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Orexin/hypocretin receptor 2 (HCRTR2) in alcohol dependence diagnosis and severity: An exploratory investigation in the role of HCRTR2 rs2653349 polymorphism

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OBJECTIVES/SPECIFIC AIMS: The preliminary analysis sought to retrospectively characterize the role of hypocretin receptor 2 (HCRTR2) in the development and prognosis of AD along with associated behavioral measures including smoking, self-reported drinking history, and neuroticism. Given the results in this study along with the paucity of information regarding the functional significance of rs2653349, we intend to comprehensively characterize HCRTR2 using haplotype analyses. We will then identify relationships between our haplotype analysis and IV alcohol self-administration using the Computer-Assisted Infusion System, and phenotypes identified in a sleep study. Furthermore, we aim at identifying functional loci in the hypocretin/orexin system by investigating differential allele expression in the orexin receptors in hippocampus tissue obtained from postmortem human brains. METHODS/STUDY POPULATION: This study examined 1569 European American and African American individuals between 18 and 65 years old, 922 of whom with a current diagnosis of AD. Participants were genotyped for HCRTR2 rs2653349 and ancestry was determined via a genome-wide panel of ancestry informative markers. AD was diagnosed using the Structured Clinical Interviews for DSM-IV (SCID-IV) for psychiatric disorders and recent alcohol use was assessed by 90-day Timeline Follow-back (TLFB) interviews. Smoking was assessed using the Fagerström Test for Nicotine Dependence and neuroticism was measured using the NEO Personality Inventory. RESULTS/ANTICIPATED RESULTS: In European Americans, a significant difference was found in current AD diagnosis between AX carriers and GG carriers ($z = -2.390$, $p = 0.017$). This relationship remained significant in a logistic regression model controlled for age and gender ($R^2 = 0.269$, $p = 0.015$). TLFB drinking measures were compared based on the median values to correct for the ceiling effect resulting from the assessment covering the past 90 days. Total drinks ($U = 8.280$, $p = 0.004$), number of drinking days ($U = 6.983$, $p = 0.008$), and average drinks per days ($U = 7.221$, $p = 0.007$) were all noted to significantly differ between the two allele groups among Caucasians. The associations between rs2653349 and total drinks ($R^2 = 0.115$, $p = 0.023$) and heavy drinking days ($R^2 = 0.190$, $p = 0.015$) remained significant in linear regressions controlled for age and gender. Furthermore, Caucasian AX carriers had a higher median number of drinking days relative to GG homozygotes among current AD positive subjects ($U = 6.937$, $p = 0.012$) and a lower median number of drinking days among current AD negative subjects ($U = 4.430$, $p = 0.035$). Among Caucasian AD negative subjects, there was a significantly greater frequency of smokers ($\chi^2 = 3.550$, $p = 0.046$). In African American participants, there were no significant differences in AD diagnosis and in measures of AD severity by genotype. African American males diagnosed with current AD had higher rates of smoking in the AX group ($\chi^2 = 4.969$, $p = 0.017$). No significant associations were found between rs2653349 and neuroticism in any of the cohorts analyzed in this sample. DISCUSSION/SIGNIFICANCE OF IMPACT: The results suggest that, among Caucasians, AX carriers have an increased risk to develop AD independently of their age and gender. In addition, among individuals with a diagnosis of AD, AX carriers reported a greater number of drinking days, as measured by the TLFB, suggesting that this polymorphism also exerts an effect on the severity of the disease. This effect on increased alcohol consumption was absent in Caucasian AX carriers without current AD diagnosis. In future analysis, we will explore how different genetic profiles in HCRTR2, and also HCRTR1, may alter the orexin signaling pathway and how such alterations may predispose patients to develop AD and exacerbate AD once it develops.

