



# Impaired Learning and Behavioral Adaptation in the SSBN.13 Consomic Rat

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## Abstract

Chromosomal substitution strains of rat, termed consomic rats, are powerful rodent models for dissecting multigenic traits and positional cloning of complex human disease genes. The consomic rats are created by introgressing a single chromosome from a "normal" strain onto the genetic background of a "disease" strain. In the present experiment, we compared performance of the SS.BN13 strain of consomic rats with the Dahl salt sensitive (SS) and Brown Norway (BN) parent strains as well as Long Evans (LE) Hooded rats on a battery of learning tasks. The battery of tasks included the Morris Water Maze (MWM) to assess visuospatial learning, the Porsolt Swim Test as a measure of depression and learned helplessness, and the Rotorod to evaluate motor skill learning and motor coordination. The first phase of the MWM consisted of three days of acquisition training in which the rats utilized abundant visuospatial cues to locate a hidden platform. The second phase of the MWM tested over a period of two days whether the animals had assimilated the desired learning strategy by moving the hidden platform to a novel location. Both the SS.BN13 and LE animals acquired the initial location of the hidden platform faster than the SS or BN strains. The results from the novel location test on day 4 revealed significant perseveration on the part of the SS.BN13 consomics relative to the LE animals. For the Porsolt Swim Test, rats were placed in a 10-liter beaker of water and the duration of immobility was recorded during a 15-minute exposure and followed 24 hours later by a 5-minute test period. The SS rats were found to be the most active while the BN were found to be the most immobile. The SS.BN13 rats displayed a 2-fold increase in immobility during the testing phase. The Rotorod consisted of three trials during which the latency to fall was recorded. The SS.BN13 rats performed as well as the SS rats by trial three, however motor skill learning was smaller in the first two trials. The results of the test battery indicate that the substitution of the BN chromosome 13 into the SS genetic background significantly alters the behavioral phenotype of the SS rat. The SS.BN13 rat displays poor learning and a failure to adapt to novel conditions. Subsequent experiments will test the extent to which these behavioral alterations occur as a result of a reported resistance to vascular plasticity in the SS.BN13 rats.

## Introduction

Chromosomal substitution strains of rat, also known as consomic rats, were originally developed by researchers at the Medical College of Wisconsin as a rapid way to map quantitative trait loci throughout the genome and then to facilitate positional cloning of causal genes for complex human diseases such as cardiovascular disease, hypertension and end-stage renal disease. Consomic rats can be considered "natural" models used to study complex disease because all genes are intact (no gene knock-outs or knock-ins) and surgical and chemical induced lesions may not be required to elicit a desired phenotype. provide a useful tool for the positional cloning of human disease states. This substitution can be accomplished by introgressing a single chromosome from a "normal" animal into the genetic background of a "disease" strain.

Previous research has shown an impairment of vascular plasticity in Dahl SS animals (Lee et al., 2007). Additional research has found that chromosome 13 contains several genes involved in the plasticity of the vascular system such as VEGF, angiotensin II, and renin (Amaral, Roman, & Greene, 2001). Thus, a behavioral phenotype of the SS.BN13 animals – in which chromosome 13 of the Brown Norway animals had been introgressed onto the genetic background of the Dahl SS animal - was desired to investigate the possibility that any behavioral alterations are the result of this vascular impairment. In this experiment, the SSBN.13 consomic rat was compared to the Dahl salt sensitive (SS) and Brown Norway (BN) parent strains as well as to the Long Evans Hooded rats (LE), in a battery of behavioral tasks including the Rotorod (RR), Porsolt Swim Test (PST), and the Morris Water Maze (MWM). It is hypothesized that there will be significant differences between the SS parent strain and the SS.BN13 consomics on all behavioral tests.

## Methods

### Procedure

#### Subjects

Four strains of rats were used in the following experiment. There were 25 male Long Evans hooded rats (Harlan), 25 male BN, 20 SS, and 10 SS.BN13 (Charles River) All rodents were maintained on a 0.4% salt diet (Harlan-Teklad) and housed individually in standard shoebox cages. Food and water were available ad libitum. Rodents were maintained on an 12 hour light/dark cycle.

#### Morris Water Maze

The MWM was used to assess visuospatial learning. Training took place in a 5 foot wide, 3 foot deep pool filled with approximately 26 inches of water. Submerged 3 cm below the surface of the water was the platform which was hidden from view by packing peanuts. Rats were given four trials each day, one from each cardinal direction (N, S, E, W). There were approximately five minutes between trials. During the acquisition phase of training (first 3 days), the platform was placed in the NE quadrant (defined as the target quadrant). Following three days of acquisition, the platform was moved to the SW quadrant. Animals were tested for two days with the platform in the novel location. For each day of training, the success to find the platform, latency to find the platform, total quadrants entered, and entries into the target quadrant were recorded.

#### Porsolt Swim Test (PST)

Animals were exposed to the PST as an indicator of learned helplessness and depression. Testing took place in a 10-liter beaker of water separated by wooden partitions. Animals were given two trials, one day apart. During the first (exposure) trial, animals were placed in the beaker for 15 minutes. On the second day – the testing trial – animals were placed in the beaker for 5 minutes. During the exposure trial, the amount of time of immobility was timed for only the first 5 minutes; during the testing trial, all 5 minutes were scored.

