Beyond Aggression: Characterising the Phenotype of the BALB/cJ Mouse

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Children with conduct disorder (CD) show high levels of aggression. Common comorbidities of CD are attention-deficit-hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). Here, we further characterised the behavioural and physiological phenotype of BALB/cJ mice, known for their increased levels of aggression. We investigated if they show symptoms of ASD (social withdrawal) and ADHD (hyperactivity and low temperature). In experiment 1, social withdrawal was investigated with a modified version of the three-chamber social interaction test. In experiment 2, telemetric devices were implanted to measure locomotion and body temperature for a period of 86 hours. In the social interaction test, BALB/cJ mice showed less interest in an unfamiliar mouse compared to BALB/cByJ mice. Experiment 2 showed that BALB/cJ mice had increased locomotor activity during the active period and a lower body temperature in the non-active period compared to control mice. In this study, we have further characterised the behavioural and physiological phenotype of BALB/cJ mice, demonstrating that these mice show symptoms of CD, and its associated comorbidities, ASD and ADHD. The model can be used to study brain structures that might give rise to the linked symptoms of CD, ADHD and ASD.

Keywords: conduct disorder, autism spectrum disorder, attention-deficit hyperactivity disorder, comorbidities, animal model, BALB/cJ mouse

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From an evolutionary perspective, aggression increases an individual's chance of survival. It facilitates the competition for resources and protection of the individual or its offspring (Coppens, De Boer, Buwalda, & Koolhaas, 2014). However, to avoid negative consequences, aggressive behaviour needs to be proportionate to the provocation or context and is subjected to strong inhibitory mechanisms (De Boer, van der Vegt, & Koolhaas, 2003). If these inhibitory mechanisms fail, aggressive behaviour can quickly escalate and lose its adaptive function in social interaction (De Boer, Caramaschi, Natarajan, & Koolhaas, 2009). Aggressive behaviour is among the most common causes of referrals to child and adolescent psychiatrists (Gurnani, Ivanov, & Newcorn, 2016) and often, conduct disorder (CD) is the diagnosis (Blair, 2013; Finger et al., 2012; Serper, Beech, Harvey, & Dill, 2008).

CD is characterised by a persistent pattern of disruptive behaviour, violating the basic rights of others and societal norms (Loeber, Burke, Lahey, Winters, & Zera, 2000). Children with CD show high amounts of aggression, fighting, bullying or being cruel to others and animals (Finger et al., 2012). They are less empathic, force someone into sexual activity or run away from home. Often, they already show these symptoms at a young age (Blair, 2013). Prognosis is poor; children diagnosed with CD show high rates of domestic violence, unemployment and homelessness in adulthood, and about 54% receive a diagnosis of antisocial personality disorder (APD) later in life (Blair, 2013; Loeber et al., 2000; Noordermeer, Luman, & Oosterlaan, 2016). Multiple brain circuits have been implicated in the aetiology and maintenance of CD, for instance, the basic threat circuitry (amygdala–hypothalamus–periaqueductal grey [PAG]), the hypothalamus–pituitary–adrenal system (HPA axis), as well as frontal circuits (anterior cingulated cortex [ACC] and ventromedial prefrontal cortex [vmPFC]). However, not all patients with CD demonstrate the same pathophysiology as different symptom clusters and different comorbidities can be present.

Common comorbidities of CD are autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD). About 30% of children with CD receive a diagnosis of ASD and even more than 50% receive a diagnosis of ADHD. ASD is characterised by severe and pervasive deficits in social interaction and repetitive or stereotyped behaviours (Glickman, 2010; McDougle, Stigler, & Posey, 2003). ADHD is characterised by a deficit in attention, increased impulsivity and hyperactivity, and in children with ADHD that develop CD, the age of onset of CD is considerably lower than in children with CD only (Loeber et al., 2000). Furthermore, children with comorbid ADHD demonstrate higher levels of physical aggression, delinquency and more severe symptoms of both CD and ADHD than children with CD or ADHD only (Noordermeer et al., 2016).

In the last two decades, increasingly more knowledge on structural and functional abnormalities in CD, ADHD and ASD has been generated but effective treatments are sparse and long-term prognosis is poor (Casanova, 2007; Esbensen, Greenberg, Seltzer, & Aman, 2009; Matthys, Vanderschuren, & Schutter, 2012; Nestler & Hyman, 2010). It is hypothesised that CD, ADHD and ASD share a common underlying aetiology and this is supported by genetic studies demonstrating that about 50–72% of the contributing genetic factors overlap (Leitner, 2014; Thapar, Harrington, & McGuffin, 2001). Furthermore, several brain structures, such as amygdala, ACC and prefrontal circuits, are affected in all three disorders (Blair, 2013; Brieber et al., 2007). However, it is unknown if these brain structures underlie the co-occurrence of CD, ADHD and ASD. Human neuroimaging studies can guide in identifying neural structures and neuro-circuitry that co-occur in all three disorders but causal relationships between neural structures and behaviour cannot be examined in such studies. Animal models enable us to experimentally manipulate neural structures, observe the effects on behaviour and gain detailed insights into pathophysiological mechanisms (Markou, Chiamulera, Geyer, Tricklebank, & Steckler, 2009; Nestler & Hyman, 2010). An animal model that shows symptoms of CD, ADHD and ASD would enable us to investigate which neural structure(s) might underlie the comorbidity of these three disorders.

