Impaired neurobehavioral function in Belgrade rats with iron loading anemia, a model of thalassemia

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Summary
Thalassemia (Cooley’s anemia) is a hereditary anemic disease due to mutations in globin synthesis and therefore requires blood transfusion. This treatment increases iron levels in the circulation and promotes iron loading anemia. Although iron is essential for brain development and monoamine homeostasis, iron loading in the brain is implicated in several neurological problems. However, whether or not iron loading anemia modifies cognitive and emotional behavior is largely unknown. In order to characterize the neurobehavioral performance, we used Belgrade rats, a model of iron loading anemia that resembles thalassemia. Our results suggest that iron loading anemia is associated with decreased anxiety and impaired motor coordination, possibly due to altered dopamine metabolism and elevated D1DR signaling.

Background and Significance
- Iron is an essential element for blood production, cell growth and development of the brain, including myelination and neurotransmitter synthesis. Proper iron transport is critical in maintaining homeostasis. A strong relationship exists between iron status and neurobehavioral functions [1-2].
- The Belgrade rat carries DMT1 mutation and exhibits severe anemia with hepatic iron overload. The complexity of iron metabolism in Belgrade rats triggered many investigations associated with hepatic iron absorption and hemoglobin characteristics.
- Iron transport

Methodology
- Animals: Homozygous Belgrade rats (b/b) and control heterozygous rats (+/b) (11-12 weeks old) were fed iron-supplemented diet (500 mg iron/kg diet) for 5 weeks and during which were subjected to a battery of behavioral tests.
- Behavioral tests:
  - Novel Object Recognition (NOR)

Results

Elevated plus maze (EPM)

Figure 2. EPM consists of two open arms and two closed arms. More anxious rats spent more time in closed arm and took longer time to explore the open arm.

Rotarod

Figure 3. Rotarod is a rotating treadmill to test the motor functions of rodent animals.

Figure 4. Physiological parameters in Belgrade rats

A Body weight

B Hematocrit

C Blood Fe

D Liver Fe

Figure 5. NOR

A Time spent with novel object

B Entries of novel object zone

C Time spent with novel object

D Entries of novel object zone

Figure 6. EPM

A Time spent in the open arm

B Time spent in the closed arm

C Total distance

Figure 7. Rotarod

A Time of first drop

B Speed of first drop

Figure 8. Dopamine-related proteins in the striatum of b/b rats

Figure 9. Monoamine transporters in the striatum of b/b rats

Conclusions
- Homozygous Belgrade rats (b/b) which display iron loading anemia, are associated with decreased anxiety-like behavior and impaired motor function.
- Western blotting analysis indicated that dopaminergic pathway and monoamine metabolisms were altered in b/b rats. D1 signaling was elevated by increasing D1R in b/b. On the other hand, b/b rats showed decreased expression levels of NET, VMAT and COMT, indicating altered dopaminergic pathway and monoamine metabolisms in b/b rats.

Future studies
- Besides iron loading control, include dietary anemia heterozygous rats to examine if the changes in behavior of Belgrade are due to anemia, gene effect or interaction.
- To investigate changes in extracellular dopamine levels using microdialysis.

Reference: