Differences in BTBR T+tf/J and C56BL/6J mice in various social behavioural paradigms

The social withdrawal battery

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Abstract

Mental disorders are still as prevalent as they were a decade ago. There have been no breakthroughs regarding new drugs for these disorders. The PSRISM project aims to find new biomarkers for mental disorders such as Alzheimer's disease, major depressive disorder and schizophrenia, by looking at a shared symptom: social withdrawal. In this study, we aim at validating a social behavioural testing battery directed at screening for social withdrawal. To accomplish this, we make use of an animal model that is commonly used in autism research, the BTBR T+tf/J (BTBR) mouse. This mouse is known to show reduced amounts of sociability. We used it to verify certain social behavioural tests, the three-chamber test, social conditioned place preference test, social discrimination task and a resident intruder test. BTBR mice displayed similar sociability in the three-chamber test as the C57BL/6J (B6) control mice. BTBR did not show associative memory in the social conditioned place preference test, whereas the control did. Both BTBR and the control did not show a preference for a novel mouse in the social discrimination task. In the resident intruder test the BTBR test group was split in high aggressive mice and low aggressive mice. Later in the behaviour analyses the high aggressive BTBR showed aberrant aggressive behaviour by displaying a highly reduced amount of threats preceding an attack. Overall our test battery provides a way to screen different aspects of social behaviour and to identify which of these aspects cause the deviating social behaviour.

Introduction

Major depressive disorder (MDD) was estimated by the world health organization (WHO) to affect over 300 million people in 2015, that is approximately 1 in every 20 persons. MDD is now ranked by WHO to be the single largest contributor to global disability (World Health Organization, 2017). According to the WHO, SZ and AD both are responsible for respectively 46 and 21 million patients worldwide (Prince, M. 2015). These numbers are starting to add up and apparently keep growing ever more. Current treatment of these diseases focuses on treating the symptoms of the disease rather than the disease itself. If the patient stops taking the medicine the disease will resurface again.

If you take a good look in the DSM-V you will find that in order to confirm MDD with a patient you need to rule out schizophrenia (SZ) first, see list of symptoms beneath. But in order to rule out SZ you also need to rule out Alzheimer's disease (AD) (American Psychiatric Association, 2013). In the list below stating the criteria to diagnose these different diseases, you can see in bold that the other diseases have to be ruled out. Also, it becomes clear that all patients who suffer from these diseases become dysfunctional in their daily lives by a decrease in cognitive skills. Because these people are becoming increasingly dysfunctional they withdraw themselves from daily activities. The PRISM Project aims to use this symptom of social withdrawal as a means to find new biomarkers for these neuropsychiatric diseases. And by doing so being able to come up with more effective treatments.

MDD is diagnosed when the patient has 5 out of 9 of the following symptoms:

- depressed mood during most of the day,
- diminished interest or pleasure in most activities of the day,
- significant weight loss or extreme weight gain
- insomnia or hypersomnia nearly every day
- psychomotor agitation or retardation
- fatigue or loss of energy
- feelings of worthlessness
- diminished ability to concentrate or indecisiveness
- recurrent thoughts of death

The symptoms must cause significant distress in social, occupational or other areas of functioning.
The episode is not attributable to other physiological effects of a substance or medical condition.

AZ is diagnosed when the following criteria are met:

- there is a significant cognitive decline from a previous level of cognitive function
    - concern of the subject or informant that there has been a decline in cognitive functioning
    - substantial impairment in cognitive performance, preferably measured by standardized tests.
- The cognitive deficits interfere with independence in everyday activities
- The cognitive deficits do not occur exclusively in the context of a delirium.
- **The cognitive deficits are not better explained by MDD or SZ.**

SZ is diagnosed when these criteria are met:

- Two or more of the following symptoms are present for a significant portion of time during a 1 month-period. At least one of these must be 1, 2 or 3.
    - Delusions
    - Hallucinations
    - Disorganized speech
    - Grossly disorganized or catatonic behaviour
    - Negative symptoms (i.e., diminished emotional expression or avolition).
- For a significant portion of time since the onset of the disturbance there is failure to achieve expected level of interpersonal, academic or occupational functioning.
- Continuous signs of the disturbance persist for at least 6 months which includes at least 1 month of symptoms.
- **Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out.**

A lot of these symptoms such as, loss of appetite, sleepiness, withdrawal from normal social activities and fatigue are also symptoms of many other diseases and they are described as “Sickness behaviour”. This sickness behaviour is caused by proinflammatory cytokines including interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-alpha.
After an infection, these cytokines are released by the immune system and form the primary mediators in the communication between the brain and the immune system. In the brain, they are responsible for cellular responses to the infection but also for behavioural changes that are needed for recovery. Sickness behaviour promotes the recovery of the infection and the withdrawal aspects makes sure that an individual does not infect others.
When a disbalance in the immunes system arises and a subject is chronically exposed to a high dose of cytokines this might results in developing neuropsychiatric diseases such as MDD, AD and SZ(Capuron & Miller, 2011).
In Figure 1, the different pathways by which cytokines pass the blood brain barrier are displayed and explained. All of these different pathways can be active at the same time and cause a high concentration of proinflammatory cytokines. This gives rise to the question of what causes a chronically high dose of proinflammatory cytokines.

