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Sex-specific behavioral traits in the *Brd2* mouse model of Juvenile Myoclonic Epilepsy

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Abstract

Idiopathic generalized epilepsy represents about 30–35% of all epilepsies in humans. The bromodomain *BRD2* gene has been repeatedly associated with the subsyndrome of juvenile myoclonic epilepsy. Our previous work determined that mice haploinsufficient in *Brd2* (*Brd2*^{+/-}) have increased susceptibility to provoked seizures, develop spontaneous seizures and have significantly decreased GABA markers in the direct basal ganglia pathway as well as in the neocortex and superior colliculus. Here we tested male and female *Brd2*^{+/-} and wild type littermate mice in a battery of behavioral tests (open field, tube dominance test, elevated plus maze, Morris water maze and Barnes maze) to identify whether *Brd2* haploinsufficiency is associated with the human behavioral patterns, so-called juvenile myoclonic epilepsy personality. *Brd2*^{+/-} females but not males consistently displayed decreased anxiety. Further, we found a highly significant dominance trait (aggression) in the *Brd2*^{+/-} mice compared to the wild type,

more pronounced in females. *Brd2*^{+/-} mice of either sex did not differ from wild type mice in spatial learning and memory tests. Compared to wild type littermates, we found decreased numbers GABA neurons in the basolateral amygdala, which is consistent with the increase in aggressive behavior. Our results indicate that *Brd2*^{+/-} haploinsufficient mice show no cognitive impairment but have behavioral traits similar to those found in patients with juvenile myoclonic epilepsy (recklessness, aggression). This suggests that either the *BRD2* gene is directly responsible for influencing many traits of juvenile myoclonic epilepsy or it controls upstream regulators of individual phenotypes.

Keywords

aggression; anxiety; cognition; idiopathic generalized epilepsy; personality; *Brd2* haploinsufficiency; Morris Water Maze; Barnes maze; open field

Introduction

Idiopathic generalized epilepsy (IGE) represents about 30–35% of all epilepsies (Annegers, 1994). Previous and current research indicates that the patients with juvenile myoclonic epilepsy (JME; a subform of IGE) have personality disorders that include impulsive personality traits and higher novelty seeking behavior (Moschetta *et al.*, 2011, Wandschneider *et al.*, 2013) that are not associated with other forms of epilepsy. Patients with JME also show less self-control than matched healthy subjects (Plattner *et al.*, 2007). Magnetic resonance spectroscopy and imaging studies suggest the involvement of prefrontal cortex in these epilepsy patients, and impairments in the prefrontal cortex may also be involved in associated behavioral traits (De Araujo Filho *et al.*, 2009a, De Araujo Filho *et al.*, 2009b, Koepf *et al.*, 2013, Piazzini *et al.*, 2008). On the other hand, JME patients usually do not have serious cognitive deficits (Moschetta & Valente, 2013) although, JME has been reported to be associated with verbal and visual memory deficits (Sonmez *et al.*, 2004) related to the frontal lobe (Koepf, 2005, Piazzini *et al.*, 2008). Although IGE is mostly genetic in origin (Greenberg *et al.*, 1992), few IGE genes have been identified. Among those genes, *BRD2* has been repeatedly both linked and associated with JME (Cavalleri *et al.*, 2007, Durner *et al.*, 1991, Greenberg *et al.*, 1988a, Greenberg *et al.*, 1988b, Greenberg *et al.*, 2000, Pal *et al.*, 2003, Sander *et al.*, 1995, Weissbecker *et al.*, 1991), specifically in Caucasian populations (Greenberg *et al.*, 2000, Sander *et al.*, 1997).

We generated a mouse model with the null mutation of the murine *Brd2* gene (Shang *et al.*, 2009). The homozygous mutation (*Brd2*^{-/-}) is incompatible with life but *Brd2*^{+/-} heterozygotes are viable. The heterozygotes have increased susceptibility to provoked seizures, develop unprovoked spontaneous seizures, and have significantly decreased GABA markers (such as GAD67 and/or parvalbumin) along the direct basal ganglia pathway, including in the neocortex and superior colliculus, indicating a GABA deficiency within the endogenous seizure-controlling pathway (Deransart & Depaulis, 2002). However, the hippocampus shows no such decrease in GABA markers (Velíšek *et al.*, 2011).

In this study we investigated behavioral differences between *Brd2*^{+/-} mice and wild type (*wt*) littermates with an emphasis on anxiety, cognition and aggression. We also determined

the numbers of GAD67 and parvalbumin immunopositive cells in the basolateral amygdala, a structure strongly related to the control of aggression and fear (Mchugh *et al.*, 2004, Schumann *et al.*, 2011, Wang *et al.*, 2011). In our mouse studies, we looked at the sexes separately because JME patients are predominantly female (Camfield *et al.*, 2013, Kleveland & Engelsen, 1998, Pedersen & Petersen, 1998), an observation that is echoed in the mouse seizure susceptibility experiments (Velíšek *et al.*, 2011).

Material and Methods

All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The respective protocols were approved by the IACUC of the Columbia University (New York, NY), New York Medical College (Valhalla, NY), and Nationwide Children's Hospital (Columbus, OH). *Brd2*^{+/-} mice and *Brd2*^{+/+} littermates (controls; referred here to as *wt*) were generated most frequently by mating *wt* *Brd2*^{+/+} females to heterozygous *Brd2*^{+/-} males. All mice used in this study were 6–9 months old, both males and females, and at least the 7th generation of backcrossing onto a C57BL/6J background strain, so that their genetic background would be considered on average 99% C57BL/6J (Mouse Nomenclature, jaxmice.jax.org). The genotypes of the mice were determined by PCR using primers that spanned the gene-trap vector junction inserted into the *Brd2* gene (Shang *et al.*, 2009). The mice were kept in groups of three in a cage (same sex) on regular light:dark cycle (lights on at 07:00) with free access to food and water. All experiments were performed during the light phase of the cycle preferable between 10:00–14:00. Two separate cohorts of animals were used for behavioral testing (open field, elevated plus maze, Morris Water maze OR Barnes maze) with at least three day between individual tests. Tube dominance was tested in an additional cohort of mice. Immunohistochemistry was performed on brains of animals not subjected to any behavioral testing.

Behavioral testing

Initially, we investigated the mice in the **open field** to determine if there were any differences in normal behavioral patterns, in the velocity of walking, trajectory traveled, grooming, etc. (Goldstein *et al.*, 2012, Velíšek, 2006). During this test, the mouse is placed in the center of an arena (42 × 42 cm) with translucent walls and 3D infrared diode arrays. The mouse is left to explore the environment freely for 5 minutes; all movements are recorded. Average speed, distance traveled, central zone entries and time spent in the central zone (centrally positioned 20 × 20 cm square) were calculated by means of software (Activities; Med-Associates, St. Albans, VT). In addition to motor performance, the open field test also provides information about anxiety level: Mice prefer to spend more time in the safety of the walls (thigmotaxis) than in the center of the arena. Relative increases in time spent close to the walls of the arena are consistent with increased anxiety, while more time spent in the center is consistent with decreased anxiety (Choleris *et al.*, 2001).

As a corollary test for anxiety, we used the **elevated “plus” maze** (Pellow & File, 1986, Velíšek, 2006). During this test, the mouse is placed at the crossing of the two open and two closed arms of the device, facing the open arm. Number of entries into either arm and the

time spent in the arms is recorded (videotracking using Stoelting's ANY-Maze software; Wood Dale, IL) for total 3 minutes. Mice typically prefer the subjectively safer closed arms, although they tend to explore new areas.

Observations during routine handling of the mice had suggested that +/- mice were more aggressive than *wt*. Therefore, we used the **dominance tube test** to test and quantify aggressive behaviors, i.e., whether the heterozygotes are more aggressive compared to *wt*. The enclosed space of the tube represents a desired environment for the mice and the tube test is a non-fighting social dominance test. The tube diameter is chosen to allow the mouse to move either forward or backward without any obstacles, but not to be able to turn around (Kovacsics & Gould, 2010). Social dominance is determined by allowing a heterozygote and a *wt* of the same sex to enter the clear acrylic tube (diameter 2.5 cm; length 30 cm) from opposite ends (Korade *et al.*, 2013). Both mice usually proceed in walking the tube until they meet in the middle followed by a non-aggressive exchange of olfactory information. Then, the more dominant mouse starts pushing back the less dominant mouse. This is considered to reflect the aggressive tendency controlled by the prefrontal cortex (Molina & O'donnell, 2008, Wang *et al.*, 2011). We have tested each mouse three times (three sessions) allowing one hour between the sessions, always with a different sex-matched partner mouse of the opposite genotype and always from a different cage. We recorded which mouse pushed the less dominant one out of the tube (Harrison *et al.*, 2009). The trial concluded when one mouse had its forepaws out of the tube (Semple *et al.*, 2012). The tube test is a reliable method for hierarchical rating of mice as well as an indicator of significant prefrontal cortex involvement (Wang *et al.*, 2011). All testing was done blind to genotype.

