Sex differences in depressive, anxious behaviors and hippocampal transcript levels in a genetic rat model

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Major depressive disorder (MDD) is a common, debilitating illness with high prevalence of comorbid anxiety. The incidence of depression and of comorbid anxiety is much higher in women than in men. These gender biases appear after puberty and their etiology is mostly unknown. Selective breeding of the Wistar Kyoto (WKY) rat strain, an accepted model of adult and adolescent depression, resulted in two fully inbred substrains. Adult WKY more immobile (WMI) rats of both sexes consistently show increased depression-like behavior in the forced swim test when compared with the control WKY less immobile (WLI) strain. In contrast, here we show that while adult female WMIs and WLIs both display high anxiety-like behaviors, only WLI males, but not WMI males, show this behavior. Moreover, the behavioral profile of WMI males is consistent from early adolescence to adulthood, but the high depression- and anxiety-like behaviors of the female WMIs appear only in adulthood. These sex-specific behavioral patterns are paralleled by marked sex differences in hippocampal gene expression differences established by genome-wide transcriptional analyses of 13th generation WMIs and WLIs. Moreover, sex- and age-specific differences in transcript levels of selected genes are present in the hippocampus of the current, fully inbred WMIs and WLIs. Thus, the contribution of specific genes and/or the influence of the gonadal hormonal environment to depression- and anxiety-like behaviors may differ between male and female WMIs, resulting in their distinct behavioral and transcriptomic profiles despite shared sequences of the somatic chromosomes.

Keywords: Anxiety behavior, comorbidity, depression animal models, early adolescence, FST, gene expression, hippocampus, OFT, RT-qPCR, sex differences

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heavily implicated in depression and anxiety (Bannerman et al. 2004; Campbell et al. 2004; Sapolsky 2000), possesses an abundance of sex hormone receptors and its function is known to be affected by gonadal hormone alterations. Here, we sought to determine sex-specific differences in the abundance of targeted hippocampal transcripts, before and after puberty, which may have behavioral relevance in the adult and early adolescent WMIs compared with the WLIs. Hippocampal transcripts to be measured in the fully inbred WMIs and WLIs were selected from genome-wide hippocampal transcriptional analyses using adult male and female WMIs and WLIs of the 13th generation. We hypothesized that if the behavioral profile from the 13th generation was maintained to the current, fully inbred generation, transcript abundance differences will be consistent between them.

Materials and methods

Animals

The Institutional Animal Care and Use Committee of Northwestern University approved all animal procedures. Animals were group housed in a temperature- and humidity-controlled vivarium under a 12-h light–dark cycle, with lights on at 0600 h. Food and water were available for ad libitum. The WMIs and WLIs strains have been maintained in our vivarium with continuous brother–sister mating. To measure depression- and anxiety-like behaviors, FST and open field test (OFT), respectively, were conducted on animals from the 24th to 25th generation. Early adolescent animals were tested on postnatal day (PND) 30–33 (n = 20–35 sex/strain) and adults at 3–5 months of age (n = 18–40 sex/strain). Approximately half of the adults were also tested as early adolescents in the OFT and FST. No significant differences in adult immobility score in the FST or percent time spent in the center of the open field were found between adult animals that performed the tests as adolescents compared with naive adults; therefore, the adult data for each behavior were combined. As another measure of anxiety-like behavior, elevated plus maze (EPM) test was conducted on adult male and female 18th generation WMIs and WLIs (n = 6–9 sex/strain).

Hippocampal dysfunction has been implicated both in the etiology and consequence of MDD (Davidson et al. 2002; MacQueen & Frodl 2011). We measured genome-wide hippocampal transcriptomic changes with microarray on brains collected from adult female WMIs and WLIs from the 13th generation (n = 9/strain). Selected genes were chosen for quantification by real-time reverse transcription-polymerase chain reaction (RT-qPCR) in 23rd–26th generation animals. The RT-qPCR was performed on PND 31 brains collected from naive early adolescents (n = 5–8 sex/strain) and adult brains collected after at least 4 weeks rest following behavioral testing (n = 5–10 sex/strain).

Behavioral tests

All behavioral testing was performed between 1000 h and 1400 h. The FST was performed on adult animals as described previously (Solberg et al. 2004). Briefly, on the first day of the 2-day test, adult animals were placed in a tank filled with 22–24 ºC water for 15 min. On day 2, animals were again placed in the tank for a 5-min session, which was videotaped and scored by a trained, blind observer for climbing, floating, swimming and diving as described previously (Detke et al. 1995). The FST was modified for the early adolescents as the 15-min pretest schedule does not affect behavior in the FST. Although no differences in adult immobility score were observed between the early adolescents and the adult group, the early adolescent animals were placed in a smaller, 58-cm-diameter arena and the time and distance in the center 36-cm-diameter arena were measured for 5 min. Distance data from the early adolescents were excluded owing to problems with movement tracking data. The FST was performed 2 days before the FST, allowing 1 day for rest. Unpublished data from our laboratory show that this testing schedule does not affect behavior in the FST.