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Soluble adenylyl cyclase (sAC) regulates melanogenesis and melanocyte response to UVB

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OBJECTIVES/SPECIFIC AIMS: Our objective is to study the role of soluble adenylyl cyclase in the melanocyte regulation of pigment in response to ultraviolet radiation. Melanocytes are specialized cells that produce melanin in organelles called melanosomes, and melanin determines the pigmentation of hair and skin. cAMP is a master regulator of pigmentation and transmembrane class of adenylyl cyclases are essential for expression of important enzymes involved in melanogenesis. However, pigmentation is also controlled by melanosomal pH, which regulates melanogenesis, tyrosinase activity, and melanosome maturation. The relationship between melanosomal pH and cAMP has been elusive. Soluble adenylyl cyclase is a noncanonical source of cAMP that is not responsive to G proteins but rather functions as a pH sensor. We recently demonstrated that loss of soluble adenylyl cyclase (sAC) activity leads to increased melanosomal pH as well as increased pigmentation in cells and hair. We expanded our research to investigate the role of sAC in the intrinsic response of melanocytes to ultraviolet radiation. METHODS/STUDY POPULATION: We utilized sACfl/fl (wild type) and sACKO mouse melanocytes and compared their change in pigmentation in response to ultraviolet radiation. Melanin was used as a measure of pigmentation. We irradiated these cells at differing doses of UVB (0, 1, 2, or 3 mJ/cm^2) daily for 3 days. After UVB treatment, cells were observed and the surviving cell numbers were determined. Cells were then analyzed for melanin content using spectroscopy. RESULTS/ANTICIPATED RESULTS: We found that while both sACfl/fl and sACKO cells had increased melanin content in response to UVB, the melanin content of sACKO cells increased more compared with sACfl/fl cells ($p = 0.001$ at daily dose of 3 mJ/cm^2). In addition, sACKO cells required less UVB dose to induce a response. We also observed that sACKO cells show increased cell death compared with sACfl/fl cells. DISCUSSION/SIGNIFICANCE OF IMPACT: Although both sACfl/fl and sACKO cells can induce melanin production in response to UV, our results suggest that sACKO cells are more sensitive. We believe that this increased response in sACKO cells is due to increased melanosomal pH. In addition, sACKO cells show increased cell death, suggesting that sAC is important in the damage response secondary to UV exposure. UV plays a wide range of roles in skin biology such as contributing to cancer risk and pigmentation. Since pigmentation is essential for the protection of the skin from UV insult, further investigation of possible mechanisms in which sAC can influence pigmentation in response to UV is warranted.

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Doxorubicin exposure in vitro stimulates ROS production and directly suppresses cardiac fibroblast proliferation

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OBJECTIVES/SPECIFIC AIMS: Our research strives to understand the pathophysiology of doxorubicin cardiotoxicity, focusing on the understudied non-myocyte cardiac cells. Our understanding will enable researchers to develop protective or alternative therapies for cancer patients and treatments for cancer survivors. METHODS/STUDY POPULATION: Early studies have been carried out in isolated primary cardiac fibroblasts. Cells were treated with varying doses of doxorubicin. Cell viability, proliferation, and reactive oxygen species generation have all been studied. Future studies will focus on mitochondrial assessment in treated cells and confirmation of findings in animal models. Potential therapies discovered in these studies will also be conducted in animal models. RESULTS/ANTICIPATED RESULTS: Our results show a direct effect of doxorubicin on cardiac fibroblasts in vitro. Treated cells show a decreased rate of proliferation and increased production of reactive oxygen species. Similarly to cardiomyocytes, we hypothesize that reactive oxygen species damage the mitochondria of cardiac fibroblasts thereby altering their function and playing a role in doxorubicin cardiotoxicity. DISCUSSION/SIGNIFICANCE OF IMPACT: Current therapies have not been able to adequately protect patients from the cardiotoxicity of doxorubicin and other anthracyclines. A complete understanding of how doxorubicin damages cardiac tissue will only be possible by studying all cell types of the heart. With a better understanding, alternative therapies can be developed to prevent or treat doxorubicin cardiotoxicity without sacrificing the efficacy of doxorubicin in treating cancer.

