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BXD recombinant inbred strains participate in social preference, anxiety and depression behaviors along sex-differences in cytokines and tactile allodynia

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Abstract

Depression and anxiety are the most common psychiatric disorders, representing a major public health concern. Dysregulation of oxidative and inflammatory systems may be associated with psychiatric disorders, such as depression and anxiety. Due to the need to find appropriate animal models to the understanding of such disorders, we queried whether 2 BXD recombinant inbred (RI) mice strains (BXD21/TyJ RI and BXD84/RwwJ RI mice) and C57BL/6 wild-type mice show differential performance in depression and anxiety related behaviors and biomarkers. Specifically, we assessed forced swim, elevated plus maze, social preference and Von Frey tests at 3-4 months-of-age, as well as activation of cytokines and antioxidant mRNA levels in the cortex at 7 months-of-age. We report that (1) the BXD84/RwwJ RI strain exhibits anxiety disorder and social avoidance-like behavior (2) BXD21/TyJ RI strain show a resistance to depression illness, and (3) sex-dependent cytokine profiles and allodynia with elevated inflammatory activity were inherent to male BXD21/TyJ RI mice. In conclusion, we provide novel data in favor of the use of BXD recombinant inbred mice to further understand anxiety and depression disorders.

1. Introduction

Depression and anxiety are the most common psychiatric disorders, representing a major public health concern (Galvez-Contreras et al., 2016). Stressors or traumatic environmental events during lifetime play key roles in the etiology of several psychiatric disorders, such as depression and anxiety (Get al., 2017). These disorders are characterized by mood and social

disabilities, and lack of motivation, which may interfere with daily life qualities. Elevated depressive/anxiety symptoms have been linked to altered immunity and markers of inflammation (Beydoun et al., 2016). The causes of psychiatric disorders have yet to be entirely understood, but seem to involve complicated interactions between environmental, genetic and immunological factors (Nazimek et al., 2016).

Experimental studies in animals have noted inherent dysregulation of oxidative and inflammatory systems in psychiatric disorders. These effects are mediated by Nuclear factor erythroid 2 related factor 2 (Nrf2) pathway dysregulation (Zhang et al., 2013). Nrf2, a transcription factor, plays a central role in cellular defenses against oxidative stress (Ma, 2013), regulating the expression of genes whose protein products attenuate reactive oxidant activity (Nguyen et al., 2009). When bound to its inhibitory protein Kelch-like ECH-associating protein 1 (Keap1), Nrf2 is targeted for ubiquitination. Upon exposure to oxidative stress, Nrf2 dissociates from Keap1, translocates to the nucleus where it binds to an antioxidant response element (ARE) that activates antioxidant signaling, thus attenuating and maintaining cellular redox homeostasis (Nguyen et al., 2009; Ma, 2013; Muramatsu et al., 2013; Zhang et al., 2013). Imbalance in redox homeostasis under stress conditions plays a role in neuroinflammatory processes (Rammal et al., 2008).

Oxidative stress is linked to anxiety-related behavior in mice. Rammal and collaborators (2008) detected the presence of oxidative stress in the hippocampus, cerebellum and cerebral cortex of anxious male mice. In addition, iNrf2-silenced [with short interfering RNA molecules (siRNA)] male rats displayed anxiety-like behavior (Khalifeh et al., 2015). Other studies have used growth factors and cytokines as biomarkers for diagnosis of several psychiatric disorders, focusing on the role of growth factors and their relationship to anxiety-related behaviors (cognitive, emotional and social) (Galvez-Contreras et al., 2016). Furthermore, the potential anti-inflammatory properties of Nrf2 have emerged as a promising approach for neuroprotection in the face of depression and anxiety disorders (Martín-de-Saavedra et al., 2013; Muramatsu et al., 2013; Zhang et al., 2013; Khalifeh et al., 2015; Mendez-David et al., 2015). In male mice, the effects of anxiety/depression have been reversed by fluoxetine, a selective serotonin reuptake inhibitor (SSRI), directly in response to the activation of Nrf2 signaling, both in the cortex and hippocampus (Mendez-David et al., 2015). Consistent with these observations, Martín-de-Saavedra and collaborators (2013) identified Nrf2 as a target for the development of novel antidepressive drugs.

