Autistic behavioral differences between female mouse strains BTBR T+tf/J and C57BL/6 mutant

Advisior:

Bong-Kiun Kaang

A dissertation submitted to the
Graduate Faculty of Seoul National University in
partial fulfillment of the requirement for the
Degree of Master of Science

Junehee Son

Department of Brain and Cognitive Sciences
Graduate School of Natural Sciences
Seoul National University

Contents

List of Figures

Abstract

Autistic behavioral differences between female mouse strains BTBR T+tf/J and C57BL/6 mutant

Junehee Son

Department of Brain and Cognitive Sciences

Graduate School of Natural Sciences

Seoul National University

Autism is a neurodevelopmental disorder that is characterized by aberrant social interaction and communication deficits, and repetitive behavior (American Psychiatric Association, 2013). As increasing number of children has been diagnosed with autism, the awareness on autism and importance of researches are also growing.

In most mouse behavioral studies, male mice are frequently used while behavioral studies with female mice are rare. As result, there is only insufficient information on female mouse behavioral

phenotypes, and more information is needed.

In this research, we used female autistic spectrum disorder model mice (BTBR T+tf/J and Shank2 *Δ*e6-7 knockout and wild type) and naïve female C57BL/6 mice to find out autistic behavioral phenotypes of female mice. Analysis from open field test shows that both autism model mouse groups have significantly more hyperactivity than their control groups, but anxiety level was not statistically meaningful between experimental and control groups. Increased mobility in autism models agrees with previous researches on male autism model mice. In ultrasonic vocalization test, BTBR T+tf/J tended to emit smaller amount of vocalizations than its comparison group. This observation reflects a typical behavioral phenotype of main autism symptoms. Both autism model groups showed impairment in retrieving pups. It is interesting to note that both BTBR T+tf/J and Shank2 *Δ*e6-7 knockout(KO) mice showed decrease repetitive behavior than their controls in marble burying test, and this result contrasts previous reports on male mice with autism like behaviors (Spencer et al., 2011; Amodeo et al., 2012). This observation may imply less sustained behavioral pattern, however we suggest it may reflect a lack of motivation, which is one of important autism symptoms. From water based Y-maze test, we found out BTBR T+tf/J, Shank2 *Δ*e6-7 wild type (WT) and C57BL/6 mice have normal learning abilities while Shank2 *Δ*e6-7 KO exhibited a severe impairment in learning.

Key words: autism, female mouse behavior, ultrasonic vocalizations, BTBR T+tf/J, Shank2 Δe6-7, ASD

Student number: 2009-23854

Introduction

Autism is a neurodevelopmental disorder that is characterized by lack of social interaction and communication, and abnormal repetitive and restricted behavior (American Psychiatric Association, 2013). According to the US centers for disease control and prevention, increasing number of children are diagnosed to have an autistic spectrum disorder (Centers for Disease Control and Prevention, 2014). The fact that more children are identified as having autistic spectrum disorder does not necessarily mean that the prevalence of autism is increased, at lease it shows an increasing number of people has developed awareness of autism and its identification.

Over the past few decades, researchers have developed rodent models of autism, such as BTBR T+tf/J and Shank2 *Δ*e6-7 knockout (KO) mice. BTBR is an inbred strain, which are known to exhibit all three behavioral symptoms of autism (Silverman et al., 2013), and their lack of sociability is not dependent of their interaction partner's strain (Yang et al., 2012). Therefore, they are often used in various autism studies (Moy et al., 2006; Amodeo et al., 2012; Yang et al., 2012; Meyza et al., 2013; Silverman at al., 2013; Martin et al., 2014). Shank2 *Δ*e6-7 KO is also one of most widely used mouse model for autism (Schmeisser et al., 2012; Won et al., 2012).

Although autistic spectrum disorders are more common in boys than girls, female behavioral investigation should not be neglected. In most rodent behavioral studies, male rodents are mostly used as subjects rather than females, except for a few experiments that are specifically designed for female subjects such as pup retrieval test, and therefore there are not sufficient reports on female mouse behaviors, more information is needed. Hence, in this study, we performed several behavioral experiments to investigate autistic behavioral phenotypes of female autism model mice and seek better understanding of fundamental mechanisms of autistic spectrum disorders.

Experimental Procedure

Animals

Twenty six 10 to12 weeks old BTBR T+tf/J, twelve C57BL/6 mice at the equivalent age, five 8 to 10 weeks old Shank2 *Δ*e6-7 knockout mice, and eight Shank2 *Δ*e6-7 wild type (WT) littermates at the same age were used as subjects in this study. Also, six 5 to 6 weeks old C57BL/6 mice were used in the ultrasonic vocalization test as an intruder, and twelve postnatal day one to two (P1-P2) C57BL/6 pups were used in the pup retrieval test. All animals used in this experiments were female.

There were two different housing conditions were used in this study; group caging and double caging. Three to four mice shared one cage for the group caging, while two mice were caged together for the double caging. After completing open field test and marble burying test, mice were transferred to double caging condition and kept in the clean lack for a week to make sure animals get used to the new caging environment. After a week in double caging condition, animals performed ultrasonic vocalization test, pup retrieval test and water based Y-maze test (**Figure 1**).

All the cages were kept in a clean rack which the temperature was maintained at 22-23°C, the humidity was 50-60%, and a 12-h light/dark cycle (9a.m. to 9p.m.) was kept. Food and water were provided *ad libitum*. All the experiments were performed during the dark cycle, and recent study reported that light cycle does not affect social behavior in BTBR mice (Yang et al., 2007).

Before each experiment, mice were placed on the shelf for an hour for stabilization, and all the experiments were processed in dim light.