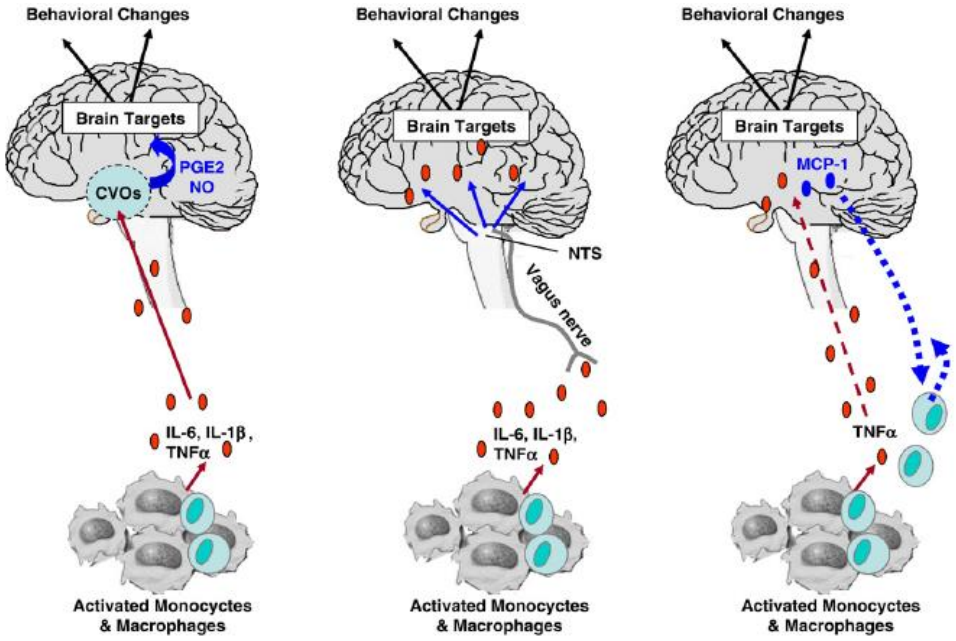

Figure 1. Communication pathways from the periphery to the brain. Different pathways by which cytokine signals access the brain have been identified. A) Humoral pathway: Proinflammatory cytokines released by activated monocytes and macrophages access the brain through leaky regions of the blood-brain barrier. Within the brain parenchyma, the activation of endothelial cells is responsible for the subsequent release of second messengers (e.g., prostaglandins [PGE2] and nitric oxide [NO]) that act on specific brain targets. B) Neural pathway: Pro-inflammatory cytokines released by activated monocytes and macrophages stimulate primary afferent nerve fibers in the vagus nerve. Sensory afferents of the vagus nerve relay information to brain areas through activation of the nucleus of the tractus solitarius (NTS) and area postrema. C) Cellular pathway: A cellular pathway has been recently described by which pro-inflammatory cytokines, notably TNF-alpha, are able to stimulate microglia to produce monocyte chemoattractant protein-1 (MCP-1), which in turn is responsible for the recruitment of monocytes into the brain (D'Mello et al., 2009). Abbreviations: interleukin-6: IL-6; interleukin-1β: IL-1β; tumor-necrosis factor: TNF (Capuron & Miller, 2011).

The project consists of two parts, a human study and an animal study.

For the human study patients are pooled together in one big group. Based on a broad scale examination with various tests a certain “social withdrawal level” is then found. These patients are rearranged in groups based on their level of social withdrawal rather than their diagnosed mental disorder. Figure 1 provides an illustration of how these patients will be categorized based on social withdrawal levels.

Figure 2. an illustration of the rearrangement of mental disorders into levels of social withdrawal (PRISM project).

After these patients are assigned to a specific group, further testing is done such as genome wide association studies and other genetic studies. These genetic studies will hopefully provide targets for the animal study of this project.

Until new targets have been found in the human study, the animal study relies on animal models that show forms of social withdrawal. For the screening of these models they will be tested in a colony-based test to screen for aberrant social behaviours. When aberrant social behaviours are observed these animals will be put through a battery of social tests, *the social withdrawal battery*.

This battery consists of the following tests:

- Three chamber test (Silverman, 2010), this tests measures sociability and social motivation of the mice.
- Social conditioned place preference (Dölen et al., 2013), this test measures social associative memory.
- Social discrimination test (Molenhuis et al, 2014), this test measures social memory and novelty.
- Resident-intruder paradigm (Koolhaas et al., 2013), this test measures aggression towards a stimulus animal.

Each of these tests measure a different aspect of sociality and each of these aspects can result in the subject showing a type of social withdrawal in the group test.

One of the animal models is the BTBR T +tf/J (BTBR) mouse, BTBR mice are used as a model for autism-like behaviour. This is because BTBR show reduced reciprocal social interactions as compared with a C57BL/6J (B6) control mouse and repetitive behaviours in the form of high levels of self-grooming in both juvenile and adult BTBR mice (McFarlane et al., 2008).