Additionally, we investigated whether the *Brd2*+/- genotype affects hippocampal learning and spatial memory using the **Morris Water Maze (MWM)** (Schenk & Morris, 1985). In this test, the mouse is released from the side of a circular water tank and will seek and swim to the rescue platform (hidden about 1/2 cm under the water level in fixed position in the tank). The mouse has constant visual cues to orientation in the tank because the platform itself is invisible. We did four training trials per day (with a one hour inter-trial interval) for four days; the starting quadrants were pseudo-randomly varied. At 24 hours after the last training trial, a retrieval test was performed: We placed the mouse in the tank but without the platform, and we recorded the time the mouse spent in the quadrant where the platform had been located during the training trials. A similar test was performed 7 days after the training phase. The swimming trajectory was always followed by videotracking (ANY-maze).

To confirm the spatial learning and memory results obtained in the MWM (Patil *et al.*, 2009), we tested different groups of mice in the **Barnes maze** (Barnes, 1979, Goldstein *et al.*, 2012), which does not involve the significant stressor of swimming. In the Barnes maze, the mouse is released from the starting box in the center of the circular platform around which 20 holes are spaced evenly in the periphery. One of the holes contains the escape box, the other 19 are blanks. The mouse needs to find the escape box. A fluorescent (not heat producing) light source provided 800 lx of illumination directed on the platform as an aversive stimulus. There were 4 days of training with four training trials per day with a one hour inter-trial interval (Goldstein *et al.*, 2012). Similar to the MWM protocol, there was a

24 hour and a 7 day retrieval test in which the escape box is replaced with a blank for the test. During the retrieval test, we recorded the length of time the mouse sniffs around the former location of the escape box (± 1 box).

Immunohistochemistry

We evaluated the expression of GABA markers (GAD67 and parvalbumin) in the amygdala and in the prefrontal cortex of mice *not* subjected to behavioral tests. The amygdala is involved in the control of anxiety and aggressive behaviors (Hale *et al.*, 2010) and GABA has a key role in the control of anxiety (Smith & Rudolph, 2012). For free-floating immunohistochemical staining, 40 μ m thick coronal hemi-sections were cut using a cryostat. To identify markers of GABAergic neurons, alternating sections were collected for separate parvalbumin (PVA) and GAD67 immunostaining. GAD67 is an isozyme of the GABA synthesizing enzyme glutamic acid decarboxylase that tags almost the entire population of GABAergic neurons. We used anti-parvalbumin (1:5000, Sigma, St. Louis, MO) and anti-GAD67 (1:4000; Millipore, Temecula, CA) antibodies. The immunostaining was visualized using the avidin-biotin horseradish peroxidase method (Vectastain AB kit, Vector Laboratories, Burlingame, CA) (Velíšek *et al.*, 2011, Velíšková & Velíšek, 2007).

Counting of immunopositive cells

Section images were digitally captured. Counts centered on the basolateral nucleus of amygdala (defined as the cross-section of the nucleus) or the prefrontal cortex (defined as a rectangle in the area of prelimbic cortex and area 1 of the cingulate cortex). For counting, a minimum of three position-matched sections for each structure were selected from the brain of each of *Brd2*^{+/-} and *Brd2*^{+/+} mice. There were four mice in each subgroup (genotype/sex) for the amygdala counts and three mice per each subgroup for the prefrontal cortex counts. All immunopositive neurons in the area of interest were counted (Ravizza *et al.*, 2003, Rieux *et al.*, 2002). Counts from three sections were averaged for each subject (mouse) and the average used for statistical evaluation. Our goal was to compare the relative number of immunopositive cells in the *Brd2*^{+/-} mice to the number in *wt* mice (Velíšek *et al.*, 2011).

Statistical Analysis

Initially, data were evaluated for distribution and variance. Data following a Gaussian distribution and with similar variance were analyzed using parametric tests (Student's t-test for two groups, ANOVA for multiple groups). Proportions in two groups were evaluated using Fisher's Exact Test. Data collected over time in spatial learning tests were analyzed using two-way ANOVA with repeated measures as one factor. Level of significance was preset to $p < 0.05$.

Results

Open Field (Figure 1)

In female mice, the average traveling speed was independent of the genotype. Both *Brd2*^{+/-} (n=14) and *wt* (n=12) mice had average speeds around 22 cm/s (Student's t-test; $t(24)=0.251$; $p=0.804$). This indicates that movement of the *Brd2*^{+/-} mice is not impaired.

There was no difference between the two groups of mice in total distance traveled (Student's t-test; $t(24)=1.090$; $p=0.287$; not shown). However, when we compared the distance traveled in the periphery and in the central zone of the open field, we found that *Brd2*^{+/-} females traveled significantly longer distance in the central area compared to *wt* females (Student's t-test; $t(24)=2.498$; $p=0.048$). This finding could be associated either with significantly more entries of *Brd2*^{+/-} females into the central zone and/or more time spent in the central zone. Statistics revealed that female *Brd2*^{+/-} mice entered the central zone more frequently (Student's t-test; $t(24)=2.314$; $p=0.030$) and stayed there longer (Student's t-test; $t(24)=2.403$; $p=0.024$) compared to *wt* female mice. These observations of *Brd2*^{+/-} females indicate significantly decreased preference for the peripheral parts of the open field (decreased thigmotaxis) compared to *wt*, a conclusion supported by 1) the longer time spent in the central area of the open field, 2) the longer distance traveled there, and 3) the increased number of entries. Decreased thigmotaxis is a sign of decreased anxiety (Simon *et al.*, 1994). In male mice we did not find differences in open field behaviors between *Brd2*^{+/-} (n=17) and *wt* (n=11) except for the time spent in the central zone. *Brd2*^{+/-} males spent *less* time in the central zone than *wt* (Student's t-test; $t(26)=2.229$; $p=0.044$), which might be suggestive of increased anxiety.

Elevated Plus Maze (Figure 2)

The findings in the elevated plus maze, confirmed the findings of decreased anxiety in *Brd2*^{+/-} female mice compared to *wt* females. Overall, female *Brd2*^{+/-} mice (n=15) spent significantly less time in the closed arms of the maze (Student's t-test; $t(27)=2.374$; $p=0.025$) but spent significantly more time on the open arms compared to female *wt* mice (n=14; Student's t-test; $t(27)=2.112$; $DF=27$; $p=0.044$). The remaining time (up to the 3 min session limit) was spent on the central square. However, we did not find any differences for elevated plus-maze behaviors between male *Brd2*^{+/-} vs. *wt* mice. Thus, this test does *not* confirm the indication of increased anxiety in males that was seen in the previous test.

Tube dominance test (Figure 3)

We had noted enhanced aggression in *Brd2*^{+/-} mice in normal handling. We used tube dominance test to confirm and quantify this observation. We tested the mice in three trials. For each trial a different male-male or female-female pair was chosen. A total of 10 *Brd2*^{+/-}/*wt* male pairs and 10 female pairs were tested (40 mice total). Figure 3A shows number of "winners" (dominant mice) in each category, i.e. male *Brd2*^{+/-}, female *Brd2*^{+/-}, male *wt* and female *wt*. The results unambiguously show that *Brd2*^{+/-} females were consistently dominant over *wt* in this test across the trials. On the other hand, *Brd2*^{+/-} males required repeated encounters with *wt* opponents to develop clear dominance. Figure 3B summarizes data from trials 1–3. Out of 60 encounters, *Brd2*^{+/-} mice prevailed in 41, while *wt* prevailed in 19 (Fisher's Exact test; * $p=0.0001$).

Morris Water Maze (MWM; Figure 4)

We asked whether the GABAergic neuron marker deficits in *Brd2*^{+/-} mice that are seen along a) the direct basal ganglia pathway, b) in the neocortex, and c) in the superior colliculus, would impair spatial learning and memory. In the water maze (MWM), we found

no difference in learning the platform location between *Brd2*^{+/-} female mice (n=16) and *wt* females [n=12; repeated measures ANOVA, between factor $F_{(1,26)}=0.167$; $p=0.687$]. Indeed, learning (i.e., the gradual decrease in time needed to find the platform over the days of training; i.e., an eventual non-zero slope of the learning curve) was present and similar in both groups [repeated measures ANOVA within factor $F_{(3,78)}=21.384$; $p<0.0001$; no significant interaction between the factors]. Similarly, there was no difference between *Brd2*^{+/-} and *wt* female mice in the memory retrieval test at 24 hours or 7 days after completion of training. The results for males were similar to females. We did not find any differences in spatial learning in the MWM between *Brd2*^{+/-} males (n=12) and *wt* males (n=11; repeated measures ANOVA between factor $F_{(1,21)}=0.001$; $p=0.980$) while, again, male mice were capable of significant learning (a gradual decrease in the time to entry into the rescue box over the days of training; repeated measures ANOVA within factor $F_{(3,63)}=16.004$; $p<0.001$). There were no differences between *Brd2*^{+/-} and *wt* males in memory retrieval tests at 24 hours or 7 days after training.