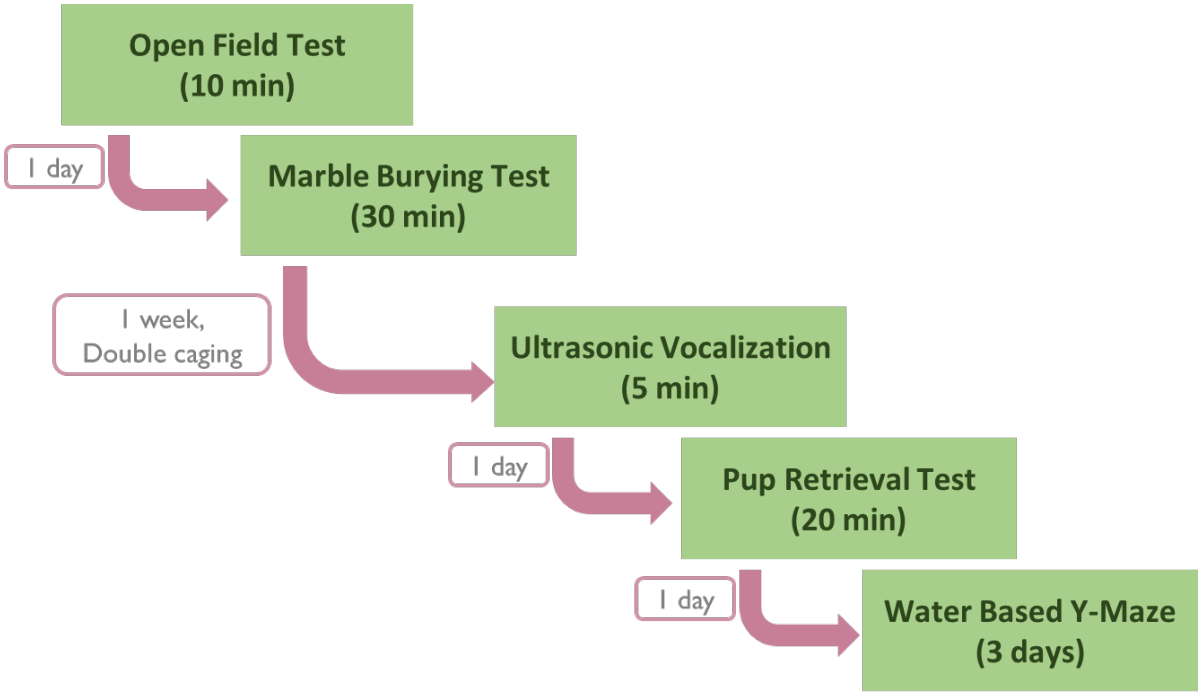

Figure 1. Sequence of the experiments.

Open field test

A mouse was placed in the clean, empty arena that is 40 cm L × 40 cm W (**Figure 2A**), for 10 minutes. There were two zones (20 for center, 40 for peripheral) in the area. Subject animal's movement was monitored with a tracking program, EthoVision 3.1 (Noldus). The arena was cleaned with 70% ethanol and distilled water after each trial.

Marble burying test

The day after the open field test, marble burying test was performed. A mouse was placed in a cage, which was filled with 3cm of beta chip bedding and twenty black glass marbles (1.5 cm in diameter) were arranged on it in 5 rows of 4 (**Figure 2B**). After 30 minutes of exploration, the mouse was removed from the arena. Marbles that were covered more than two third of the surface was considered to be buried (Thomas et al., 2009; Amodeo et al., 2012). Beta chip bedding were disposed and glass marbles were cleaned with 70% ethanol, after each trial.

Ultrasonic vocalization test

The main function of ultrasonic vocalizations in male mice is thougt to be a sort of courtship song to lure female mice (Hammerschmidt et al., 2009; Sugimoto et al., 2011), while female ultrasonic vocalization thought to play a role in social communictions (Scattoni et al., 2008; Merten et al., 2013). There is a report that female ultrasonic vocalization reflects positive affects (Wang et al., 2008), as well. Thus in this study we measured number of ultasonic vocalization call as a prarameter for social communication.

After a week of double caging environment, ultrasonic vocalization test was performed. The subject animal's cage mate was temporarily moved to a clean cage, and the subject mouse left in her

home cage alone. The home cage was put inside a sound attenuating styrofoam box, and the room where the experiment was carried was also a sound proof room. The home cage was covered with a transparent acrylic lid with a hole for the microphone in the center. An Ultrasonic condenser microphone (CM16/CMPA, Avisoft) that was connected to a recording device (UltraSoundGate 116H, Avisoft) was place on the center of the lid. After being habituated in her home cage for 15 to 20 minutes, a five to seven weeks old stranger mouse was introduced to her home cage, while ultrasonic vocalizations and video were being record for 5 minutes (**Figure 2C**). Sampling rate was set at 250 kHz; format 16 bit. The recorded data was processed with a software, SASLab Pro (Avisoft). After each trial, female body smear was obtained using saline to identify the estrus phase of the subject (Champlin et al., 1973; Byers et al., 2012). Although there are four stages of mouse estrus cycle, in this study we reorganized them into two group: estrus and non-estrus, which includes proestrus, metestrus and diestrus phases.

Pup retrieval test

The subject animal's cage mate was temporarily moved to another cage, and the subject mouse left in her home cage alone to get habituated for about 15 minutes. After the habituation, three P1-P2 pups were placed at three different corners apart from the subject. The subject mouse was given 30 minutes to retrieve the pups, while video was recording (**Figure 2D**). The latency to retrieve each pup and the number of pups retrieved was manually counted. Only virgin mice that do not have any experiences with giving birth or nurturing pup were used in this experiment.

Water based Y-maze test

Water based Y-maze test was performed in the end of the battery since it causes most physical and psychological distress to the subject. Water based Y-maze is made of while acrylic material and

composed of three arms in 120°; a starting arm, left arm, and right arm. The maze is filled with water and non-toxic, fragrant free white paint was diluted in order to keep the water opaque so the platform is not visible to the animals. The temperature of the water was maintained at 26±1 °C. This experiment takes three days; habituation on day 1, 20 trials of training session on day 2, test and 20 trials of reversal learning session on day 3 (**Figure 2E**). On the first day, mice explore the maze without platform for 1 minute, for 3 times. The day after the habituation, platform is place 70mm below the surface of the water in one arm, and mice were trained to find a platform. In each trial mice were allowed 20 seconds to make an arm choice, and when the subject made an incorrect arm choice, she had to trapped in the wrong arm for 20 seconds. The inter-trial interval was 5 to 10 minutes. On next day, in the maze where the platform is placed in the same arm as previous day of training, mice were tested 5 times, and only those which passed 4 tests out of 5 continued to reversal learning session. Reversal learning session is basically the same as training session except that the platform is place in the opposite arm. There performances were manually recorded while video was being recorded.

Data analysis

For ultrasonic vocalization data, SASLab Pro (Avisoft) was used to analyze the data. Frequencies below 35kHz were filtered, and removed the back ground noise manually. For element separation, “whistle tracking” was used, and any element that shorter than 3ms was not counted, hold time was set at 20 ms.

Statistically analyses were performed using GraphPad Prism 5.01. All data are represented as means±SEM.

