dsRed-synaptobrevin fusion protein with NMUR2 on synaptic inputs into the medial prefrontal cortex. Following quantification of pre- and post- treatment events using the InScopix data acquisition software, total events during the pre- and post-treatment time periods were calculated. In these studies, both animals demonstrated a clear increase in calcium transient activity between pre- and post- treatment evaluations, suggesting that NMU administration increases the neuronal activity of neurons in the prefrontal cortex. DISCUSSION/SIGNIFICANCE: This research provides a new site of action for the known therapeutic effects of NMU. We demonstrate the presence of presynaptic NMUR2 in the mPFC and show that systemic administration of NMU increases mPFC neuronal activity. This illustrates NMU may act as a top-down mediator for substance use disorders and binge eating behaviors.

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Diffusion MRI to investigate atypical corticospinal tract microstructure and motor impairments in hemiplegic cerebral palsy

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OBJECTIVES/GOALS: Hemiplegic cerebral palsy (HCP) limits the functional ability of one side of the body, but motor impairments are very heterogeneous among children with this diagnosis. The purpose of this study was to evaluate the CST using DTI and tractography analyses as it relates to quantitative measures of the severity of weakness and mirror movements in HCP. METHODS/STUDY POPULATION: Preliminary results include five participants with HCP (2M, 16Â±7.8 years) and six controls (2M, 12Â±3.5 years). DTI data were collected using a spin-echo echo-planar imaging sequence with diffusion weighting of b=1000 s/mm2 in 60 different directions and 8 scans without diffusion weighting (b=0 s/mm2). Images were processed with steps of brain extraction, denoising, motion and eddy current correction, and fit with tensors to generate maps of diffusivity metrics. Anatomical landmarks were used to guide probabilistic tractography of the CST for analyses in both the lesioned and non-lesioned hemispheres. To quantify grasp weakness and mirroring severity, participants completed a bilateral assessment of grip strength using handheld force measurement devices and custom MATLAB data acquisition software. RESULTS/ANTICIPATED RESULTS: DTI is a feasible method to evaluate CST microstructure in HCP and typically developing pediatric participants. Spearman correlation analyses, using age and sex as covariates, revealed that for the lesioned hemisphere CST, there were significant positive correlations between grasp weakness severity and mean diffusivity (MD) (Ï =0.66, p=0.038) and between grasp weakness severity and axial diffusivity (AD) (Ï =0.68, p=0.030). There was not a significant correlation between grasp weakness severity and fractional anisotropy (FA) (Ï =-0.47, p=0.166). For the non-lesioned hemisphere CST, there was a significant positive correlation between mirroring severity and radial diffusivity (RD) (Ï =0.70, p=0.023). There was not a significant correlation between mirror movement severity and FA (Ï =-0.41, p=0.2361). DISCUSSION/SIGNIFICANCE: The correlations demonstrated here show a potential relationship between CST microstructure and the severity of hand impairments in HCP. While these relationships between CST diffusivity properties and hand function are preliminary, they provide the first steps to better understand underlying neural mechanisms for motor impairments in HCP.

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Mild Maternal Undernutrition Results in a Premature Neonatal Leptin Surge and Resistance in Male Offspring to a High Fat Diet†