Barnes Maze (Figure 5)

Swimming may represent a confounder in the MWM because mice are not fond of swimming. Thus, stress effects of swimming might have overridden any possible (subtle) differences between +/- and *wt* mice of both sexes. Therefore, using a different sets of animals than in the MWM test, we validated our MWM data in the Barnes Maze, the dry version of the spatial learning and memory test. For female mice, there was again no difference in spatial learning between *Brd2*^{+/-} (n=16) and *wt* (n=14; repeated measures ANOVA between factor $F_{(1,28)}=0.206$; $p=0.653$); both groups displayed significant acquisition of the task (repeated measures ANOVA within factor $F_{(3,84)}=33.912$; $p<0.0001$). There was no difference between *Brd2*^{+/-} female mice and *wt* controls in memory retrieval at 24 hours and 7 days after learning. In male mice, we also did not see any difference between the groups of *Brd2*^{+/-} (n=17) and *wt* mice (n=12; repeated measures ANOVA between factor $F_{(1,27)}=0.439$; $p=0.573$). Similar to the female results, and similar to results collected in MWM, there was significant learning in both groups over 4 days of training (repeated measures ANOVA within factor $F_{(3,81)}=21.079$; $p<0.0001$). There was no difference in memory retrieval after 24 hours or 7 days between male *Brd2*^{+/-} and *wt* mice.

These results indicate that the spatial learning and memory are affected neither as a result of *Brd2* haploinsufficiency nor as a result of the earlier-reported GABA marker decrease in the basal ganglia and neocortex. It is noteworthy that there were no differences in GABAergic neuron markers in the hippocampus (Velíšek *et al.*, 2011). Thus, the finding that genotype did not affect spatial learning and memory supports the observation that *Brd2* haploinsufficiency does not affect hippocampal function.

Expression of markers for GABAergic neurons in the basolateral amygdala and prefrontal cortex (Figure 6)

We determined expression of GAD67 and PVA (GABA markers) in amygdala since amygdala function is associated with fear and with aggressive behaviors (Mchugh *et al.*, 2004, Schumann *et al.*, 2011, Treit *et al.*, 1993). We found that both markers we examined, GAD67 and PVA, were significantly decreased in *Brd2*^{+/-} mice, irrespective of sex. In

GAD67 expression, there was a significant effect of genotype (ANOVA $F_{(1,12)}=24.735$; $p=0.0003$), however there was no effect of sex (ANOVA $F_{(1,12)}=0.685$; $p=0.424$) or interaction between the factors (ANOVA $F_{(1,12)}=1.330$; $p=0.271$). Similar to GAD67, in PVA expression, there was a significant effect of genotype (ANOVA $F_{(1,12)}=10.946$; $p=0.0062$), but there was no main effect of sex (ANOVA $F_{(1,12)}=0.030$; $p=0.866$) or interaction between the effects (ANOVA $F_{(1,12)}=0.009$; $p=0.930$).

We also investigated expression of GABA markers in prefrontal cortex because of the role prefrontal cortex plays in dominance and aggression (Wang *et al.*, 2011). We determined the number of GAD67 and PVA immunopositive neurons in the perilimbic and cingulate portions of the prefrontal cortex. First, we found that the numbers of GAD67 and PVA immunopositive cells are virtually the same in this area (paired t-test; $t(11)=0.834$; $p=0.422$), indicating that most, if not all, GABAergic cells express PVA in this particular part of the prefrontal cortex. Second, in counts of GAD67 immunopositive cells, we found no main effect of genotype (ANOVA $F_{(1,9)}=1.170$; $p=0.310$) or sex (ANOVA $F_{(1,9)}=0.065$; $p=0.805$). There was no main effect of genotype in counts of PVA-immunopositive cells (ANOVA $F_{(1,10)}=0.0034$; $p=0.990$) or main effect of sex (ANOVA $F_{(1,10)}=0.079$; $p=0.784$). No interactions between the main effects were found in either cell type.

Discussion

Our data show that *Brd2*^{+/-} mice have sex-specific alterations in anxiety as compared to *wt* mice. We found decreased anxiety traits in *Brd2*^{+/-} female mice but these traits were not observed in *Brd2*^{+/-} males. Further, both *Brd2*^{+/-} female and male mice displayed increased aggression compared to *wt*, aggression that was more pronounced in females. In either sex, *Brd2* haploinsufficiency was not associated with any problems in spatial learning and memory. Finally, we also found a significant decrease in GABA neuronal markers (GAD67 and PVA) in basolateral amygdala in *Brd2*^{+/-} mice compared to *wt*, yet no difference was found in the prefrontal cortex. These findings correspond to our previously published study in *Brd2*^{+/-} mice that showed sex-specific increases in susceptibility to provoked seizures, the occurrence of spontaneous seizures in females, and decreased numbers of GAD67 immunopositive neurons along the basal ganglia pathways compared to *wt* mice (Velíšek *et al.*, 2011).

Our behavioral findings show decreases in anxiety in female *Brd2*^{+/-} mice and increases in aggression in all *Brd2*^{+/-} mice compared to *wt*. There are studies indicating that patients with JME (repeatedly linked to, and associated with, the *BRD2* gene (Greenberg *et al.*, 2000, Sander *et al.*, 1997)) also show certain behavioral problems. These behavioral problems include increased risk-taking behaviors (Wandschneider *et al.*, 2013) and impulsive personality traits (Moschetta *et al.*, 2011). Indeed, in our mouse experiments, the enhanced exploration of the central area (the risk zone) of the open field and the increase in time spent on the open arms of the elevated plus maze, are indicators of increased risk-taking behaviors in the mice (Olsen *et al.*, 2013). Interestingly, a study investigating deficits of a gene controlling circadian rhythms (*Clock*) (Easton *et al.*, 2003) also found more robust increases in risk-taking behavior specifically in females. The findings suggest that some genes such as

Clock (or *Brd2* in this study) may interact with gonadal steroid hormones or sex to produce behavioral changes.

We did not find any cognitive deficits (determined in the Barnes maze or Morris Water Maze) in *Brd2*^{+/-} mice compared to controls. Similarly, clinical data do not show significant cognitive deficiencies in patients with JME (Moschetta & Valente, 2013). The cognitive tasks used in our study in the mice depend on intact hippocampal function (Stewart *et al.*, 2011) and, in fact, we did not find any changes in GABA markers in the hippocampus of *Brd2*^{+/-} mice compared to *wt* in our previous work (Velíšek *et al.*, 2011). This is also in accordance with neuropsychological studies, which generally report normal IQ in JME patients; the functions of the temporal lobes specifically (verbal and nonverbal episodic memory) seem to be unaffected (Delgado-Escueta *et al.*, 2013, Roebeling *et al.*, 2009, Wandschneider *et al.*, 2010). These parallel findings in patients with JME and in *Brd2*^{+/-} mice indicate that hippocampal structure and function are unaffected in both JME patients and in our mouse JME model.

On the other hand, in the *Brd2*^{+/-} mice we did find increased social dominance in the tube test, which tests dominance as well as aggression (Johns *et al.*, 2010). The main structures responsible for these behaviors involve circuitry among prefrontal and orbital cortices, striatum and amygdala (Cho *et al.*, 2013, Molina & O'donnell, 2008, Nelson & Trainor, 2007, Wang *et al.*, 2011). Previously, we found impairment in the striatal number of GAD67 and parvalbumin immunopositive neurons in *Brd2*^{+/-} mice (suggesting a deficit in GABA neurons (Velíšek *et al.*, 2011)). In the current study, we expanded those findings and we show, in addition, that there are decreased GABA markers in the amygdala of the *Brd2*^{+/-} mice as well. In patients with JME, changes in fronto-striatal circuitry have been demonstrated, with abnormalities in the dorsolateral prefrontal cortex, premotor cortex, basal frontal cortex, thalamus, and putamen (Koepp *et al.*, 2013, Woermann *et al.*, 1999) associated with decreased cognitive flexibility (McDonald *et al.*, 2006, Van Schouwenburg *et al.*, 2014, Wandschneider *et al.*, 2012). Although JME's major pathology is a form of generalized epilepsy, EEG recordings and current imaging techniques provide evidence of the major involvement of the frontal lobes (Koepp *et al.*, 2013, Wandschneider *et al.*, 2013), all in accordance with the findings of behavioral and neuropsychological studies (De Araujo Filho *et al.*, 2013, Schmitz *et al.*, 2013). Another study of JME patients investigated risk-taking behaviors and determined that JME patients with ongoing seizures display increased risk-taking traits as well as activation of the prefrontal cortex compared to controls without JME or JME patients without ongoing seizures (Wandschneider *et al.*, 2013). While we did not find any modifications of GABA markers directly in the prefrontal cortex of the *Brd2*^{+/-} mice, we found that, in addition to increased aggression, these mice have decreases in anxiety traits, which can be related to risk-taking behaviors (Easton *et al.*, 2003, Keers *et al.*, 2012). These behaviors also involve prefrontal cortex, but we hypothesize that a neurotransmitter system other than the GABA system is involved (Vialou *et al.*, 2014).

