HPLC-MS/MS. Power density was determined in EEG bands using a custom algorithm. A two-compartment link PKPD model was developed to describe the relation between ALLO plasma concentration and change in EEG power in the alpha, beta, delta and theta bands. RESULTS/ANTICIPATED RESULTS: ALLO caused a rapid increase in absolute power density in all EEG bands measured (1-4, >4 – 8, >8 – 12, >12 – 25, and >25 – 100 Hz). The onset of effect was rapid (1-3 min) and demonstrated by frequency band and dose analysis. Concentration-EEG data were best fit by a two-compartment PK model and sigmoidal Emax PD indirect link model. The beta frequency band was most sensitive, showing increases in power at the lowest ALLO concentrations. The EC50 concentration for the beta frequency was ~270 ng/mL. The EC50 values for effects on the other frequency bands were ~500-700 ng /mL. In conclusion, IV ALLO causes a rapid effect on EEG that can be used to determine minimal plasma concentrations associated with target engagement. DISCUSSION/SIGNIFICANCE OF IMPACT: Dose selection for future clinical trials will use the effective concentrations determined here in conjunction with studies in animal status epilepticus models. Studies are planned in client owned dogs with epilepsy to evaluate clinical efficacy in dogs and as nonclinical proof-of-concept evidence supporting translational studies in people. CONFLICT OF INTEREST DESCRIPTION: Michael Rogawski and Dorota Zolkowska are named as inventors on patent applications claiming use of neuroactive steroids including allopregnanolone and ganaxolone in the treatment of status epilepticus.

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alpha-Synuclein Induced Reactive Gliosis†

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OBJECTIVES/GOALS: Reactive gliosis is a hallmark of neurodegenerative disease and is characterized by the release of pro-inflammatory cytokines and physiologic changes to glial cells. Our work identifies a novel inflammatory glial-glial cell interaction and role for mGluR5 that has the potential to provide novel insight into the mechanisms of neurodegeneration. METHODS/STUDY POPULATION: **Cell Culture:** Mouse primary astrocytes and microglia were isolated from P0-P3 C57BL/6 or Cx3cr1$^{GFP/+}$ mice.[1] **Treatment:** Glia were treated with oligomeric α-synuclein 1μg/mL or mGluR5 agonist CHPG 100 μM.[2,3] **ELISA:** Glia culture media was collected and analyzed according to the manufacturer. **qRT-PCR:** TaqMan™ probes were used according to manufacturer on extracted glia mRNA. **ICC:** Microglia were labeled with 1:750 Rb x Iba1 (Wako) and 1:500 Alexa Fluor 488 Gt x Rb. **Phagocytosis Assay:** Primary glia were treated with α-synuclein or astrocyte-conditioned culture media for 24-48hrs. For treatment of microglia with conditioned media, astrocytes were washed with PBS and fresh media was added to prevent carry over of α-synuclein to microglia. The number of fluorescent microbeads per microglia was quantified. RESULTS/ANTICIPATED RESULTS: Mouse primary cortical astrocytes simulated with α-synuclein aggregates adopt a reactive A1 phenotype independent of microglial stimulation. This A1 phenotype is characterized by release of pro-inflammatory cytokines including Complement Component 3 and the monocyte chemoattractant CCL2. Reactive astrocyte media induces a phagocytic phenotype in primary mouse microglia. Along with this, α-synuclein-directed microglial phagocytosis was attenuated with the addition of the mGluR5 agonist CHPG. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest that oligomeric α-synuclein is capable of inducing a reactive phenotype in astrocytes independent of microglia and implicate crosstalk between glia as an important mediator of inflammation and microglial phagocytosis in synucleinopathies.

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Angiopoietin F-domain valency determines outcome of Tie2 receptor engagement and accelerates angiogenesis in tissue regeneration

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OBJECTIVES/GOALS: Lack of blood vessels remains a major obstacle in tissue regeneration. Angiopoietin 1 and 2 modulate angiogenesis through the Tie2 receptor tyrosine kinase. Ang1 activates pAKT to promote endothelial cell survival while Ang2 antagonizes these effects. We aim to dissect the Ang/Tie2 pathway to uncover the molecular basis for these opposing effects. METHODS/STUDY POPULATION: Ang1 and Ang2 bind Tie2 via nearly identical F-domains (Fd). To investigate the molecular basis regulating the Tie2 pathway, we generated a series of computationally designed self-assembling protein scaffolds presenting F-domains in a wide range of valencies and geometries using Rosette Molecular Modeling Suite. We examined the protein kinase activation, cell migration, and blood vessel formation produced by the designed proteins in human umbilical vein endothelial cells. RESULTS/ANTICIPATED RESULTS: Two phenotypic classes were demonstrated by the number of presented F domains: scaffolds presenting 3 or 4 Fd have Ang2 like activity, upregulating pFAK and pERK but not pAKT and failing to induce cell migration and tube formation; scaffolds presenting more than 6 Fd have Ang1 like activity, upregulating the three signaling branches and enhancing cell migration and tube formation. Scaffolds with 8 or more Fd show superagonist activity, producing significantly stronger phenotypes than Ang1. These results suggest that Fd valency largely determines Ang1 vs Ang2 signaling outcomes, and our designed superagonists can outperform Ang1 in vascularization and wound healing. In *in vivo* experiments, nanoparticles displaying 60 copies of Fd produce significant revascularization in hemorrhagic brains. DISCUSSION/SIGNIFICANCE OF IMPACT: Targeting the Tie2 pathway is a new paradigm in regenerative medicine. Our designed constructs will enable us to generate high-affinity Tie2 agonists and antagonists as drugs to control angiogenesis, enabling tissue regeneration that recapitulates the biological architecture of the native tissue physiology, improving organ transplant outcome.

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Anibiotic-Resistant Organism Acquisition in Nursing Facility Patients

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OBJECTIVES/GOALS: We investigated the association between gut microbiota features in newly admitted nursing facility (NF) patients and the acquisition of vancomycin-resistant *Enterococcus* (VRE)

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