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OBJECTIVES/GOALS: Maternal undernutrition, a form of malnutrition, can alter neonatal leptin signaling and result in metabolic dysfunction in adulthood. We developed a mild undernutrition model to relate more to societys nutritional challenges and to test the hypothesis that a shift in the neonatal leptin surge would result in sex-specific metabolic changes. METHODS/STUDY POPULATION: We studied pups from undernourished dams which were calorically restricted by 20% (CR20) from embryonic day 15 until postnatal day (PND) 21. We tested 216 offspring from 11 Fed dams and 13 undernourished dams (CR20), detecting a leptin surge in control fed progeny at PND11. At 3 months of age, offspring from 3 dams per maternal nutrient status were either exposed to a 45% high fat diet (HFD) or control diet (10% fat) for 16 weeks. Anterior pituitary hormones were analyzed in the pituitary and serum of neonates and adults. To determine the mechanism of the phenotype observed in male adult offspring on the HFD, single cell RNA sequencing was used to analyze the pituitary, fat and liver. RESULTS/ANTICIPATED RESULTS: Offspring of CR20 dams had an early leptin surge peaking at PND8 and GH levels at PND1 were higher in CR20 progeny. Weights of both male and female CR20 offspring were lower and body lengths were shorter than controls. As adults, Fed mice from both sexes had increased weight gain with HFD. However, although CR20 females gained weight on the HFD, male progeny from CR20 dams did not gain weight on the HFD and appeared protected from impact. We found sex-specific changes in pituitary Gh, Ghrhr, and Ghsr mRNA levels. Single cell RNA sequencing of pituitary, fat and liver of male offspring showed significant regulation of transcripts in fat of male offspring from Fed dams that was not found in CR20 males when compared to control fed mice. DISCUSSION/SIGNIFICANCE: Mild undernutrition causes a prematurely high leptin surge and sex-specific growth responses to a HFD, including resistance to a HFD in underfed males. Transcript analysis in fat of males resistant to HFD induced obesity may reveal mechanisms that provide protection against HFD induced weight gain.

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Essential role for the neurodevelopmental disorder-linked gene, MEF2C, in inhibitory neuron function and neurotypical behaviors*

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OBJECTIVES/GOALS: The MEF2 family of transcription factors regulate gene expression controlling cell differentiation and synapse development. Mutations or deletions in the MEF2C gene cause a neurodevelopmental disorder that includes symptoms of autism spectrum disorder. In this study, we aim to study the role of MEF2C in GABAergic populations using an animal model. METHODS/

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STUDY POPULATION: MEF2C Haploinsufficiency Syndrome (MCHS) occurs when there is one functional allele and one disrupted allele of MEF2C. To study the role of MEF2C in GABAergic populations during mouse development, we bred Vgat (vesicular GABA transporter)-Cre mice, which express cre recombinase broadly in early developing GABAergic neurons, with a floxed Mef2c loss-of-function mouse to create offspring that are GABAergic cell-specific Mef2c heterozygous mutants (Mef2c cHetVgat-cre). We then subjected these mutants and littermate controls to a battery of tests measuring MCHS-relevant phenotypes, including spatial working memory, anxiety-like behavior, social preference, sensory sensitivity, and Pavlovian learning and memory. RESULTS/ANTICIPATED RESULTS: Mef2c cHetVgat-cre mice showed significant deficits in spatial working memory, social preference, and contextual fear memory, all of which are prefrontal cortex (PFC)-dependent behaviors. Interestingly, we noted that conditional Mef2c knockout mice (Mef2c cKOVgat-cre) showed embryonic and early postnatal lethality, probable seizures, and severe motor coordination problems, highlighting the importance of MEF2C function in GABAergic populations. DISCUSSION/SIGNIFICANCE: We hypothesize that MEF2C plays a cell-autonomous role in GABAergic cells to control the balance of excitatory and inhibitory synaptic transmission in the developing and mature brain, which in the Mef2c cHet mice might be critical for PFC-dependent learning and memory and sociability.

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Cortisol cut off point to diagnose adrenal insufficiency (AI) using a monoclonal antibody immunoassay*