Previously, we found sex-specific phenotypic differences between *Brd2*^{+/-} and *wt* mice in both seizure susceptibility and in the expression of GABA markers (Velíšek *et al.*, 2011), and now we see such differences in behaviors as well. It is striking that many of these observations in the mouse are similar to observations in humans (Craiu, 2013), [although, to

our knowledge, no one has investigated sex-specific differences in human JME patients]. Sex-specific occurrence of traits may indicate: (a) A possible role of sex chromosome differences and their interaction with the partial *Brd2* deficit in heterozygotes. (b) Interactions of *Brd2* function with the effects of gonadal sex steroids, as shown previously for the interaction of the *Clock* gene with sex in anxiety behaviors (Easton *et al.*, 2003). (c) The role of circulating sex steroids (specifically progesterone) during the ovarian cycle on anxiety behaviors in females (Galeeva *et al.*, 2003, Gangitano *et al.*, 2009, Maguire *et al.*, 2005). Our ongoing experiments in animals during phases of postnatal development will further elucidate the first two possibilities of genetically-based sex differences versus interaction with early developmental organizational effects of steroid hormones (sexually dimorphic effects) (Velíšková & Moshé, 2001). Testing both *Brd2*^{+/-} and *wt* females at random time points throughout the ovarian cycle speaks against the last option.

The finding that the decreased anxiety in female *Brd2*^{+/-} mice is associated with decreased expressions of GABA markers in the basolateral amygdala seems counterintuitive, as anxiolytic effects are usually dependent on strengthening GABA-mediated inhibition (Smith & Rudolph, 2012); for example, benzodiazepines, which strengthen such inhibition, have significant anxiolytic properties (Griebel & Holmes, 2013). However, mice with high anxiety traits have increased levels of GAD65 and GAD67 mRNAs and protein, as well as increased levels of GABA in the amygdala (Tasan *et al.*, 2011). Lesions of basolateral amygdala, on the other hand, lead to decreased anxiety traits in rats (Treit *et al.*, 1993). Finally, one study directly and positively links the number of active PVA-containing neurons in the amygdala to the level of anxiety (Hale *et al.*, 2010). A very recent study in patients with JME, using Magnetic Resonance Spectroscopy with ultra short echo time, determined that there is a decrease in thalamic GABA neurotransmission with an increase in N-acetyl-aspartate. These observations together suggest damage to thalamic GABAergic neurons (Hattingen *et al.*, 2014). Unfortunately, relatively low resolution of this technique does not make possible to investigate relatively small brain structures such as dorsolateral striatum, substantia nigra pars reticulata or amygdala.

Our data suggest that the *Brd2* gene (in addition to governing seizure susceptibility) plays a role in the expression of sex-specific behavioral traits. Since the *Brd2* gene product is likely a transcription factor controlling production of many different transcripts (Belkina *et al.*, 2013) (*ergo* regulating many different functions), these transcripts may participate in brain development and control of seizure susceptibility, cell proliferation, and behavior, and also may be responsible for differential outcomes in males and females. Since these control functions, or interactions, of *Brd2* with other transcripts may also occur at certain specific developmental stages, the data indicate that JME is a developmental disorder (De Nijs *et al.*, 2013).

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References

- Annegers JF. Epidemiology and genetics of epilepsy. *Neurol Clin.* 1994; 12:15–29. [PubMed: 8183207]
- Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol.* 1979; 93:74–104. [PubMed: 221551]
- Belkina AC, Nikolajczyk BS, Denis GV. BET protein function is required for inflammation: Brd2 genetic disruption and BET inhibitor JQ1 impair mouse macrophage inflammatory responses. *J Immunol.* 2013; 190:3670–3678. [PubMed: 23420887]
- Camfield CS, Striano P, Camfield PR. Epidemiology of juvenile myoclonic epilepsy. *Epilepsy Behav.* 28(Suppl 1):S15–S17. [PubMed: 23756473]
- Cavalleri GL, Walley NM, Soranzo N, Mulley J, Doherty CP, Kapoor A, Depondt C, Lynch JM, Scheffer IE, Heils A, Gehrmann A, Kinirons P, Gandhi S, Satishchandra P, Wood NW, Anand A, Sander T, Berkovic SF, Delanty N, Goldstein DB, Sisodiya SM. A multicenter study of BRD2 as a risk factor for juvenile myoclonic epilepsy. *Epilepsia.* 2007; 48:706–712. [PubMed: 17437413]
- Cho YT, Ernst M, Fudge JL. Cortico-amygdala-striatal circuits are organized as hierarchical subsystems through the primate amygdala. *J Neurosci.* 2013; 33:14017–14030. [PubMed: 23986238]
- Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev.* 2001; 25:235–260. [PubMed: 11378179]
- Craiu D. What is special about the adolescent (JME) brain? *Epilepsy Behav.* 2013; 28(Suppl 1):S45–S51. [PubMed: 23756479]
- de Araujo Filho GM, de Araujo TB, Sato JR, Silva I, Lin K, Junior HC, Yacubian EM, Jackowski AP. Personality traits in juvenile myoclonic epilepsy: evidence of cortical abnormalities from a surface morphometry study. *Epilepsy Behav.* 2013; 27:385–392. [PubMed: 23510795]
- de Araujo Filho GM, Jackowski AP, Lin K, Guaranha MS, Guilhoto LM, da Silva HH, Caboclo LO, Junior HC, Bressan RA, Yacubian EM. Personality traits related to juvenile myoclonic epilepsy: MRI reveals prefrontal abnormalities through a voxel-based morphometry study. *Epilepsy Behav.* 2009a; 15:202–207. [PubMed: 19303459]
- de Araujo Filho GM, Lin K, Lin J, Peruchi MM, Caboclo LO, Guaranha MS, Guilhoto LM, Carrete H Jr, Yacubian EM. Are personality traits of juvenile myoclonic epilepsy related to frontal lobe dysfunctions? A proton MRS study. *Epilepsia.* 2009b; 50:1201–1209. [PubMed: 19400877]
- de Nijs L, Wolkoff N, Grisar T, Lakaye B. Juvenile myoclonic epilepsy as a possible neurodevelopmental disease: role of EFHC1 or Myoclonin1. *Epilepsy Behav.* 2013; 28(Suppl 1):S58–S60. [PubMed: 23756481]
- Delgado-Escueta AV, Koeleman BP, Bailey JN, Medina MT, Duron RM. The quest for juvenile myoclonic epilepsy genes. *Epilepsy Behav.* 2013; 28(Suppl 1):S52–S57. [PubMed: 23756480]
- Deransart C, Depaulis A. The control of seizures by the basal ganglia? A review of experimental data. *Epileptic Disord.* 2002; 4(Suppl 3):S61–S72. [PubMed: 12495876]
- Durner M, Sander T, Greenberg DA, Johnson K, Beck-Mannagetta G, Janz D. Localization of idiopathic generalized epilepsy on chromosome 6p in families of juvenile myoclonic epilepsy patients. *Neurology.* 1991; 41:1651–1655. [PubMed: 1922810]
- Easton A, Arbizova J, Turek FW. The circadian Clock mutation increases exploratory activity and escape-seeking behavior. *Genes Brain Behav.* 2003; 2:11–19. [PubMed: 12882315]
- Galeeva AY, Tuohimaa P, Shalyapina VG. The role of sex steroids in forming anxiety states in female mice. *Neurosci Behav Physiol.* 2003; 33:415–420. [PubMed: 12774846]
- Gangitano D, Salas R, Teng Y, Perez E, De Biasi M. Progesterone modulation of alpha5 nAChR subunits influences anxiety-related behavior during estrus cycle. *Genes Brain Behav.* 2009; 8:398–406. [PubMed: 19220484]
- Goldstein LE, Fisher AM, Tagge CA, Zhang XL, Velíšek L, Sullivan JA, Upreti C, Kracht JM, Ericsson M, Wojnarowicz MW, Goletiani CJ, Maglakelidze GM, Casey N, Moncaster JA, Minaeva O, Moir RD, Nowinski CJ, Stern RA, Cantu RC, Geiling J, Blusztajn JK, Wolozin BL, Ikezu T, Stein TD, Budson AE, Kowall NW, Chargin D, Sharon A, Saman S, Hall GF, Moss WC,