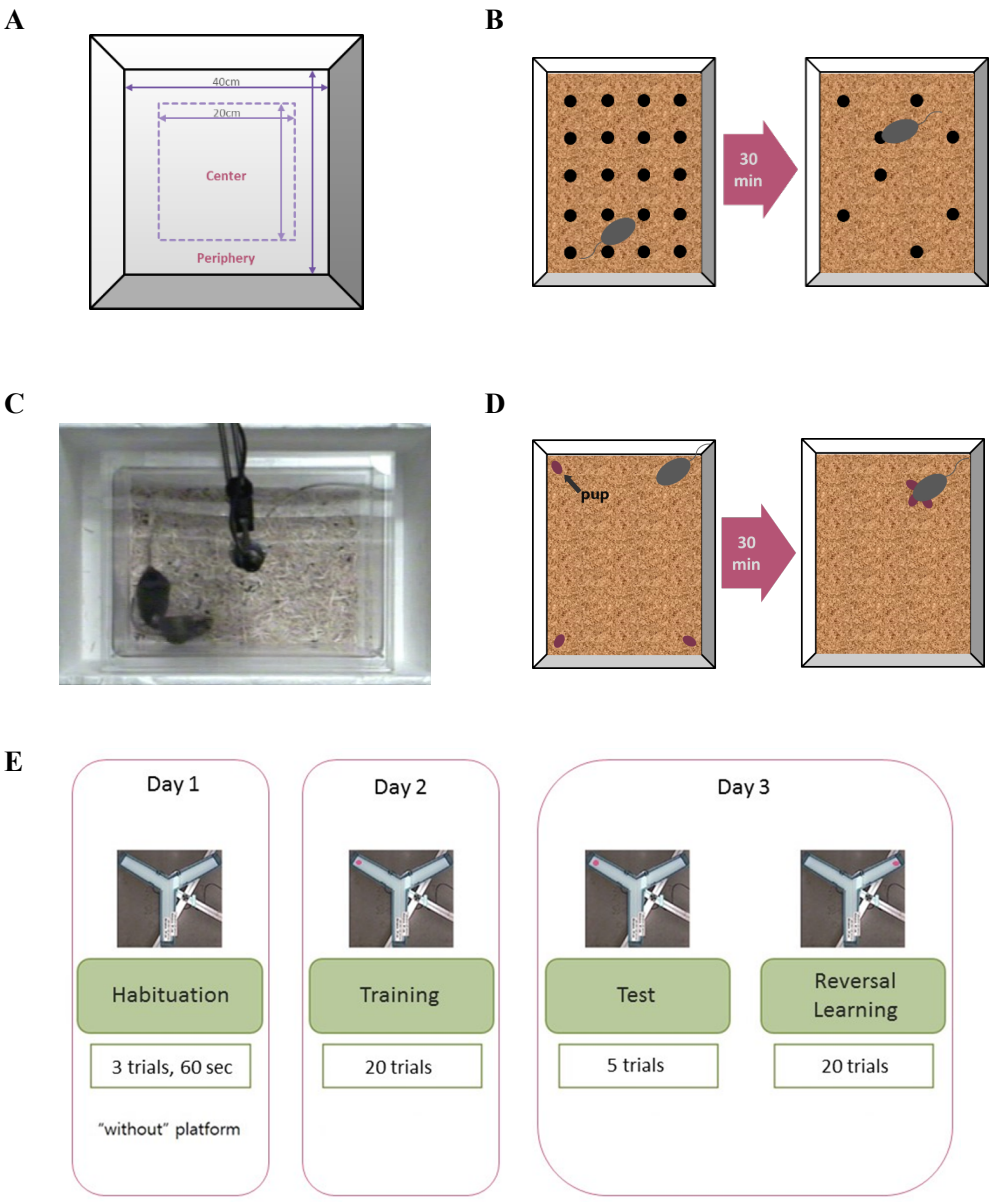

Figure 2. Experimental settings (A) Open field test. (B) Marble burying test. (C) Ultrasonic vocalization test. (D) Pup retrieval test. (E) Water based Y-maze test.

Results

BTBR T+tf/J and Shank2 Δe6-7 showed hyperactivity in open field test.

Open field test is commonly used in rodent behavioral studies to assess the locomotion and basal anxiety. There were four groups; Shank2 *Δ*e6-7 (n=26), naïve C57BL/6 (n=12), Shank2 *Δ*e6-7 KO (n=5), and Shank2 *Δ*e6-7 WT (n=8). Both autism mouse model groups, BTBR T+tf/J (7858±572.3 cm for BTBR T+tf/J, 5003±161.1 cm for C57BL/6) (p=0.0020, unpaired t-test) and Shank2 *Δ*e6-7 KO (10010±1482 cm for Shank2 *Δ*e6-7 KO, 6162±314.5 cm for Shank2 *Δ*e6-7 WT) (p=0.0087, unpaired t-test), showed significantly more distance moved than their control groups (**Figure 3A, C**). However, the basal anxiety level of both experimental groups were not different from their control groups. BTBR T+tf/J spent slightly more time in the center zone than C57BL/6 (58.97±5.2 sec for BTBR T+tf/J, 42.50±3.9 sec for C57BL/6) (n.s, p=0.0516, unpaired t-test), but it was not statistically meaningful (**Figure 3B**). Shank2 *Δ*e6-7 KO spent similar time in the center zone with Shank2 *Δ*e6-7 WT (46.73±18.5 sec for Shank2 *Δ*e6-7 KO, 36.24±7.2 sec for Shank2 *Δ*e6-7 WT) (n.s, p=0.5486, unpaired t-test) (**Figure 3D**). Overall, autism model groups showed increased locomotor activities than controls but basal anxiety level for both groups did not differ from controls.

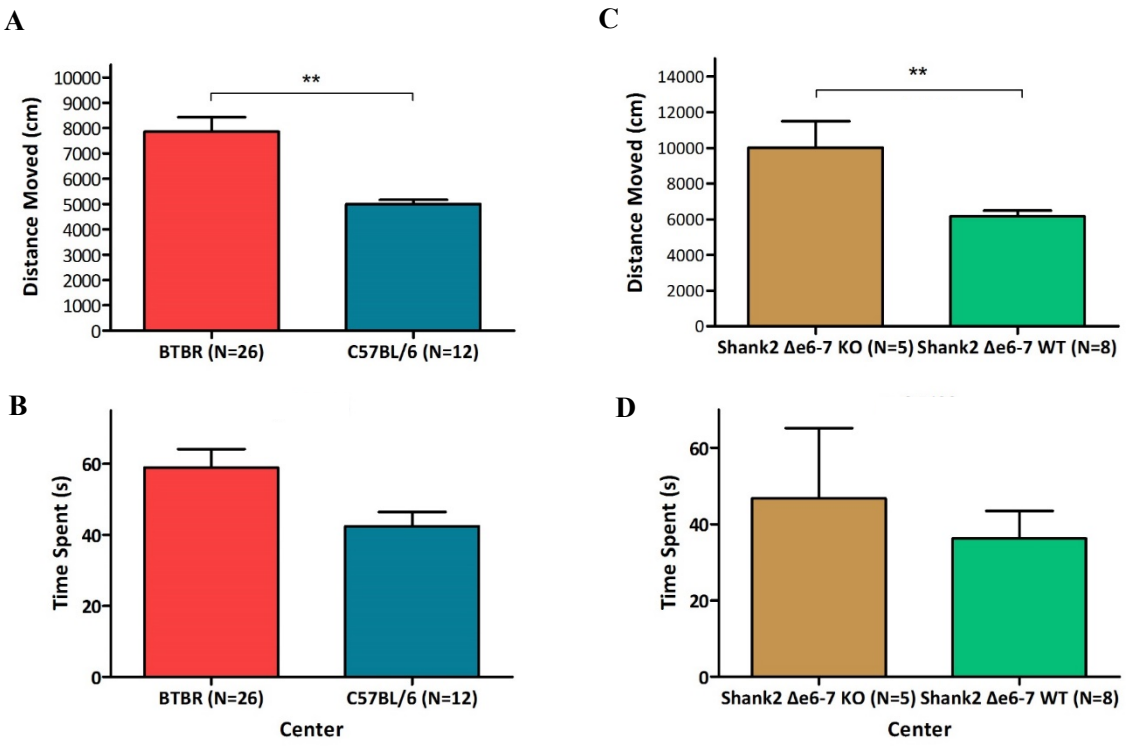

Figure 3. Comparison of open field test results. (A) Distances moved and (B) Time spent in the center area were compared between BTBR T+tf/J, and control group. (C) Distance moved (D) Time spent in the center area were compared between Shank2 *Δ*e6-7 KO and WT

