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Mixed Mood State Behaviors and Circadian Dysfunction following Homocysteic Acid Treatment: Potential Animal Model for Bipolar Disorder

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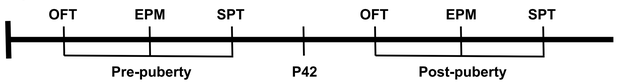
Background

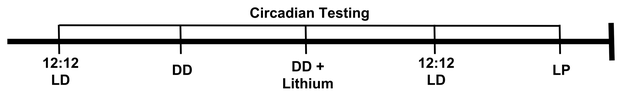
- Bipolar Disorder (BPD), which affects 3% of the world's population, is characterized by manic and depressive episodes, along with abnormal sleep-wake patterns (4).
- Elevated levels of homocysteine and its metabolite homocysteic acid (HCA) have been associated with schizophrenia, depression, and bipolar disorder (2).
- An animal model for BPD with face, construct, and predictive validity has yet to be established (1).
- The working hypothesis was that the administration of HCA to rat pups would result in an animal model with mixed mood state behaviors.
- Prepubescent emergence of manic and depressive-like behaviors have not been previously examined (1), and may illuminate important developmental markers for early recognition of BPD.
- In the present study, behavior and circadian rhythms were analyzed in rats injected with either HCA or saline solution from postnatal days P3-P21.
- Rats were tested in the following apparatuses:
  - Open field test (OFT) for locomotion and anxiety-like behaviors
  - Elevated plus maze (EPM) for risk-taking behaviors
  - Saccharin preference test (SPT) for reward-seeking behaviors
- Behaviors were measured pre- and post-puberty to assess the effects of HCA on the emergence of BPD-like behaviors across development.
- BPD patients present abnormalities in circadian rhythmicity and sleeping patterns. HCA-treated rats exhibit disrupted *CLOCK* expression in the prefrontal cortex (3).
  - Circadian analyses of singly-housed rats using infrared sensors were used to examine any treatment-specific differences in activity levels and circadian rhythmicity.
- We predicted that HCA treated rats would exhibit significant abnormalities in circadian rhythmicity, characterized by changes in circadian period and alpha.

Methods

- From P3-P21, Sprague Dawley rat pups received daily intraperitoneal injections of HCA (0.8 mmol/kg) or saline solution (0.8 mmol/kg)
- The following behavioral tests were performed to assess BPD-like behaviors. Prepubescent behavioral tests were performed prior to P42, and post-pubescent behavioral tests were performed after P42.
- Open Field Test (OFT); Exploration of apparatus for 5 minutes
- Elevated Plus Maze (EPM): Exploration of apparatus for 5 minutes
- Saccharin Preference Test (SPT) for 6 days
- Days 1-2: Both bottles contain water
- Days 3-4: Choice between water and 0.033% saccharin
- Days 5-6: Choice between water and 0.066% saccharin



- After P42, HCA and saline female rats were individually housed in cages with overhead Infrared Motion Detectors. Activity was counted in one minute intervals.
- Rats were placed in 12:12 light-dark conditions (LD) for 3 weeks
- Placed under constant darkness conditions (DD) for 2 weeks
- Placed under constant darkness conditions (DD) + Lithium for 4 weeks
  - Lithium: 2 g/1000 g of food
- Return to 12:12 light-dark conditions for 2 weeks
- 2 hour light pulse (LP) administered, from Zeitgeber time (ZT) 13-15 during 12:12 LD



Anxiety is not affected by HCA treatment

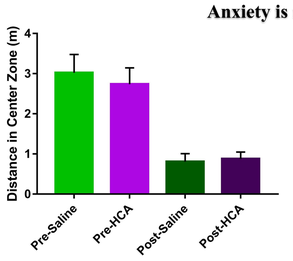


Figure 1. Distance in center zone of Open Field Test. The distance in the center zone of the OFT was not affected by HCA-treatment either pre-puberty (t_{66} = .462, p = .645) or post-puberty (t_{66} = -.264, p = .793).

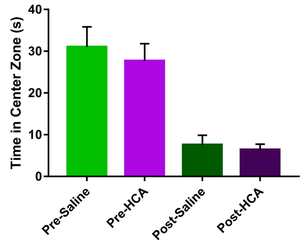


Figure 2. Time in center zone of Open Field Test. The time in the center zone of the OFT was not affected by HCA-treatment either pre-puberty (t_{66} = .530, p = .598) or post-puberty (t_{66} = .450, p = .654).

Risk-taking behavior is affected by HCA treatment

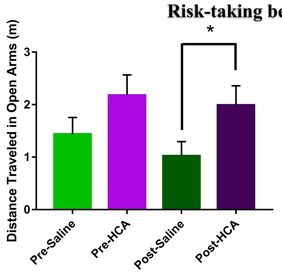


Figure 3. Distance in open arms of Elevated Plus Maze. Distance in open arms of EPM was significantly affected by HCA-treatment post-puberty (t_{66} = -2.081, p < .05) but not pre-puberty (t_{66} = -1.453, p = .151).

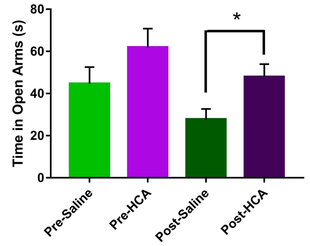


Figure 4. Time in open arms of Elevated Plus Maze. Time in open arms of EPM was significantly affected by HCA-treatment post-puberty (t_{66} = -2.543, p < .05) but not pre-puberty (t_{66} = -1.435, p = .156).