Despite of the evidence on the relationship between anti-oxidative and anti-inflammatory defenses and psychiatric alterations, their etiology is not well understood. Several issues have yet to be fully addressed: (1) the above-mentioned studies have used male animals to assess the role of oxidative stress or inflammatory system in psychiatric symptoms. Thus, it has yet to be determined whether sex-specific differences in the activation of Nrf2 and/or cytokines under depression/anxiety symptoms exist. (2) The studies referred to above took advantage of Nrf2 knockdown experimental models, with complete Nrf2 silencing. Whether, differential expression of Nrf2 can modify responsiveness to various stressors indicative of depression and anxiety disorders was tested here, taking advantage of 2 BXD recombinant inbred (RI) mouse strains (BXD21/TyJ RI and BXD84/RwwJ RI mice). Per Philip and collaborators (2009), only a few BXD RI strains have been studied for behavioral

phenotypes. Thus, we tested whether BXD21/TyJ RI, BXD84/RwwJ RI and C57BL/6 wild-type strains (considered suitable for genetics of behavioral phenotypes) show analogous performance in forced swim, social preference, elevated plus maze and Von Frey tests, as well as activation of cytokines and antioxidant mRNA levels.

2. Experimental procedures

2. 1. Animals

All experimental protocols were conducted in accordance with the NIH Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee at Albert Einstein College of Medicine (Bronx, NY). BXD RI strains and C57BL/6 wild-type mice (5–6 weeks) were provided by the Jackson Laboratory (Bar Harbor, ME). Mice were housed in groups of three to five per cage in an environmentally controlled animal facility with a 12 h light/dark cycle and given free access to regular food and water. Animals were acclimated for 15 days to the local conditions prior to experimentation. Two BXD RI mouse strains, BXD21/TyJ RI, BXD84/RwwJ RI and C57BL/6 wild-type mice were used with 12 animals per strain and 6 animals per sex. BXD RI lines represent a genetic reference population derived from a cross between C57BL/6J mice (B6) and DBA/2J mice (D2) using a strategy of advanced intercrosses (Peirce et al., 2004). These filial 1-cross progeny were mated to create an isogenic F2 population (F2s inbred for >20 generations). BXD RI strains have been used to study the genetics of behavioral phenotypes including alcohol and drug addiction, stress, impulsivity, pain sensitivity and nociception.

The BXD21/TyJ and BXD84/RwwJ RI strains were selected for their levels of Nrf2 mRNA expression on postnatal day 21 (PND 21), based on a previous pilot study (data not showed). This previous study evaluated the relationship between Nrf2 heritability in brain in 4 different BXD RI mouse strains. The results revealed a high expression of Nrf2 mRNA levels in BXD21/TyJ RI strain (mean of relative expression = 0.090) and low Nrf2 mRNA levels in BXD84/RwwJ RI strain (mean of relative expression = 0.020) at PND 21. Then, we considered BXD21/TyJ RI strain as high Nrf2 and BXD84/RwwJ RI strain as low Nrf2 expressors.

2. 2. Behavioral testing

Each animal was tested in three different models of anxiety/depression (elevated plus maze, forced swim and social preference tests) at 3 months of age and in one model of nociceptive sensitivity (von Frey test) at 4 months of age. The behavioral battery evaluation was performed in the Behavioral Core facilities at Rose F. Kennedy center at Albert Einstein College of Medicine. Animals were acclimated for 15 days to the local conditions prior to experimentation. All mice were subjected to all behavioral tests. Each mouse was placed in the testing room for 30–40 minutes prior to testing in order to acclimatize the animal. In all behavioral tests, the tester was blind to the condition of the mice.