BTBR T+tf/J and Shank2 Δe6-7 KO buried significantly less marbles than their controls

Marble burying test is often used to investigate repetitive or preservative behaviors which is one of characteristics of autism symptoms. However, in this study, both autism model groups showed significantly less burying behaviors than the control groups. The total number of marbles buried after30 minutes was 4.1±0.8 for BTBR T+tf/J, 10.0±1.3 for C57BL/6 (p=0.0003, unpaired t-test) (**Figure 4A**). It is interesting to note that BTBR T+tf/J group showed an opposite result from another report, which observed significantly increased burying behavior of BTBR T+tf/J male mice (Spencer et al., 2011; Amodeo et al., 2012). The observation in current study reflects decreased repetitive behaviors in out autism model groups, but it also can reflect an increased lack of interest or motivation of autism model groups, especially in Shank2 *Δ*e6-7 KO case (0.2±0.2 for Shank2 *Δ*e6-7 KO, 9.5±1.0 for Shank2 *Δ*e6-7 WT) (p<0.0001, unpaired t-test), which all mice hardly buried any marbles (**Figure 4A**). Several studies have reported the lack of motivation is a crucial indicator for autistic spectrum disorder (Dawson at al., 1998; Assaf at al., 2009; Chevallier et al., 2012; Kohls et al., 2012; Assaf et al., 2013). Significantly decreased marble burying behavior observed in autism mouse models may be considered as a sign of the absence of motivation.

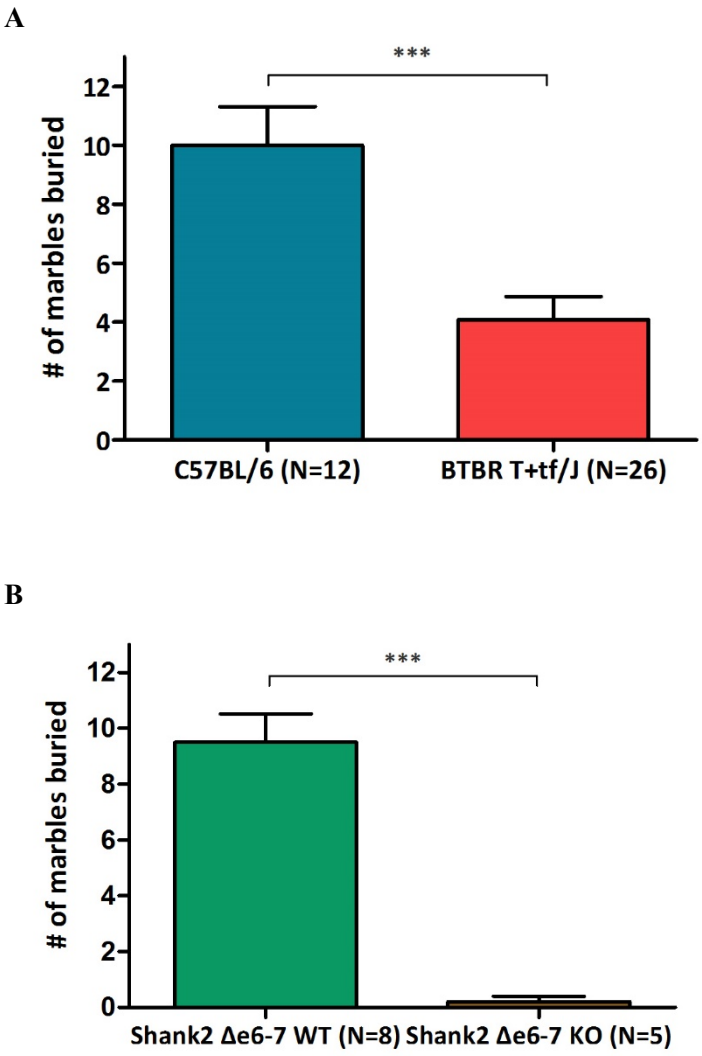

Figure 4. Results from marble burying test. The number of marble buried in 30 minutes was compared between (A) BTBR T+tf/J and control mice, and also (B) Shank2 *Δ*e6-7 KO and WT mice

BTBR T+tf/J mice show decitis in communications than naïve C57BL/6 mice when encounted an intruder

Since there are some evidences that ultrasonic vocalizaiton emission of female mice during a stranger encounter can be a parameter of sociability (Maggio & Whitney, 1985; Mole at al., 2007; Yang et al., 2013), we added ultrasonic vocalization test in our series of experiments. When encounted an intruder, BTBR T+tf/J mice emitted significantly fewer number of ultrasonic calls than C57BL/6 mice (340.2±56.7 for BTBR T+tf/J, 600.6 ±135.80 for C57BL/6) (p=0.0423, unpaired t-test) (**Figure 5A**). However, the number of calls that Shank2 *Δ*e6-7 KO mice emitted was not statistically different with Shank2 *Δ*e6-7 WT mice (624±326.0 for Shank2 *Δ*e6-7 KO, 580.6±153.7 for Shank2 *Δ*e6-7 WT) (n.s, p=0.8969, unpaired t-test) (**Figure 5B**). Since there is a report that phase of estrus cycle mostly does not affect behavior of C57BL/6J female mice, some strain differences may apply in some strains such as BALB/cByJ (Meziane et al., 2007), and female estrus cycle may affect male courtship vocalizations during male-female interaction (Hanson & Hurley, 2012), we had to make sure if the estrus phase of mice, especially in our experiment, BTBR T+tf/J mice, has any influence on the amount of ultrasonic vocalizations, the body smear of all the mice in all four groups was obtained right after each trial. Since there was only one estrus mouse in B6 control group, estrus cycle within BTBR group, which is the only group that has sufficient samples, was compared (**Figure 6**). Our data indicated that there is no correlation between estrus phases and the number of ultrasonic vocalization calls (333.7±57.5 for non-estrus BTBR mice, 358.0±150.7 for estrus BTBR mice) (n.s, p=0.8535, unpaired t-test).

