POPULATION: To achieve the objective, we evaluated mice with pancreas lineage Kras-mutation (KC mice), which are predisposed to develop the full spectrum of pancreas cancer precursor lesions (pancreatic intra-epithelial neoplasia or PANIN-1, 2, 3) and PDAC. We subjected KC mice to a light-dark phase shift protocol known to induce circadian disruption (KCCD, n = 18), and another group to standard lighting conditions (KCNC, n = 31), with equal numbers of males and females in each group. The mice were allowed access to food and water ad libitum until sacrifice at age 9 months. Histopathologic evaluation of the pancreas was then performed to assess for pancreatic inflammation, pancreatic precursor lesions (PANIN) and PDAC. Fisher's Exact Test was used to evaluate differences in incidence. RESULTS/ANTICIPATED RESULTS: As expected, both groups of mice demonstrated 100% incidence of chronic pancreatitis and PANIN-1 (low-grade precursor lesion) at age 9 months. This is consistent with the KC phenotype. However, the KCCD mice demonstrated a significant increase in acute pancreatic inflammation (61.1% vs 19.4%, $p = 0.005$) compared to KCNC mice. Furthermore, intermediate grade precursor lesions (PANIN-2) were also significantly increase in the KCCD mice (38.9% vs 6.5%, $p = 0.006$). Incidence of high-grade precursor lesions (PANIN-3, or carcinoma in situ: 22.2% vs 9.7%) and PDAC (27% vs 19%) were also increased, but these were not statistically significant. These results are notable given the established progression from higher grade premalignant PANIN lesions (PANIN-2, PANIN-3) to PDAC. DISCUSSION/SIGNIFICANCE OF FINDINGS: Insight into how circadian disruption leads to increased PANIN-2 formation and increase in acute inflammation may be advantageous for understanding circadian disruption in PDAC carcinogenesis. The circadian clock is present in immune cells and disruption can induce immune dysregulation. This mechanism will be evaluated in follow up studies.

15727

K_{ATP} channel prodrugs as therapeutics for chronic pain and substance abuse disorders

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ABSTRACT IMPACT: Pharmacological activation of K_{ATP} channels may provide analgesia and attenuate opioid tolerance and withdrawal OBJECTIVES/GOALS: Our long term goal is to develop therapeutics for the treatment of the overuse of opioids. The objective of this application is to test novel K_{ATP} channel-targeting prodrugs in rodent models of neuropathic and inflammatory pain in addition to opioid tolerance after chronic morphine administration. METHODS/STUDY POPULATION: In one study, two different measures for chronic pain were implemented in mice. Male and female mice (n=10) were subjected to spinal nerve ligation (SNL) or intraplantar injection of Complete Freund's Adjuvant (CFA) to induce neuropathic and inflammatory pain, respectively. Administration of K_{ATP} channel prodrugs (60ug, it) attenuated mechanical hypersensitivity after SNL or CFA compared to vehicle (saline). In a separate study, changes in mechanical hypersensitivity were tested while mice undergo chronic morphine treatment (15mg/kg, 2x, 5 days) with administration of the prodrugs. Tolerance was measured as the loss of antinociception, and withdrawal is measured ~24 hours after the final morphine injection. RESULTS/ANTICIPATED RESULTS: Intrathecal administration of either K_{ATP} channel prodrugs significantly attenuated mechanical hypersensitivity after SNL and significantly attenuated mechanical hypersensitivity after CFA in mice. We predict that intrathecal administration of these prodrugs will also attenuate morphine tolerance and withdrawal in mice. This hypothesis is based off our previous data indicating non-water soluble K_{ATP} channel agonists produce analgesia and attenuate morphine tolerance in mice. DISCUSSION/SIGNIFICANCE OF FINDINGS: Pharmaceutical strategies to utilize K_{ATP} channels for therapeutics have been hindered due to the low solubility and low ability to cross the neurovascular unit. Newly developed, water-soluble K_{ATP} channel openers could be useful pharmaceutical strategy to reduce chronic pain, opioid tolerance, and withdrawal in human populations.