2. 2. 1. Elevated plus maze test—Animals were evaluated in elevated plus maze (Lafayette Instrument Company, Lafayette, CA, USA) during a single session/trail of 10 minutes of duration for each mouse. The elevated plus maze apparatus consists of two

enclosed, opaque, arms ($50 \times 10 \times 40$ cm) and two open arms (50×10 cm) elevated 50 cm above the floor. The testing session was initiated of placing the animal in the center of the apparatus and recording the following behaviors: total time (in seconds) spent in the open arm and open arm entries using Viewer automated tracking software (Biobserve, Bonn, Germany). The maze was cleaned with 70% ethanol after every trial/mouse to avoid possible bias due to odors and/or residues left by mice tested earlier. Decreased time spent in the open arm generally indicates higher levels of anxiety (Gulinello et al., 2002; Pellow et al., 1985).

2. 2. 2. Forced swim test—Depression-like behavior was assessed by a single session/trail in a forced swim test. Animals are placed in a round glass cylinder (diameter 25 cm) of water ($25\text{--}26^\circ\text{C}$) from which they cannot escape will typically swim for up to 10 minutes for each mouse. The duration of immobility (in seconds), which was defined as the absence of escape-oriented behaviors, such as struggling, climbing and swimming, was recorded and manually scored. The water in the cylinders was replaced after every trial/mouse and the cylinders will be cleaned (by 70% ethanol) at the completion of the experiment. After removal from the cylinder, wet animals were dried and provided with access to heating pads/lamps (for 15 min), before to return to their home cages. Results were expressed as percentage of time each animal was immobile (%immobility) (Li et al., 2015). Increased time spent immobile reflects higher levels of depression-like behavior (Porsolt, 1979).

2. 2. 3. Social preference—The preference to explore and interact with conspecifics rather than inanimate objects was assessed during a single session/trail of 5 minutes of duration for each mouse. Animals were placed in the start arm of an open field chamber divided into three sections and allowed to choose freely between two arms that contain either an inanimate object or an ovariectomized female conspecific stimulus animal, both behind Plexiglas barriers that contain many air holes. The use of ovariectomized female animals as stimulus animals minimizes aggressive and sexual interactions. The amount of exploration (defined as sniffing, rearing, whisking, orientation and physical contact) of each area was recorded using Viewer automated tracking software (Biobserve, Bonn, Germany). Results are expressed as social preference scores: $100 \times (\text{exploration of stimulus animal} / \text{total exploration})$ (Li et al., 2015). The social avoidance is a hallmark sign of anxiety, depression and sickness behavior (Dantzer et al., 1987).

2. 2. 4. Von Frey test—Von Frey hairs were used to establish the presence or absence of tactile allodynia during a single session/trail for each mouse. The mice are placed on a wire grid platform through which von Frey filaments, in a series of ascending forces from 0.02 to 8.0 grams (Stoelting, Wood Dale, IL, USA). The filaments are inserted from below, and applied to the plantar surface of hind paw with just enough force so that the filaments buckle. A plexiglass holder is placed over the animal with 3-5 cm clearance above the head and 3-5 cm clearance around the body to restrict the animal's movement sufficiently to permit accurate placement of the filaments. This does not restrain the mice or interfere with paw withdrawal responses. The filament was applied only when the mouse was stationary, standing on all paws and holding its head straight. Each filament was tested ten times per paw. A response to a given stimulus was defined as rapid withdrawal of the head or hind paw

when stimuli were applied and the number of responses to each stimulus-intensity was determined (Hanstein et al., 2010). Tactile threshold was defined as a withdrawal response in 8 of the 10 trials to a given stimulus intensity. A higher number of responses across stimulus intensities indicates a lower tactile threshold and is interpreted as increased tactile sensitivity. Some degree of allodynia is a frequent symptom of disease and may persist long after the initial cause for pain, even when injury or inflammation had completely is completely ameliorated (Hanstein et al., 2010; Sandkühler, 2009).