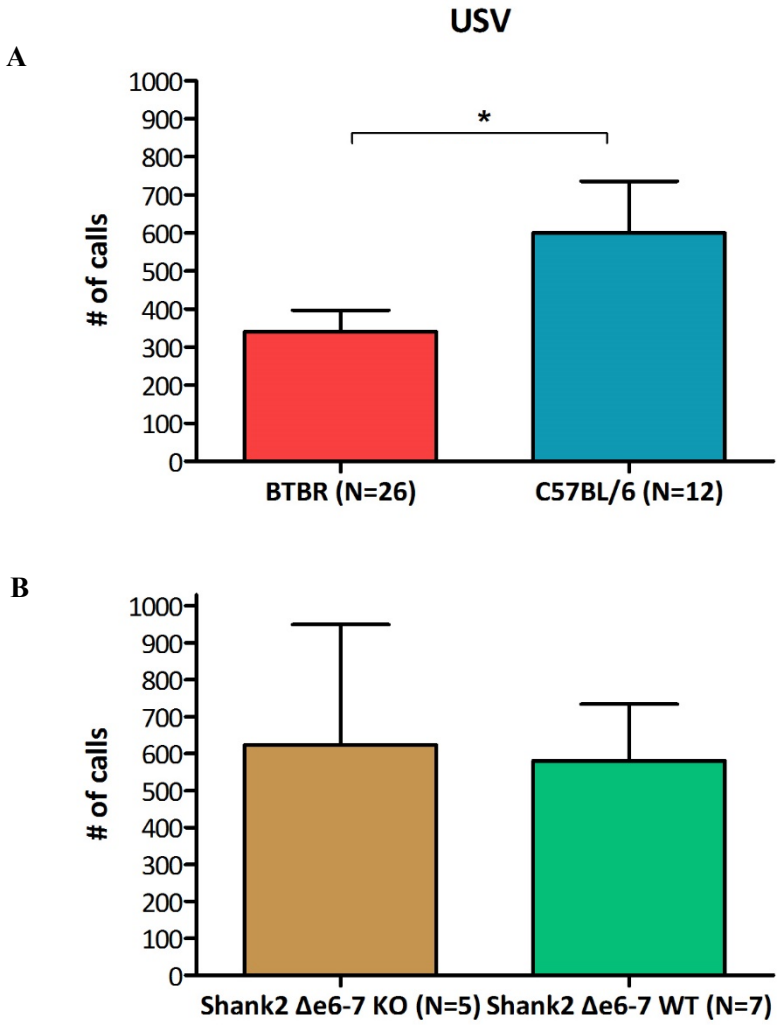

Figure 5. Results from ultrasonic vocalization test. The number of ultrasonic vocalization calls emitted during 5 minutes of stranger incounter was measured and compared between (A) BTBR T+tf/J and control mice, and (B) Shank2 *Δ*e6-7 KO and WT mice, as well.

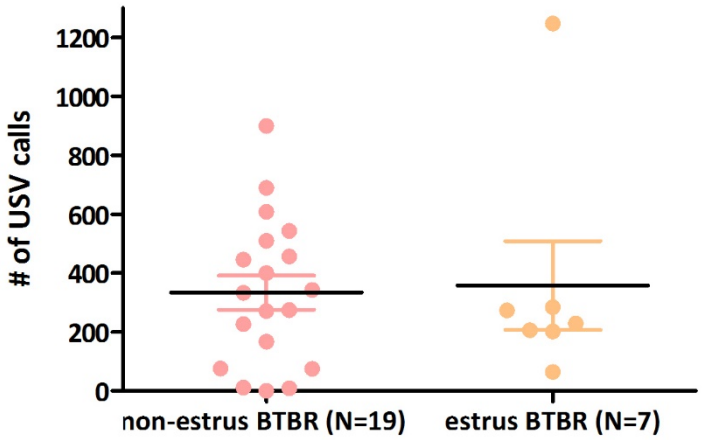

Figure 6. Comparison of the number of ultrasonic vocalization between estrus BTBR T+tf/J mice and non-estrus BTBR T+tf/J mice.

BTBR T+tf/J mice retrieve less pups and take more time than the control mice.

Pup retrieval test is often used to investigate the maternal behavior and social interaction. To see the differences in innate characteristics between autism model mice and their control groups, we performed this test. Out of 26 BTBR T+tf/J mice, only 13(50%) mice successfully retrieved all three pups, while 91.6% (11 out of 12) C57BL/6 mice succeeded in retrieving all the pups. Overall, BTBR mice retrieved significantly less pups than controls (2.15±0.21 for BTBR T+tf/J, 2,92±0.08 for C57BL/6) (p=0.0226, unpaired t-test) (**Figure 6B**). Moreover, BTBR mice showed significantly delayed latency to retrieve each. It took 492.2±84.96 sec for BTBR T+tf/J mice to retrieve the first pup, while the time taken for C57BL/6 was 94.73±24.61sec (p=0.0004, unpaired t-test). The latency to retrieve the second pup (570.2±82.68sec for BTBR, 257.5±52.53sec for C57BL/6) (p=0.0058, unpaired t-test) and the third pup (848.0±123.4sec for BTBR, 505.9±70.38sec for C57BL/6) (p=0.0319, unpaired t-test) were significantly delayed in BTBR group (**Figure 6A**).

Meanwhile, out of 5 Shank2 *Δ*e6-7 KO mice, none of them retrieved any pup at all, while 3 out of 7 Shank2 *Δ*e6-7 WT mice retrieved all three pup, and the mean number of pup that Shank2 *Δ*e6-7 WT retrieved was 1.86±0.5 pups. Our result suggests both autism model mouse exhibited have significantly impaired maternal behavior and social interaction in pup retrieval test. The observation that significantly more number of autism model mice failed at collecting all three pup and also significantly delayed latency to collect pups may be a sign of decreased motivation, as well as the lack of maternal instinct and sociability.