- Cleveland RO, Tanzi RE, Stanton PK, McKee AC. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci Transl Med.* 2012; 4:134ra160.
- Greenberg DA, Delgado-Escueta AV, Maldonado HM, Widelitz H. Segregation analysis of juvenile myoclonic epilepsy. *Genet Epidemiol.* 1988a; 5:81–94. [PubMed: 3136050]
- Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman L, Maldonado HM, Park MS, Terasaki PI. Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. *Am J Med Genet.* 1988b; 31:185–192. [PubMed: 3146924]
- Greenberg DA, Durner M, Delgado-Escueta AV. Evidence for multiple gene loci in the expression of the common generalized epilepsies. *Neurology.* 1992; 42:56–62. [PubMed: 1574177]
- Greenberg DA, Durner M, Keddache M, Shinnar S, Resor SR, Moshe SL, Rosenbaum D, Cohen J, Harden C, Kang H, Wallace S, Luciano D, Ballaban-Gil K, Tomasini L, Zhou G, Klotz I, Dicker E. Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. *Am J Hum Genet.* 2000; 66:508–516. [PubMed: 10677311]
- Griebel G, Holmes A. 50 years of hurdles and hope in anxiolytic drug discovery. *Nat Rev Drug Discov.* 2013; 12:667–687. [PubMed: 23989795]
- Hale MW, Johnson PL, Westerman AM, Abrams JK, Shekhar A, Lowry CA. Multiple anxiogenic drugs recruit a parvalbumin-containing subpopulation of GABAergic interneurons in the basolateral amygdala. *Prog Neuropsychopharmacol Biol Psychiatry.* 2010; 34:1285–1293. [PubMed: 20647026]
- Harrison FE, Hosseini AH, McDonald MP. Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. *Behav Brain Res.* 2009; 198:247–251. [PubMed: 18996418]
- Hattingen E, Luckerath C, Pellikan S, Vronski D, Roth C, Knake S, Kieslich M, Pilatus U. Frontal and thalamic changes of GABA concentration indicate dysfunction of thalamofrontal networks in juvenile myoclonic epilepsy. *Epilepsia.* 2014
- Johns JM, McMurray MS, Joyner PW, Jarrett TM, Williams SK, Cox ET, Black MA, Middleton CL, Walker CH. Effects of chronic and intermittent cocaine treatment on dominance, aggression, and oxytocin levels in post-lactational rats. *Psychopharmacology (Berl).* 2010; 211:175–185. [PubMed: 20526586]
- Keers R, Pedroso I, Breen G, Aitchison KJ, Nolan PM, Cichon S, Nothen MM, Rietschel M, Schalkwyk LC, Fernandes C. Reduced anxiety and depression-like behaviours in the circadian period mutant mouse afterhours. *PLoS One.* 2012; 7:e38263. [PubMed: 22719873]
- Kleveland G, Engelsen BA. Juvenile myoclonic epilepsy: clinical characteristics, treatment and prognosis in a Norwegian population of patients. *Seizure.* 1998; 7:31–38. [PubMed: 9548223]
- Koepp MJ. Juvenile myoclonic epilepsy--a generalized epilepsy syndrome? *Acta Neurologica Scandinavica. Supplementum.* 2005; 181:57–62. [PubMed: 16238711]
- Koepp MJ, Woermann F, Savic I, Wandschneider B. Juvenile myoclonic epilepsy--neuroimaging findings. *Epilepsy Behav.* 2013; 28(Suppl 1):S40–S44. [PubMed: 23756478]
- Korade Z, Folkes OM, Harrison FE. Behavioral and serotonergic response changes in the Dhcr7-HET mouse model of Smith-Lemli-Opitz syndrome. *Pharmacol Biochem Behav.* 2013; 106:101–108. [PubMed: 23541496]
- Kovacsics CE, Gould TD. Shock-induced aggression in mice is modified by lithium. *Pharmacol Biochem Behav.* 2010; 94:380–386. [PubMed: 19800363]
- Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci.* 2005; 8:797–804. [PubMed: 15895085]
- McDonald CR, Swartz BE, Halgren E, Patell A, Dames R, Mandelkern M. The relationship of regional frontal hypometabolism to executive function: a resting fluorodeoxyglucose PET study of patients with epilepsy and healthy controls. *Epilepsy Behav.* 2006; 9:58–67. [PubMed: 16713363]
- McHugh SB, Deacon RM, Rawlins JN, Bannerman DM. Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. *Behav Neurosci.* 2004; 118:63–78. [PubMed: 14979783]

- Molina Y, O'Donnell S. Age, sex, dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Dev Neurobiol*. 2008; 68:950–959. [PubMed: 18361403]
- Moschetta S, Fiore LA, Fuentes D, Gois J, Valente KD. Personality traits in patients with juvenile myoclonic epilepsy. *Epilepsy Behav*. 2011; 21:473–477. [PubMed: 21683658]
- Moschetta S, Valente KD. Impulsivity and seizure frequency, but not cognitive deficits, impact social adjustment in patients with juvenile myoclonic epilepsy. *Epilepsia*. 2013; 54:866–870. [PubMed: 23621878]
- Nelson RJ, Trainor BC. Neural mechanisms of aggression. *Nat Rev Neurosci*. 2007; 8:536–546. [PubMed: 17585306]
- Olsen D, Kaas M, Schwartz O, Nykjaer A, Glerup S. Loss of BDNF or its receptors in three mouse models has unpredictable consequences for anxiety and fear acquisition. *Learn Mem*. 2013; 20:499–504. [PubMed: 23959707]
- Pal DK, Evgrafov OV, Tabares P, Zhang F, Durner M, Greenberg DA. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet*. 2003; 73:261–270. [PubMed: 12830434]
- Patil SS, Sunyer B, Hoger H, Lubec G. Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. *Behav Brain Res*. 2009; 198:58–68. [PubMed: 19022298]
- Paxinos, G.; Franklin, KBJ. *The mouse brain in stereotaxic coordinates*. Amsterdam ; Boston: Elsevier Academic Press; 2004.
- Pedersen SB, Petersen KA. Juvenile myoclonic epilepsy: clinical and EEG features. *Acta Neurol Scand*. 1998; 97:160–163. [PubMed: 9531431]
- Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav*. 1986; 24:525–529. [PubMed: 2871560]
- Piazzini A, Turner K, Vignoli A, Canger R, Canevini MP. Frontal cognitive dysfunction in juvenile myoclonic epilepsy. *Epilepsia*. 2008; 49:657–662. [PubMed: 18177360]
- Plattner B, Pahs G, Kindler J, Williams RP, Hall RE, Mayer H, Steiner H, Feucht M. Juvenile myoclonic epilepsy: a benign disorder? Personality traits and psychiatric symptoms. *Epilepsy Behav*. 2007; 10:560–564. [PubMed: 17466599]
- Ravizza T, Friedman LK, Moshé SL, Velišková J. Sex differences in GABA(A)ergic system in rat substantia nigra pars reticulata. *Int J Dev Neurosci*. 2003; 21:245–254. [PubMed: 12850057]
- Rieux C, Carney R, Lupi D, Dkhissi-Benyahya O, Jansen K, Chounlamountri N, Foster RG, Cooper HM. Analysis of immunohistochemical label of Fos protein in the suprachiasmatic nucleus: comparison of different methods of quantification. *J Biol Rhythms*. 2002; 17:121–136. [PubMed: 12002159]
- Roebeling R, Scheerer N, Uttner I, Gruber O, Kraft E, Lerche H. Evaluation of cognition, structural, and functional MRI in juvenile myoclonic epilepsy. *Epilepsia*. 2009; 50:2456–2465. [PubMed: 19490045]
- Sander T, Bockenkamp B, Hildmann T, Blasczyk R, Kretz R, Wienker TF, Volz A, Schmitz B, Beck-Mannagetta G, Riess O, Epplen JT, Janz D, Ziegler A. Refined mapping of the epilepsy susceptibility locus EJM1 on chromosome 6. *Neurology*. 1997; 49:842–847. [PubMed: 9305351]
- Sander T, Hildmann T, Janz D, Wienker TF, Neitzel H, Bianchi A, Bauer G, Sailer U, Berek K, Schmitz B, et al. The phenotypic spectrum related to the human epilepsy susceptibility gene "EJM1". *Ann Neurol*. 1995; 38:210–217. [PubMed: 7654068]
- Schenk F, Morris RG. Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Exp Brain Res*. 1985; 58:11–28. [PubMed: 3987843]
- Schmitz B, Yacubian EM, Feucht M, Hermann B, Trimble M. Neuropsychology and behavior in juvenile myoclonic epilepsy. *Epilepsy Behav*. 2013; 28(Suppl 1):S72–S73. [PubMed: 23756486]
- Schumann CM, Bauman MD, Amaral DG. Abnormal structure or function of the amygdala is a common component of neurodevelopmental disorders. *Neuropsychologia*. 2011; 49:745–759. [PubMed: 20950634]