2. 3. Gene expression assay

At 7 months old the animals were humanely sacrificed under deep anesthesia with isoflourane. The brain was extracted and the cortex regions were dissected and subsequently flash-frozen in liquid nitrogen and stored at -80°C for RNA analyses. Quantitative reverse transcription PCR (qRT-PCR) was performed on antioxidant genes (Nrf2, Keap1 and glutathione peroxidase 1 (Gpx1)), as well as on proinflammatory genes (interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF α)).

2. 3. 1. TaqMan gene expression assay—Total RNA was obtained from frozen cortex region using the Trizol reagent (Invitrogen). Briefly, following 1 mL of Trizol (Life Technologies) was added to each tube containing cortex section and after the tissue was homogenized and incubated for 10 minutes at room temperature. Next, 200 mL of chloroform was added to each tube and spin for 15 minutes (15,000 revolutions per minute) at 4°C . Next the upper aqueous phase was transferred to a new tube followed by precipitation using isopropanol and glycogen (Ambion). The tissue was incubated at -20°C overnight. Next, after spin the tube and discard the supernatant, the pellet was washed with 75% ethanol. The RNA isolated was dissolved in nuclease-free water (Ambion) and heated at 55°C during 10 minutes. Following isolation, the RNA concentration and purity was checked using a spectrophotometer NANODROP 2000 (Thermo Scientific). RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Life Technologies), per manufacturer's instructions. cDNA samples were stored at 4°C . Quantitative reverse-transcription PCR (BioRad CFX96) was conducted in triplicate wells using TaqMan Gene Expression Assay probes (Life Technologies) for each gene, using the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) homolog housekeeping gene for normalization after determining the fold difference using the comparative $2^{-\text{Ct}}$ method (Livak and Schmittgen, 2001). The following probes were used: Nrf2 (assay ID: Mm00477784m1); Keap1 (assay ID Mm00497268_m1); Gpx1 (assay ID: Mm00656767_g1); IL-6 (assay ID: Mm00446190_m1) and TNF α (assay ID: Mm00443259_g1).

2. 4. Statistics

Factorial analysis of variance (ANOVA) was used for the behavioral and genetic data. All analyses were performed using the statistica and SPSS software packages. The graphics were completed using GraphPad PRISM 6.0. The following dependent variables were analyzed: time (in seconds) in open arms (elevated plus maze test), % immobility time (forced swim test), % social preference (social preference test), Nrf2, Keap1, Gpx1, IL-6 and TNF α mRNA levels. As independent variables (or fixed factors) STRAIN (BXD21/TyJ RI,

BXD84/RwwJ RI and C57BL/6 wild type control) and SEX (male and female) were used. For the analyses of von Frey test (threshold responses in grams), one-way was used analyzing SEX separately and STRAIN as independent variable. *Post hoc* analysis was performed with Bonferroni test. The accepted level of significance for all tests was $p = 0.05$.

3. Results

3.1. Behavioral results

BXD84/RwwJ RI strain spends less time in elevated open spaces during the elevated plus maze test, attesting to anxiety-like behavioral alteration—

Decreased exploration of the open arms in elevated plus maze tests reflects increased anxiety-like behavior. Statistical analyses showed a STRAIN effect ($F_{(2, 28)} = 7.051$; $p = 0.003$). At the age of 3 months, BXD84/RwwJ RI mice displayed less amount of time in the open arm during the first 5 minutes of test relative to C57BL/6 wild type control ($p = 0.003$) (Fig. 1). This result suggests higher anxiety-like behavior in BXD84/RwwJ RI mice (low Nrf2 expressing strain at PND 21) compared to the C57BL/6 wild type strain. No differences were noted in sex analyses between strains. No differences were seen in the BXD RI 84 mice compared to BXD21/TyJ RI mice.

