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Characterization of BTBR T+tf/J Mouse Model for Autism Spectrum Disorder

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BACKGROUND

Autism spectrum disorders (ASD) are severe neurodevelopmental disorders, which typically emerge early in childhood. Animal models of ASD include various genetically modified strains as well as inbred strains of mice expressing traits relevant to autism and targeted mutations in candidate genes. One of the used models is a BTBR T+tf/J mouse model which reportedly exhibits several symptoms of ASD including reduced social interactions, low exploratory behavior, unusual vocalizations and high anxiety as compared to other inbred strains. Also, it has been shown that in the model, mouse brain anatomy has profound abnormalities when compared to regular strains, such as C57Bl/6.

PURPOSE OF THE STUDY

The purpose of this study was to characterize BTBR T+tf/J (BTBR) model phenotype. Mice were tested in various behavioral tests and also taken through MRI/MRS measurements.

MATERIALS AND METHODS

Animals

Male and female BTBR T+ Itpr3tf/J (JAX strain #002282) and C57Bl/6J (JAX strain # 000664) mice (9-10 weeks) (JAX, USA) were be used. Animals were housed at a standard temperature (22 ± 1°C) and in a light-controlled environment (lights on from 7 am to 8 pm) with *ad libitum* access to food and water. All animal experiments were approved by the National Animal Experiment Board, Finland.

Experimental Groups

Following experimental groups were used: Group 1: 8 male C57Bl/6J mice; Group 2: 8 female C57Bl/6J mice; Group 3: 8 male BTBR mice; Group 4: 8 female BTBR mice

Elevated Plus Maze

The Elevated plus maze (EPM) was made of dark plastic. The arms were 30 cm length, 5 cm wide and the size of center area was 5 cm x 5 cm. The height of the walls were 0.25 cm (open) and 15 cm closed. The stand was about 50 cm long (= height from the floor). After a 5 minute period, a mouse was transferred back to its home cage. The number of entries to and time spent in closed and open arms was measured.

Open field Motor Activity

Open field (OF) test was conducted using activity chambers (Med Associates Inc, St Albans, VT; 27 x 27 x 20.3 cm) equipped with IR beams. Testing was done under red light conditions. The mouse was placed in the center of empty arena for 30 min. Following parameters were calculated: distance moved, number of vertical rearings, velocity and % resting time.

Contextual Fear Conditioning

Contextual fear conditioning (CFC) test was conducted using a Coulbourn FreezeFrame system (Coulbourn, Whitehall PA, USA). The mouse was placed on the grid floor in the empty arena and challenged with 16 inescapable electro-shocks each of 0.2 mA x 3 sec and delivered at inter-e-shock intervals (ITI) of 50 sec. Mean % time spent freezing during ITIs were calculated.

Rotarod

Mice were tested for 2 consecutive days. Each daily session included a training trial of 5 min at 4 RPM on the rotarod apparatus (AccuScan Instruments, Columbus, USA). One hour later, the animals were tested for 3 consecutive accelerating trials of 6 min with the speed changing from 0 to 40 RPM over 360 seconds and with an inter-trial interval at least 30 min. The latency to fall from the rod was recorded.

Sociability and Preference for Social Novelty

The apparatus for sociability and preference for social novelty test was comprised a rectangular, three-chamber box. Each chamber was 19 x 45 cm and the dividing walls were made from clear Plexiglas, with an open middle section, which allowed free access to each chamber. Two identical, wire cup-like containers with removable lids that large enough to hold a single mouse were used. These were placed vertically inside the apparatus, one in each side chamber, and contained the naíve/unfamiliar mouse. Mice were placed at the center of the middle chamber for adaptation for 5 minutes. Session 1: One control mouse ("Stranger 1") was placed inside a wire containment cup that was located in one of the side chambers. Following parameters: were recorded: duration and number of direct contacts between the subject mouse and the containment cup housing or not housing the Stranger 1 mouse. Session 2: The second control mouse ("Stranger 2") was placed inside an identical wire containment cup in the opposite side chamber (that had been empty during the Session I). Stranger 1 was kep at same location. Same parameters were analyzed.

