Exposure to Early-Life Stress Exacerbates the Effects of Inflammation on Amyloid-β and Cognitive Dysfunction

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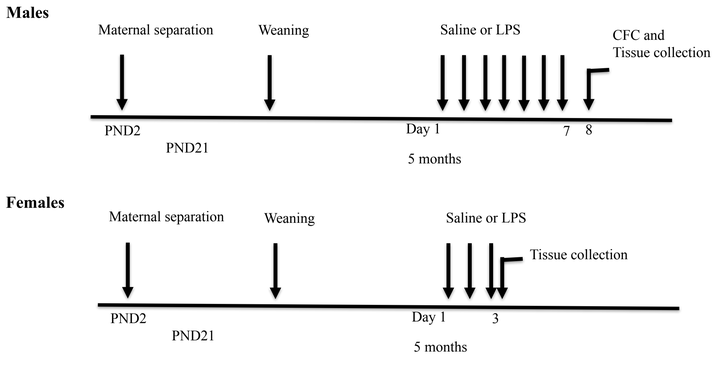
Alzheimer's Disease (AD) is the most common form of dementia and is currently estimated to affect over 5 million Americans. There is no treatment for AD, and the incidence is expected to increase as our population grows older. Many risk factors for AD have been identified, several of which involve stress and inflammation. Our lab has shown previously that repeated injections of lipopolysaccharide (LPS) produce inflammation and exacerbate Alzheimer's pathology through increasing amyloid-beta (Aβ), one of the pathological hallmarks of AD, as well as resulting in deficits in cognition. Our study aims to explore how an early life stressor, maternal separation, can impact AD pathology in adulthood. Mouse pups were separated from their mothers daily early in life and then were allowed to age normally into adulthood. Mice were then injected with LPS and cognition was assessed utilizing contextual conditioning. Tissue was collected and Aβ levels were measured. Maternal separation significantly impaired cognitive function, and exacerbated LPS-induced accumulation of Aβ. Overall, the results suggest that early-life stress exacerbates inflammation-induced AD pathologies.

Introduction

- Alzheimer's disease (AD) is a neurodegenerative disorder that affects nearly 5.5 million Americans, and there is currently no cure (1).
- Our lab has shown that 7 consecutive daily injections of lipopolysaccharide (LPS) result in an increase in hippocampal expression of amyloid beta (Aβ), along with deficits in learning and memory (2).
- Clinical research suggests that a stress can be a risk factor for the development of AD, with individuals prone to psychological stress being two times more likely to develop the disease (4).
- Maternal separation is a model of early-life stress that has been shown to have detrimental effects across the entire lifespan (3).
- We hypothesize that early-life stress will exacerbate hippocampal Aβ production in response to LPS injections and result in cognitive deficits in the C57BL6/J, non-transgenic mice.

Methods

- **Maternal Separation:** From postnatal day (PND)2, animals were separated from their mothers for 3 hours daily until PND21 in order to model early-life stress.
- Following PND21, animals were weaned, sexed, and allowed to age in a normal group-housed setting until 5 months of age.
- **Intraperitoneal Injections:** Male animals were given 7 consecutive days of LPS injections to model an acute immune insult; female animals were given 3 consecutive days of LPS injections; control groups were given either 3 or 7 days of saline injections.
- **Contextual Fear Conditioning:** After the final injection, male mice were trained in contextual conditioning (CFC) and tested the following day to assess cognitive function.
- **Tissue Collection:** 4 hours following the final injection (females) or CFC (males) hippocampal tissue was isolated.
- **Aβ Quantification:** In order to quantify the amount of Aβ in hippocampal tissue samples, an Aβ ELISA was performed.