- Semple BD, Canchola SA, Noble-Haesslein LJ. Deficits in social behavior emerge during development after pediatric traumatic brain injury in mice. *J Neurotrauma*. 2012; 29:2672–2683. [PubMed: 22888909]
- Shang E, Wang X, Wen D, Greenberg DA, Wolgemuth DJ. Double bromodomain-containing gene *Brd2* is essential for embryonic development in mouse. *Dev Dyn*. 2009; 238:908–917. [PubMed: 19301389]
- Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav Brain Res*. 1994; 61:59–64. [PubMed: 7913324]
- Smith KS, Rudolph U. Anxiety and depression: mouse genetics and pharmacological approaches to the role of GABA(A) receptor subtypes. *Neuropharmacology*. 2012; 62:54–62. [PubMed: 21810433]
- Sonmez F, Atakli D, Sari H, Atay T, Arpacı B. Cognitive function in juvenile myoclonic epilepsy. *Epilepsy Behav*. 2004; 5:329–336. [PubMed: 15145302]
- Stewart S, Cacucci F, Lever C. Which memory task for my mouse? A systematic review of spatial memory performance in the Tg2576 Alzheimer's mouse model. *Journal of Alzheimer's disease : JAD*. 2011; 26:105–126.
- Tasan RO, Bukovac A, Peterschmitt YN, Sartori SB, Landgraf R, Singewald N, Sperk G. Altered GABA transmission in a mouse model of increased trait anxiety. *Neuroscience*. 2011; 183:71–80. [PubMed: 21458543]
- Treit D, Pesold C, Rotzinger S. Noninteractive effects of diazepam and amygdaloid lesions in two animal models of anxiety. *Behav Neurosci*. 1993; 107:1099–1105. [PubMed: 8136064]
- van Schouwenburg MR, Onnink AM, ter Huurne N, Kan CC, Zwiers MP, Hoogman M, Franke B, Buitelaar JK, Cools R. Cognitive flexibility depends on white matter microstructure of the basal ganglia. *Neuropsychologia*. 2014; 53:171–177. [PubMed: 24291339]
- Velíšek L. Prenatal exposure to betamethasone decreases anxiety in developing rats: Hippocampal neuropeptide Y as a target molecule. *Neuropsychopharmacology*. 2006; 31:2140–2149. [PubMed: 16407893]
- Velíšek L, Shang E, Velíšková J, Chachua T, Macchiarulo S, Maglakelidze G, Wolgemuth DJ, Greenberg DA. GABAergic neuron deficit as an idiopathic generalized epilepsy mechanism: the role of *BRD2* haploinsufficiency in juvenile myoclonic epilepsy. *PLoS One*. 2011; 6:e23656. [PubMed: 21887291]
- Velíšková J, Moshé SL. Sexual dimorphism and developmental regulation of substantia nigra function. *Ann Neurol*. 2001; 50:596–601. [PubMed: 11706965]
- Velíšková J, Velíšek L. Beta-estradiol increases dentate gyrus inhibition in female rats via augmentation of hilar neuropeptide Y. *J Neurosci*. 2007; 27:6054–6063. [PubMed: 17537977]
- Vialou V, Bagot RC, Cahill ME, Ferguson D, Robison AJ, Dietz DM, Fallon B, Mazei-Robison M, Kus SM, Harrigan E, Winstanley CA, Joshi T, Feng J, Berton O, Nestler EJ. Prefrontal cortical circuit for depression- and anxiety-related behaviors mediated by cholecystokinin: role of DeltaFosB. *J Neurosci*. 2014; 34:3878–3887. [PubMed: 24623766]
- Wandschneider B, Centeno M, Vollmar C, Stretton J, O'Muircheartaigh J, Thompson PJ, Kumari V, Symms M, Barker GJ, Duncan JS, Richardson MP, Koepp MJ. Risk-taking behavior in juvenile myoclonic epilepsy. *Epilepsia*. 2013
- Wandschneider B, Kopp UA, Kliegel M, Stephani U, Kurlemann G, Janz D, Schmitz B. Prospective memory in patients with juvenile myoclonic epilepsy and their healthy siblings. *Neurology*. 2010; 75:2161–2167. [PubMed: 21048200]
- Wandschneider B, Thompson PJ, Vollmar C, Koepp MJ. Frontal lobe function and structure in juvenile myoclonic epilepsy: a comprehensive review of neuropsychological and imaging data. *Epilepsia*. 2012; 53:2091–2098. [PubMed: 23106095]
- Wang F, Zhu J, Zhu H, Zhang Q, Lin Z, Hu H. Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science*. 2011; 334:693–697. [PubMed: 21960531]
- Weissbecker KA, Durner M, Janz D, Scaramelli A, Sparkes RS, Spence MA. Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. *Am J Med Genet*. 1991; 38:32–36. [PubMed: 1901452]

Woermann FG, Free SL, Koeppe MJ, Sisodiya SM, Duncan JS. Abnormal cerebral structure in juvenile myoclonic epilepsy demonstrated with voxel-based analysis of MRI. *Brain*. 1999; 122:2101–2108. [PubMed: 10545395]

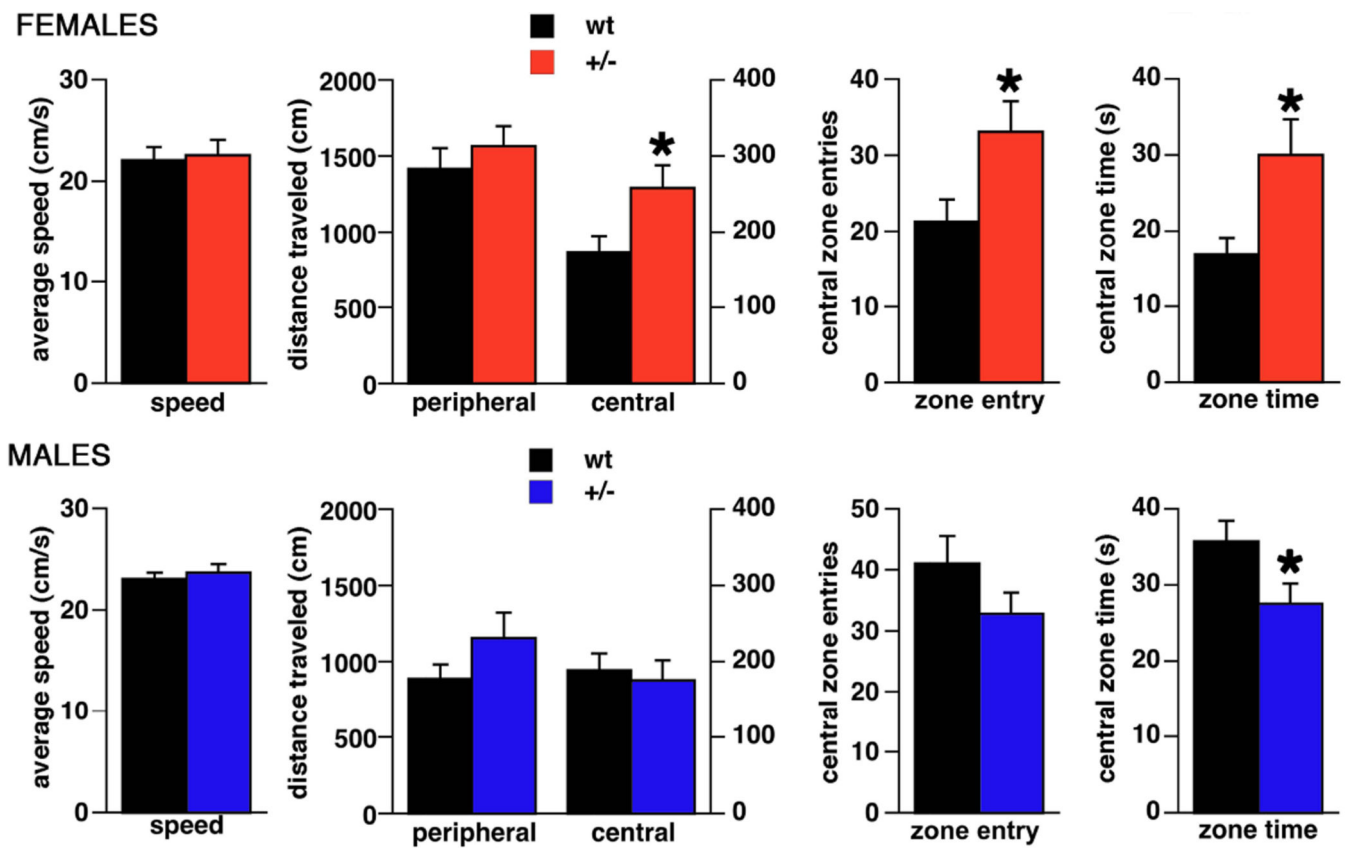


Figure 1. Performance of *Brd2*^{+/-} mice and wt littermates in the open field

Top – females (n^{+/-}=14; n^{wt}=12), bottom – males (n^{+/-}=17; n^{wt}=12). We determined speed, distance traveled in the central versus peripheral zone of the open field, number of entries in the central zone and the time spent in the central zone. Female *Brd2*^{+/-} mice traveled longer distance in the central field, made more entries to the central field and spent more time there compare to *wt* female littermates (*p<0.05). None of these outcomes was recorded in male *Brd2*^{+/-} mice. In contrast, *Brd2*^{+/-} male mice spent less time in the central area of the open field compared to the *wt* male littermates.