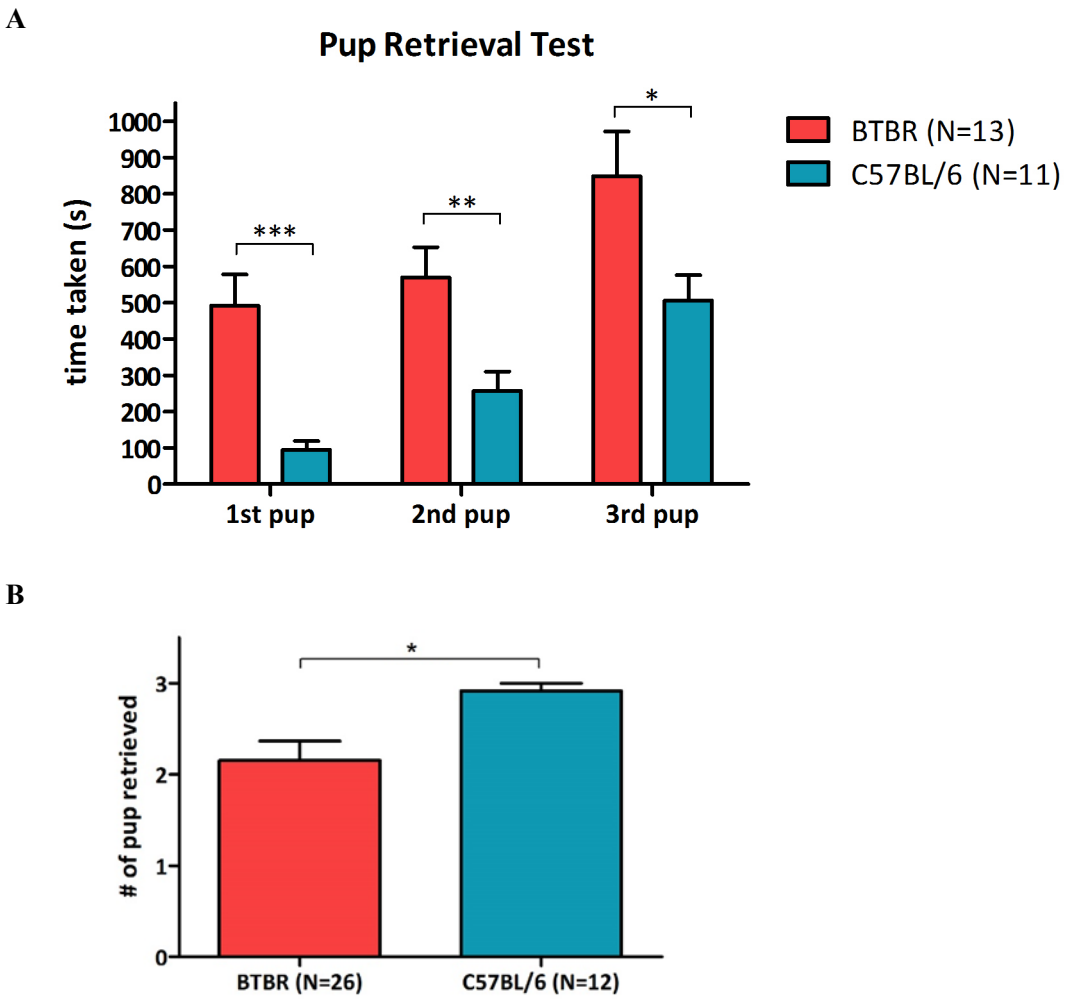

Figure 7. Results from pup retrieval test. (A) Pup retrieval latency was compared between BTBR T+tf/J and control mice. (B) Number of pups retrieved was compared BTBR T+tf/J and control group.

BTBR T+tf/J showed general performances in learning, but deficits in reversal learning ability.

BTBR T+tf/J mice did not show any statistically meaningful differences during the training session. While 73.1% (19 out of 26) BTBR mice passed the test and continued to reversal learning session, 83.3% (10 out of 12) C57BL/6 mice passed the test (**Figure 8**). In general, reversal learning of BTBR T+tf/J mice seemed hindered, but statistical difference was only observed in reversal learning block 3 ($p<0.05$), which represents 11th to 15th trials. This result somewhat agrees with another research that reported BTBR mice performed normal at learning but showed deficits in reversal learning in Morris water maze test (Yang et al., 2011). Shank2 *Δ*e6-7 KO mice did not show any sign of learning at all. Only one mouse out of five passed the test, but even that mouse did fail at reversal learning, hence it was unable to plot the result of Shank2 *Δ*e6-7 KO and WT mice on a graph.

The learning session alone was compared in **Figure 9**, Shank2 *Δ*e6-7 KO mice show serious deficits in information acquisition. This impaired learning was significantly severe even when Shank2 *Δ*e6-7 KO group was compared with BTBR T+tf/J, which is also an autism mouse model.

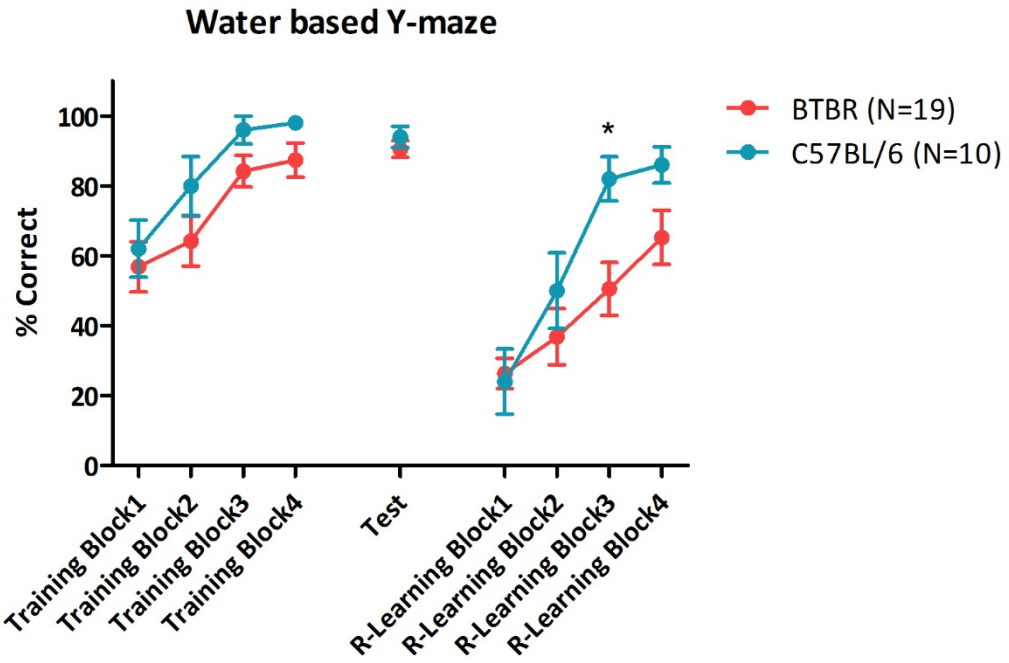

Figure 8. Results from water based Y-maze test of BTBR T+tf/J and control mice. Each block is consist of five trials in this graph.

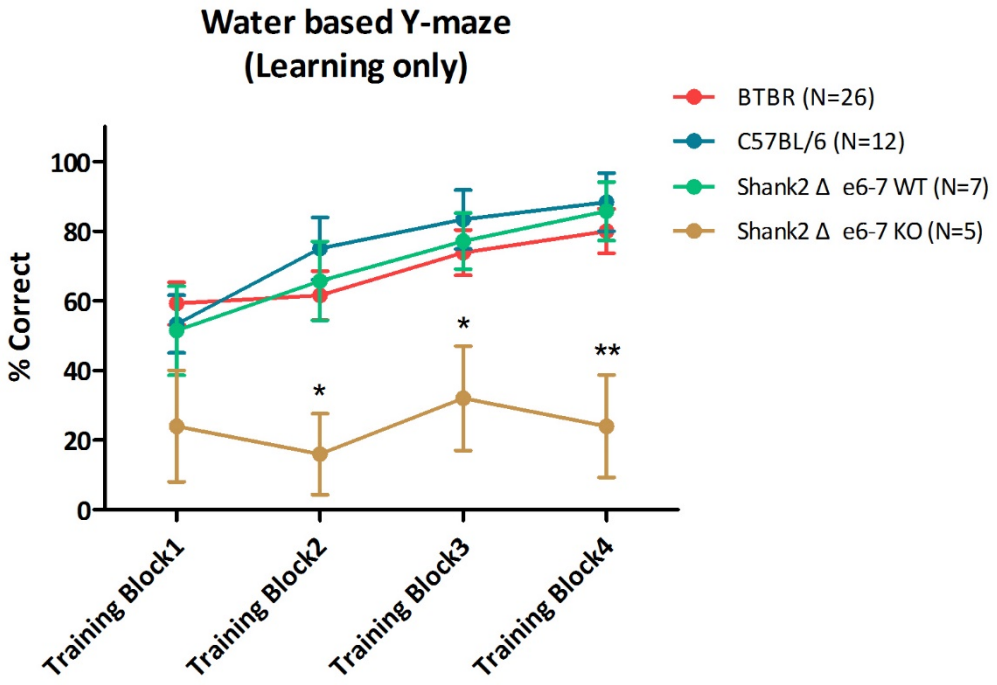

Figure 9. Spatial learning ability of Shank2 *Δ*e6-7 KO mice was significantly impaired. The impaired learning was severe even when Shank2 *Δ*e6-7 KO group was compared with BTBR T+tf/J, which is also an autism mouse model.