We used the BTBR mouse as a means to validate our *social withdrawal battery* in its capacity to screen for the underlying cause of social deficits. BTBR was chosen as a model because of its known deficits in social behaviour. And we used B6 mice as our control strain, this strain is also used as a control in other comparative studies of the BTBR mice(Martin et al., 2014)(Chalovich & Eisenberg, 2011; Pearson et al., 2012; Pobbe et al., 2010).

Materials and methods

Animals

For this experiment, we used three different strains of mice, C56BL/6JRj(B6), BTBR tF/ tJ(BTRB), A/J. the B6 mice were procured from *Janvier Labs*, Le Genest Saint-Isle(Foxn, 2013).
The BTBR and A/J mice were procured from *The Jackson laboratory, Maine*(The Jackson Laboratory, 2017).
Of the B6 and BTBR strain a total of 10 males and 10 females per strain were used, of the A/J strain we used 20 males to act as stimulus animals. Animals at the time of testing were between 120-174 weeks old.
Because we want to look at sociability of the different strains, they are group-housed in 2-4 animals per cage. Males and females were kept in separate rooms to prevent any interference for the experiment. All of the animals were kept under the same housing conditions. They followed a 12/12 light dark cycle with a light intensity of 26 lux, measured at the bottom of the floor in the middle of the room. The animals were given ad libitum food, standard chow, and water. Once a week the cages were cleaned, if they were not being used in an experiment at that time.

Experiments,

All of our experiments were performed during the beginning of the dark cycle. This period was chosen because this is said to be to active period of mice. Because of testing in the dark, we used an infrared camera *from Basler vision technologies,* which was compatible with Media recorder XT from *Noldus information technology*. The movies were later analysed either manually or automatic by The Observer XT or Ethovision XT from Noldus information technology, respectively.

Three-chamber test

For the three-chamber test we used the sociability cage from *Noldus Information Technology*, figure 2. This cage is specially designed to work well with the tracking software ethovision XT which was also provided by *Noldus Information Technology*. All three compartments are of the same size and the outer compartments contain a wire cage. The entire box and wire cages are crafted out of Plexiglas.
The testing was done in a separate room than the room in which the animals were housed. We did this to rule out effects of other male mice that were also housed there. The test animals were transported to the testing room half an hour prior testing. This was done so that the animals could habituate to the new smells and surroundings.

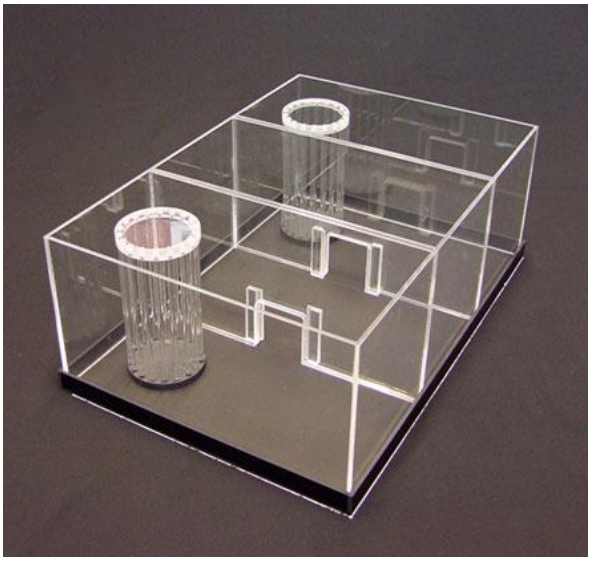

Figure 3. Noldus sociability cage, three-chamber test designed for tracking software. http://www.noldus.com/content/sociability-cage

The test begins with a 10-minute habituation in the centre chamber, which is closed off by slide doors. After the first 10-minute window the slide doors are taken out and the mouse can habituate for another 10 minutes, but now in the full test-box. When the subject is fully habituated after 20 minutes, the mouse is again confined to the centre chamber for a brief moment. In this moment, we introduce the wire cups in the right and left chamber. One of these wire cups contains a stimulus animal of the A/J strain and the other cup is empty. The side on which the cup with the stimulus animal stands, is alternated between subjects. This way we want to exclude external differences in the room. The slide doors are taken out and the subject can explore the box with the wire cages for 10 minutes. We measured the time that the mouse was in either chamber of the box and we measured the time the subject interacted with the wire cage. The test was analysed using the Ethovision XT software from *Noldus Information Technology*. Figure 3 shows how we defined an interaction with the wire cup. The wire cup was defined as the blue zone and around that we drew another zone, orange. If the mouse was in the orange zone and his nose was directed to the blue zone, this was counted as interaction with the wire cup. Once the final 10 minutes had expired the subject and stimulus animal were taken out and the box was cleaned with acetic acid (.01 M).

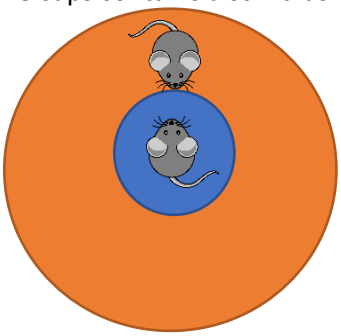

Figure 4. schematic example of the detection setting used in Ethovision XT.