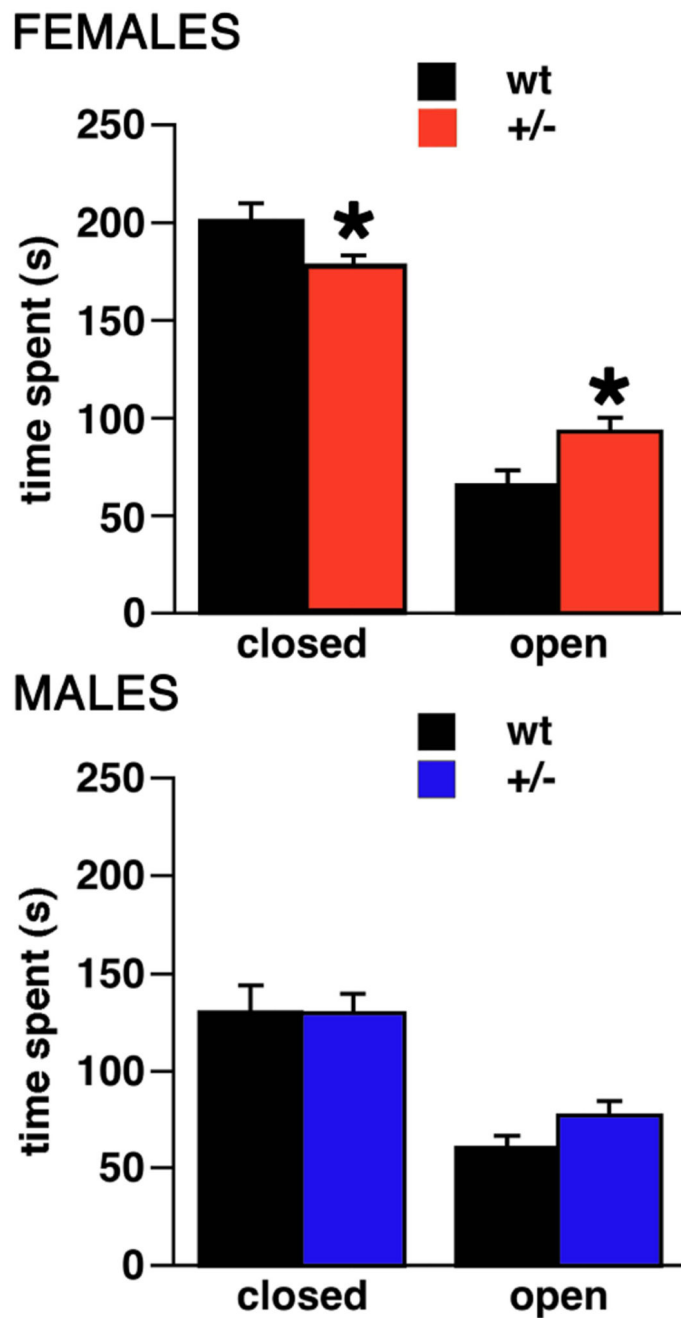


Figure 2. Activity of *Brd2*^{+/-} mice and wt littermates in the elevated plus maze

Top – females (n^{+/-}=15, n^{wt}=14), bottom – males (n^{+/-}=17, n^{wt}=12). In this test *Brd2*^{+/-} females spent more time in the open arms and proportionally less time in the closed arms of the open field compared to their *wt* female littermates (*p<0.05). This was not the case in male *Brd2*^{+/-} mice.

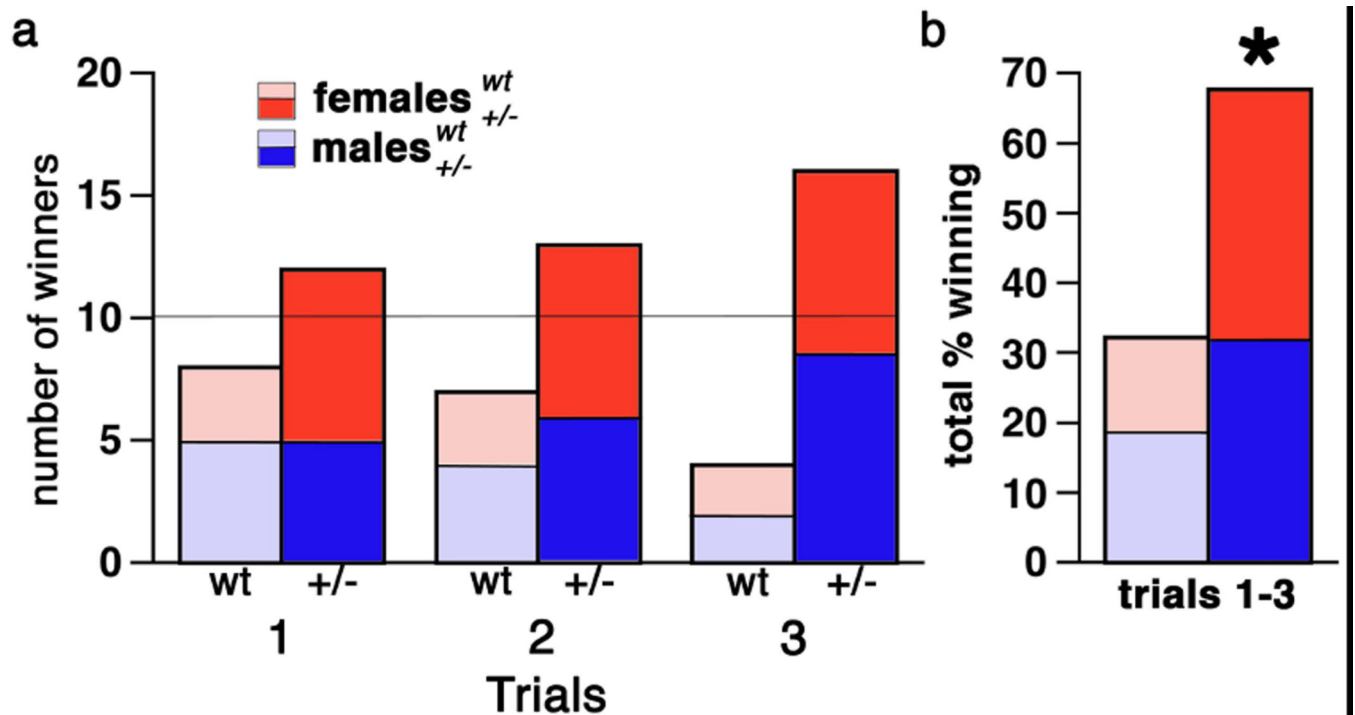


Figure 3. Increased dominance in *Brd2*^{+/-} mice compared to *wt* littermates tested in the tube
 a. Overview of three sequential trials (1 hour apart), during which pairs (+/- male – *wt* male or +/- female – *wt* female; n=10 per each subgroup) were placed in the opposite ends of clear acrylic tube. A “winner” was assigned to the mouse who after the encounter in the mid tube overpowered the other mouse having it back up out of the tube. Columns represent number of wins for individual subgroups separated by sex. For each trial, the pairs were built differently, so there was no repeated interaction with a former tube mate. Columns clearly indicate prevailing dominance of *Brd2*^{+/-} females over *wt* females from the beginning, while +/- males developed their dominance over continuing trials 1–3.
 b. Combined data from trials 1–3 indicating proportion of wins per in *Brd2*^{+/-} and *wt* mice split by sex. *Brd2*^{+/-} mice were clearly dominant in this test (*p=0.0001).