MRI & MRS

MRI acquisitions were performed using a horizontal 11.7T magnet with a bore size of 160 mm, equipped with a gradient set capable of max. gradient strength of 750 mT/m and interfaced to a Bruker Avance III console (Bruker Biospin GmbH, Ettlingen, Germany). 1H-MRS data were collected using TE 10 ms PRESS sequence. Voxel of 2.0x1.8x1.8 mm^3 was placed in the striatum of the mouse based on collected T2-weighted images. Peak area for major metabolites (NAA, Cho, Tau, Ins, Glu, Gln, Cr+PCr) were analyzed using LCModel (Stephen Provencher Inc., Oakville, Canada) and results are given relative to water content in tissue.

CONTEXTUAL FEAR CONDITIONING

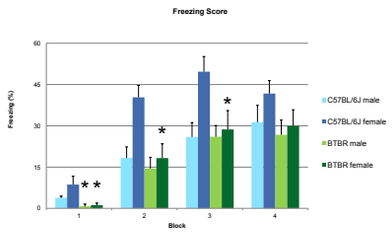

Figure 1. Contextual fear conditioning freezing %. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: * p < 0.05, vs. respective C57Bl/6J (Student's t-test).

OPEN FIELD

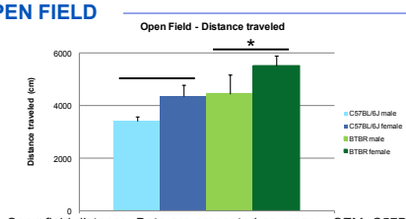

Figure 2. Open field distance. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: no differences in genders separated data. When data presented with pooled genders, BTBR mice had significantly increased distance (*p < 0.05) vs. C57Bl/6J mice (Student's t-test).

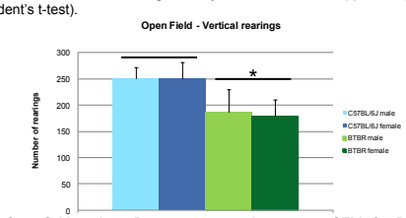

Figure 3. Open field rearings. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: no differences in genders separated data. When data presented with pooled genders, BTBR mice had significantly decreased number of rearings (*p < 0.05) vs. C57Bl/6J mice (Student's t-test).

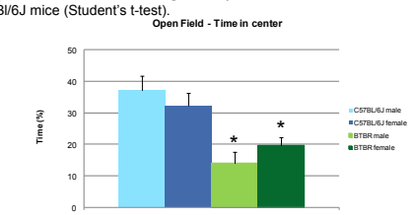

Figure 4. Open field time in center. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: * p < 0.05, vs. respective C57Bl/6J mice (Student's t-test).

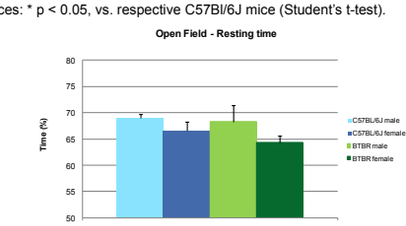

Figure 5. Open field resting time. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. No statistical significances.

MRI & MRS

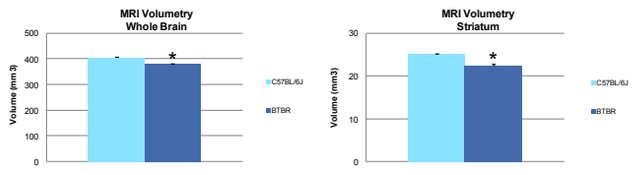

Figure 6. Whole brain and striatum volume. Data are presented as mean + SEM. C57Bl/6J, n = 15; BTBR, n = 16. Statistical significances: * p < 0.05, vs. C57Bl/6J (Student's t-test).

