Effects of Chronic Unpredictable Stress on Cognition and AD-like Pathology in C57BL/6 Mice

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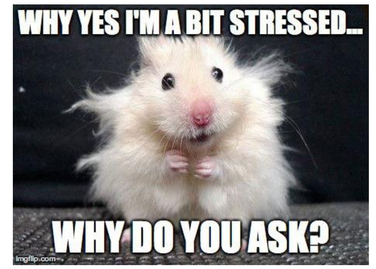
Alzheimer's disease (AD) is a progressive form of dementia marked by decline in cognitive functioning and memory loss due to protein abnormalities in the brain, and there is evidence suggesting that chronic stress can be a risk factor for exacerbating this pathology. Deficits in cognitive function often precede or coincide with the aberrant deposition of proteins such as amyloid-beta (Aβ), specifically within the hippocampus. The present study aimed to explore the relationship between chronic unpredictable stress and AD-like pathology in mice. Results showed that 21 days of chronic unpredictable stress led to cognitive deficits and increased Aβ compared to the control group, indicating that chronic stress should be investigated further as a risk factor for AD. Given that more than 77% of US adults report experiencing some form of significant chronic stress, understanding how this could impact the increasingly prevalent AD pathology is of vital importance.



Introduction

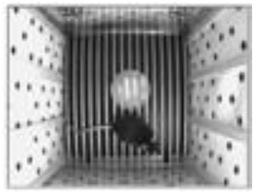
- An estimated 5.8 million people currently have Alzheimer's disease (AD), and that number is only expected to rise in the coming years.
- Aggregation of neurofibrillary tau tangles as well as deposits of amyloid-beta (Aβ) are both hallmarks of AD.
- Well over 2/3 of adults in the US report significant chronic stress.
- Psychological stress has been identified as a risk factor for numerous neurodegenerative processes, including inflammation, amyloid pathology, and hippocampal atrophy.
- Our lab has previously demonstrated that mice administered 7 consecutive days of lipopolysaccharide (LPS), a bacterial mimetic, exhibit increases in Aβ and proinflammatory cytokines in the hippocampus, as well as cognitive dysfunction.
- It is hypothesized that chronic unpredictable stress will exacerbate LPS-induced AD-like pathology in C57BL/6 mice.

Methods

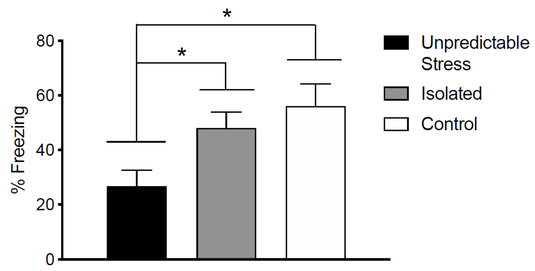
Stressors (presented in a random order each day from 8:00am-6:00pm):

- Tilted Cage (2 hours)	- Restraint Stress (30 mins)	- Empty Cage (1 hour)
- Wet Bedding (2 hours)	- Forced Swim (5 mins)	- No nestlet (overnight)

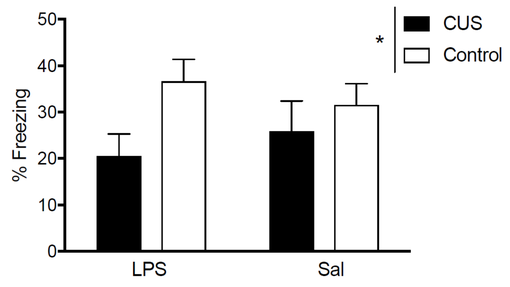
- Pilot data show that 7 days of unpredictable stress (US) is associated with impaired cognitive function in contextual fear conditioning compared to isolation stress and a group-housed control condition.
- Male C57BL/6 mice were divided into two groups – chronic unpredictable stress (CUS) and group housed controls.
- Mice in the CUS group were housed in isolation and exposed to six different stressors (listed above) presented at random for 21 consecutive days. Mice in the control group remained in their group-housed cages without any stressors.
- During the final week of the experiment, all mice received 7 days of LPS or saline injections.
- All mice underwent contextual fear conditioning after the 21-day paradigm to examine learning and memory.
- Hippocampal tissue was collected to quantify Aβ, a protein which aggregates to form plaques that disrupt neuronal communication in AD.



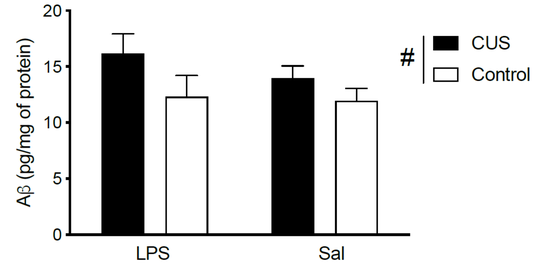
Results



Pilot Data - 7 Days of US or Isolation: Contextual Fear Conditioning. A one-way ANOVA revealed a significant effect of stress condition ($p = 0.015$). Post-hoc tests revealed that mice that underwent US froze significantly less compared to isolated mice ($p = 0.040$) and group-housed control mice ($p = 0.005$). Bars represent ± SEM. Significant differences are represented by *.



21 Days of CUS: Contextual Fear Conditioning. A 2X2 ANOVA revealed a significant main effect of stress condition, such that the mice that underwent CUS froze less compared to group-housed controls ($p = 0.045$). Bars represent ± SEM. Significant differences are represented by *.



21 Days of CUS: Hippocampal Aβ. A 2X2 ANOVA revealed a main effect of stress condition that was approaching significance, such that the mice that underwent CUS had higher levels of Aβ in the hippocampus compared to controls ($p = 0.062$). Bars represent ± SEM. Differences approaching significance are represented by #.

Conclusions

- Mice that underwent 21 days of CUS demonstrated less freezing behavior compared to the group-housed control mice, indicating cognitive dysfunction in the stressed mice.
- An effect of stress condition that was approaching significance was observed, such that mice in the CUS group had slightly elevated levels of hippocampal Aβ compared to the group-housed control mice.
- There were no observed effects of the LPS administration on cognition or hippocampal Aβ.
- 21 days of CUS alone was associated with AD-like pathology in male C57BL/6 mice.

Future Directions

- Future efforts will include repeating this 21-day CUS experiment using a different batch of LPS, as the batch used in this study contained lower endotoxin units, likely making the batch less potent (thus, less effective) compared to batches used in our lab's previous research.
- Additionally, further investigation is necessary to explore the mechanisms that could be driving the cognitive deficits and increased hippocampal Aβ observed in this study. Possible targets could include hippocampal beta-secretase (BACE1), brain-derived neurotrophic factor (BDNF), indicators of microglial activation, and proinflammatory markers in the brain and periphery.
- In order to better understand the cognitive impairments observed as a result of the stress paradigm, future experiments will include an object location task that we are currently optimizing for our lab's use in addition to contextual fear conditioning.

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