Depressive-like behavior is affected by HCA treatment

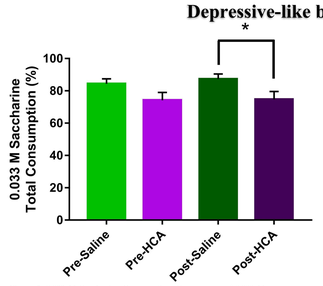


Figure 5. 0.033 M Saccharine Consumption. Consumption of 0.033 M saccharine was significantly affected by HCA-treatment post-puberty (t_{66} = 2.065, p < .05) but not pre-puberty (t_{66} = 1.699, p = .094).

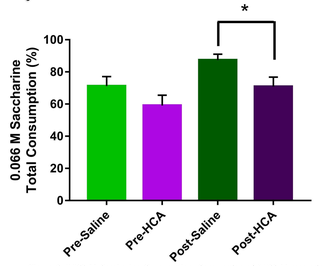


Figure 6. 0.066 M Saccharine Consumption. Consumption of 0.066 M saccharine was significantly affected by HCA-treatment post-puberty (t_{66} = 2.266, p < .05) but not pre-puberty (t_{66} = 1.391, p = .169).

Period is affected by HCA treatment, but can be reversed with Lithium treatment

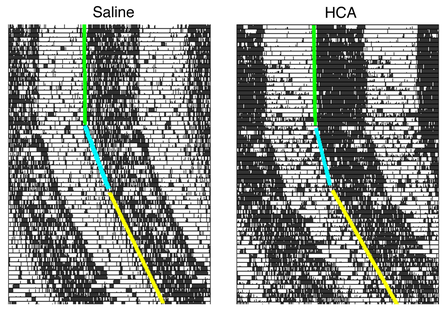


Figure 7. Representative Actograms. The actograms present 3 weeks of 12:12 light/dark (LD, green line), 2 weeks of constant darkness (DD, blue line), and 4 weeks of lithium treatment in DD (yellow line) for a Saline female and HCA female.

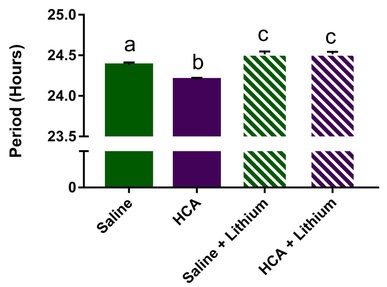


Figure 8. Circadian Period Between Treatment Groups. Female HCA-treated rats had a significantly shorter circadian period in constant darkness conditions than saline controls (t_{34} = -6.777 , p < 0.001), and saline + lithium along with HCA + lithium rats had a significantly longer circadian period than without lithium (t_{23} = 3.212 , ps < 0.005).

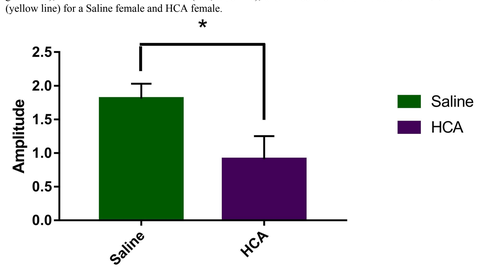


Figure 9. Amplitude of Circadian Rhythm. HCA-treated females had a significantly lower circadian amplitude when compared to the saline group (t_{34} = -4.524 p < .001). Amplitude measurements were averaged over 7 days of constant darkness conditions using ClockLab software.

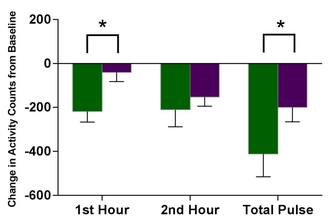


Figure 10. Change in Activity from Baseline Day During 2 Hour Light Pulse. HCA-treated female rats exhibited a significantly dampened response during the 1st hour of a 2 hour light pulse during 12:12 LD beginning at ZT13 as compared to Saline-treated rats (t_{34} = 3.92, p <.001).

Discussion

- Prior to puberty, mixed mood state behaviors were not observed in HCA-treated rats.
- After puberty, HCA-treated rats displayed an increase in risk-taking behaviors in the Elevated Plus Maze (EPM).
- BPD patients in manic episodes present increases in risk-taking and exploratory behavior (1).
- On days 4 and 6 of the post-puberty saccharin preference test, HCA-treated rats consumed a significantly lower proportion of 0.033% and 0.066% saccharin solution respectively compared to Saline-treated rats, indicating anhedonic-like behavior.
- During depressive episodes, BPD patients present decreases in reward-seeking behaviors or anhedonia (1).
- HCA-treated female rats display a lower circadian amplitude and period compared to Saline-treated rats during constant darkness (DD), indicating an effect of HCA-treatment on the circadian clock. Lithium treatment reversed this phenotype.
- BPD patients often present with abnormalities in circadian rhythmicity and sleeping patterns (3).
- HCA-treated female rats present a decrease and delay in the suppression of activity as an acute response to light during a 2-hour light pulse at ZT13 during 12:12 light/dark.

Conclusions

The effects of HCA treatment on circadian function and post-pubescent behavior in rats align with a mixed mood state, similar to symptoms seen in Bipolar Disorder patients. Thus, providing face validity for the HCA animal model of Bipolar Disorder.

Future Work

- Analyze male circadian patterns
- Use brain samples to analyze abnormalities caused by the HCA treatment on the SCN and hippocampus
- Microarray to determine genetic effects of HCA

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