The BALB/cJ mouse model of CD

BALB/cJ mice have been repeatedly used as a model for aggressive behaviour (Dow et al., 2011; Velez, Sokoloff, Miczek, Palmer, & Dulawa, 2010). These mice were derived from an initial BALB/c stock, which was established in 1935. Several other laboratories acquired mice of the initial BALB/c stock, maintained them and bred them as independent stocks including BALB/cJ, BALB/cN, and BALB/cByJ. Due to breeding errors introducing new alleles and/or spontaneous mutations, the substrains started to exhibit genetic and phenotypic differences (Velez et al., 2010). For example,

BALB/cJ and BALB/cByJ show differences in eleven copy number variants (CNVs) and 38 mRNAs (Jager et al., unpublished data; Velez et al., 2010). BALB/cJ and BALB/cByJ mice also demonstrate differences in their behavioural phenotype; BALB/cJ mice are more aggressive than BALB/cByJ mice. They show high levels of intermale aggression, a shorter latency to attack and a higher incidence of attack in comparison to BALB/cByJ mice and other mouse strains in the resident intruder paradigm (Dow et al., 2011; Velez et al., 2010). In comparison to BALB/cByJ mice, BALB/cJ mice demonstrate decreased structural connectivity and decreased gamma-aminobutyric acid (GABA) inhibition in the ACC, changes that have been linked to aggressive behaviour (Jager et al., 2015). The BALB/cJ mouse model thus reproduces the core symptom of CD – increased aggression – and it also shows brain pathology comparable to the human situation (Teng et al., 2016). However, symptoms of common comorbidities of CD, such as ASD and ADHD, have been insufficiently studied in BALB/cJ mice. Here, we further characterised the behavioural and physiological phenotype of BALB/cJ mice by investigating if these mice show social withdrawal and hyperactivity, major symptoms of ASD and ADHD.

Experiment 1. In order to verify social withdrawal behaviour as a symptom of ASD in the BALB/cJ mouse model, we tested them in a social interaction test. We used a modified version of the three-chamber social preference test. The original test consists of a rectangular arena with three chambers and in one of the end chambers a stimulus mouse is restrained within a clear Plexiglas cylinder. For a period of five minutes a second mouse, the test mouse, can explore the whole arena. In the past, BALB/cJ mice have been reported to spend less time staying close to the cylinder with the stimulus mouse compared to other mouse strains; this behaviour has been interpreted as social withdrawal (Brodkin, Hagemann, Nemetski, & Silver, 2004; Fairless et al., 2008). A reduced size of the corpus callosum and increased brain size in these mice has been linked to social withdrawal (Brodkin, 2007; Fairless et al., 2008). However, there are two specific problems associated with previous studies. First, BALB/cJ mice have never been compared to other BALB/c substrains with a similar genetic background. It is known that mice of the BALB/c strain are less social than C57BL/6 mice; therefore, comparing BALB/cJ mice to C57BL/6 mice might lead to an overestimation of effects. Second, the use of a three-chamber apparatus might have a large influence on the behaviour of BALB/cJ mice. The mice are placed into a novel environment and are exposed to an unfamiliar stimulus mouse. Both the stress and novelty may alter the social behaviour of the test mouse, and this may be particularly of importance in BALB/cJ mice, as these mice display high levels of anxiety and are more sensitive to stress than other mice (Crawley et al., 1997; Fairless et al., 2013). Therefore, we created a modified version of the three-chamber test that consists of an arena with a single chamber and cylinders to the right and left side. This arena is similar to the homecage of mice and allows testing of social behaviour in a more familiar environment. We tackled the following hypothesis: BALB/cJ mice demonstrate less social interest than BALB/cByJ mice, a behaviour that can be interpreted as social withdrawal.

Experiment 2. In this experiment, we investigated if BALB/cJ mice show signs of hyperactivity commonly associated with ADHD, more specifically, increased locomotor activity. We implanted radio telemetry transmitters that could measure locomotor activity, temperature and heart rate in freely moving animals (Butz & Davisson, 2001). It has been observed that children with ADHD have a decreased core body temperature compared to healthy control children (Bijlenga et al., 2013; Dahl & Lewin, 2002). Therefore, we also investigated if a decreased core body temperature is seen in BALB/cJ mice. We aimed to tackle the following two hypotheses: (1) BALB/cJ mice demonstrate increased locomotor activity in comparison to BALB/cByJ mice, and (2) BALB/cJ mice show a decreased core body temperature in comparison to BALB/cByJ mice.

