Title: Modeling Autistic-Like Behavior In The BTBR T+Tf/J Mouse: Testing Juvenile Play Behaviors In Females.

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Introduction

Autism is a neurodevelopmental disorder characterized by three cardinal traits:

- Deficits in social interactions
- Deficits in communication
- Repetitive and stereotyped behaviors.

Previous work has established that the BTBR mouse strain has good face validity to the cardinal traits of autism (McFarlane et al., 2008). However, the previous work exploring the BTBR mice was done in males because there is a 4:1 male: female ratio in the disorder. In order to strengthen the model, we need to assess the females' behaviors relevant to the disorder. Therefore, the present study assessed play and social behavior in BTBR females using the same protocols that we used for the males. We expected that unlike the males, the females should perform similarly to controls (B6 mice) on tests of play and social behavior.

If our results show that the females performed similarly to controls on most measures of social and play behavior they would demonstrate that the social and play deficits seen in the male BTBR mouse are not as prevalent in the females of the strain. This sex difference is similar to what is seen in the autism. Therefore, these results would support and strengthen the use of the BTBR mouse as a model strain for testing both the etiology and novel therapeutics for autism.

Measures of Play

Investigative

Nose-to-nose sniff: sniffing of the head and snout region of the partner.
Anogenital sniff: sniffing the partner's anogenital region.


Affiliative

Social grooming: allogrooming, one mouse grooms the other mouse on any part of the body.
Social inactive: close physical contact, while lying or standing still.

Play soliciting

Crawl over: traverses the partner's body by crawling over the back from one side to the other.
Crawl under: traverses the partner's body by crawling under from one side to the other.

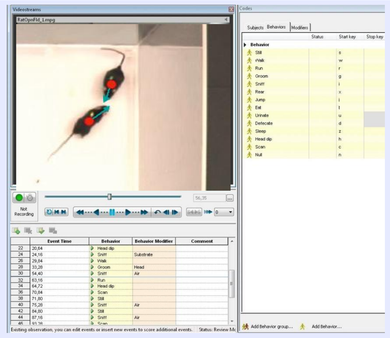
Nonsocial behaviors

Self-grooming: mouse grooms any part of its own body.

Do BTBR Female mice have deficits in social interactions?

Statistics

The data were analyzed using multivariate analysis of variance (MANOVA), with a post hoc ANOVA. Significance was set at $p = 0.05$.



Procedure

- Fifty-six mice from two strains (BTBR T+tf/J and C57Bl/6J) bred at Kenyon College were used.
- Juvenile play testing were carried out on PND 21.
- Experiments took place during the first half of the dark phase.
- Test rooms were lit with a single red light. Test chambers were cleaned with 70% alcohol between test subjects
- One day before testing the mice were brought into the testing room to acclimate and habituate to the experimental conditions and procedures.
- The next day the mice were brought to the testing room again, weighed, marked, and housed individually.
- Behavior with a non-sibling, matched mouse was recorded for 30 minutes, by the Noldus Phenotyper.
- Frame-by-frame analysis of the recorded videos was conducted after the testing was completed.
- The video files were coded so that the investigator was blind to the strain of the play pair while doing the video analysis.