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Receptor for advanced glycation end-products: Mitigating the persistent effects of particulate matter induced airway injury

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OBJECTIVES/SPECIFIC AIMS: Obstructive lung disease following particulate matter (PM) exposure is a major health concern. Coexisting metabolic

syndrome (MetSyn) often occurs. Receptor for advanced glycation end products (RAGE) is highly expressed in the lung, is a strong predictor of FEV1, and is a key mediator of MetSyn. To determine if the loss of RAGE protects from the persistence of effects of particulate associated lung injury in a murine model. METHODS/STUDY POPULATION: Wild type (WT) and RAGE knockout (RKO) mice were exposed to 100 μg of PM (WTC-Aggregate, PM53) or PBS control by oropharyngeal aspiration. Lung function, methacholine challenge, and bronchoalveolar lavage (BAL) were quantified 28 days after PM exposure using flexiVent (Scireq Montreal, QC). BAL was obtained and cell differentials, cytokines and transcription factors were assayed. Bio-volume to airspace ratio and mean chord length were measured (Image J and Adobe Photoshop). RESULTS/ANTICIPATED RESULTS: WT mice were hyper-reactive to methacholine compared with their PBS controls 28 days after a single exposure to PM. They recovered from increased neutrophilia, loss of FEV, decreased compliance, and increased resistance, which were previously observed 24-hours after exposure. RKO were not hyper-reactive when compared with their own PBS controls. Lung histology shows persistence of loss of alveolar space in WT mice exposed to PM after 28 days. Area fraction was significantly higher in PM exposed WT mice after 28 days which was not significant after 24 hours. Mean chord length was significantly shorter for PM exposed at both time points for WT mice. The relative expression of phosphorylated to total CREB and ERK1/2 proteins was lower in RKO PM exposed mice compared with WT PM while STAT3 expression was lower in WT PM compared with WT PBS. PM induced a lower fold change of total proteins from the PBS controls in RKO for CREB, p38, ERK1/2, STAT3, and STAT5. JNK and p70S6k total proteins expressed a decreased fold change in WT PM exposed mice compared with WT PBS controls. DISCUSSION/SIGNIFICANCE OF IMPACT: A single dose of PM can produce persistent airway hyper-reactivity after 28 days of exposure. This PM induced injury is alleviated in the absence of RAGE, similar to what was seen at 24 hours. Inhibiting RAGE may be key to limiting the persistent inflammatory effects of high intensity PM exposure.

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The role of gut microbiota in the susceptibility of Parkinson disease development

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OBJECTIVES/SPECIFIC AIMS: Several clinical studies have established a correlation between changes in relative bacterial populations in the gut and Parkinson disease. However, few published experiments have been able to parse out whether these associations are causative or correlative. Our aim is to determine how bacteria in the gut may impact the health and resilience of dopaminergic signaling. Our experiment is designed to serve as a proof-of-principle that controlled alterations to the gut microbiome alters mechanisms in dopamine homeostasis in the midbrain. METHODS/STUDY POPULATION: Bacterial inoculation 8–10-week-old germ-free male mice (C57BL/6) were exclusively used in this experiment. Mice were orally gavaged every 3 days (D0, 3, 6, and 9) with 100 μL novel bacterial suspension (~108 CFU resuspended in PBS with 1.5% $NaHCO_3$) or vehicle and were sacrificed on D11. Tissue preparation—brains were quickly extracted and the striatum was isolated and homogenized in either RIPA buffer with protease inhibitors (for Western blot analysis) or in 0.1 N $HClO_4$ (for HPLC processing). The homogenates were processed through fractional centrifugation to remove cellular debris. Lysate samples were frozen at −80°C until ready for analysis. Protein expression quantification—expression of proteins were measured using intensity of bands from Western blots. Lysates were denatured prior to loading with LB with 10% β-mercaptoethanol and 30-minute incubation at 37°C. All immunoblots were normalized to immunoreactivity to α-tubulin. Immunoblot intensity was determined using the ImageJ software. Dopamine/dopamine metabolite quantification HPLC analysis was used to determine dopamine and dopamine metabolite concentration. Aliquots of the lysate were injected onto a C18 column using a mobile phase consisting of 50 mM $H_2NaO_4P{\cdot}H_2O$, 0.72 mM sodium octyl sulfate, 75 μM Na_2 EDTA, and 10% acetonitrile (pH 3.0). The mobile phase was pumped through the system at 0.3 mL/minute. RESULTS/ANTICIPATED RESULTS: Measured total dopamine concentration through HPLC analysis in the striatum showed no significant differences in the bacteria-treated group relative to the control group. The metabolites DOPAC and HVA had an elevated measured concentration in the bacteria-treated group relative to the control group. Western blot analysis showed decreased immunoreactivity for DAT and TH in the bacteria-treated group compared with the control group. There was no significance difference in the immunoreactivity for VMAT2. DISCUSSION/SIGNIFICANCE OF IMPACT: This study demonstrates that dopamine signaling dynamics in the midbrain can be altered by changes in the gut flora in mice. These results further substantiate the impact of the gut-brain axis and may even point to a potential avenue of bolstering the resilience of dopaminergic neurons in preventing the onset of PD. Further experiments must be performed to understand the mechanism of the observed changes and to determine if these changes have any salutary effect.