The EPM test was performed on adults as described previously (Nosek et al. 2008) but the animal movement data were collected by the TSE Videomot 2 version 5.75 software (Technical & Scientific Equipment, Bad Homburg, Germany). Briefly, animals were placed in the center of an 82-cm-diameter arena, with an internal, central illumination of 77 lux. The test lasted 10 min, and the time and distance in the center 50-cm-diameter arena were measured. For OFT on the early adolescents, animals were placed in a smaller, 58-cm-diameter arena and the time and distance in the center 36-cm-diameter arena were measured for 5 min. Data from the early adolescents were excluded owing to problems with movement tracking data. The OFT was performed 2 days before the FST, allowing 1 day for rest. Unpublished data from our laboratory show that this testing schedule does not affect behavior in the FST.

The EPM test was performed on adults as described previously (Nosek et al. 2008) but the animal movement data were collected by the TSE Videomot 2 version 5.75 software. Briefly, an elevated maze with two open and two closed arms and a central platform with a light measurement of 60 lux was used. Animals were placed in the maze to explore for 5 min and time and distance in the open arms were measured.

Affymetrix microarray gene expression analysis

The hippocampal gene expression analyses of adult female WMIs and WLIs were performed at the same time as that of the males, described previously (Andrus et al. 2012), but the results have not been published before. Briefly, adult female WMIs and WLIs were killed by fast decapitation immediately after removal from the home cage. Brain regions were dissected immediately and stored in RNA later (Ambion, Austin, TX, USA) at ~80 ºC. RNA was isolated from individual hippocampi using the TRIzol method (Invitrogen, Carlsbad, CA, USA) and treated with DNase1 (Qiagen, Valencia, CA, USA). To synthesize double-stranded cDNA, reverse transcription was performed on total RNA from each sample with Invitrogen's SuperScript® III First-Strand kit (18080-051). Double-stranded cDNA from adult female WMIs and WLIs was synthesized, linearly amplified and labeled with biotinylated nucleotides and hybridized onto Rat Genome 230 2.0 GeneChip arrays (Affymetrix, Santa Clara, CA, USA). Probe intensity data of the array were determined in the R environment using the R affy package (http://www.R-project.org). Data were normalized and processed as described previously (Andrus et al. 2012).

Real-time reverse transcription-polymerase chain reaction

The RT-qPCR was performed as described previously (Andrus et al. 2012). Male and female early adolescent and adult WMIs and WLIs were killed by fast decapitation immediately after removal from the home cage. Hippocampi were dissected using Paxinos coordinates (dorsal hippocampus (anterioposterior (AP) = −2.12 to −4.16, mediolateral (ML) 0–5.0 and dorsoventral (DV) 5.4–7.6) and ventral hippocampus (AP = −4.2 to −6.0, ML 0–5.0 and DV 5.4–7.6)). Tissue from the right dorsal and ventral hippocampus was used for analysis. RNA was isolated and cDNA was synthesized as in the microarray study above. ABI 7900HT real-time cycler was used to amplify 5ng cDNA using SYBR green reaction mix (ABI, Carlsbad, CA, USA). Primers were designed to amplify 80–150 bp products, preferentially within the region of the microarray probe, using default settings of ABI’s Primer Express software (version 3.0). The primer pairs used for each gene are listed in Table 1. Reactions were performed in triplicate and reached threshold amplification within 32 PCR cycles. Transcript levels were determined relative to 18S (primers commercially available from ABI, Foster City, CA, USA) and a general calibrator using the 2−A/ACT method.

Statistical analysis

Data are displayed as means ± SEM. Because of differences in the behavioral test paradigms for adolescents and adults, FST and OFT data were analyzed by two-way analysis of variance (ANNOVA, sex and strain). The RT-qPCR data were analyzed by three-way ANNOVA. Bonferroni-adjusted post hoc tests were used to identify differences
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Table 1: Quantitative RT-PCR primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence 5′–3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhdds</td>
<td>F: GGAACCTTTGTGAGGCAATCC</td>
</tr>
<tr>
<td></td>
<td>R: GTTCGGGCCCTTGCAGGTA</td>
</tr>
<tr>
<td>Dhx36</td>
<td>F: AGGCTCTATCTTATCTGCACAAAG</td>
</tr>
<tr>
<td></td>
<td>R: AACTGSGACAACGACTGTCA</td>
</tr>
<tr>
<td>Galnt1</td>
<td>F: TCCGGTCATTGTCAGAAG</td>
</tr>
<tr>
<td></td>
<td>R: TGACAGATGCTCTGCTATCA</td>
</tr>
<tr>
<td>Igf2</td>
<td>F: CGGTACCTCCGGGACACTCT</td>
</tr>
<tr>
<td></td>
<td>R: CGTCGCCGCGACTCTGCT</td>
</tr>
<tr>
<td>Psmc6</td>
<td>F: AGCTTTCAGATGGCTTTAATTGGA</td>
</tr>
<tr>
<td></td>
<td>R: TGCAAAATCACTGCTTCAATCA</td>
</tr>
<tr>
<td>Slc4a5</td>
<td>F: GTCATGTACCCTGGCCAAATAC</td>
</tr>
<tr>
<td></td>
<td>R: GTCACTGCGAGGAAAATACTAATG</td>
</tr>
<tr>
<td>Slc29a3</td>
<td>F: ACCTACTGCTGCCCAACTG</td>
</tr>
<tr>
<td></td>
<td>R: AAACACGTGGATTGAGGACAC</td>
</tr>
<tr>
<td>Usf1</td>
<td>F: CAGGAAACAGCAGATGAGA</td>
</tr>
<tr>
<td></td>
<td>R: CGCGCTCCCTCGTTA</td>
</tr>
<tr>
<td>Usp40</td>
<td>F: AACATAGAATGGCTTGGAAAAGGA</td>
</tr>
<tr>
<td></td>
<td>R: GGACTCTCCGTCTTCTTTTCT</td>
</tr>
<tr>
<td>Yipf3</td>
<td>F: TCACCGGCACATGCTCTCT</td>
</tr>
<tr>
<td></td>
<td>R: GAGTCTACCTGCCACCTT</td>
</tr>
</tbody>
</table>