Methods

General methods

Housing conditions. All mice were housed individually in an enriched environment (High Makrolon® cages with Enviro Dri® bedding material and Mouse Igloo®) and had free access to dry food and water. They were kept at a reversed 12/12 day/night cycle with sunrise at 7.00 pm. Efforts were taken to restrict the number of mice and to keep the discomfort as minimal as possible. All animal procedures, including behavioural tests and surgical procedures were conducted in strict compliance with the European regulations for animal experimentation. The study was approved by the Ethics Committee on Animal Experimentation of Radboud University (RU- DEC).

Experiment 1: Social withdrawal

Animals. Fifteen-week-old male BALB/cJ (n = 5) and BALB/cByJ (n = 4) mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and used as test mice. Male C57BL/6 (n = 2, Charles River Laboratories, Erkrath, Germany) were used as stimulus mice.

Experimental procedure. Social behaviour was assessed with a modified version of the three-chamber test. The arena (50 cm × 43 cm) consisted of a single chamber with cylinders at the centre of the right and left side (see Fig. 1). Testing was done in the dark and behaviour was video-recorded. At the start of the test a stimulus mouse was randomly placed in one of the cylinders ("social cylinder"). We randomly assigned a stimulus mouse to each test mouse. The test mouse was then placed in the middle arena and was allowed free exploration of the arena and cylinders for a period of five minutes. Both cylinders had many holes, so that the test mouse could sniff the stimulus mouse. The side of the arena with the stimulus mouse was labelled as "social side" and the side with an empty cylinder as "non-social side". There was no barrier or line between the sides. Mice were sacrificed two weeks after the social interaction test.

Data Analysis. We manually scored "social cylinder investigation", defined as the amount of time (in seconds) that the test mouse sniffed, reared against, and climbed on the walls of the cylinder with the stimulus mouse inside. Climbing on the walls occurred very rarely in both groups and sniffing of the social cylinder was the predominant behaviour. For manual scoring The Observer XT software (Noldus Information Technology BV, Wageningen, The Netherlands) was used. Social cylinder investigation was analysed with a one-way ANOVA. All statistical analyses were performed using SPSS21- software (SPSS inc., Chicago, USA).

Experiment 2: Hyperactivity and temperature

Animals. Six-week-old BALB/cJ (n = 5) and BALB/cByJ (n = 6) mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Upon arrival, mice were housed individually. Surgery was performed at seven weeks of age and mice were allowed two weeks of recovery. Experimental recordings were performed at nine weeks of age.

Surgical Procedure. Transmitters were purchased from Data Sciences International (DSI™, St. Paul, MN, USA). The transmitter model PhysioTel ETA-F10 was used, allowing simultaneous recordings of heart rate, temperature and locomotor activity. These transmitters operate on radio frequency, have a battery life of about 2 months and are placed intraperitoneally. The transmitters consist of an insulated red (positive) and an insulated white (negative) electrode wire. Before surgery mice were weighed and transferred to a clean homecage. Surgery was performed under sterile conditions. Anaesthesia was induced and maintained using isoflurane (3% and 1.5-1.8%, respectively). The mouse was fixated with tape on all four legs ventral side up on a temperature-controlled surgery stage, adjusted to 36.5 °C. The abdomen and chest of the mice were shaved and the skin was disinfected

