Neural Progenitor Cells Show Sex-specific Differences in Autophagy related proteins when exposed to Hyperoxia and Fetal Zone Steroids

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INTRODUCTION

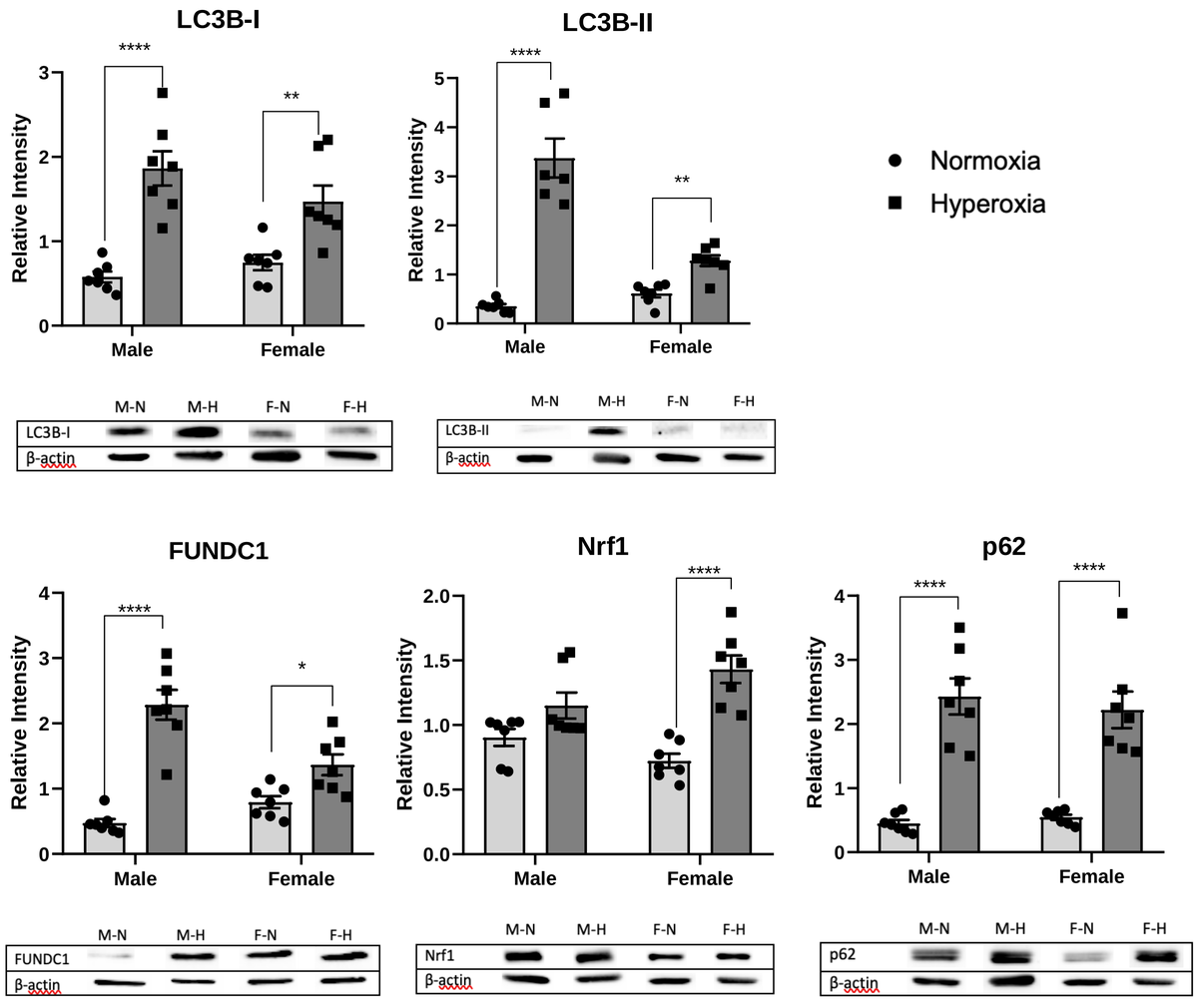
Preterm babies are highly susceptible to neurodevelopmental impairments, with male neonates disproportionately affected [1]. A key factor is the sudden increase in oxygen tension after birth, which leads to supraphysiological levels of reactive oxygen species (ROS) and oxidative stress. Neural progenitor cells (NPCs), which are still abundant and actively proliferating at this stage, are especially vulnerable to such stressors [2]. To maintain cellular homeostasis under stress, NPCs rely on pathways like autophagy and mitophagy, which help eliminate damaged organelles and proteins. Disruption of these processes has been linked to abnormal brain development and long-term neurological deficits [3]. During fetal development, high concentrations of adrenal-derived fetal zone steroids (FZS), such as dehydroepiandrosterone (DHEA), are present. These neuroactive steroids may interact with oxidative stress pathways and influence autophagy in a sex-dependent manner [4]. In this study, we examined how hyperoxia affects autophagy and mitophagy in male and female NPCs, and whether FZS modulate these responses in a sex-specific manner.

