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**Neonatal morphine administration (P1-14) in outbred CFW mice induces enhanced behavioral signs of withdrawal in females and a distinct brainstem transcriptomic profile compared to males**

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The Opioid Use Disorder epidemic has led to higher incidence of Neonatal Opioid Withdrawal Syndrome (NOWS). NOWS-affected infants born to opioid-dependent mothers display body weight deficits, inconsolability, insomnia, and increased pain sensitivity. The neurobiological basis of NOWS is largely unknown, but mouse models will help facilitate mechanistic discovery. We treated neonatal outbred Cartworth Farms White (CFW) mice (Swiss Webster) with morphine sulfate (15.0 mg/kg, s.c.) twice daily from postnatal day 1 (P1) to P14, the approximate third trimester-equivalent of human gestation. Behavioral symptoms were measured on P7 and P14 at 16 h post-morphine. Brainstem (containing pons and medulla) was collected on P14 and processed for transcriptome analysis via mRNA sequencing (RNA-seq). Morphine-induced weight loss was observed from P2 to P14 and sustained at P21 and P50. Morphine also induced a delayed self-righting latency at P4 and a female-specific delay at P7. Morphine-treated females emitted more ultrasonic vocalizations (USVs) on P7, and both morphine-treated sexes showed increases in USVs on P14. Furthermore, thermal nociception via hot plate and tail withdrawal assays indicated thermal hyperalgesia in morphine mice on P7 and P14, with females showing greater hyperalgesia (tail withdrawal) on P7. Morphine-treated mice also exhibited anxiety-like behavior at P21 (open field). Interestingly, brainstem transcriptome analysis identified a canonical gene set relevant to opioid signaling in males and a distinct gene set in females that was enriched for ribosomal proteins, mitochondrial function and neurodegenerative disorders. A sex-combined gene set more broadly implicated disrupted development, cell cycle regulation, and innate immune signaling.