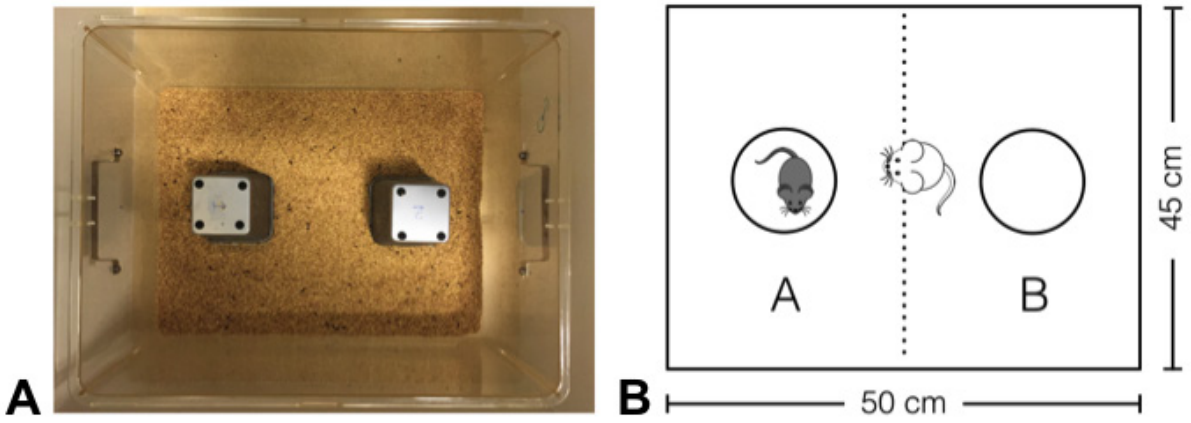

Fig. 1. A. The arena consisted of a single chamber and two metallic cylinders. The bottom was covered in corn pops to create a more familiar environment. **B.** Schematic of the arena with the stimulus mouse restrained in the cylinder in the social side (A) and an empty cylinder in the non-social side (B). In this example, the test mouse would be scored as being in the social side.

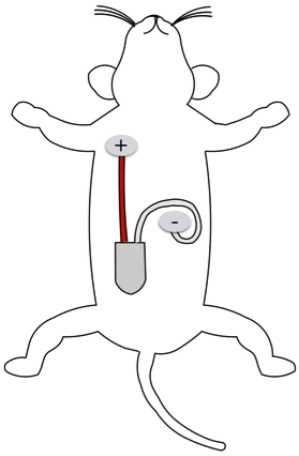

Fig. 2. Schematic of the transmitter placement. The transmitter was placed abdominally. The positive wire (red) was placed at chest-level; the negative wire (light-grey) was placed in a loop just below the costal arch.

with iodine sterilisation solution. Next, a longitudinal and medial abdominal skin incision of 1.8-2 cm was performed. The incision extended from the navel area to about 1 cm below the caudal tip of the sternum. Using a blunt metal probe moistened with physiological solution (0.9% sterile NaCl), skin and underlying muscle tissue were separated. Then, a subcutaneous tract towards the right front leg and the left hind leg was opened for the electrode wires. According to the first skin cut, a cut of muscle tissue was performed (about 1.5 cm long). The transmitter was then placed into the abdominal cavity with the electrode wires positioned anteriorly. Next, using a sharp probe, a little opening left from the abdominal cut, at chest-level, was made (through the muscle tissue). Through this opening the red electrode wire was guided and non-absorbable threads were used to fixate the electrode on the muscle tissue. The muscle tissue was then closed with absorbable threads. The white wire was placed in a loop under the skin and fixated to the muscle tissue below the costal arch (using non-absorbable threads; see Fig. 2 for a schematic). The two plastic fixation straps of the transmitter were connected to the muscle tissue using non-absorbable threads. Then, the muscle tissue was closed with absorbable threads. Before closing the skin, the transmitter was switched on with a magnet to assure proper function and signal. The skin was closed using three to four wound clips. Analgesia was provided by subcutaneously injecting rimadyl (Carprofen 5% with Ethanol 10%; 5-10 mg/kg) directly after surgery and twice a day for two days post-surgery. After surgery, mice were kept overnight in a warming chamber (38.5 °C) for recovery. The weight and the general condition of the mice were checked daily. Wound clips were removed 10 days post-surgery.

Experimental Procedure. Two weeks after surgery, mice were transferred to a separate room (same reversed day/night cycle as in the housing room, with sunrise at 7.00 pm). Their cages were placed on receiver plates (PhysioTel®, DSI™) that collected the signal from the transmitters. To initiate data collection, the transmitter was switched on by touching the mouse with a magnet (possible through the cage). Ponemah Software (DSI™) was used for the detection, collection and initial analysis of signals. This program collects data signals sent to the computer from the receiver plates via a Data Matrix (Matrix 2.0, DSI™). Data were collected at regular intervals (every 5 seconds). Recordings were started at 5.00 pm and lasted 86 hours. During this period no one was allowed entrance to the room, preventing any effect of the experimenter on the mice. Mice were provided with enough food and water for this period. At the end of the experiment, mice were brought back to their original room and were sacrificed two days after the recordings.

Data Analysis. The first two hours of the recordings were not analysed to account for possible stress due to transportation. Data were analysed with start of the non-active phase (7.00 pm, lights on) for a total of 84 hours (3.5 days). Ponemah Software (DSI™) initially pre-processed the data in data intervals of five minutes (the user can manually change these intervals). Temperature was measured in °C per minute and locomotor activity in counts per minute. Initially, for each hour we calculated a mean for temperature and locomotor activity. The data were analysed with repeated-measures ANOVAs (hour as within-subject factor and group as between-subject factor) and post-hoc tests were done when necessary. All statistical analyses were performed using SPSS21- software (SPSS inc., Chicago, USA).