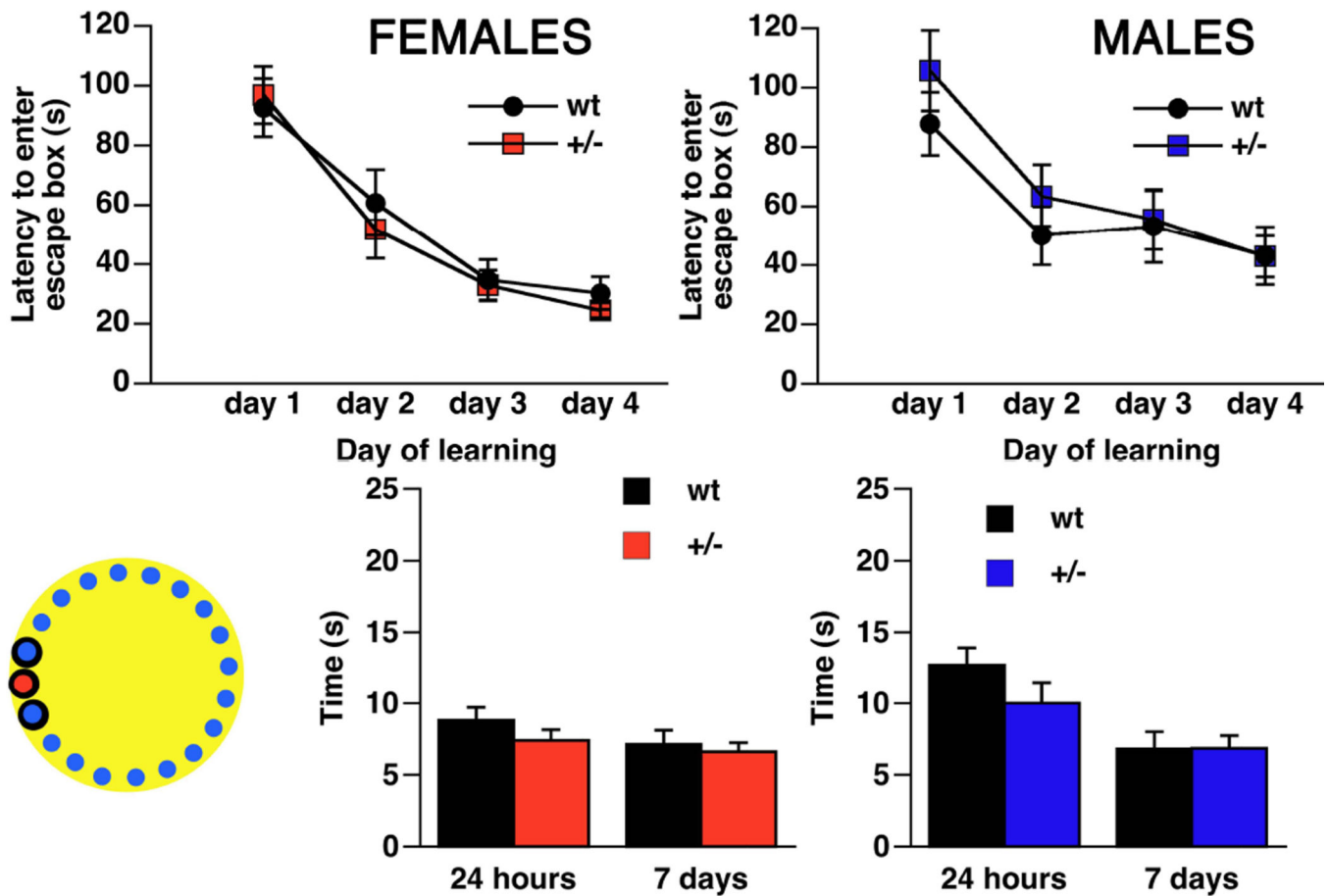


Figure 5. Learning and memory retrieval of *Brd2*^{+/-} mice and *wt* littermates in the Barnes maze
 Left inset – a scheme of Barnes maze with 19 blank boxes (blue), one full escape box (red) and area of the adjacent boxes (encircled black) to the escape box evaluated as positive outcome during memory retrieval test. Red – females (n^{+/-}=16; n^{wt}=14); blue – males (n^{+/-}=17; n^{wt}=12). In the Barnes maze, bright light (800 lx) serves as motivation to escape from the open platform and seek a hiding place in the full escape box. While both males and females were capable of learning of the position of escape box over four days of training, there was no difference between +/⁻ and *wt* littermates. Similarly, there was no difference between +/⁻ and *wt* littermates in memory retrieval at either 24 hours or 7 days after learning has been completed.

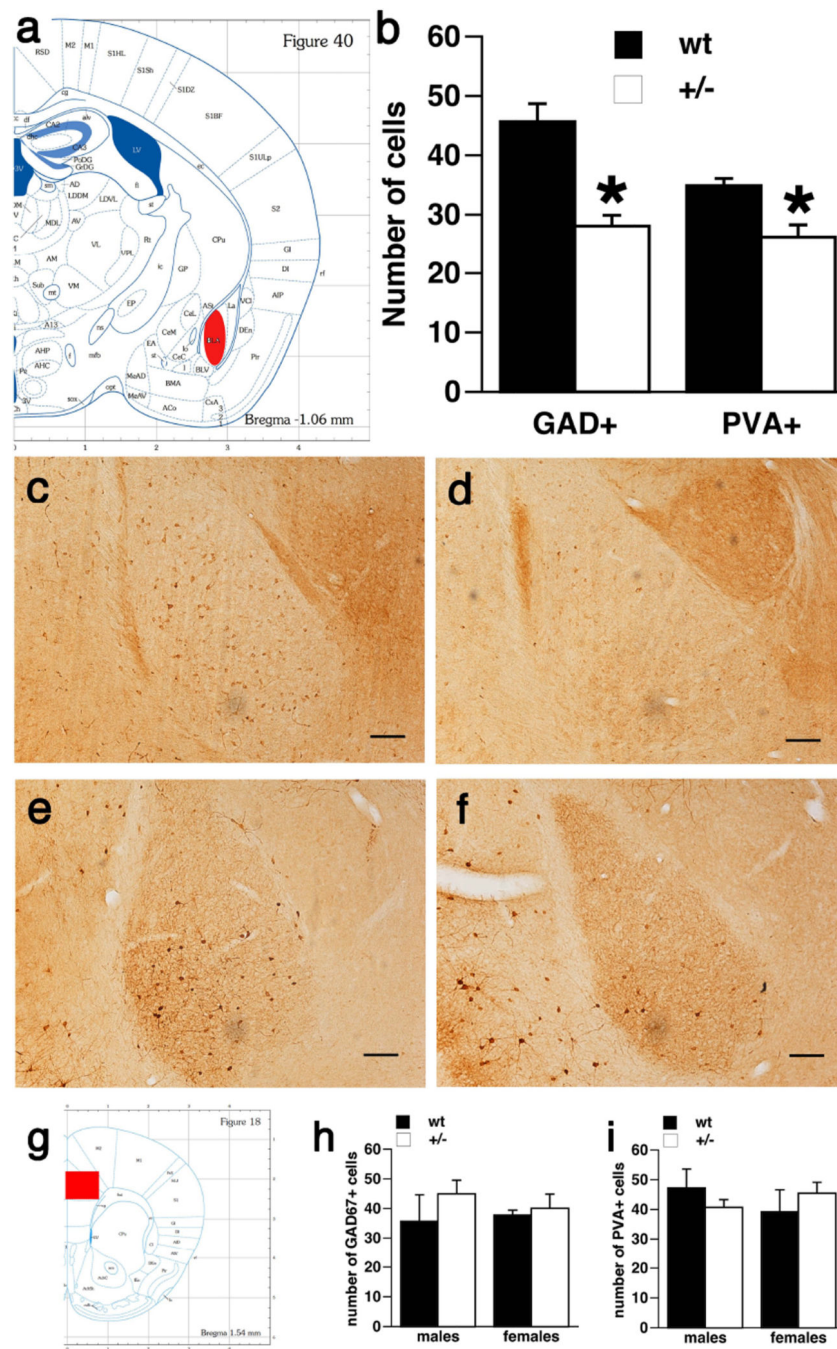


Figure 6. Decreased number of GAD67 and parvalbumin immunopositive neurons in the basolateral amygdala of *Brd2*^{+/-} mice compared to *wt* littermates

a. Plate 40 from (Paxinos & Franklin, 2004) showing the area of basolateral amygdala where the counts were performed (red fill).

b. Numbers of both GAD67-immunopositive cells and parvalbumin immunopositive cells were decreased in *+/-* mice irrespective of sex compared to *wt* littermates (two way ANOVA; * $p < 0.05$).

- c. Microphotograph of basolateral amygdala in a wt female mouse with immunostaining of GAD67 cells. Scale bars always 100 μ m.
- d. Microphotograph of basolateral amygdala in a *Brd2*^{+/-} female mouse with immunostaining of GAD67 cells. Please note that number of dark stained cells is very low, in contrast to wt female.
- e. Microphotograph of basolateral amygdala in a wt female mouse with immunostaining of parvalbumin cells.
- f. Microphotograph of basolateral amygdala in a *Brd2*^{+/-} female mouse with immunostaining of parvalbumin cells. Please note that number of dark stained cells is very low, in contrast to wt female.
- g. Plate 18 from (Paxinos & Franklin, 2004) showing the area of prefrontal cortex (frame dimension) where the counts were performed (red fill).
- h. There was no difference in the expression of GAD67 immunopositive cells in the prefrontal cortex area both between *Brd2*^{+/-} and *wt* mice and between males and females.
- i. There was no difference in the expression of PVA immunopositive cells in the prefrontal cortex area both between *Brd2*^{+/-} and *wt* mice and between males and females.