational purposes.

Clinical Epidemiology

Basic Science

60941

Vaginal pH predicts cervical intraepithelial neoplasia-2 regression in women living with human immunodeficiency virus

Katherine Michel[1], Christine Colie[1], Robert D. Burk[2], L. Stewart Massad[3], Cuiwei Wang[1], Michaeline Hebron[1], Charbel Moussa[1], Gypsyamber D'Souza[4], Lisa Rahangdale[5], Lisa Flowers[6], Joel Milam[7], Joel M. Palefsky[8], Howard Minkoff[9], Howard D. Strickler[2] and Seble G. Kassaye[1]
[1]Georgetown University, [2]Albert Einstein College of Medicine, [3]Washington University in St. Louis, [4]Johns Hopkins University, [5]University of North Carolina School of Medicine, [6]Emory University, [7]University of California Irvine, [8]University of California San Francisco and [9]Maimonides Medical Center

ABSTRACT IMPACT: The potential to use vaginal pH as a low cost, non-invasive diagnostic test at the point of CIN2 diagnosis to predict

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