Results

Experiment 1: Social withdrawal

BALB/cJ mice showed decreased (M = 42.47, SD = 10.36) social cylinder investigation compared to BALB/cByJ mice (M = 75.28, SD = 14.12, $F(1, 7) = 16.28$, $\eta^2 = .69$ $p = .004$). The data are presented in Figure 3.

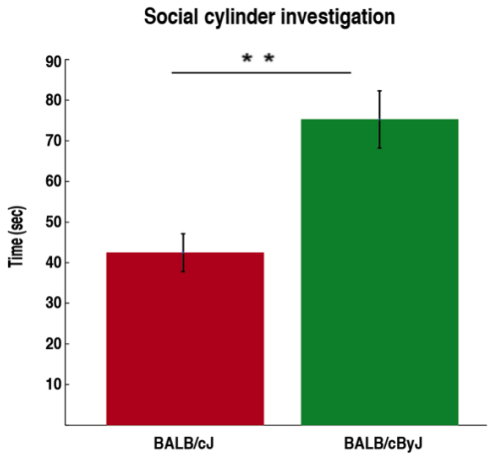

Fig. 3. Bar diagram showing the mean and standard error of the mean (SEM) of social cylinder investigation for BALB/cJ and BALB/cByJ mice. BALB/cJ mice spent significantly less time investigating the social cylinder than BALB/cByJ mice. $^{**}p < .01$

Experiment 2: Hyperactivity and temperature

Locomotor activity. In both groups, locomotor activity was higher during the active than the non-active hours ($F(23, 207) = 7.55$, $\eta^2 = .46$, $p = .000$). There was an interaction effect of hour × group ($F(23, 207) = 2.53$, $\eta^2 = .22$, $p = .036$), demonstrating that BALB/cJ mice were more active than BALB/cByJ mice during the active phase (all $p < .05$). The data are presented in Figure 4.

Temperature. In both groups, temperature was lower during the non-active hours than the active hours ($F(23, 207) = 9.81$, $\eta^2 = .53$, $p = .000$). BALB/cJ mice showed a lower temperature than BALB/cByJ mice (trend, $F(1, 9) = 3.29$, $\eta^2 = .26$, $p = .07$). There was an interaction effect of hour × group ($F(23, 207) = 2.99$, $\eta^2 = .25$, $p = .025$), demonstrating a lower temperature of BALB/cJ mice compared to BALB/cByJ mice in the non-active phase (all $p < .05$). The data are presented in Figure 5.

Discussion

Experiment 1: Social withdrawal

The results of this experiment confirm our hypothesis that BALB/cJ show social withdrawal. We demonstrated that BALB/cJ mice are less interested in a stimulus mouse than BALB/cByJ mice. By comparing BALB/cJ mice to a proper control group, the BALB/cByJ strain, we avoided an overestimation of effects as frequently observed in literature. BALB/cJ mice are usually compared to C57BL/6 mice, although it is known that C57BL/6 mice are more social than most other strains and that mice of the BALB/c strain in general show less social interest than other mice (Brodkin, 2007; Fairless et al., 2013). The arena we used closely resembled the homecage environment and did not require the mice to explore three different chambers. It has been repeatedly demonstrated that BALB/cJ mice