19233

Basis profile curve identification to understand electrical stimulation effects in human brain networks

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ABSTRACT IMPACT: Brain networks can be explored by delivering brief pulses of electrical current in one area while measuring responses in other areas, and this describes an open-source novel algorithm to carry out this exploration. OBJECTIVES/GOALS: If we focus on a single brain site and observe the average effect of stimulating each of many other brain sites, visually-apparent motifs in the temporal response shape emerge from adjacent stimulation sites. There are no existing approaches to identify and quantify the spatiotemporal structure of these motifs. METHODS/STUDY POPULATION: Individual stimulation trials are correlated with one another, then a correlation-significance matrix quantifying similarity between stimulation sites is decomposed with non-negative matrix factorization, in which the inner dimension is iteratively reduced. The dimensionality reduction identifies stimulation sites that produce a common elicited temporal response, and linear kernel PCA is applied to obtain the robust profile of this response cluster. RESULTS/ANTICIPATED RESULTS: We describe and illustrate a data-driven approach to determine characteristic spatiotemporal structure in these response shapes, summarized by a set of unique 'basis profile curves' (BPCs). Each BPC may be mapped back to underlying anatomy in a natural way, quantifying projection strength from each stimulation site using simple metrics. Our technique is demonstrated for an array of implanted brain surface electrodes in a human patient, and our code is shared at https://purl.stanford.edu/rc201dv0636. DISCUSSION/SIGNIFICANCE OF FINDINGS: This framework enables straightforward interpretation of single-pulse brain stimulation data, and can be applied generically to explore the diverse milieu of interactions that comprise the connectome.

19734

L-type calcium channels in cerebellar neuron development and motor learning

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ABSTRACT IMPACT: We aim to understand how LTCCs impact cerebellar function. OBJECTIVES/GOALS: L-type calcium channels

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(LTCCs) are important in activity-dependent neurite outgrowth, which comprises neurite initiation and elongation. We used cerebellar granule neurons (CGNs) to differentiate between LTCC effects on neurite initiation vs elongation. We also tested cerebellar function in mice lacking specific LTCCs with behavioral assays. METHODS/STUDY POPULATION: CGNs were cultured from 129SvEv mouse pups at P4-P6. Potassum chloride (50mM) was used to stimulate neuronal cultures for 24 hours. Isradipine (20nM) was added to culture medium to inhibit all LTCCs for 1 hour. For Cav1.2 deletion, we crossed Cav1.2 conditional knockout mice (Cav1.2-cKO) to Syn-Cre mice (for deletion in most neurons) or Atoh1-Cre mice (for deletion in CGNs). The Cav1.2-cKO line was maintained on a 129SvEv background. For constitutive Cav1.3 deletion, mice were maintained on a C57BL/6NTac. Behavioral tasks included open field, rotarod, and Erasmus Ladder. Data were analyzed with sexes combined and separated to assess for sex as a biological variable. Studies were analyzed by one-way ANOVA, two-way ANOVA, or generalized linear mixed model, where appropriate. RESULTS/ANTICIPATED RESULTS: CGNs exhibited an increase in neurite initiation but not elongation when stimulated with potassium chloride, consistent with previous reports of activity-dependent neurite outgrowth in this cell type. LTCC inhibition with isradipine blunted KCl-induced neurite initiation. We observed no change in the length of either primary or secondary neurites with isradipine treatment with or without KCl stimulation. In our behavioral experiments, we observed no deficits in open field, rotarod, or Erasmus Ladder when Cav1.2 was deleted in most neurons (driven by Syn-Cre expression) or in cerebellar granule neurons (driven by Atoh1-Cre expression). In contrast, loss of Cav1.3 was associated with impaired motor learning in the rotarod task without evidence of ataxia on Erasmus Ladder. DISCUSSION/SIGNIFICANCE OF FINDINGS: We show a specific role for LTCCs in activity-dependent CGN neurite initiation. While loss of Cav1.2 does not affect motor learning, loss of Cav1.3 does impair motor learning. Our results help expand our understanding of LTCC function in cerebellar neurodevelopment and function.