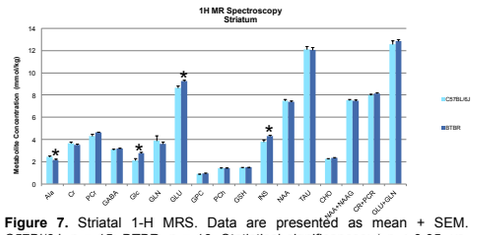

Figure 7. Striatal 1-H MRS. Data are presented as mean + SEM. C57Bl/6J, n = 15; BTBR, n = 16. Statistical significances: * p < 0.05, vs. C57Bl/6J (Student's t-test).

ROTAROD

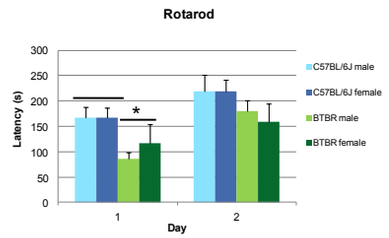

Figure 8. Rotarod latency. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: no differences in genders separated data. However, when data were presented with pooled genders, BTBR mice had significantly decreased drop latency on Day 1 (*p < 0.05) vs. C57Bl/6J mice (Student's t-test).

ELEVATED PLUS MAZE

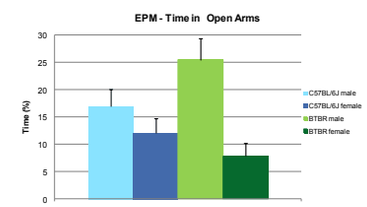

Figure 9. Elevated plus maze, time in open arms. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: No differences.

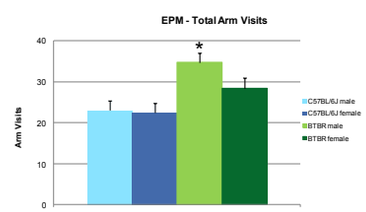

Figure 10. Elevated plus maze, number of arm visits. Data are presented as mean + SEM. C57Bl/6J male, n = 8; C57Bl/6J female, n = 8; BTBR male, n = 8; BTBR female, n = 8. Statistical significances: Statistical significances: * p < 0.05, vs. respective C57Bl/6J mice (Student's t-test).

SOCIABILITY & PREFERENCE FOR SOCIAL NOVELTY

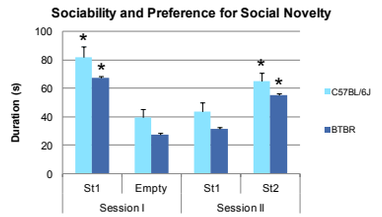

Figure 11. Social affiliation and sociability & social memory and novelty - duration of contact time. Data are presented as mean SEM. C57Bl/6J, n = 15; BTBR, n = 16. Session I: stranger 1 (St1) vs. empty chamber; both genotypes demonstrate a preference for social proximity by spending more time with St1. Session II: stranger 1 (St1) vs. Stranger 2 (St2); both genotypes demonstrate a preference for social novelty by spending more time with St2. Statistical significances: * p < 0.05, St1 vs. Empty; St1 vs. St2 (paired t-test).

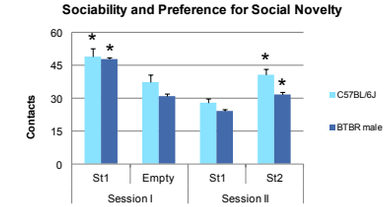

Figure 12. Social affiliation and sociability & social memory and novelty - number of contacts. Data are presented as mean SEM. C57Bl/6J, n = 15; BTBR, n = 16. Session I: stranger 1 (St1) vs. empty chamber; both genotypes demonstrate a preference for social proximity by having more contacts with St1. Session II: stranger 1 (St1) vs. Stranger 2 (St2); both genotypes demonstrate a preference for social novelty by having more contacts with St2. Statistical significances: * p < 0.05, St1 vs. Empty; St1 vs. St2 (paired t-test).

SUMMARY

- BTBR mice showed increased horizontal activity in open field test and made more arm entries in elevated plus maze test
- BTBR mice showed less freezing behavior in contextual fear conditioning test
- BTBR mice were impaired in rotarod test
- BTBR mice had decreased whole brain and striatum volumes and changed striatal MRS metabolite levels
- BTBR mice did not show differences in test for social affiliation and sociability & social memory and novelty