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OBJECTIVES/GOALS: AI is diagnosed when peak cortisol level after a cosyntropin stimulation test is <18 mg/dL using polyclonal antibody (pAb) immunoassay. However, the polyclonal assay is being replaced by a specific monoclonal antibody (mAb) immunoassay which yields lower cortisol levels, leading to the over diagnosis of AI and use of unnecessary steroid use. METHODS/STUDY POPULATION: We obtained 36 samples from patients undergoing 1 mcg cosyntropin stimulation tests for diagnosis of AI. Samples were analyzed using pAb immunoassay (Abbott Architect Cortisol), mAb immunoassay (Roche Elecsys Cortisol II), and mass spectrometry (MS). AI was diagnosed if serum cortisol level was <18 using the pAb immunoassay. Measurements by MS and mAb immunoassay were individually used in simple logistic regression models to predict AI. For each model, we calculated a cortisol level corresponding to a 50% probability (median) of AI and used the delta method to determine the standard error and 95% confidence interval of the median. We used receiver operator characteristic (ROC) curve, area under the curve, sensitivity, and specificity to evaluate the potential of the median values as thresholds for each predictor. RESULTS/ANTICIPATED RESULTS: Data showed a mean cortisol level of 17 mcg/dL using the pAb immunoassay, 12 mcg/dL using the mAb immunoassay, and 12.96 mcg/dL using MS. The mean difference in cortisol level between the mAb immunoassay and the pAb immunoassay was 5.12 mcg/dL (p-value <0.01). The ROC curve model indicated an area under the curve of 0.997 with a median value of 11.2 mcg/dL for the mAb immunoassay. This provides a sensitivity of 95%, specificity of 95%, positive predictive value of 95%, and negative predictive value of 94%. This new threshold has a Kappa coefficient of 0.89 when compared to the pAb immunoassay. DISCUSSION/SIGNIFICANCE: New and highly specific mAb immunoassays are being used more widely but yield lower cortisol results. This reflects the need for further studies to determine new cut off points for highly specific cortisol immunoassays. A cut off level of 11.2 mcg/dL would provide a sensitivity of 95% and specificity of 95%.

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Behaviors and Quality of Life in Children with Neurodevelopmental Disorders Undergoing Refractive Surgery*

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OBJECTIVES/GOALS: The goal of this project is to characterize the adaptive and social behaviors of ametropic children with neurodevelopmental disorders (NDD) including Autism Spectrum Disorder (ASD) undergoing refractive surgery due to spectacle intolerance. Eye-related quality of life and visual behaviors will also be measured. METHODS/STUDY POPULATION: This is an ongoing prospective cohort study of children with NDD who are spectacle-intolerant and undergoing refractive surgery at St. Louis Childrens Hospital starting July 2020. The method of refractive surgery (photorefractive keratectomy (PRK), phakic intraocular lens implantation (phIOL), and refractive lens exchange (RLx)) is chosen based on degree and type of refractive error, astigmatism, and characteristics of the cornea and anterior chamber. Behaviors are characterized using the Adaptive Behavioral Assessment System, 3rd edition (ABAS-3) and Social Responsiveness Scale, 2nd edition (SRS-2), completed by parents/caregivers. Eye-related quality of life is measured using the pediatric Eye Questionnaire (PedEyeQ). Visual behaviors are assessed with optokinetic nystagmus tracking. RESULTS/ANTICIPATED RESULTS: In a preliminary sample of 9 patients, 4 have undergone PRK, 3 phIOL, and 2 are awaiting surgery. Pre-surgical SRS-2 scores had a median t-score of 70 (range 57 to 90), indicating a clinically significant deficiency in reciprocal social behaviors as this score is consistent with moderately severe ASD. Pre-surgical ABAS-3 scores had a median General Adaptive Composite (GAC) of 51 (range 48 to 74.5). This corresponds to a percentile of 0.100 (range <0.1 to 19th percentile for age). As rated by the Parental PedEyeQ, the domains most affected were "Parent Worry About Childs Eye Condition" (median 42.0, range 15.0 to 80.0) and "Parent Worry About Functional Vision" (median 18.8, range 3.2 to 68.7), where 0 represents the worst quality of life or functional vision and 100 the best. DISCUSSION/SIGNIFICANCE: Prior research has shown that refractive surgery can improve observed visual awareness, attentiveness, or social interactions in children with NDD. This is the first study to characterize the baseline adaptive/social behaviors and quality of life using validated surveys, with the goal of subsequently identifying patients most likely to benefit.

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