BXD21/TyJ RI strain displays decreased immobility time in the forced swim test, attesting to resistance to depression-like behavior—

Mice were tested in the forced swim test in a 10-minute single session. The time spent immobile reflects depression-like behavior. BXD21/TyJ RI mice have a lower duration of time spent immobile ($F_{(2, 29)} = 8.075$; $p = 0.002$) relative to BXD84/RwwJ RI and C57BL/6 wild type control at 3 months old ($p = 0.024$ and $p = 0.002$ respectively) (Fig. 2). This result suggests that high-expressors of Nrf2 (BXD21/TyJ) might be resistant to depression induced by acute forced-swim stress. No sex differences were noted between strains.

BXD84/RwwJ RI strain exhibits social avoidance behavior, reflecting

emotional disorders—Mice prefer to explore and interact with conspecifics rather than inanimate objects. Lack of such social preference indicates social withdrawal, a hallmark of anxiety, depression and sickness behavior. Statistical analyses showed a STRAIN effect ($F_{(2, 30)} = 5.439$; $p = 0.010$) at 3 months old, indicating that time in the social interaction zone was significantly lower in BXD84/RwwJ RI mice compared to C57BL/6 wild-type controls ($p = 0.008$) (Fig. 3) during the 5-minute test period. No differences were seen in BXD RI 84 mice compared to BXD21/TyJ RI mice. This result indicates social avoidance-like behavior in BXD84/RwwJ RI strain (low Nrf2 mice), suggesting that this strain might be sensitive to emotional alterations. No sex differences were noted between strains.

Male-specific sensitivity, referred to as allodynia, is noted in BXD21/TyJ RI mice—

Tactile sensitivity was examined with a series of von Frey filaments from 0.02 to 8.0 grams applied to the plantar surface of the hind paw. Tactile threshold is defined as a withdrawal response in 8 of the 10 trials to a given stimulus intensity. At 4-months-of-age, two-ways analyses didn't show any statistical difference. However, one-way ANOVA was used analyzing SEX separately and STRAIN as independent variable. Analyses showed a

STRAIN effect ($F_{(2, 15)} = 3.786$; $p = 0.046$) in male mice leading to a high number of withdrawal responses for BXD21/TyJ RI mice compared to BXD84/RwwJ RI ($p = 0.052$) and C57BL/6 wild type control ($p = 0.051$) (Fig. 4). A higher number of responses across stimulus intensities is interpreted as increased tactile sensitivity for BXD21/TyJ RI strain (high Nrf2 expressing mice at PND 21). The nociceptive sensitivity to mechanical stimuli was not altered in any of the female mice strains analyzed.

3. 2. Gene expression results

The BXD21/TyJ RI strain shows alterations in cortical Nrf2 mRNA levels absent changes in Keap1 mRNA levels—We performed qRT-PCR to examine Nrf2 and Keap1 gene expression at 7 months of age. Unexpectedly, factorial ANOVA analyses showed a significant effect on STRAIN ($F_{(2, 29)} = 3.973$; $p = 0.030$) on Nrf2 mRNA levels, different from the levels observed at PND 21 in the BXD21/TyJ RI strain (data from pilot not showed). Although, the slight significant effect seen in STRAIN is lost when we revised the *post hoc* results. At 7-months-of-age, BXD21/TyJ RI strain has the tendency to a lower level of Nrf2 mRNA compared to C57BL/6 wild-type control, but it doesn't reach statistical significance ($p = 0.066$) (Fig. 5A). No difference in levels of the transcription of Keap1 were noted in the strains tested (Fig. 5B). No differences were seen in BXD RI 84 mice compared to BXD21/TyJ RI for either Nrf2 or Keap1 mRNA levels. These results suggest that Nrf2 and Keap1 mRNA levels in the cortex of BXD21/TyJ, BXD84/RwwJ and C57BL/6 wild-type strains at 7 months of age are similar. No sex differences were noted between strains.