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Impacts of a long-term community-university partnership on investigator-initiated research at an Urban Research University

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OBJECTIVES/SPECIFIC AIMS: Engaging Richmond is a community-university partnership, made up of local residents and university faculty and staff that was established in 2011 with an NIH supplement to a Clinical and Translational Science Research Award at Virginia Commonwealth University (VCU). The primary aims of the supplement were to (1) to conduct community-based participatory research (CBPR) on the leading causes of health disparities perceived by the Richmond community and (2) to thereby highlight community needs and assets and build capacity for future community-engaged research (CEnR). The goal was to prepare a community-focused, community-prioritized, health equity report while building capacity, strengthening relationships, and discovering local barriers to CEnR, and therefore to stimulate, facilitate, and inform future CEnR at VCU. METHODS/STUDY POPULATION: This is a case study exploring the impact of 1 community-university partnership on investigator-initiated research using historical and qualitative data. RESULTS/ANTICIPATED RESULTS: Although Engaging Richmond received only 12 months of support from the NIH supplement that provided its initial funding, the community-university partnership has worked continuously since its formation in 2011. This work has not only helped to build connections with the community and key stakeholders, it has also contributed substantially to the resources available to university faculty pursuing CEnR. Specifically, we find that Engaging Richmond has contributed to investigator initiated research in the following ways, either working as co-investigators or in a consultative capacity: consultation on proposal development (5 projects); assisted with instrument development (4 projects); participant recruitment (7 projects); data collection and analysis (6 projects); dissemination (5 projects). In addition to collaboration on projects, Engaging Richmond has increased institutional capacity for CEnR through its contributions to the Annual Community Engaged Institute at the university and the Center of Clinical and Translational Science's Community Review Board (CRB). The CRB helps researchers work successfully in a community setting, enhance the research design, help to improve study implementation and assist with translation and dissemination of findings. DISCUSSION/SIGNIFICANCE OF IMPACT: Although community-university partnerships have become much more common over the past several decades, there remains a gap in research evidence on the impact of these partnerships. In their 2004 review, Viswanathan *et al.* note that community-based participatory research studies infrequently document improved capacity of researchers and research organizations as an outcome, despite the expectation that such improvement will accrue through investment in CEnR. A more recent study assessing the range of community-university partnerships across a research university also noted the lack of processes in place to assess impacts (Holton *et al.*, 2015). While assessments of CEnR impact on communities have become increasingly common as demand for evidence about the effectiveness of community-engaged partnerships has mounted, there does not appear to be a similar trend in assessing the impact of these efforts on faculty research and institutional capacity. By focusing on the impact of 1 community-university partnership that has been sustained for over 5 years, we highlight the ways in which having ongoing partnerships in place can support and strengthen investigator-initiated research, reflecting the flexible, "2-way approach" (Weerts and Sandmann, 2010) at the heart of CEnR.

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Effects of cortical stimulation of the noninfarcted Versus peri-infarcted motor cortex

Serena-Kaye Kinley-Cooper and DeAnna Adkins

OBJECTIVES/SPECIFIC AIMS: The objectives of this study are to determine whether high-frequency ipsi-lesion or low-frequency contra-lesion ECS improves forelimb function following experimental stroke in aged animals with focal and large strokes. We also want to investigate whether ECS-induced improvements in motor function are related to an enhancement of neural structural plasticity (dendrites and synapses) and changes in growth promoting (BDNF) and growth inhibiting (NOGO-A) expression in the infarcted motor cortex in young and aged animals. METHODS/STUDY POPULATION: We will