METHODS

Primary mouse derived NPCs were obtained from newborn male and female mice and grown for 6 days with 3% oxygen (normoxia) until spheroids were formed. As well, human ReNcell VM cell line was used. Male and female derived NPCs were treated with 80% oxygen (hyperoxia) and steroids for 24h. Supernatant was collected, and protein was extracted from the cells using M-PER lysis buffer. After which, Western Blot analysis was performed, focusing on autophagic marker proteins, including LC3B-I and LC3B-II, Nrf1, FUNDC1, p62. Also, ROS measurement with DCFDA ROS assay kit and mitochondrial potential measurement with TMRE assay kit was performed at different time points.

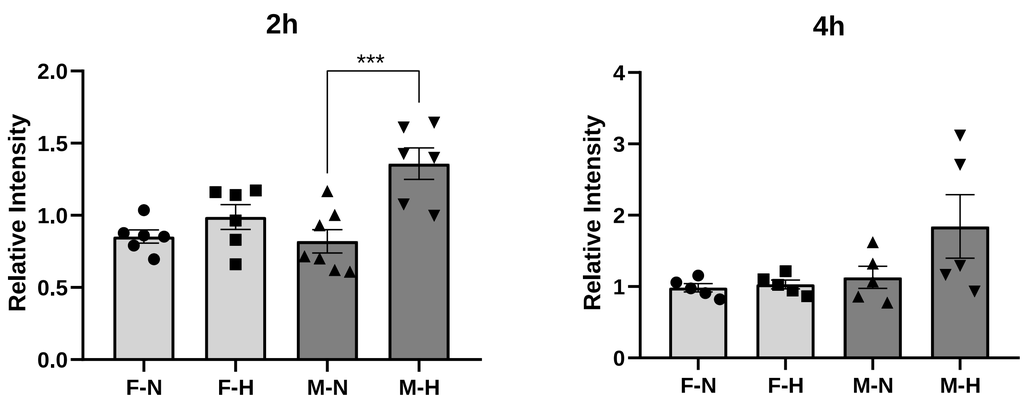
RESULTS

NPCs exhibit sex-dependent changes in specific autophagic protein levels following hyperoxia treatment.