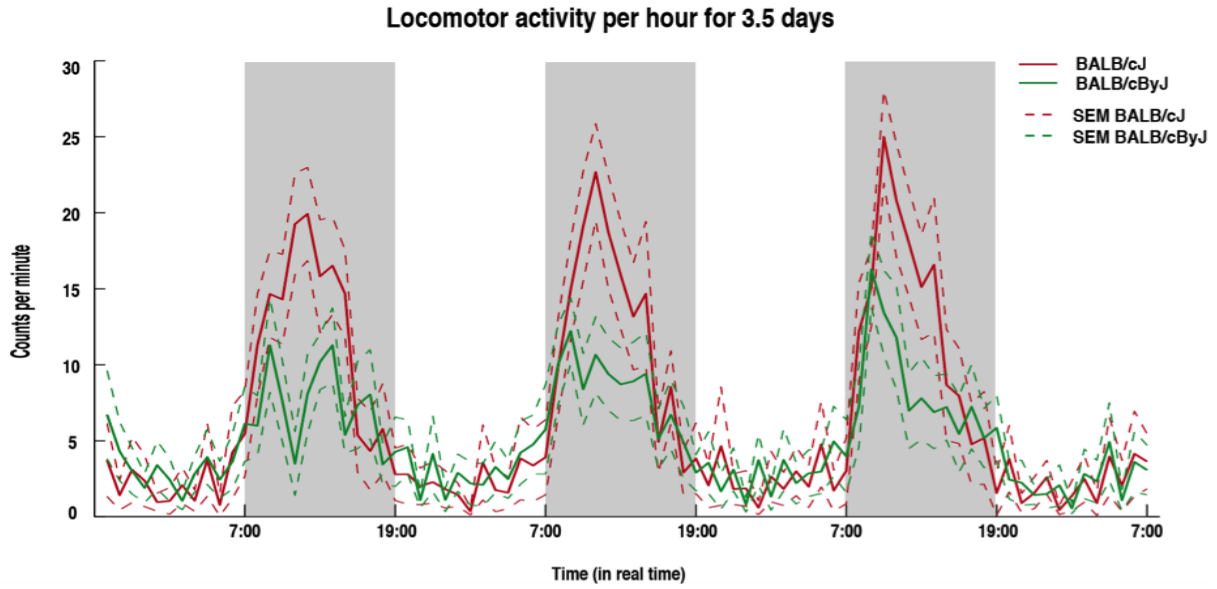

Fig. 4. Graph showing the mean and SEM of locomotor activity per hour for 3.5 days. Grey shaded areas indicate the active hours (lights off). Both groups show a higher locomotor activity in the active hours than the non-active hours. BALB/cJ mice are more active in the active hours than BALB/cByJ mice.

show high levels of anxiety (e.g., Brodkin, 2007). Therefore, we aimed to reduce anxiety induced by a new environment. The fact that BALB/cJ mice did not differ from BALB/cByJ mice in exploration behaviour (both groups investigated both sides of the arena), illustrates that BALB/cJ mice were not too anxious to investigate the arena. In this way, we were able to demonstrate that reduced social interest of BALB/cJ mice, interpreted as social withdrawal, is not (only) due to being too anxious to investigate the arena.

We need to note that apart from the utilised arena, there are a few other methodological differences between our study and previous reports. We housed both BALB/cJ and BALB/cByJ mice individually while previous studies housed mice in groups. By housing them individually we prevented high levels of intermale aggressive behaviour in BALB/cJ mice and to account for individual housing effects we also housed our control group individually. It is known that individual housing can increase aggressive behaviour but it is unknown how individual housing affects behaviour in tests where test and stimulus mouse cannot interact freely, as the three-chamber social preference test and the arena used in this study (Beery & Kaufer, 2015). Follow-up studies should investigate if individual housing has any effects on social behaviour in tests as the three-chamber social preference test. Previous studies usually tested BALB/cJ mice at young age (around 4 weeks, corresponding to pre-pubescence) whereas we have chosen to test our mice at a later stage (15 weeks), corresponding to adulthood (Kumar et al., 2012). In patients with ASD, deficits in social interaction persist into adulthood (Bejerot, Eriksson, & Mörtberg, 2014). Here, we have not only demonstrated that BALB/cJ mice show social withdrawal but also that they demonstrate decreased social interest in adulthood, recapitulating the human situation.

Experiment 2: Hyperactivity and temperature

In experiment 2 we investigated if BALB/cJ mice show hyperactivity (increased locomotor activity) and a lower body temperature, symptoms observed in patients with ADHD. We implanted transmitters and took measurements for a period of 86 hours. We found that BALB/cJ mice showed increased locomotor activity in comparison to BALB/cByJ mice and a lower (nocturnal) body temperature than BALB/cByJ. Multiple lines of research point toward a role of dopamine in ADHD, and more concretely, a dopamine dysfunction in the mesocortical, mesolimbic, and nigrostriatal pathways (Sonuga-Barke, 2005). In patients with ADHD, there seems to be increased dopamine reuptake by dopamine transporters (DAT), resulting in decreased extracellular dopamine (Gold, Blum, Oscar-Berman, & Braverman, 2014; Volkow et al., 2001). Symptoms of hyperactivity have been related

