

# **Sexual differences in stress-responsive behavior and resiliency of adult mice exposed to maternal separation during infancy.**

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Maternal separation is an early-life adversity that can cause long-term brain and behavioral changes, increasing vulnerability to stress-related disorders later in life. This study aimed to investigate sex-specific responses to adult stress in mice exposed to early adversity. Female and male C57BL/6J mice underwent 3-hour daily maternal separation (MS) for 21 consecutive days. At day 60, they were exposed to a single 2-hour restriction stress (RS) and assessed 24 hours later through a 3-day behavioral test protocol: Nest Building, Open Field and Forced Swimming Tests. The experimental groups included Control, RS, MS, and MS+RS.

Stress responses differed between sexes. RS males performed better in the Nest Building Test, while for females, this behavior was only observed in the MS+RS group. In the Open Field Test, RS males spent less time in the center, while MS females showed increased movement velocity. These variables were unaltered in the MS+RS groups for both sexes. In the Forced Swimming Test, MS+RS females showed increased swimming time but this was not observed in the MS-only group. No significant results were found for males in this test.

Principal component analysis (PCA) revealed that RS males developed maladaptive coping strategies (e.g., faster nest building and increased grooming). MS females were hyperactive in low-threat situations (Open Field) but exhibited passive behavior in high-threat situations (Forced Swimming). However, the MS+RS group exhibited a reduction in maladaptive coping behaviors in both sexes, with females showing increased motivation for nest building and prolonged swimming. In males, the MS+RS combination diminished stereotypic behaviors.

In conclusion, these sex-specific behavioral responses to stress provide insights into the neurobiological basis of susceptibility or resilience to disorders such as depression.

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