Social conditioned place preference test

The social conditioned place preference test is much like a classic place preference test that is drug-based. For our test-setup we use the same box as we used in the three-chamber test. Via a pulley-system we devised a way that we can open the slide doors from outside the room and in this way minimizing any disturbances in the room. As a conditioning cue, different types of bedding are used, cellu dri and corncob(LBS-Biotech). These two types of bedding are highly distinguishable because of e.g. colour, hardness and grain size, see figure 4, 5.

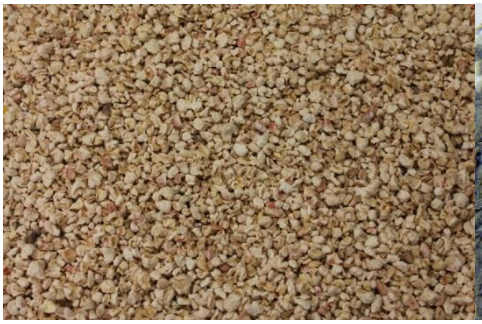

Figure 6 Corncob bedding.

Figure 5 Cellu Dri bedding.

The left and the right chamber were filled with opposing types of bedding, which was rotated with every next trial. 30 minutes prior to testing the subjects were put in the experimental room to habituate to the different environment. On the first day subjects were put in the box for 30 minutes to habituate to the box and the different types of bedding. After 30 minutes, the subject was taken out and put back with his cagemates. On the second day, the subjects were group-housed with their cagemates on either the corncob bedding or the cellulose bedding in a regular cage for a period of 24

hours. The next day the subjects were individually housed on the opposing bedding of that on which they were group-housed, again for a period of 24-hours. The conditioning for the beddings with housing conditions was done in the experimental room and not in the housing room. The fourth and final day the animals were placed in the three-chamber box for 30 minutes. The bedding types were rotated between each trial opposite of the first day. The whereabouts of the subject in the box were manually scored after the test with The Observer XT. This was necessary because the automated tracking software had difficulties with the darker bedding and therefor losing the animal sometimes. A preference index was calculated using the formula tG/(tG+tI), were tG is the time spent on the bedding associated with being in a group and tI the time spent on the bedding associated with being isolate. If the preference index exceeded 0.5 the animals had a preference towards the group-housing associated bedding.

Social discrimination test

The social discrimination test measures the short- and the long-term memory in a social context. Half an hour prior to testing the subject and stimulus animals were taken from the housing room and put in the testing room to habituate to the new environment. The subject is put in a standard housing cage alone for 5 minutes to habituate to the cage. On top of the cage a plexiglass cover was put to prevent the mouse from escaping. After 5 minutes, the subject was exposed to unfamiliar stimulus mouse from the A/J strain for 2 minutes. After 2 minutes, the A/J mouse was taken out again. The subject is now left alone in the cage again for 5 minutes. Then, the previous A/J mouse is reintroduced plus another unfamiliar A/J mouse. This is repeated 24 hours later to test the long-term memory. This time with a different A/J stimulus mouse as unfamiliar, because the one used before is no longer an unfamiliar. The familiar stays the same as day 1.

The time that the subject interacted with either of the stimulus animals was manually scored using the observer XT (noldus information technology, Wageningen, The Netherlands). The discrimination capacity was calculated by the formula tN/(tN+tF), where tN is the time spent exploring the novel animal and tF the time spent exploring the familiar animal. A ratio higher than 0.5 shows a preference for the novel stimulus animal. When an aggressive interaction occurred, the test was stopped and taken out of later calculations, because these aggressive behaviours interfere with reliable measurements of the social discrimination capacity

Resident Intruder paradigm.

~~De Boer 2000~~

We used the resident-intruder paradigm to test the aggressiveness of our subjects. The test is based on the natural instincts to protect one's territory. To establish such a territory the subjects were given a large home cage with nesting material and a hiding tunnel. To further instigate aggressive behaviour towards intruders, the subject was given a female conspecific of the same strain. The female underwent surgery to sterilize her by cutting the oviducts. This way the female will still display normal behaviour and become in eustress.
The subject and the female are left in this cage for a period of 7 days to fully establish territory. After this week, the attack latency was measured on 3 consecutive days. 30 minutes before testing the female and any enrichment were taken out. The resident intruder box comes with a compartment that can be closed off and opened from the side. This compartment was used to put the intruder animal in and allow the resident to see the intruder. We used an A/J male mouse as intruder, because these are more docile animals and we are not interested in the intruder animal(van Gaalen & Steckler, 2000). For a period of 10-minutes the compartment stayed closed off in order to let the resident prime and build aggression. After priming the slide door was taken out and the intruder exposed to the resident. From the moment the slide door opens, the time was measured until the resident attacks the intruder. After the first clinch attack, the intruder was taken out to prevent further injury. If the resident did not attack the intruder for a period longer than 600 seconds the trial was cut off. This was repeated on the second and third day, but with a different intruder. On the fourth and final day, the same procedure was carried out as on the first three days, but now we let the intruder be in the cage for 10 minutes after the first clinch attack. This 10-minute window was later manually analysed using Observer XT. The behaviours that we scored are: upright offensive, chase, lateral threat, tail rattle, attack, non-social exploration, social exploration, immobility, body care, feeding. In later analysis upright offensive, chase, lateral threat, tail rattle and attack were pooled as offensive behaviour. Again if the resident did not attack in the first 10 minutes the trial was cut off.