Results

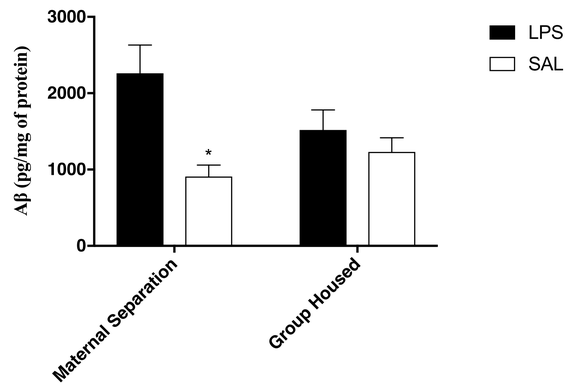


Figure 1: Amyloid-Beta Protein Concentration in Hippocampal Tissue – Females. There was a significant interaction of condition on treatment wherein maternally separated animals had significantly more Aβ following LPS injections compared to saline treated controls.

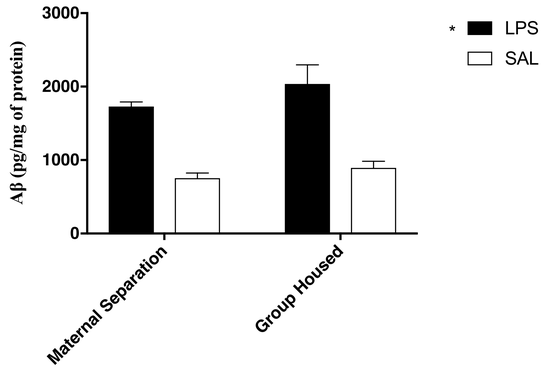


Figure 2: Amyloid-Beta Protein Concentration in Hippocampal Tissue – Males. LPS treated animals had significantly more Aβ than did saline treated controls.

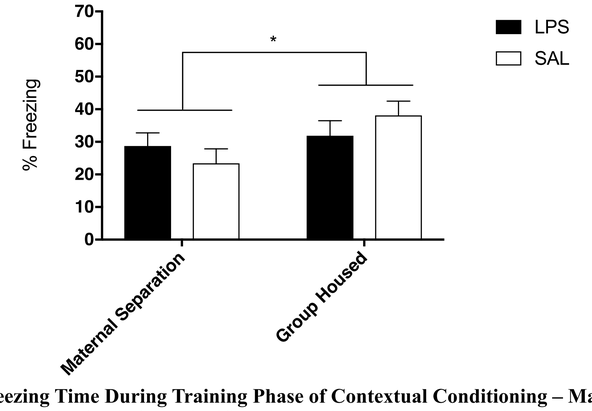


Figure 3: Freezing Time During Training Phase of Contextual Conditioning – Males. Maternally separated animals froze significantly less than did group housed controls.

Conclusions

- Cognition was impaired in animals exposed to early-life stress
- MS alone was insufficient to increase Aβ levels, but MS interacted with 3 days of LPS exposure to exacerbate Aβ accumulation in the hippocampus
- 7 days of LPS exposure increased hippocampal Aβ, but there was no significant difference between the LPS treated groups, suggesting that a ceiling in Aβ accumulation was reached.
- The overall effects of maternal separation on Aβ and cognitive function support our hypothesis, but more research is needed to understand the interaction between between early-life stress and these markers and to determine the mechanism(s) by which MS produces these effects.

Future Directions

- We plan to follow up this experiment wherein females will receive 7 days of LPS or saline, and males will receive 3 injections. CFC and Aβ quantification will be completed as described so those data can be compared. That will allow for sex-mediated differences in the response to stress to become apparent. This will also allow for the possible ceiling effect of 7 consecutive days of LPS administration to be elucidated.
- Another study will implement MS as described, followed by a single injection of LPS. We will then quantify the amount of TNF-α and IL-1β, two pro-inflammatory cytokines, in the isolated brain tissue. This will allow us to determine if the exacerbation in Aβ following LPS injections in the maternally separated animals is due to an increased inflammatory response to LPS.

References

1 – 2016 Alzheimer's Disease facts and figures

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Acknowledgments

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