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In Utero Opioid Exposure Induced Vulnerability to Later Life Brain Injury

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Opioid misuse during pregnancy is a rising crisis in the United States, with rates of children born with in utero opioid (IUO) exposure having doubled in the last decade. While several works reported IUO associated neuro-pathophysiology, a large gap in the field regarding the potential post-natal vulnerability of IUO exposed infants still exists. Particularly, during peri-adolescence, a key neurodevelopmental period, little is known how IUO exposure impacts the later in life vulnerability to brain injury. Interestingly, the most reported emergency room (ER) case for juvenile trauma is mild traumatic brain injury (mTBI), most often caused by slips and falls. Moreover, a distinct neuroanatomical decrease in the precentral gyrus, an area responsible for the control of voluntary motor movement has been reported in IUO children. These data collectively suggests that, potentially, IUO exposed children are not only more vulnerable to mTBI but are also at a greater risk of suffering mTBI due to neurodevelopmental impairment of motor cortices. Here, we seek to understand if IUO exposure causes later life vulnerability to mTBI. The opioid epidemic has seen a dramatic increase in the number of babies born with neonatal abstinence syndrome (NAS). Recent investigation found a 52% increase from 2012-2016 of children born with NAS and an associated $2.5 billion in related costs in 2016. Brain injuries are not as well reported as NAS cases, but data from the Center for Disease Control and Prevention in a 2014 report to congress found a 56% increase in ER visits related to mTBI from 2007-2010. The direct costs of mTBI in 2000 was $9.2 billion with indirect costs of $51.21 billion. mTBI and IUO are growing epidemics in the United States. With both epidemics rapidly growing, research addressing long-term challenges is needed. Given the predisposition for NAS sufferers to have increased rates of ER visit for injuries and trauma there is an urgent need to understand the potential vulnerabilities of this disadvantaged sub-population to later life injuries. Mild traumatic brain injuries, commonly called concussions, are the third most common injury/trauma related ER admittance in children under 10. IUO exacerbates physiological deficits caused by mild-traumatic brain injury suffered during peri-adolescence. Nulliparous dams were orally gavaged with clinically relevant doses of oxycodone (oxy) or saline during mating and through weaning of pups. Pups were weaned and at post-natal day 28 (P28), consequently, subjected to a free weight drop model of mTBI or a sham procedure. Physiological measurements and righting reflex were recorded at P28. Rats were assessed two days post-injury (DPI) to measure acute effects on the cortex using magnetic resonance imaging (MRI). At DPI 2 cortical purified synaptosomes were isolated using density gradient ultracentrifugation for high-throughput mass-spectrometry proteomics. Additionally, cortical purified synaptosomes and tissue lysate samples were tested using western blotting for hits identified in proteomics. Our results revealed that IUO+mTBI groups displayed significant impairment in righting reflex post-mTBI when compared to Sal+mTBI. Further, proteomics data revealed changes in several key molecular pathways in the IUO+mTBI groups. Interestingly, mitochondrial dysfunction was one of the top hits when comparing both IUO groups to both Saline groups. Using western blotting, key proteins associated with the mitochondrial transport chain were

found to be significantly altered in response to injury in purified synaptic fractions, synaptosomes. However, while differences were found in cortical tissue lysate expression of these proteins, the levels were unique when compared to synaptosomes. Furthermore, these findings were complemented with *in vivo* H-MRI assisted metabolite measurements in the cortex that revealed significant deficits in the IUO+mTBI group. Our data suggest that infants exposed *in utero* to opioids are significantly more vulnerable to mTBI due to impaired mitochondrial proteins. Specifically, distinct molecular aberrations were seen in synaptic mitochondrial proteins of IUO exposed rats suffering mTBI. These aberrations correlate with the impaired righting reflex and cortical metabolite pathophysiology. Mitochondrial impairment caused by IUO exposure in response to mTBI suggests that IUO can have not only measurable pathophysiological impacts but also deficits that do not potentiate until an insult is delivered. These findings could lead to further understanding of the role of gestational-developmental insults and how to not only better educate providers but also lead to the discovery of novel therapeutics. Both outcomes will serve to reduce the economic and social burden of the rising opioid epidemic.