Results

Three chamber-test.

B6 mice and BTBR mice both show a strong preference to interact with the wire cage that contains a stimulus (A/J) mice. Figure 6 shows the time that B6 and BTBR interacted with the social cage, containing stimulus mouse, and the empty cage. To test for normality a standard shapiro Wilkinson test was performed as well as looking at Q-Q plots. The outcome of this showed that all four groups were normally distributed. We compared the time spent in the novel mouse-chamber vs the novel object-chamber using a paired samples t-test. Both BTBR and B6 showed significant preference for the novel mouse-chamber respectively, $p< 0.0001$ and $p< 0.00001$.
In figure 7 a preference index is shown towards the social cage containing the stimulus animal. This preference was compared, using a one-sample t-test, with a hypothetical value of 0.5 where the animal has no preference. Both B6 and BTBR showed significant deviation from 0.5 with respectively $p < 0.0001$ and $p < 0.0001$.
Both strains show equal levels of sociability towards the stimulus mouse.

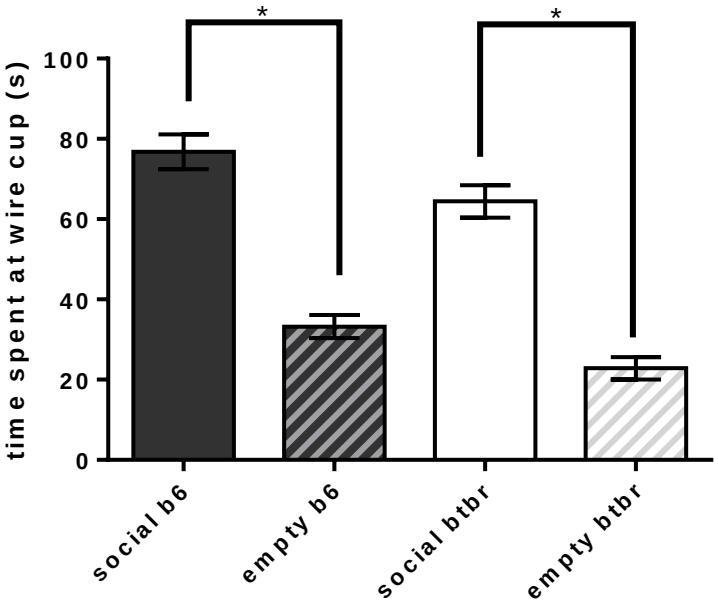

Figure 7.The total amount of time spent interacting with the social cage and with the empty cage. N=10 For both groups. There is a Strong preference for the social cage for both B6 and BTBR.

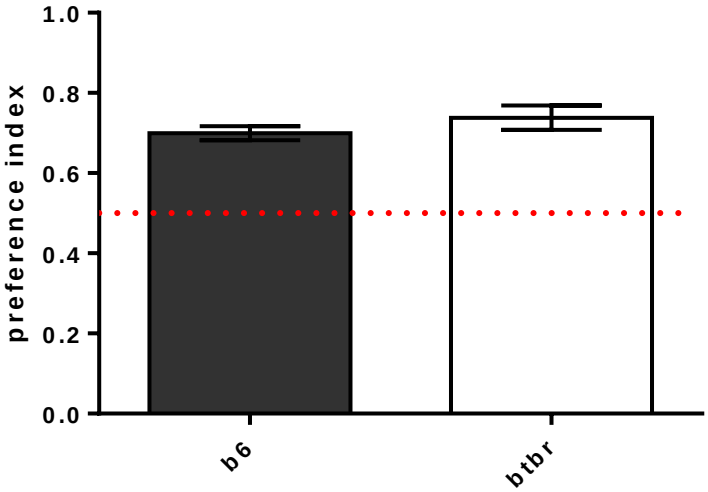

Figure 8. preference index for interaction with the stimulus animal. Calculated as the time spent interacting with the stimulus animal divided by the total time spent interacting with either wire cage. Both groups show a strong preference for the social cage. N=10.

Social conditioned place preference.

Shapiro Wilkinson and QQ-plots were used to test for normality and showed a normal distribution. To compare the time spent in the social chamber with the isolation chamber we used a paired t-test. B6 showed a significant higher preference for the social chamber $p < 0.5$, BTBR however did not show a significant difference $p > 0.2$.
In figure 10 a baseline of 0.5 is added and a comparison with a one sample t-test of our data with the baseline was done. B6 showed a significant preference for the social chamber, $p < 0.5$, BTBR showed no difference with the baseline preference, $p > 0.3$. These results show that B6 mice are more capable to associate the different types of bedding with the housing condition, social or isolate.