Discussion

We performed five experiments to find out the similarities and differences in behavioral phenotype of female autism mouse models and naïve C57BL/6 mice. Like reported in many other male mouse studies, both BTBR and Shank2 Δe6-7 KO mice showed hyperactivities in open field test.

A significantly fewer ultrasonic vocalization calls in BTBR T+tf/J mice reflect their well-known characteristic; a lack of communication. However it was surprising that the number of ultrasonic vocalization calls of Shank2 Δe6-7 KO was not only statistically different from Shank2 Δe6-7 WT, but KO mice emitted slightly more calls than WT. However, the number of subjects in Shank2 Δe6-7 group was not sufficient enough for drawing any conclusion, and more quantity of data should be collected for more stable information.

There was an interesting result that BTBR T+tf/J and Shank2 Δe6-7 KO buried significantly less marbles than their controls. This result is conflicting with previous reports that male BTBR mice buried more marbles than control mice (Amodeo et al., 2012). Marble burying test was first started to used as an anxiety measurement (Njung'e & Handley, 1991), however recently it is often used in autism studies to observe repetitive behavior (Thomas et al., 2009), which is one of three main clinical symptoms for autistic spectrum disorder. The observation in current study reflects decreased repetitive behaviors in our autism model groups, and the marble burying test certainly is a usuful method for measuring repetitive activities, but our result also may reflect an increased lack of interest or motivation in autism model groups.

Deficits in motivation are considered to be one of crucial characteristics of autistic spectrum disorders, nowadays (Dawson at al., 1998; Assaf at al., 2009; Chevallier et al., 2012; Assaf et al., 2013). In line with this marble burying test result, we suggest the results from pup retrieval test may be due to the low motivation, as well. In pup retrieval test, autism model mice retrieved significantly

smaller number of pups compared to their controls, and the latency to retrieve each pup was greatly delayed than the comparison groups for all three pups. This result does not differ from many previous reports, and certainly this result reflects disrupted motherhood of female autism model mice, it should be considered if such behavior is just from the lack of maternal instinct and sociability, or it is originated from the absence of motivation, which is one of important autistic traits. Although there are evidences that there are some genetic factors (Noor et al., 2010; Filges et al., 2011) for reason why male autism spectrum disorder patients are almost five times common than female autism patients, some still argue etiology of female autistic spectrum disorder is much lower because female may tend to show less phenotypic symptoms than males, even though they have autism, and that's why female autistic spectrum disorder patients are detected less than males.

Many results from our female behavioral experiments agreed with the previous reports from male studies, and data from female mice yielded rather stable results when sample size is large enough. Female mice are often neglected in behavioral studies because they tend to produce fluctuating results due to their estrus cycle. However, the influence of estrus cycle may not that significant in many cases (Meziane et al., 2007), and our result also supports this idea. In addition, especially in autism studies, female behavioral phenotype should be further investigated to find out whether morbidity differences between male and female is just due to a genetic factor, or if differences in behavioral phenotype between females and male may play a role in such disparity.

References

American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders 5th edition (Washington, DC: American Psychiatric Association)

Amodeo, D. A., Jones, J. H., Sweeney, J. A., & Ragozzino, M. E. (2012). Differences in BTBR T+ tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors. Behavioural Brain Research, 227(1), 64–72.

Assaf, M., Hyatt, C. J., Wong, C. G., Johnson, M. R., Schultz, R. T., Hendler, T., & Pearlson, G. D. (2013). Mentalizing and motivation neural function during social interactions in autism spectrum disorders. NeuroImage. Clinical, 3, 321–331.

Assaf, M., Kahn, I., Pearlson, G. D., Johnson, M. R., Yeshurun, Y., Calhoun, V. D., & Hendler, T. (2009). Brain Activity Dissociates Mentalization from Motivation During an Interpersonal Competitive Game. Brain Imaging and Behavior, 3(1), 24–37

Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. PloS One, 7(4), e35538.

Centers for Disease Control and Prevention (2014). Community Report on Autism 2014 (Atlanta, GA: Centers for Disease Control and Prevention)

Champlin, A. K., Dorr, D. L., & Gates, A. H. (1973). Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. Biology of Reproduction, 8(4), 491–494.

Chevallier, C., Kohls, G., Troiani, V., & Brodkin, E. S. (2012). The social motivation theory of autism. Trends in Cognitive ….

Dawson, G., Meltzoff, A. N., Osterling, J., Rinaldi, J., & Brown, E. (1998). Children with Autism Fail to Orient to Naturally Occurring Social Stimuli. Journal of Autism and Developmental Disorders, 28(6), 479–485

Filges, I., Röthlisberger, B., Blattner, A., & Boesch, N. (2011). Deletion in Xp22. 11: PTCHD1 is a candidate gene for X-linked intellectual disability with or without autism. Clin Genet, 79(1):79-85

Hammerschmidt, K., Hammerschmidt, K., Radyushkin, K., Radyushkin, K., Ehrenreich, H., Ehrenreich, H., et al. (2012). The structure and usage of female and male mouse ultrasonic vocalizations reveal only minor differences. PloS One, 7(7), e41133.

Hanson, J.L., Hurley, L.M. (2012). Female Presence and Estrous State Influence Mouse Ultrasonic Courtship Vocalizations. PLoS ONE 7(7), e40782

Kohls, G., Chevallier, C., Troiani, V., & Schultz, R. T. (2012). Social "wanting" dysfunction in

autism: neurobiological underpinnings and treatment implications. Journal of Neurodevelopmental Disorders, 4(1), 10.

Martin, L., Sample, H., Gregg, M., & Wood, C. (2014). Validation of operant social motivation paradigms using BTBR T+tf/J and C57BL/6J inbred mouse strains. Brain and Behavior, 4(5), 754–764,

Merten, von, S., Hoier, S., Pfeifle, C., & Tautz, D. (2014). PLOS ONE: A Role for Ultrasonic Vocalisation in Social Communication and Divergence of Natural Populations of the House Mouse (Mus musculus domesticus). PloS One, 9(5), e97244.