Cerebral cortex Gpx1 mRNA levels are indistinguishable in BXD21/TyJ RI, BXD84/RwwJ RI and C57BL/6 wild-type mice—Factorial ANOVA analyses showed no differences Gpx1 mRNA levels between BXD21/TyJ RI, BXD84/RwwJ RI and C57BL/6 wild-type mice. The three strains also displayed analogous levels of cerebrocortical Gpx1 mRNA levels at 7 months-of-age (see table 1).

Male BXD21/TyJ RI mice show sex-specific overexpression of cerebral cortex proinflammatory cytokines, IL-6 and TNF α —To determinate whether BXD21/TyJ RI and BXD84/RwwJ RI strains exhibit differential neuroinflammatory response, cerebral cortex IL-6 and TNF α mRNA levels were measured at 7-months-of -age. Factorial ANOVA analyses showed a significant effect on STRAIN for IL-6 mRNA levels ($F_{(2, 29)} = 23.179$; $p = 0.000$) and TNF α mRNA levels ($F_{(2, 29)} = 12.061$; $p = 0.000$) (Fig. 6A and Fig. 6C). Increased IL-6 and TNF α mRNA levels were noted in the BXD21/TyJ RI strain compared to the other strains ($p = 0.000$). In addition, a significant effect on STRAIN by SEX was found for both IL-6 and TNF α mRNA levels ($F_{(2, 29)} = 4.384$; $p = 0.022$ and $F_{(2, 29)} = 4.049$; $p = 0.028$ respectively) (Fig. 6B and Fig.6D). *Post hoc* analyses revealed increased proinflammatory response in males BXD21/TyJ RI compared to BXD84/RwwJ RI and C57BL/6 wild-type control for levels of IL-6 and TNF α mRNA ($p = 0.000$ and $p = 0.000$). Moreover, this increase in cytokine levels was noted in male BXD21/TyJ RI mice, but not in females of the same strain, leading to a sex-dependent effect, which was absent in the other strains.

4. Discussion

The relationship between Nrf2, sex-dependent inflammatory processes and psychiatric disorders has yet to be fully elucidated. To address these issues we performed a battery of behavioral assessments and determined the status of oxidative stress and inflammatory responses in 2 BXD RI strains (BXD21/TyJ RI mice, selected as higher brain Nrf2 mRNA expressors versus BXD84/RwwJ RI mice at PND 21).

We expected to see a difference in nrf2 mRNA levels at 7-months-of-age, based on a previous pilot studies in our lab where differences were noted on PND21. However, the mRNA levels of Nrf2 in BXD84/RwwJ RI mice (low Nrf2 expression at PND 21) and BXD21/TyJ RI (high Nrf2 expression at PND 21) were statistically indistinguishable from C57BL/6 wild-type controls (Fig. 5) (opposite to levels observed at PND 21). Despite of absence of correspondence between the data on Nrf2 mRNA levels on PND 21 and 7-months-of age, we consider the data of importance. In an attempt to explain the discrepancy in Nrf2 mRNA levels, we considered that it may reflect compensatory mechanisms or adaptive regulation in the Keap1- Nrf2 system (Baxter and Hardinghamat, 2016; Davies, 2016) from early age to 7 months-of-age. Nrf2 levels can rapidly fluctuate in response to environmental changes, such as stress induced by behavioral tests (Zhang et al., 2013). Thus, the up- or down-regulation of Nrf2 appears to closely depend upon the nature of the stress or type of disease, and is likely to involve positive and negative feedback loops (Zhang et al., 2013). We point to the need for future experiments to address how the Nrf2 pathway might vary at different developmental stages under normal and altered conditions.