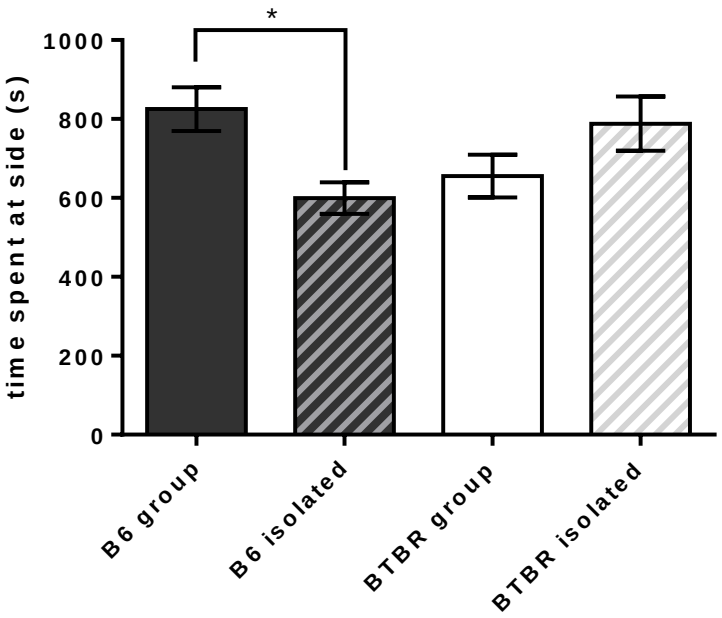

Figure 9. Time spent on the bedding associated with either social housing or individual housing. The B6 mouse show a significant preference for the social bedding. for both groups N=10.

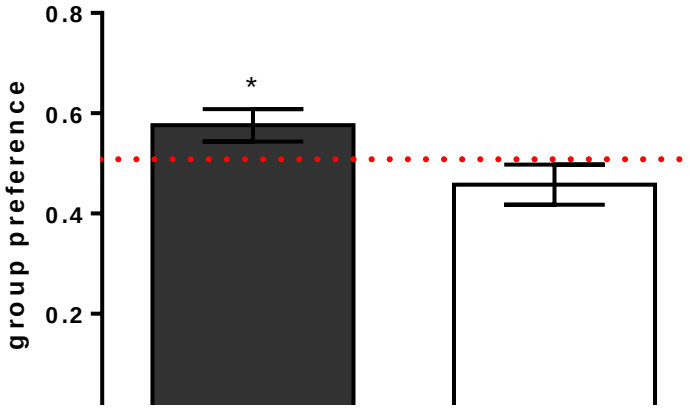

Figure 10. preference index of both B6 and BTBR, N=10 for both groups. The dashed red line indicates the hypothetical value of 0.5 at which there is no preference. The B6 mice differ significantly from this value and have a preference for the social bedding. BTBR mice however show no significant deviation from 0.5.

Social discrimination

For a 2-minute window of time our subjects were allowed to interacts with a familiar animal and a novel animal, both of the A/J strain. This 2-minute window was then manually scored to assess the interaction time of our subject with the stimulus animals. This interaction time was then expressed as a preference towards the novel animal by dividing the interaction time with the novel animal by the total interaction time with either animal, see figure 11. Animals were excluded when they fought during the test. After normality checks, we compared our preference to a baseline preference of 0.5 using a one-sample t-test. For both B6, day 1 p=.094 day 2 p= .331 and BTBR, day 1 p=.224 day 2 p=.053, there is no significance to be found.

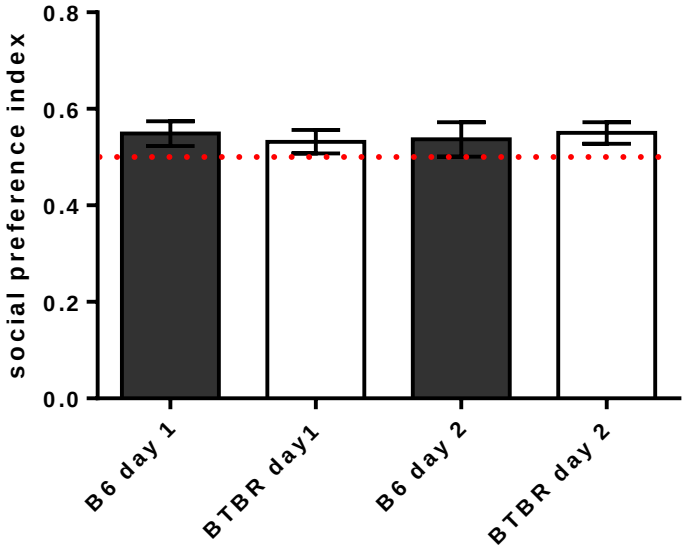

Figure 11. Preference index of both B6 and BTBR, N=10, for the novel animal. The dashed line represents the hypothetical value of 0.5. There are no differences between the groups. N per group is respectively N=9, N=10,

Resident intruder paradigm.

The results of the resident intruder paradigm are displayed in two different figures, figure 11 and figure 12. Figure 11 shows the attack latency of the two strains towards an intruder, the intruder was of the A/J strain. Due to the large skew in the BTBR group the data was not normally distributed and we had to do a non-parametric test. Mann Whitney U test then confirmed what is clearly visible with the eye, B6 and BTBR are significantly different. B6 mice are more prone to attack immediately when the intruder enters the cage, whereas the BTBR group is divided in two groups with high aggression and low aggression.