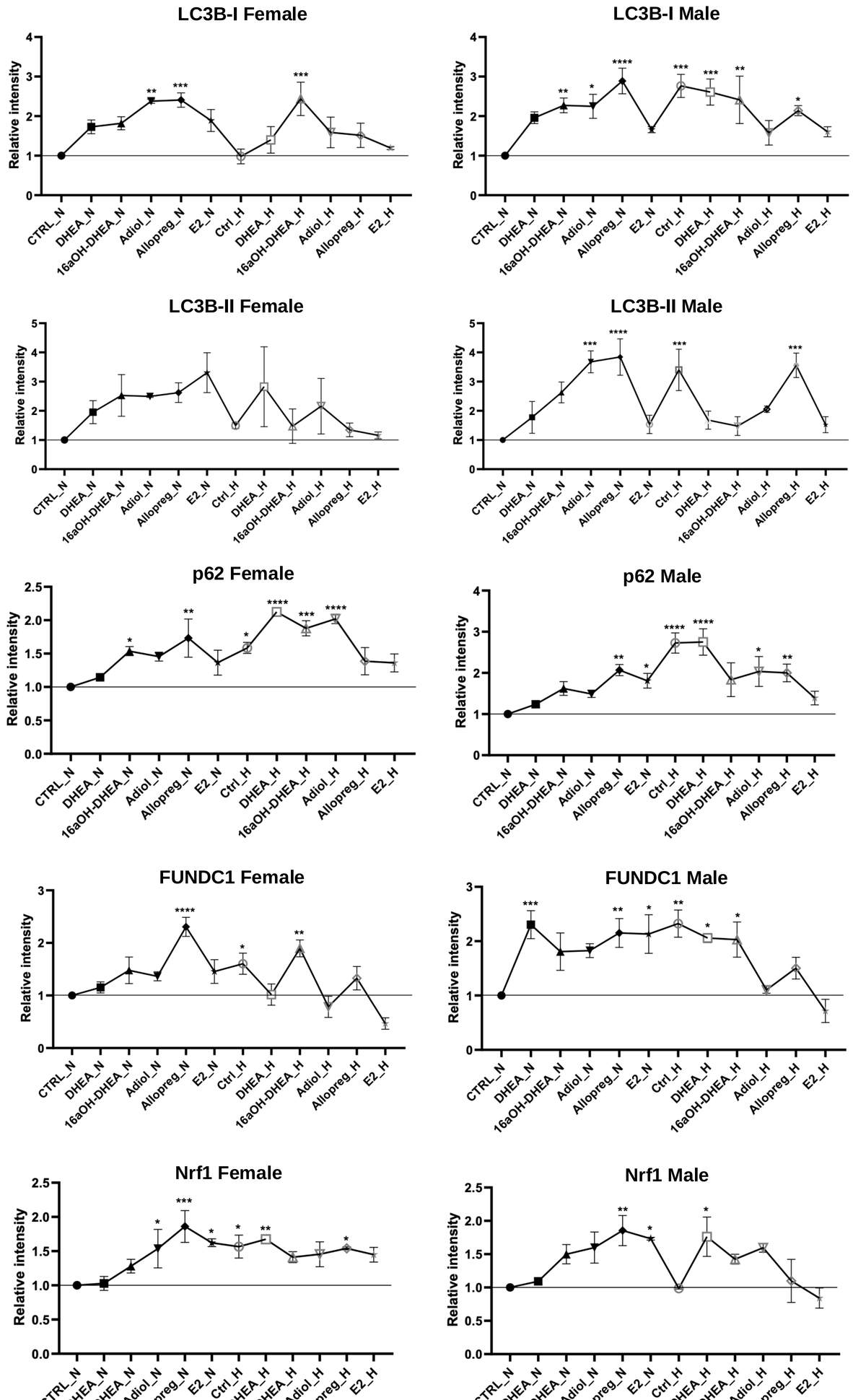
Western blot analysis of male and female NPCs with anti-LC3B-**I** and **II**, anti-p62, anti-FUNDC1, anti-Nrf1 and anti-Nup133 antibodies under normoxic conditions (3%O_2) and post 24h 80%O_2 treatment, showing significant changes in expression levels in male and female cells. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 (Sidak's multiple comparison test). Values are means ±SEM.

Male NPCs exhibit a greater increase in reactive oxygen species (ROS) within two hours of hyperoxia exposure compared to female NPCs.



DCFDA ROS assay results of male and female NPCs under normoxic conditions at 3%O_2 and 24h of treatment with 80% O_2 (F-N: female normoxia; F-H: female hyperoxia; M-N: male normoxia; M-H: male hyperoxia), showing significant or trending upregulation in male NPCs, while female NPCs show no change in ROS. ***p<0.001 (Tukey's multiple comparison test). Values are means ±SEM.

Male NPCs show an overall stronger increase in autophagic marker proteins under steroid treatment than females.



Western Blot analysis of male and female NPCs under normoxic conditions (3%O_2), post 24h 80%O_2 treatment and steroid treatment (dehydroepiandrosterone, 16α-hydroxydehydroepiandrosteron, adiol, allopregnanolone and estradiol). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 (Dunnette's multiple comparison test). Values are means ±SEM.

CONCLUSIONS

- **Male NPCs seem to be under higher oxidative stress as indicated by increased ROS and may compensate by increasing both bulk autophagy and mitophagy to remove damaged proteins and mitochondria.**
- **The lack of Nrf1 upregulation suggests that they possibly do not engage the proteasomal pathway, potentially making them more vulnerable to proteotoxic stress if autophagy becomes overwhelmed which could lead to greater vulnerability under prolonged oxidative conditions.**
- **Male cells show a stronger and distinct reaction to specific FZS and, also suggests a shift in autophagic dynamics and oxidative stress response.**
- **Minimal ROS increase in female NPCs suggests better oxidative stress resilience. Female NPCs appear to handle oxidative stress more efficiently, likely through early activation of proteasomal degradation (Nrf1-driven) and mitophagy (Fundc1-driven) before significant ROS accumulation occurs.**
- **A balanced autophagic response (moderate LC3B-I/II increase) suggests they might maintain homeostasis more effectively, preventing excessive stress build-up. This dual activation of autophagy and proteasomal degradation could contribute to female resilience under hyperoxia.**
- **In female cells, FZS show distinct responses. DHEA treatment seems to bring about a disruption in autophagic flux and could indicate a potential shift towards proteasomal degradation through Nrf1 upregulation.**

REFFERENCES

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