

Could Stereotypies be Caused by Oxidative Stress?

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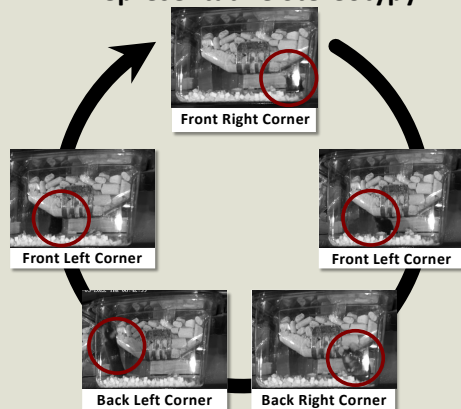
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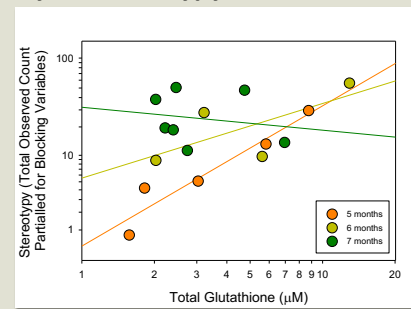
Background and Rationale

- Imagine living trapped in a body that acts on its own, often against your will. This is 'Knowledge-Action-Dissociation' and accompanies all abnormal repetitive behaviors (e.g. compulsive behaviors and stereotypies).
- Stereotypy (repetitive, unvarying, and seemingly goal-less behavior) is documented in many animal species^{1,3} and in humans with neuropsychiatric conditions.^{2,3}
- In humans, stereotypy is correlated with knowledge-action dissociation and distressing affective states.⁵
- There is strong evidence of shared pathophysiology between spontaneous stereotypy in animals and stereotypy in humans (e.g. dysregulation of the indirect pathway of the premotor corticostriatal loop and basal ganglia dysfunction in general).^{3,5}
- However, despite evidence of shared pathophysiology in animals and humans, the neurodevelopmental etiology of stereotypies remains unclear.
- Compulsive behaviors, which involve dysfunction of an adjacent corticostriatal loop³ are strongly correlated with REDOX imbalance (oxidative stress) and symptom severity is reduced via targeted antioxidant therapy⁴.
- Therefore, given the conservation of the corticostriatal loops, we hypothesized that stereotypies would also be predicted by REDOX balance.
- As glutathione is the most abundant antioxidant in the brain, here, we tested if REDOX balance (measured via plasma-based glutathione levels) predicted stereotypies in mice.

Representative Stereotypy



Do Measures of Redox Balance Predict Severity of Stereotypy?



- In the log-log multiple regression, both reduced ($F_{1,8}=7.805$; $P=0.0234$) and oxidized ($F_{1,8}=6.189$; $P=0.0311$) plasma-based glutathione positively predicted stereotypy, but each relationship weakened with age (reduced glutathione: $F_{1,8}=7.4487$; $P=0.0259$; oxidized glutathione: $F_{1,8}=5.6538$; $P=0.0447$).
- Given the similar effects of reduced and oxidized plasma-based glutathione, we repeated this analysis using total plasma-based glutathione as the only predictor. Total glutathione positively predicted stereotypy ($F_{1,10}=13.50$; $P=0.0043$), but again, this relationship weakened with age ($F_{1,10}=11.54$; $P=0.0068$).
- Standardized (to creatinine) excreted antioxidant capacity did not predict stereotypies ($F_{1,10}=0.7453$; $P=0.4082$), nor was there a significant effect of age on this relationship ($F_{1,10}=0.6223$; $P=0.4485$).

Methods

- Subjects:** $N = 17$ mice (9 males; 8 females) ranging in age from 5 to 8 months old that were slated for euthanasia.
- Housing:** We singly housed mice in standard IVC cages (Innovive) on a 12hr-light/12hr-dark cycle with food and water available *ad libitum*.
- Urine Collection:** We flash-froze freely voided urine on dry ice and stored it at -80°C for future analysis.
- Blood Collection:** We collected blood via cardiac puncture into heparin tubes. We then incubated equal amounts of plasma and 5% sulfosalicylic acid for 10 min. We centrifuged this mixture and stored the supernatant at -80°C for future analysis.
- Antioxidant Capacity Kit:** Cayman Chemical colorimetric kit (item number 703002) with urine diluted 1:80.
- Creatinine Kit:** Cayman Chemical colorimetric kit (item number 500701) with urine diluted at 1:20.
- Glutathione Kit:** Arbor Assay fluorescent kit (item number K-006F) with plasma diluted 1:5 with assay buffer.
- Behavioral Recordings and Coding:** We unobtrusively recorded home-cage video of mice. A trained observer scored 24 hours of footage.
- Statistical Analysis:** All analyses were performed using GLM in JMP16 Pro for Windows. All analyses were blocked for sex, strain, and age, and tested for effects of analyte and the interaction of analyte and age. Stereotypy is the sum of periods in which jumping, bar-mouthing, or route tracing were observed. All measures were log-transformed to meet the assumptions of linear methods. The ratio of antioxidant capacity to creatinine was tested independently. For reduced glutathione and oxidized glutathione, we first performed a multiple regression with the log of both measures as predictor variables. This allows us to ask whether both reduced glutathione and oxidized glutathione are elevated (in which case both would have positive coefficients); or if the ratio of the two is more important (in which case oxidized glutathione should have a negative coefficient). Given that reduced glutathione and oxidized glutathione both had positive coefficients, we did not look at the ratio of reduced glutathione to oxidized glutathione, and instead performed a secondary analysis using total glutathione.

What Does This Mean?

- Plasma-based total glutathione is predictive of stereotypies in mice.
- The relationship between total glutathione and stereotypies diminishes with age.
- The relationship between glutathione and stereotypies in mice parallels the relationship between glutathione and compulsive behaviors in mice and humans.
- These results further support the interpretation of stereotypies as an indicator of neural injury and poor animal welfare.

Why Do We Care?

- Given the established relationships between knowledge-action dissociation and stereotypies in mice and humans, and that these behaviors are ego-dystonic in humans, we hypothesize that stereotypies are also distressing for mice.
- The results from this study suggest REDOX imbalance as a potential neurodevelopmental etiology of stereotypy and highlight potential intervention strategies.

What's Next?

- Investigate therapeutic potential of antioxidants in preventing or mitigating severity of stereotypy in mice.
- Examine the relationship between stereotypies and plasma-based measures of glutathione in clinical populations.



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