The behavioral battery of tests was performance at 3-4 months of age. The results in the BXD84/RwwJ RI strain, which is implicated in anxiety disorder and social avoidance-like behavior corroborate earlier observations. It has been previously suggested that oxidative stress causes anxiety-related behaviors (Bouayed et al., 2009; Djordjevic et al., 2015; Hovatta et al., 2005; Khalifeh et al., 2015; Rammal et al., 2008). The first study, which identified a close relationship between oxidative stress and anxiety disorders, used six inbred male mouse strains showing that A/J, DBA/2J and 129S6/SvEvTac were the most anxious and FVB/NJ the least anxious strains (Hovatta et al., 2005). We found high levels of anxiety and social avoidance in BXD84/RwwJ RI mice (low Nrf2 expressing mice at PND 21) (Fig. 1 and 3), it is in agreement with other studies, which also indicated a possible role of Nrf2, especially a decrease in Nrf2 levels in male rats, in pathogenesis of anxiety disorders (Djordjevic et al., 2015; Khalifeh et al., 2015). Other studies reported that anxiety levels are linked to the oxidative status through the central and peripheral systems in male mice (Rammal et al., 2008).

The interest in identifying novel antidepressants has led to the study of the role of Nrf2 in depression (Lee et al., 2013). The efficacy of antidepressants to decrease oxidative stress via activation of Nrf2 signaling has been previously noted (Martín-de-Saavedra et al., 2013; Mendez-David et al., 2015; Uchihara et al., 2016; Yao et al., 2016; Zhang et al., 2013), leading to the hypothesis that oxidative stress contributes to the pathogenesis of depressive disorders (Mendez-David et al., 2015; Lee et al., 2013). Although our results show shorter immobility time, and thus less depression-like behavior in the BXD21/TyJ RI strain

(considered high Nrf2 expressing at PND 21) (Fig. 2), the role of Nrf2 in this context needs to be further addressed. We propose that alternative mechanisms, such as inflammatory hypothesis might underline these results.

Consistent with the inflammatory hypothesis, depression is characterized by cell-mediated immune activation and inflammation (Avolio et al., 2016; Galvez-Contreras et al., 2016; Maes, 2011). Our result indicates that both males and females demonstrate distinct inflammatory cytokine and allodynia profiles, reflecting a sex-dependent response in BXD21/TyJ RI strain (Fig. 6 and 4). We have observed, less depression in females BXD21/TyJ RI strain with absence of inflammatory process and less depression in males BXD21/TyJ RI strain along increased inflammatory cytokines and pain sensitivity or allodynia. Explaining the observed sex-differences is challenging despite the consistency with others studies. For example, male rats developed visceral and somatic hypersensitivity compared to controls following exposure to maternal separation and limited nesting, whereas females subjected to the same paradigms were normosensitive (Prusator and Meerveld, 2016). The difference in allodynia conditions between males and females might be attributed to sex hormones (Mogil, 2012; Woller et al., 2015). Female hormones may increase the sensitivity for developing chronic pain or decrease the nociception of pain, dependent upon the menses cycle (Prusator and Meerveld, 2016). In addition, it appears that the female brain might be more resistant to the inflammatory process than the male brain via Nrf2 upregulation by sex steroid hormones, such as 17 β -estradiol (Zhu et al., 2015). Sex hormones were not investigated in this experiment; hence it remains to be determined how they might affect inflammatory processes under normal mood conditions. Our findings underscore the need for further evaluation on the relationship between depression-like-behavior and inflammatory profiles in the BXD21/TyJ RI strain.

In conclusion, our studies suggest BXD21/TyJ and BXD84/RwwJ RI strains offer suitable models for further characterization of depression and anxiety disorders. Table 1 summarizes the main results in behavioral tests and mRNA gene expression. We conclude: (1) BXD84/RwwJ RI strain is implicated in anxiety disorder and social avoidance-like behavior meanwhile (2) BXD21/TyJ RI strain shows a resistance to depression illness, and (3) sex-dependent cytokine profiles and allodynia with elevated inflammatory activity are inherent to male BXD21/TyJ RI mice. Taken together, our data shed new light on psychiatric disorders and the potential for BXD RI mice to offer mechanistic insight into their underpinnings.