Meyza, K. Z., Defensor, E. B., Jensen, A. L., Corley, M. J., Pearson, B. L., Pobbe, R. L. H., et al. (2013). The BTBR T+ tf/J mouse model for autism spectrum disorders-in search of biomarkers. Behavioural Brain Research, 251, 25–34.

Maggio, J.C. & Whitney, G. (1985) Ultrasonic vocalizing by adult female mice (*Mus musculus*). *J* Comp Psychol **99**, 420–436.

Meziane, H., Ouagazzal, A. M., & Aubert, L. (2007). Estrous cycle effects on behavior of C57BL/6J and BALB/cByJ female mice: implications for phenotyping strategies. Brain and Behavior.

Moles, A., Costantini, F., Garbugino, L., Zanettini, C. & D'Amato, F.R. (2007) Ultrasonic vocalizations emitted during dyadic interactions in female mice: a possible index of sociability? Behav Brain Res **182**, 223–230

Moy, S. S., Nadler, J. J., Magnuson, T. R., & Crawley, J. N. (2006). Mouse models of autism spectrum disorders: the challenge for behavioral genetics. American Journal of Medical Genetics. Part C, Seminars in Medical Genetics, 142C(1), 40–51.

Moy, S. S., Nadler, J. J., Young, N. B., Perez, A., Holloway, L. P., Barbaro, R. P., et al. (2007). Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behavioural Brain Research, 176(1), 4–20.

Njung'e, K., & Handley, S. L. (1991). Evaluation of marble-burying behavior as a model of anxiety. Pharmacology, Biochemistry, and Behavior, 38(1), 63–67.

Noor, A., Whibley, A., & Marshall, C. R. (2010). Disruption at the PTCHD1 Locus on Xp22. 11 in Autism spectrum disorder and intellectual disability. Sci Transl Med, 2(49), 49-68

Pearson, B. L., Pobbe, R., & Defensor, E. B. (2011). Motor and cognitive stereotypies in the BTBR T+ tf/J mouse model of autism. Genes.

Scattoni, M. L., Scattoni, M. L., Ricceri, L., Ricceri, L., Crawley, J. N., & Crawley, J. N. (2010). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. Genes, Brain, and Behavior, 10(1), 44–56.

Schmeisser, M. J., Ey, E., Wegener, S., Bockmann, J., Stempel, A. V., Kuebler, A., et al. (2012). Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature, 486(7402), 256–260.

Silverman, J. L., Babineau, B. A., Oliver, C. F., Karras, M. N., & Crawley, J. N. (2013). Influence of stimulant-induced hyperactivity on social approach in the BTBR mouse model of autism. Neuropharmacology, 68, 210–222.

Silverman, J. L., Yang, M., Lord, C., & Crawley, J. N. (2010). Behavioural phenotyping assays for mouse models of autism. Nature Reviews Neuroscience, 11(7), 490–502.

Spencer, C. M., Alekseyenko, O., Hamilton, S. M., Thomas, A. M., Serysheva, E., Yuva-Paylor, L. A., & Paylor, R. (2011). Modifying behavioral phenotypes in Fmr1KO mice: genetic background differences reveal autistic-like responses. *Autism Research: Official Journal of the International Society for Autism Research*, *4*(1), 40–56. doi:10.1002/aur.168

Sugimoto, H., Okabe, S., Kato, M., Koshida, N., Shiroishi, T., Mogi, K., et al. (2011). A role for strain differences in waveforms of ultrasonic vocalizations during male-female interaction. PloS One, 6(7), e22093.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology, 204(2), 361–373.

Wang, H., Wang, H., Liang, S., Liang, S., Burgdorf, J., Burgdorf, J., et al. (2008). Ultrasonic Vocalizations Induced by Sex and Amphetamine in M2, M4, M5 Muscarinic and D2 Dopamine Receptor Knockout Mice. PloS One, 3(4), e1893.

Won, H., Lee, H. R., Gee, H. Y., Mah, W., Kim, J. I., Lee, J., & Ha, S. (2012). Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature.

Yang, M., Abrams, D. N., Zhang, J. Y., Weber, M. D., Katz, A. M., Clarke, A. M., et al. (2012). Low sociability in BTBR T+tf/J mice is independent of partner strain. Physiology & Behavior, 107(5), 649–662.

Yang, M., Scattoni, M. L., Zhodzishsky, V., Chen, T., Caldwell, H., Young, W. S., et al. (2007). Social approach behaviors are similar on conventional versus reverse lighting cycles, and in replications across cohorts, in BTBR T+ tf/J, C57BL/6J, and vasopressin receptor 1B mutant mice. Frontiers in Behavioral Neuroscience, 1, 1.

국문초록

암컷 BTBR T+ ft/J와 C57BL/6 변종의 자폐적 행동의 차이

손준희

뇌인지과학과

자연과학대학원

서울대학교

자폐증은 사회성 결여, 의사소통의 장애, 특정 행위를 반복하는 증상등을 대표적으로 보이는 발달장애이며 (American Psychiatric Association, 2013), 해마다 점점 더 많은 수의 어린이들이 자폐 스펙트럼 장애로 진단을 받고 있다고 보고되고있다 (Centers for Disease Control and Prevention, 2014). 따라서 늘어나는 자폐진단 인구와 마찬가지로 자폐증과 관련 장애에 대한 유려와 관심, 치료법의 모색 또한 증가하는 추세다.

동물 연구에서 주로 수컷들 만이 행동 실험에 쓰여 암컷 동물에 대한 정보가 많이 부족하다. 이 연구에서는 자폐적인 성향을 보이는 BTBR T+ ft/J와 C57BL/6의 변종인 Shank2 Δe6-7 KO과 그 대조군 쥐들의 암컷들만을 사용하여 행동실험을 수행하였

고, 그 결과를 비교하여 앞으로의 연구 방향을 모색하였다.

전반적으로 BTBR T+ ft/J 암컷쥐와 Shank2 Δe6-7 KO는 수컷쥐들의 연구결과와 일치하는 과다활동 증상을 보였으나, 불안감은 대조군과 유의미한 차이를 보이지 않았다. 사회성의 척도로 ultrasonic vocalization (USV)을 측정하였더니 BTBR T+ ft/J 군에서 현저히 적은 수의 USV가 관찰되어, 결여된 의사소통을 보였다. 또한 pup retrieval test 에서는 이 실험의 두가지 자폐 모델 군인 BTBR T+ ft/J 와 Shank2 Δe6-7 KO 둘 다 새끼를 한 곳에 모으기 까지 두드러지게 더 오랜 시간이 걸렸고, 총 세마리 새끼를 다 모은 쥐들의 수도 대조군인 C57BL/6와 Shank2 Δe6-7 WT에 비해 확연히 적었다. Marble burying test 에서는 두 자폐 모델 군 다 대조군보다 훨씬 더 적은 수의 구슬을 묻은것으로 나타났으며, water based Y-maze 실험에서는 Shank2 Δe6-7 KO 군의 심각한 학습능력 결여가 관찰되었다.