Fig. 1

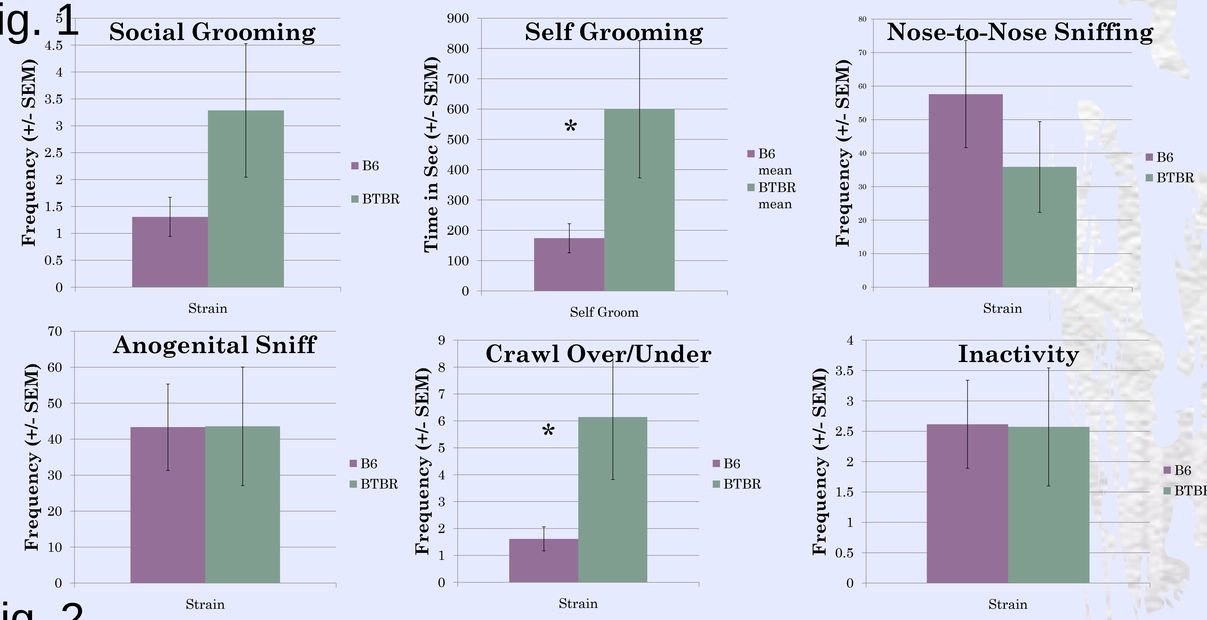


Fig. 2

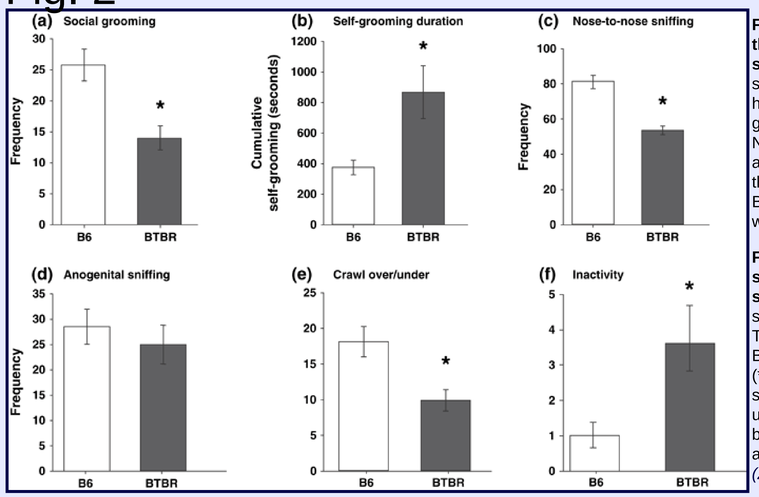


Figure 1: Juvenile play in 21-day-old female B6 and BTBR mice, of the same strain for different litters, during a 30-min videotaped test session. *n* =14 pairs of B6, 7 pairs of BTBR. (a) Number of bouts of social grooming showed trends to B6 involved in less grooming, however, was not significant to p<.05 (b) Total time spent in self-grooming was significantly higher in BTBR than B6 (*$p < 0.001$). (c) Nose-to-nose sniffing was similar (d) sniffing the anogenital region was also similar between strains. (e) Number of times that one member of the pair crawled over or under the other was significantly higher in BTBR (*$p < 0.001$). (f) Number of bouts in which one member of the pair was inactive was similar between strains.

Figure 2: Juvenile play in 21-day-old male B6 and BTBR mice, of the same strain from different litters, during a 30-min videotaped test session. *n* = 14 pairs of B6, 13 pairs of BTBR. (a) Number of bouts of social grooming was significantly less in BTBR than in B6 (*$P < 0.01$). (b) Total time spent in self-grooming was significantly higher in BTBR than B6 (*$P < 0.001$). (c) Nose-to-nose sniffing was significantly less in BTBR (*$P < 0.0001$), while (d) sniffing the anogenital region was similar between strains. (e) Number of times that one member of the pair crawled over or under the other was significantly less in BTBR (*$P < 0.001$). (f) Number of bouts in which one member of the pair was inactive within the social arena was greater in BTBR (*$P < 0.05$). *Adapted from McFarlane et al., (2008).*

Discussion

Unlike our previous study of the males, females BTBR mice did not perform significantly different from the B6 on most behaviors. Only two behaviors were significantly different in females: time spent self-grooming [F(1,18)=52.5, p<.001] and a higher frequency crawling over or under the playmate [F(1,18)=18.667, p<.001]. Unlike the males who showed decreased social behaviors, the female BTBR mice appear to spend more time soliciting play and being socially aggressive as demonstrated in the frequency of crawl under/over occurrences. All other social behaviors appear consistent with that of the controls. Although self-grooming behavior is elevated in both sexes of BTBR mice, the males spend more time on this behavior than the females. There are slight trends to other social differences in the females, however it appears that significant social deficits are much more prevalent in males. These results are in keeping with what is observed in autism; namely, that human males are four times more likely than human females to show the disorder. These data support the use of the BTBR mouse as a model for autistic-like behaviors; specifically, this strain makes a good model for studying both the etiology of the disorder as well as for testing novel treatments.

Future

Further testing of the BTBR females is necessary to ensure their results remain consistent through open field testing. In addition, it is important to evaluate whether female BTBR mice show higher self-grooming durations when alone, a behavior observed in males (*data not shown*). This would determine whether the observed elevation in self-grooming is due to the stress of social interactions.

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