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Fig. 1.

Mean ($\pm$ SEM). Time spend in open arms expressed in seconds during the performance of the elevated plus maze test at 3 month old ($n=12$). (*) Statistical analyses indicate significant strain differences by ANOVA ($p = 0.003$). Posthoc binary comparisons revealed significant differences between the BXD84/RwwJ RI mice (low Nrf2 expressing strain at PND 21) and the control group ($p = 0.05$), but not the BXD21/TyJ RI mice ($p = 0.054$).

Fig. 2. Mean ($\pm$ SEM). % Immobility time in forced swim test during 10 minutes at 3 month old ($n=12$). (*) Statistical analyses indicate significant differences between the BXD21/TyJ RI mice (high Nrf2 expressing strain at PND 21) and BXD84/RwwJ RI and the control group ($p < 0.05$).

Fig. 3.

Mean ($\pm$ SEM). % Social preference test during 5 minutes at 3 month old (n=12). (*)

Statistical analyses indicate significant differences between the BXD84/RwwJ RI mice (low Nrf2 expressing strain) and the control group ($p = 0.05$), but not the BXD21/TyJ RI mice ($p = 0.296$).

Fig. 4. Mean ($\pm$ SEM). Paw threshold responses in grams at 4 months old ($n=6$) in von Frey test. (*) Statistical analyses indicate significant differences between the BXD21/TyJ RI male mice (high Nrf2 expressing strain) compared to BXD84/RwwJ RI and the male control group ($p = 0.05$).

Fig. 5. Mean ($\pm$ SEM). **A)** Levels of Nrf2 mRNA and **B)** Keap1 mRNA in cortex in three selected strains: BXD84/RwwJ RI and BXD21/TyJ RI mice (low and high Nrf2 expressing strains at PND 21) and C57BL/6 wild type as control group at 7 months old (n=11-12).

Fig. 6. Mean ($\pm$ SEM). Levels of IL-6 and TNF α mRNA in cortex in three selected strains: BXD84/RwwJ RI and BXD21/TyJ RI mice (low and high Nrf2 expressing strains at PND 21) and C57BL/6 wild type as control group at 7 months old (n=6-12). **A)** Levels of IL-6 mRNA grouping by strains. **B)** Levels of IL-6 mRNA grouping by strains and sex. **C)** Levels of TNF α mRNA grouping by strains. **D)** Levels of TNF α mRNA grouping by strains and sex. (*) Statistical analyses indicate significant differences between the BXD21/TyJ RI strain and the control group and BXD84/RwwJ RI strain ($p < 0.001$).

Table 1

Summary of results in behavioral tests and mRNA levels

Data are expressed as % with respect to C57BL/6 wild type control.

Strains	Behavioral results (3-4 months old)				mRNA gene expression results (7 months old)					
	<i>a</i> Elevated plus maze	<i>b</i> Forced swim	<i>c</i> Social preference	<i>d</i> Von Frey	<i>e</i> Nfr2	<i>f</i> Keap1	<i>g</i> Gpx1	<i>h</i> IL-6	<i>i</i> TNFa	
C57BL/6			100%							
BXD84/RwwJ	53% *	114%	83% *	72%	100%	111%	118%	83%	93%	
BXD21/TyJ	78%	59% *	93%	223% *	87%	102%	127%	193% *	154% *	

*a*Time spend in open arms expressed in seconds by strain.

b% Immobility time by strain.

c% Social preference by strain.

*d*Paw threshold responses in grams by male strain.

*e*mRNA levels by strain.

*f*mRNA levels by strain.

*g*mRNA levels by strain.

*h*mRNA levels by male strain.

*i*mRNA levels by male strain.

* Statistically significant data.