21813

Changes in Electrophysiologic Activity in the Rat Visual Cortex following Traumatic Brain Injury (TBI)

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ABSTRACT IMPACT: This research aims to identify changes in visual network function after TBI as a way to define potential therapeutic targets for neuromodulation or neural tissue substrates. OBJECTIVES/GOALS: The objectives of this study are to compare neural activity in the visual cortex following TBI with cortical activity in the uninjured brain. This study aims to characterize functional changes in single neuron activity, spike-field relationships and oscillatory activity. METHODS/STUDY POPULATION: The effects of TBI will be studied by comparing electrophysiologic recordings from Long-Evans rats with a fluid percussion injury (FPI) to rats with a sham injury. Four days after the injury or sham procedure, a laminar probe with multiple electrode contacts will be chronically implanted in the ipsilesional primary visual cortex (V1). Afterwards, rats will be anesthetized weekly for 3 weeks (up to 4 weeks post-injury) to assess visual processing in response to drifting grating visual stimulation. To assess behavioral correlates, neural activity will also be recorded while rats perform a visual discrimination task in an operant, touchscreen chamber twice weekly. Recordings will be analyzed for visually evoked units, unit entrainment to local field potentials (LFPs) and evoked oscillatory activity. RESULTS/ANTICIPATED RESULTS: Consistent with other studies, our preliminary evidence from V1 recordings in naive rats has shown that individual neurons are responsive to visual stimuli, visual stimuli are associated with evoked oscillations and unit activity is correlated with LFPs. While activity of individual V1 neurons in injured animals is expected to recover to resemble activity in uninjured animals over time, patterns of functional organization in the two groups are expected to diverge over time. We anticipate that TBI-associated axonal damage, neuronal loss and changes in synaptic weights will lead to disruptions in the timing of neural activity in V1. These perturbations of neural communication within the visual system are expected to be associated with behavioral deficits in the awake, visual discrimination task. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study helps define how cortical network disruption after TBI. These changes are potential targets for novel TBI therapeutics, including neuromodulation and neural tissue transplantation. Thus, this work lays the groundwork for future studies aimed at mitigating the effects of TBI with rationally designed experimental therapeutics.

24088

Investigation of the Apelingeric System on Oxidative Imbalance within Cardiorenal Syndrome Type 4

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ABSTRACT IMPACT: Approximately 15% of US adults have chronic kidney disease with over 700,000 of those in the end stages where treatment options are severely limited to dialysis or kidney transplant; the research presented here will help identify novel strategies that address oxidative imbalance and preserve renal function. OBJECTIVES/GOALS: Chronic Kidney Disease patients often develop secondary cardiovascular disease - Cardiorenal Syndrome Type 4. RNA sequencing data show increased apelin receptor expression in 5/6 nephrectomy rats. The Apelinergic (APJ) system is deemed beneficial in normal physiological systems. Here we explore links between stress and the APJ system in CRS4. METHODS/STUDY POPULATION: In preliminary studies performed in NRK cells, inflammatory cytokines, IL-1β and IL-6, caused increases in apelin receptor transcripts and decreased apelin transcripts, respectfully. The literature describes inflammatory processes that contribute to degradation of many organs (kidneys, heart, and liver) suggesting an oxidative imbalance. To investigate this imbalance within CRS4, three rat cell types" H9c2 cardiomyocytes, HII4E hepatocytes, and NRK renal epithelial cells" will be used to assess the role of exogenous apelin on pro- and anti-oxidant levels. Cells will be pretreated with apelin or vitamin E 48 hours prior to the addition of toxins or cytokines (uremic: uric acid and d-galactose or hydrogen peroxide; cytokines: IL-1β and IL-6), to assess pro- and anti-oxidant protein levels via Western Blot. RESULTS/ANTICIPATED RESULTS: We anticipate with toxin or cytokine addition (either uremic, hydrogen peroxide, IL-1β or IL-6) in all cell types, an increase in protein levels for GPX" a known measure of oxidative stress" should be greater than the increases in antioxidants" SOD1 and Catalase. After pre-treatment of vitamin E, GPX protein levels should decrease compared to toxin/cytokine control, while SOD1 and catalase protein levels increase; this coincides with vitamin E inducing antioxidant activity in animals and humans. The anticipated results for this study after exogenous apelin addition should reveal in all three cells types reduced levels of GPX and increased levels of SOD1 and

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