**Title**: Brainstem Microstructural Differences are Associated with Increased Inattention and Hyperactivity in Autistic Children.

**Authors**: Monica Duran1, Olivia Surgent2, Jose Guerrero-Gonzalez1, Doug Dean1, Gregory Kirk1, Andrew Alexander1, Brittany Travers1.

**Introduction**: Attention difficulties are prevalent in autism spectrum disorder (ASD) and are often exacerbated when ASD co-occurs with attention-deficit/hyperactivity disorder (ADHD), leading to heightened behavioral challenges and reduced quality of life (Leitner, 2014; Rao & Landa, 2014). To date, human neuroimaging studies examining attention in autistic children have primarily focused on cortical structures, leaving subcortical regions like the brainstem largely unexplored. This is an alarming omission because many behavioral features of ASD such as attention deficits, autonomic dysregulation, sensorimotor challenges, and sleep disturbances, are consistent with differences in brainstem substructures and networks (Rimland, 1964; for review see Dadalko & Travers, 2018). While recent studies in autistic children have identified associations between brainstem gray and white matter regions and sensory processing and core autism features (Surgent et al., 2022; Travers et al., 2024), the brainstem’s role in inattention/hyperactivity remains poorly understood.

**Methods**: To address this gap, in this study, we applied advanced neuroimaging techniques to examine the relationship between the microstructural properties of 54 different brainstem nuclei and the prominence of inattention and hyperactivity (ADHD features) in 69 autistic children ages 6 - 11. T1 and multi-shell diffusion weighted imaging data were collected with a 3T GE scanner and TiDi-Fused processed (Guerrero-Gonzalez et al., 2022) to optimize brainstem visualization. Microstructural features derived from relaxometry (R1), free water elimination diffusion tensor imaging (FEW-DTI), and neurite orientation dispersion and density imaging (NODDI) models were used in a principal component analysis (PCA) for brainstem feature data reduction, resulting in 18 principal components. Inattention and hyperactivity features were measured with the NICHQ Vanderbilt Assessment scale. We performed multiple regression analyses to examine the relationship between ADHD feature scores and principal components of brainstem diffusivity measures, while controlling for sex, age, and scan head motion.

**Results**: The analyses revealed statistically significant relationships between ADHD features and three of the eighteen principal components. Specifically, more prominent ADHD features were associated with decreased diffusivity in the following brainstem regions after false discovery rate correction: 1) Bilateral tegmental and midbrain reticular nuclei (t(64) = -3.44, p = 0.001, r = -0.38), 2) Pontine and medullary reticular formation nuclei (t(64) = -3.49, p < 0.001, r = -0.38) and 3) Substantia nigra (t(64) = -3.25, p = 0.002, r = -0.37). These findings reveal a significant link between microstructural changes in specific brainstem regions and behavioral outcomes related to inattention and hyperactivity in autistic children.

**Discussion:** The observed decreased diffusivity in the bilateral tegmental and midbrain reticular nuclei, pontine and medullary reticular formation nuclei, and substantia nigra may indicate increased cellular density or reduced extracellular space, possibly reflecting higher neuronal packing or altered glial composition (Yi et al., 2019). Such microstructural alterations could affect neural signal transmission and processing, thereby influencing attentional control and behavior. Moreover, the identified brainstem regions are part of neural circuits that are involved in attention modulation and have been implicated in hyperactivity. The tegmental nuclei and the midbrain, pontine, and medullary reticular nuclei are key components of the ascending arousal network, which is critical for regulating arousal states and modulating alerting, orientation to salient stimuli, and attention shifting (Coull, 1998). The substantia nigra, is a critical part of the dopaminergic system and nigrostriatal pathway and has been suggested to contribute to inattention, impulsivity and hyperactivity (Elliot et al., 2021; Wang et al., 2024). Altogether, our study indicates that changes in brainstem gray matter microstructure may play a pivotal role in modulating attention and hyperactivity features in autistic children. While we acknowledge that attention control is a complex function involving multiple brain regions, these findings underscore the importance of including the brainstem in neurobiological investigations. A comprehensive understanding of attention deficits in ASD requires exploring both cortical and subcortical structures to fully elucidate the intricate neural mechanisms at play. By highlighting the brainstem's contribution, we open avenues for more holistic research approaches that could inform targeted interventions and improve outcomes for individuals with ASD with and without co-occurring ADHD.

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University of Wisconsin-Madison

2 University of California-Davis