Developmental Regulation of Protein Degradation In The Brains

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Background

- It is widely reported that sex is an important factor involved in the development of post-traumatic stress disorder (PTSD), with females about 3 times more likely to have PTSD than males.
- In the amygdala, the emotional control region of the brain, protein degradation by the ubiquitin-proteasome system has been established as a critical mechanism for the formation of fear memories that may underlie PTSD.
- We previously found that K48 polyubiquitination, a marker of protein degradation, is increased following learning and is critically involved in the process of forming fear memories in the amygdala of male and female rats.
- Surprisingly, we also previously found that 9-week-old, young adult female rats had higher levels of K48 polyubiquitination in the amygdala, pointing to a possible mechanism for predisposition to PTSD.
- In this study, we looked into if this sex difference in K48 polyubiquitination was inherited or developmentally regulated, which could provide clues to when the sex-bias for PTSD develops in humans.
- We examined K48 polyubiquitination and DNA methylation of the major ubiquitin gene, *Uba52*, in the amygdala of prepubescent (4-week-old) and postpubescent (9-week-old) male and female rats in order to understand if the sex differences in protein degradation arose due to sexual maturity.

Methods

Subjects: A total of 40 Sprague-Dawley rats from Envigo were split into 4 groups of 10 animals: 4-week-old males, 4-week-old females, 9-week-old males, and 9-week-old females

RNA Extraction: RNA was extracted from each animal using the Qiagen All-Prep kit, converted to cDNA and PCR amplified using primers against *Uba52* with *Tubulin* as a control.

Bisulfite Sequencing: DNA was extracted using the Qiagen All-Prep kit and bisulfite converted using the Epigentek Bisulfite Treatment kit. Converted DNA was then amplified using a semi-nested PCR protocol with methyl-specific primers to a CpG island in the *Uba52* promoter region.

Protein Extraction: Each region was homogenized manually using glass homogenization tubes, and a whole-cell lysate procedure was performed with denaturing buffer with NEM to preserve ubiquitin modifications, followed by centrifuging. The supernatant of each sample was collected and protein concentration determined. Normalized amounts of protein were used for western blotting assays.

Western Blotting: Samples were loaded onto acrylamide gels, ran through SDS-PAGE, and transferred onto membranes using a Turbo Transfer system. These membranes were washed, incubated in blocking buffer for 1 hour, and then incubated overnight in a primary antibody against K48 polyubiquitin. The next day, membranes were washed and then incubated in secondary antibody, followed by washing in TBS with Tween-20 before being imaged using the Li-Cor Odyssey imaging system. After imaging, the membranes were stripped in 0.1M NaOH and rewashed in TBS with Tween-20. The membranes were then incubated overnight in Actin antibody. The next day, the membranes were washed, incubated in secondary antibody (goat anti-rabbit), rewashed, and then imaged again on the Li-Cor Odyssey system.

Figure 1: Sex differences in K48 Polyubiquitination in the Amygdala of 4-week-old vs. 9-week-old animals