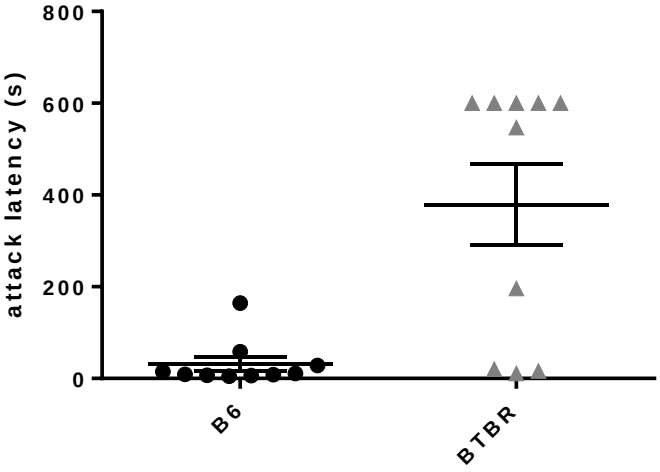

Figure 12. attack latency times of both B6 and BTBR displayed. Each mark represents an individual. For both groups N=10. A clear difference can be seen in the two groups.

On the last day of testing, a 10-minute window was analysed after the first attack. In this 10-minute window we looked at the behaviour of the subject and not the stimulus mouse. We scored aggressive behaviours (attack, chase, threat, tail rattle), social exploration, non-social exploration and body care. The results of this are displayed in figure 12. This data was normally distributed and therefore we continued with an anova. The results of this test for offensive, non-social exploration, social exploration, body care, are respectively p=.110, p= .886, p= .481, p=.740.

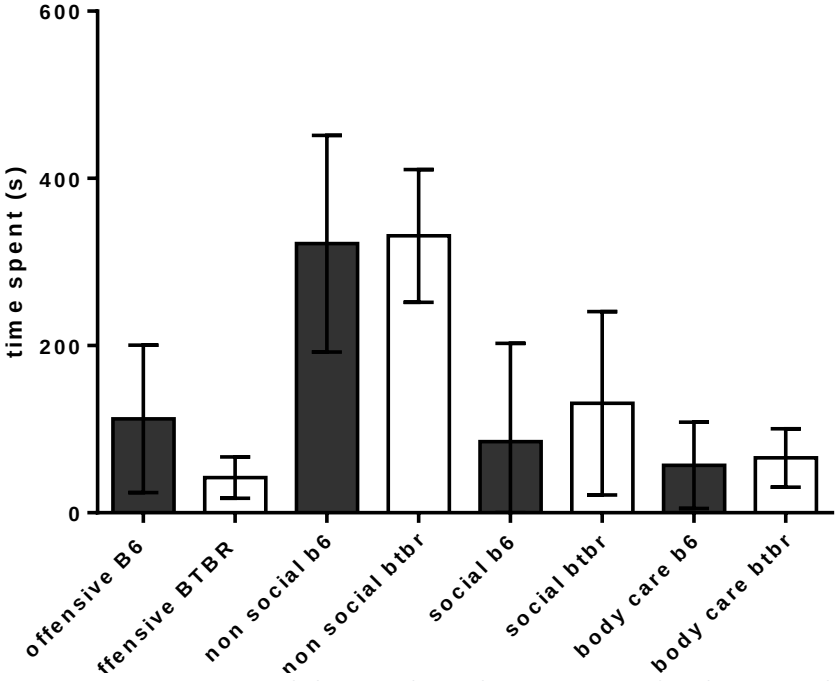

Figure 13. Time spent on certain behaviours during the 10-minute window that was analysed. For B6 N=10 and for BTBR N=5, this is because some BTBR did not attack on the final day.

One thing that stood out when we analysed the videos of the behaviour, was that the BTBR mice that did attack do not show or show less threats preceding an attack. Classically an attack is preceded by warning signs such as lateral threat or tail rattling, but this was seen far less with the BTBR mice. So, we created a ratio of the threats followed by an attack divided by the total amount of threats. this data was normally distributed and a t-test resulted in the following p value of .026. This shows that the B6 mice display significantly more threats before an attack.

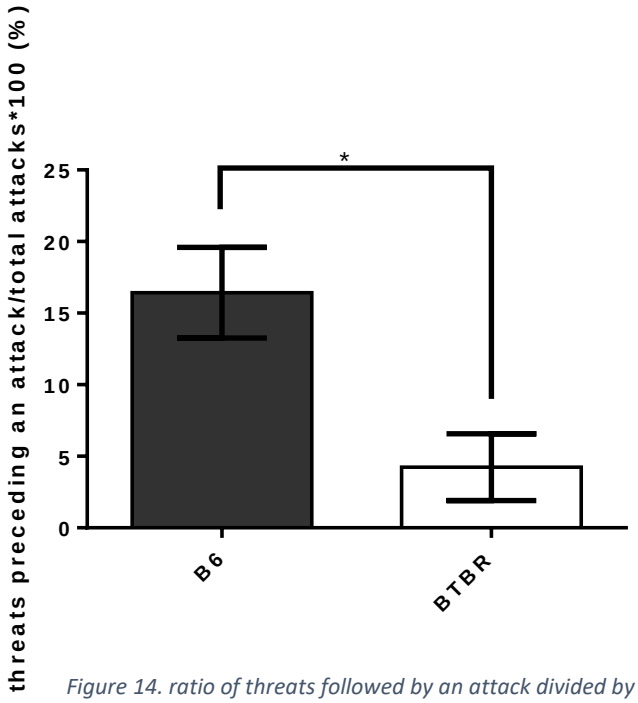

Figure 14. ratio of threats followed by an attack divided by the total amount of attacks times a hundred percent. For B6 N=10 and for BTBR N=5. A clear difference can be seen between the two groups.

