were unchanged. DISCUSSION/SIGNIFICANCE OF IMPACT: In a large cohort in cirrhosis, cognitive impairment was associated with increased HRV, in contrast to other markers of liver disease, where HRV was reduced and unrelated to other cardiovascular measures. This paradoxical relationship, if validated, could be developed as a longitudinal digital biomarker for the detection of cognitive impairment in HE.

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Bacteriophages shape intraspecies competition among clinical isolates of Pseudomonas aeruginosa

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OBJECTIVES/GOALS: The goal of this study is to explore the lysogenized bacteriophages of clinical isolates of Pseudomonas aeruginosa (Pa) for their capacity to affect intraspecies competition. This study also aims to explore the capacity of using clinical isolates as a source of new candidates for phage therapy. METHODS/STUDY POPULATION: We compiled a collection of 207 Pa isolates and screened them for the presence of lysogenic phages using polymerase chain reaction (PCR). A subset of 96 phage containing clinical isolates was tested for intra-strain infectivity. Using a representative subset of isolates producing infective and non-infective phage, we evaluated competitive dynamics in liquid co-cultures, bacterial biofilms and in a murine chronic wound model. RESULTS/ANTICIPATED RESULTS: Among all isolates, 74.3% contained detectable phages in their genome, and 63.7% contained detectable phage in their supernatants. Among those tested for intra-strain infectivity, 82.2% of strains demonstrated inhibition of growth on lawns of at least one other strain, while 94.8% of these strains were susceptible to at least one other supernatant. In *in vitro* co-culture competition experiments, strains producing infective phages outcompeted competitors more effectively than strains producing non-infective phage. Infective phage producing strains were more resistant to biofilm invasion than non-infective counterparts. *In vivo* competition experiments showed that PAO1 was significantly reduced when co-infecting with an infective phage-producing strain compared to a non-infective one. DISCUSSION/SIGNIFICANCE OF IMPACT: Collectively, these data demonstrate that clinical isolates of Pa frequently encode active bacteriophages that can mediate intraspecies competition and confer a competitive advantage during infection. Future work will center on combining these phages as therapeutic cocktails targeting acute Pa infections.

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Targeting disrupted neural circuits to treat PTSD with comorbid psychosis

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OBJECTIVES/GOALS: To define how stress alters dopamine system function in PTSD with comorbid psychosis and determine whether orexin receptor antagonism with suvorexant can normalize dopamine neuron activity by targeting the insular cortex–orbitofrontal cortex (IC–OFC) circuit. METHODS/STUDY POPULATION: We used *in vivo* electrophysiology to record ventral tegmental area dopamine neuron population activity in rats following restraint stress. Chemogenetic and pharmacological manipulations targeted the IC–OFC pathway to assess its role in stress-induced dopamine dysregulation. Suvorexant, a dual orexin receptor antagonist, was administered systemically or directly into the OFC. Fiber photometry recordings measured IC-evoked glutamatergic transmission within the OFC to determine how suvorexant modulates circuit-specific activity contributing to altered dopamine function. RESULTS/ANTICIPATED RESULTS: Foot shock stress produced robust increases in dopamine neuron population activity. Intra-OFC suvorexant infusion reversed these effects, restoring dopamine system function to baseline levels. Chemogenetic inhibition of the IC–OFC circuit produced a similar normalization, confirming this pathway's role in stress-induced dopamine dysregulation. Fiber photometry revealed that suvorexant attenuated IC-evoked glutamatergic signaling in the OFC, indicating that orexin receptor antagonism modulates excitatory input within this cortical circuit to restore balanced dopamine activity. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings identify the IC–OFC circuit as a key mediator of stress-induced dopamine dysfunction and reveal orexin receptor antagonism as a promising strategy for treating PTSD with comorbid psychosis. Targeting cortical orexin signaling may enable circuit-specific precision therapies.

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Mechanism of agmatine prodrugs for alleviating chronic pain and opioid addiction

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OBJECTIVES/GOALS: There remains an urgent need for the development of safe and effective therapeutics for the long-term management of pain. We evaluated our novel analgesic in multiple preclinical models to assess its abuse liability, analgesic efficacy in models of chronic pain, and its mechanism of action in *ex vivo* spinal cord slices. METHODS/STUDY POPULATION: We evaluated SSA3's abuse liability by utilizing an IV self-administration paradigm. In this experiment, rats are placed in a chamber and have the option to press two different levers. Abuse liability was determined based on extent of lever pressing on the drug-infusion lever. To evaluate chronic pain efficacy, mice underwent a spared nerve injury (SNI) which induces sustained hypersensitivity in their hind paw. Mice were then treated with SSA3, and their tactile hypersensitivity was assessed. Finally, the mechanism of action was investigated through patch clamp electrophysiology recordings taken from excised spinal cord slices in mice. We recorded currents from neurons involved in the pain signaling pathway before and after application of our compound to observe drug efficacy at the cellular level. RESULTS/ANTICIPATED RESULTS: Our results demonstrated that in a pre-clinical IV self-administration paradigm, SSA3 exhibited no abuse liability, indicated by a lack of drug intake or escalation of drug intake. Results from our SNI model of chronic pain reveal that when SSA3 is administered directly onto the spinal cord via intrathecal injection, it is efficacious in reversing the pain hypersensitivity established by the

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