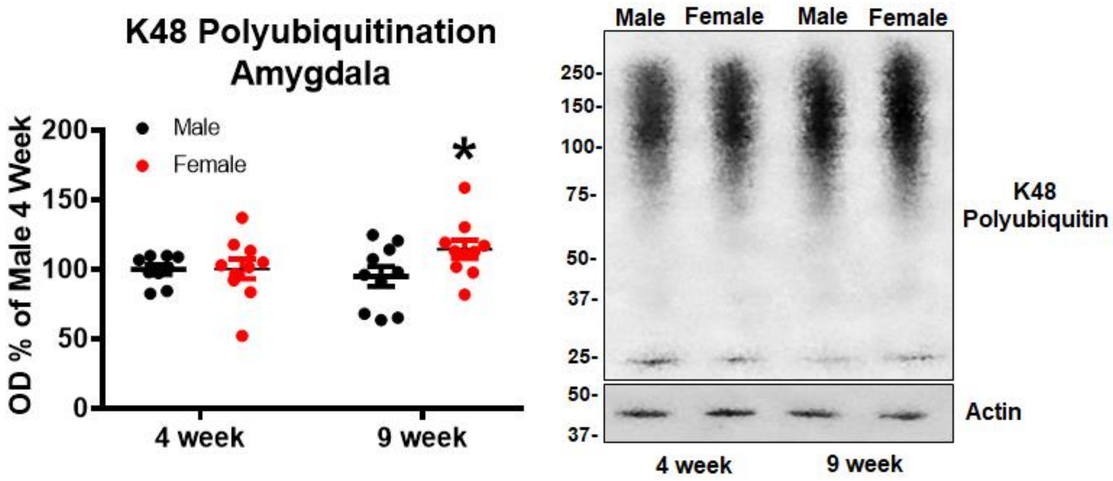
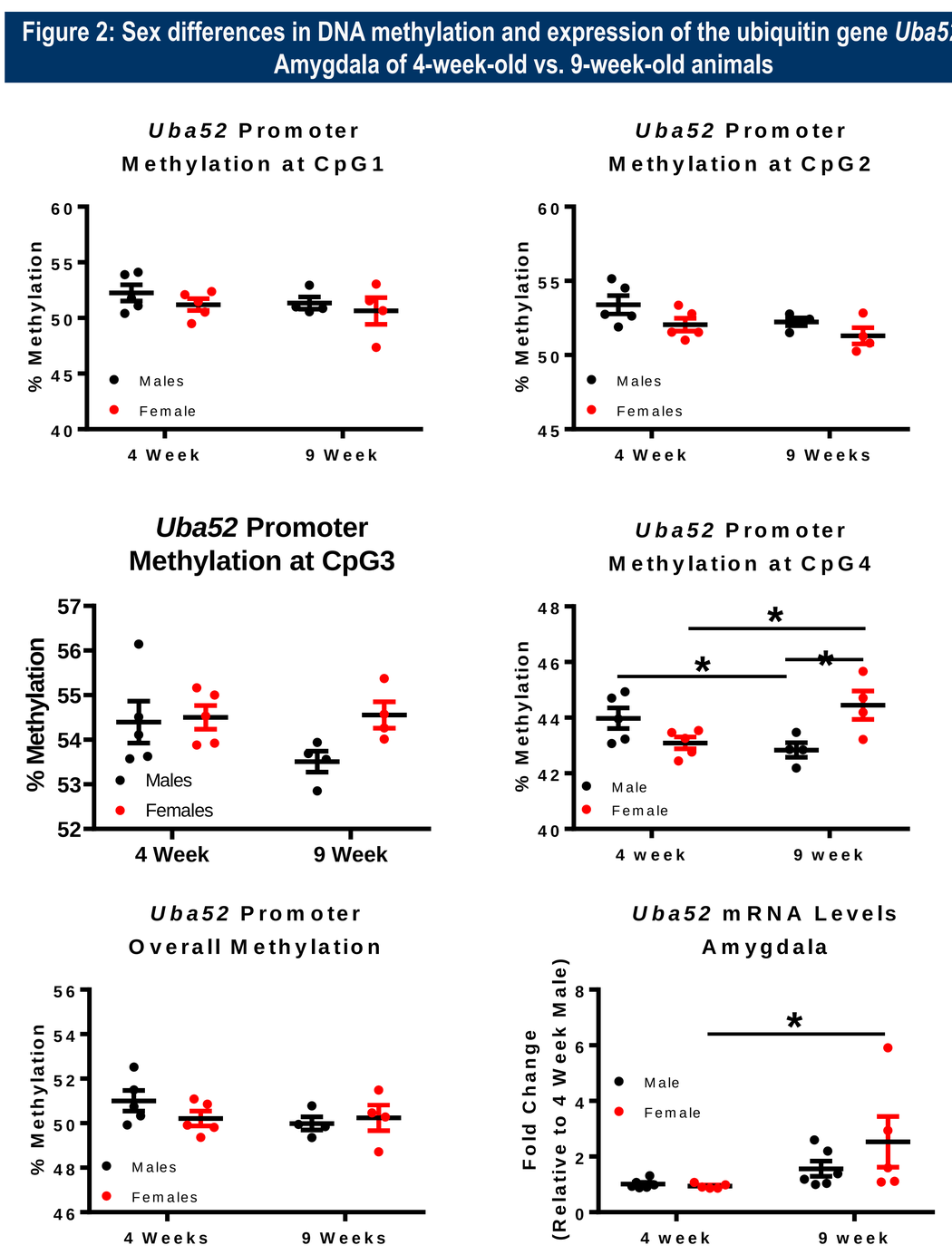


Figure 2: Sex differences in DNA methylation and expression of the ubiquitin gene *Uba52* in the Amygdala of 4-week-old vs. 9-week-old animals



Summary

- 9-week-old females, but not 4-week-old females, had increased K48 polyubiquitination in the amygdala in comparison to males.
- 4-week-old females have decreased DNA methylation of *Uba52* relative to 4-week-old males
- 9-week-old females have increased DNA methylation of *Uba52* relative to 9-week-old males and 4-week-old females
- 9-week-old females have increased expression of *Uba52* relative to 4-week-old females
- This suggests that sex differences in amygdala protein degradation occur as a result of sexual matuirty

Future Directions

- Determine if preventing sexual maturity will abolish the sex differences observed in young adulthood
- Determine sex differences in the protein targets of degradation across development – will be used to identify the functional significance of these differences

Acknowledgements

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