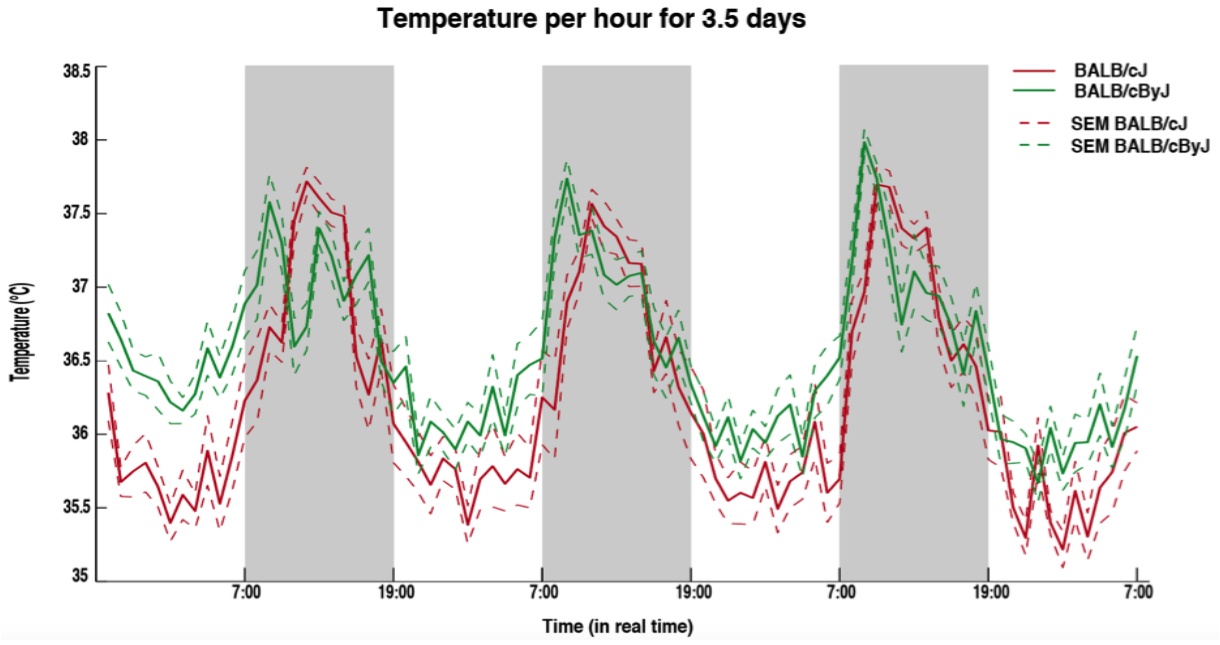

Fig. 5. Graph showing the mean and SEM of temperature per hour for 3.5 days. Grey shaded areas indicate the active hours (lights off). Both groups show a lower temperature in the non-active hours than the active hours. BALB/cJ mice have a lower temperature in the non-active hours than BALB/cByJ mice.

to the nigrostriatal pathway, which is involved in cognitive and voluntary movement control (Aguiar, Eubig, & Schantz, 2015) and projects from the substantia nigra to the striatum (Cho, Baek, & Baek, 2014).

Dopamine levels can also be regulated by serotonin, and serotonin dysfunction has been related to ADHD and hyperactivity (Quist et al., 2003). For example, studies in rodents and humans demonstrated that polymorphisms in the 5-HT1B receptor are associated with hyperactivity (Guimarães et al., 2009; Quist et al., 2003). Deletion of the 5-HT1B receptor in mice resulted in hyperactivity, increased exploratory activity and increased aggressive behaviour. Patients with ADHD frequently have the G861C polymorphism of the 5-HT1B receptor, which is associated with reduced 5HT1B receptors (Huang, Grailhe, Arango, Hen, & Mann, 1999; Quist et al., 2003). Interestingly, polymorphisms in the TPH2 gene, coding for the rate-limiting enzyme in the synthesis of 5-HT, have been associated with ADHD and BALB/cJ mice have a polymorphism in the TPH2 gene as well (Biskup et al., 2012). Therefore, it might be that a serotonin dysfunction in BALB/cJ mice influences the dopamine system, resulting in symptoms of ADHD, such as increased locomotor activity. Furthermore, the lower body temperature we observed in BALB/cJ mice is also observed in children with ADHD and has been linked to low levels of serotonin and dopamine (Catalina, Milewich, Frawley, Kumar, & Bennett, 2002). Children with ADHD often have sleep problems (Stein, 1999) and waking up during a period of low body temperature has been related to sleep deprivation and attention deficits (Dahl & Lewin, 2002).

Increased locomotor activity in BALB/cJ mice might be a method to augment their low body temperature, instead of being a sign of hyperactivity. However, one would then expect to see increased levels of locomotor activity especially during the non-active phase, as the temperature of BALB/cJ mice is significantly lower during the non-active phase. In the non-active phase there is no difference in locomotor activity between BALB/cJ mice and BALB/cByJ mice. It is known that stimulant medication used for the treatment of ADHD decreases symptoms of hyperactivity and there are indications for increases in body temperature in patients taking stimulant medication (Lakhan & Kirchgessner, 2012; Schacher, Tannock, Cunningham, & Corkum, 1997). However, patients that misuse their prescribed stimulants (e.g., take increased doses) show increased hyperactivity and increased body temperature at the same time (Lakhan & Kirchgessner, 2012), and healthy individuals that make use of stimulant medication also show increased activity and increased body temperature (Pigeau et al., 1995). Therefore, hyperactivity in ADHD does not seem to be a method to augment body temperature. To definitely test whether a low body temperature causes increased locomotor activity in BALB/cJ mice, one could house BALB/cJ mice in a heat chamber and observe if they still show signs of hyperactivity.