Discussion

We sought out to validate a battery of tests that can show us what lies at the basis of social withdrawal. These tests are the three-chamber test, social conditioned place preference test, social discrimination test and resident-intruder paradigm which respectively measure sociability, social associative memory, social memory and aggression.
In the three-chamber test we can see that there is no difference in sociability between the BTBR strain and the B6 strain. These results are backed up by a full characterization done by Puoliväli et al. in 2015. This characterization shows that the BTBR mouse is equally interested in the novel mouse as the B6 mouse and both are less interested in the novel object. Though there are also studies that report exactly the opposite of our results. Pobbe et al. found in 2011 that the BTBR mouse has a preference towards the side where there is no other mouse and claim that the BTBR has low sociability. They also report that when BTBR are treated with diazepam this effect is reversed and the BTBR show high sociability. However, as this is a drug study there is always an effect of the handling and the injection of the vehicle and the drug. This stress factor of the injection of the vehicle might have a bigger effect on the BTBR than it does on the control mouse and induce high anxiety levels for the BTBR mouse. A other study done by Silverman et al. in 2010 also finds low sociability in BTBR mice when they do a three-chamber test. In their article, they summarize different tests that can be used to screen for autism-related behaviour when studying mice and they state that different housing conditions can be chosen to maximize the outcome of these tests. What they do not state is what kind of housing conditions they used when performing their three-chamber test. It could very well be that their mice were not socially reared and therefor lack incentive to be in a social environment.
The approach that we took for our social conditioned place preference was adapted from Panksepp & Lahvis who in 2007 used this for screening in juvenile mice, Dölen et al. found in 2013 that this method works equally well with less days of conditioning. These studies are not directed at out animal of interest, the BTBR mouse. We are the first to show this method of social conditioning used for BTBR. As you can see in the results this method shows very nicely that after 2 days of conditioning the B6 have learned to associate either bedding with being in a social setting. The BTBR actually show a slight trend to favour the bedding associated with isolate housing. This can be explained by the fact that the subjects were conditioned to the isolate bedding the day before testing. Maybe, the BTBR have a preference for the bedding that they were on most recently. Pearson et al. also showed in 2012 that BTBR do not associate a certain environment with a social or isolate setting. However, they required 5 to 10 days of conditioning to reach a rather small difference between control and the BTBR strain. With our method in just two days of conditioning we found an even bigger difference.

Our social discrimination task sadly did not live up to our expectations. Molenhuis et al. showed in 2014 a nice effect where the BTBR does recognize a familiar after one hour but loses this effect after a period of 24 hours, where the control mice do not. The fact that our test did not work as expected could be due to several reasons, but we believe that the mice have experienced high levels of stress during transportation from Jackson laboratory. The test was later redone with mice that were reared in-house and they showed the same results as Molenhuis et al. did (unpublished data, Ike et al. 2017)

The resident intruder paradigm provided some interesting results regarding the BTBR strain. During the attack latency testing we saw two groups forming, one of high aggression and one of very low aggression. The group with high aggression was similar to the control group. We sought out if the animals with the low aggression also were the animals that performed worse in other tests. This was not the case and why we saw this remains unexplained. After testing brains were taken from the subject and further brain analyses might reveal if there are differences within our group of BTBR mice. In the behaviour window that was analysed we could only include those animals that actually attacked on the final day. Because of the low aggression of some BTBR the final N value for this behaviour window was only 5 animals. And due to large variation in our control group we were not

able to find any significant differences between BTBR and B6. What we did find and was very interesting is the fact that BTBR show aberrant aggressive behaviour by displaying a reduced amount of threats or warning signs before attacking. BTBR seem to very unexpectedly attack and afterwards show almost no aggression anymore. Our B6 control strain however, does show high levels of threats and tail rattling before they attack and continue to do so during the rest of the analysed behaviour window.

Overall our social withdrawal battery seems to be an effective way of screening all sorts of aspects of social behaviour. But, seeing as certain tests are not yet giving consequent results when repeated, our battery needs to be finetuned in order to make sure that we get the same results every time. This might have to do with order effects of the tests that we performed or the housing conditions that the animals were in. Also, we ordered the animals that we used from Jackson laboratory and they had to undergo shipment from the U.S. to the Netherlands. This might have induced high stress levels in the animals which caused them to perform differently than they would if they are in-house bred and reared.

Using the social withdrawal battery, we hope to pinpoint those areas which cause social withdrawal in different models. By pinpointing these areas, we might find the cause of the chronic exposure of the brain to a high concentration of proinflammatory cytokines and ultimately develop new drugs for mental disorders such as MDD, AD and SZ.

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