#### Rotorod

Motor coordination and motor learning were assessed on the rotorod. Rats were placed on the rotorod and latency to fall was recorded, with a maximum possible latency of 300 seconds. Small cohorts of animals were tested during the session, and approximately 90 minutes rest was given time between trials.

#### Analysis

All testing was assessed using the Statistical Package for the Social Sciences (SPSS) version 13 software.

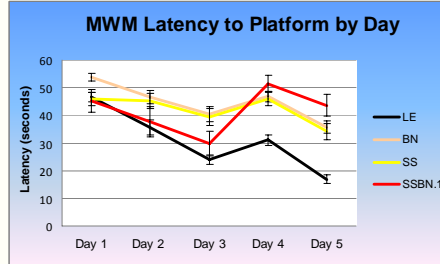


Figure 1 (left). The SS.BN13 animals successfully acquired the task, learning the location of the hidden platform during the acquisition phase of the task (days 1-3). The SS parent strain failed to acquire the task as indicated by a consistently greater latency to reach the platform compared to the SS.BN13 consomics. Upon moving the platform to a novel location, however, the SS.BN13 animals displayed severe (day 4) perseveration as indicated by a great increase in latency from day 3 to day 4 (p<.000)

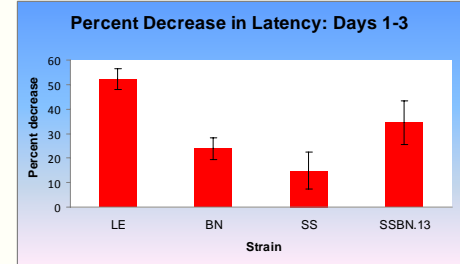


Figure 2 (right). Throughout acquisition, the SS animals displayed the smallest decrease in latency to reach the platform, whereas the SS.BN13 animals performed comparably to the LE control strain. This difference (between SS and SS.BN13 consomics) however, did not reach statistical significance.

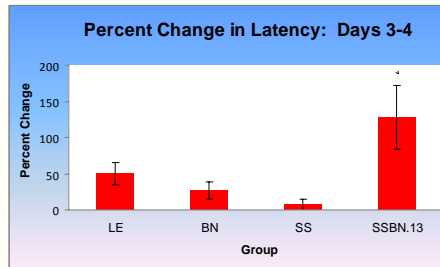
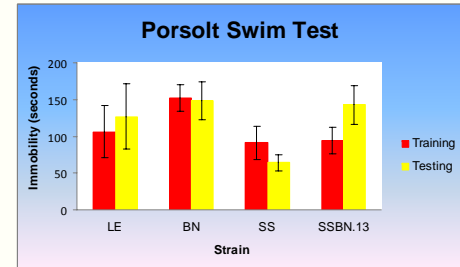


Figure 3. SS.BN13 animals showed a significantly greater percent change in latency from day 3 to day 4 compared to the SS animals (p<.01).

Figure 4 (right). During the 5-minute testing trial, the SS.BN13 animals were immobile for significantly more time than the SS parent strain (p<.05).



## Conclusions, Implications, and Future Directions

- Data indicate that insertion of the BN chromosome 13 onto the genetic background of the Dahl SS rat results in a unique behavioral phenotype.
- The Dahl SS parent animals appear to be poorer learners when compared to the SS.BN13 animals as indicated by a failure to acquire the location of the hidden platform during the acquisition phase of the MWM. Similarly, these animals fail to learn the immobility response during the testing phase of the PST.
- Insertion of chromosome 13 has resulted in a savings or a rescuing of some of the behaviors assessed – SS.BN13 animals are able to acquire the MWM task, and show an increase in the time immobile in the PST.
- However, it is notable that not all behaviors have been rescued in the SS.BN13 animals. For example, they display a severe perseveration on day 4 of the MWM, and their motor learning is delayed as shown by their performance on the RR.
- The use of chromosomal substitution rat strains, consomic rats, is an effective tool in establishing unique, characterized phenotypes.
- Future studies will investigate the physiological processes, specifically the vascular plasticity, that may underlie these changes in behavioral phenotype.

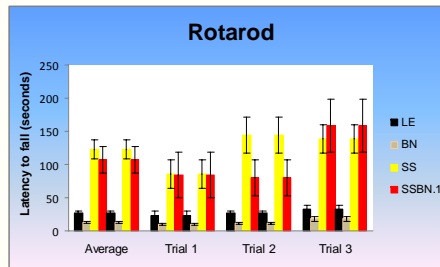


Figure 5. SS and SS.BN13 animals perform comparably on this task. However, SS.BN13 animals perseverate, being slower to learn the motor skills necessary to remain on the rotorod.

## References

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Lee, J. F., Didier, D. N., Luckett, M. R., Sealf, M., Greene, A. S., Oliver, M., et al. (2007). Characterization of vascular endothelial growth factor receptors on the endothelial cell surface during hypoxia using whole cell binding arrays. *Analytical Biochemistry*, 369, 241-247.