General discussion

The current study served to characterise the behavioural and physiological phenotype of BALB/cJ mice. These mice are highly aggressive, and aggression is a cardinal symptom of CD (Blair, 2013; Velez et al., 2010). However, it was unknown whether BALB/cJ mice also demonstrate symptoms of ADHD and ASD, common comorbidities of CD. Therefore, we further characterised the behavioural and physiological phenotype of BALB/cJ mice and designed two experiments that enabled us to explore if BALB/cJ mice show symptoms of ASD and ADHD. Our results show that BALB/cJ mice not only demonstrate increased aggression, a core symptom of CD, but also symptoms of common comorbidities such as ASD and ADHD in form of social withdrawal, hyperactivity and a lower body temperature. Having validated the behavioural and physiological phenotype of BALB/cJ mice, we can use this model to study brain structures that might give rise to the linked symptoms of CD, ADHD and ASD. Ultimately, this could aid in the discovery of new treatments for children and adolescents that suffer from CD with comorbid ADHD and/or ASD.

Children with ADHD that develop CD, show a considerably earlier onset of CD symptoms and more severe symptoms than children with CD only (Loeber et al., 2000). Furthermore, children with ASD that also receive a diagnosis of ADHD, have a higher chance to develop CD (Montes & Halterman, 2007). This suggests that symptoms of ADHD, such as impulsivity, hyperactivity or inattention, might play a role in the link between CD, ADHD and ASD. The focus has long been on impulsivity, as high levels of impulsivity in children with ADHD or CD contribute strongly to the risk of criminal involvement, even more than early symptoms of CD alone (Babinski, Hartsough, & Lambert, 1999). However, to date it is unknown if high levels of impulsivity are a common cause of ADHD, ASD and CD, explaining the high

comorbidity of these three disorders. More recently, the focus has been shifted toward inattention as a possible mediator of the relation between CD, ADHD and ASD. Attention enables us to selectively concentrate on certain aspects of information, suppressing distracting or irrelevant information (Kim, Ährlund-Richter, Wang, Deisseroth, & Carlén, 2016). Social situations in general, and even more so ambiguous social situations, require high levels of attention. If a person is non-attentive to subtle cues during interactions (e.g., tone and facial expression), situations can be interpreted as hostile leading to an (unprovoked) outburst of aggression (Evans, Fite, Hendrickson, Rubens, & Mages, 2015). It is known that patients with ASD, ADHD as well as CD have difficulty understanding social cues and that they tend to interpret ambiguous social situations as hostile (Evans et al., 2015). It might be that being inattentive to subtle cues leads to a misinterpretation of a situation, which results in aggressive behaviour. Indeed, causal modelling in a population of ADHD patients suggests that inattention is causal to aggression (Heskes, unpublished data). This also implies that inattention should precede aggression, being in line with the fact that children with ADHD, who develop CD, have an earlier onset of CD symptoms than children with CD only. Preliminary results in our group indicate that the BALB/cJ mouse model shows signs of inattention, enabling us to utilise this model to further study the role of inattention in aggression.

Future Directions: The BALB/cJ mouse and attention

Recently, Kim et al. (2016) demonstrated that gamma-aminobutyric acid (GABA-ergic) interneurons expressing parvalbumin (PV) in prefrontal cortex are involved in sustaining and directing attention. Mice performed a 3-choice-serial reaction-time task (modification of the 5-choice-serial reaction time task) and neuronal responses in prefrontal cortex were recorded. In the 3- (or 5-) choice task, the animal needs to sustain and divide its attention across a row of three (or five) screen locations to detect and respond to a brief visual stimulus in order to receive a reward. At the start of each trial the activity of the PV neurons increased and this heightened activity was sustained during the whole delay period (i.e., until presentation of the stimulus). The activity increased even more when the animal was about to perform correctly. This means that a high and sustained activity of PV neurons at the start of a trial predicts whether the animal will perform correctly, more than 2.5 seconds before presentation of the stimulus. The activity of the PV neurons neither correlated to the motivational state of the animal or motor behaviour and it can be concluded that prefrontal PV neurons are involved in attentional control. Decreased activity during tasks involving attentional processing (e.g., measures of sustained attention comparable to the 5-choice-serial reaction time task as used in rodents) in prefrontal cortex has also been observed in patients with ADHD and comorbid CD and/or ASD (Dickstein, Bannon, Castellanos, & Milham, 2006). Furthermore, prefrontal cortex is heavily interconnected and regions such as vmPFC and ACC have also been implicated in the control of aggressive behaviour, and both ACC and vmPFC have been found to be hypofunctioning in ADHD, ASD and CD (Blair, 2013; Hare, Rakimi, & Rangel, 2014). Possibly, malfunctioning prefrontal circuits cannot communicate efficiently with each other and cannot exert control over the basic aggression circuitry running from medial amygdala to medial hypothalamus and to the dorsal half of the PAG (Blair, 2013), which in turn could lead to aggressive behaviour. Future studies can utilise the BALB/cJ mouse model to investigate the role of prefrontal circuits in attention and aggression.

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