F, forward; R, reverse.

between groups; significant strain differences are shown in figures. When main effects were seen in the ANOVA, but post hoc analysis did not show significance, hypothesis testing by Student’s t-tests was carried out between strains and also reported in the figures. EPM data were analyzed by Student’s t-tests. Significance was considered at P < 0.05 for all tests. Statistical analyses were performed using Systat 11 (Systat Software, Chicago, IL, USA). Statistical comparisons of microarray expression differences between WMI and WLI were made by using ANOVA methods with the R/multtest package as described previously (Andrus et al. 2012).

Results

Depression- and anxiety-like behavior
As immobility was the functional selector for the two strains, the significant strain differences persisted in adulthood, as expected (F₁,129 = 20.9, P < 0.01). Adult immobility behavior also differed by sex (F₁,129 = 13.4, P < 0.01), specifically, adult male WMIs floated significantly more than adult female WMIs (Bonferroni post hoc; P = 0.01). As Fig. 1 indicates, strain differences in immobility behavior of early adolescent males were mirrored in adulthood, whereas female strain differences did not follow this pattern. Specifically, immobility behavior of the early adolescent female WMI was not different from that of WLI, but early adolescent male WMI showed significantly more immobility than the WLI (Sex × Strain: F₁,94 = 8.6, P < 0.01). Please note that the length of the FST test differed between adolescents and adults and therefore, direct age comparisons were not made.

In contrast to the expected strain differences of immobility in the FST, significant strain differences of the percent time in the anxiety-provoking center of the OFT were unexpectedly strain-, sex- and age-dependent, as seen in Fig. 2. Specifically, male and female early adolescent WLIs spent less time in the anxiety-provoking center of the OFT than their respective WMIs (F₁,79 = 7.9, P < 0.01). However, as adults, only male WLIs spent less time in the center (Strain: F₁,59 = 6.8, P = 0.01; Sex: F₁,59 = 10.3, P < 0.01; Sex × Strain: F₁,59 = 6.3, P = 0.01). The strain and sex differences in the time spent in the center of the open field were paralleled by the distance traveled in the center of the open field, but there were no significant strain differences in overall locomotor activity as measured by total distance traveled (data not shown).

The EPM was only conducted on adults (Fig. S1, Supporting Information). Supporting the adult OFT results, adult male WMIs spent more time in the open arm than WLIs (WLI = 0.78 ± 0.78 seconds; WMI = 27.7 ± 9.5 seconds; t(13) = 3.4, P < 0.01), whereas female WMIs spent less time in the open arm than their WLI counterparts (WLI = 10.6 ± 2.9 seconds; WMI = 3.7 ± 1.4 seconds; t(15) = 2.3, P < 0.05). Accordingly, adult male WMIs also traveled significantly more in the open arm than the WLIs (WLI = 0 cm; WMI = 58.7 ± 22.6 cm; t(13) = 3.2, P < 0.01). The adult female WMIs traveled significantly less in the open arm than the WLIs (WLI = 35.6 ± 10.9 cm; WMI = 8.3 ± 3.0 cm; t(15) = 2.6, P < 0.05).

Hippocampal gene expression
Transcript abundance differences between 13th generation adult male and female hippocampi have been reported previously (Andrus et al. 2012), and will be used here only for comparison to the unpublished female microarray data. The microarray analyses of female hippocampal RNA were carried out from the same generation animals, at the same time as the males, using identical analytical procedures. We found that a total of 72 genes had transcript abundance differences of more than 30% (fold change > 1.3) with P < 0.01, and false discovery rate (FDR) = 0.05 between the strains in female hippocampi. Using these same criteria on the published male results (Andrus et al. 2012), 85 genes had transcript abundance differences between the WMI and WLI male hippocampi (Table S1). Fifteen of these transcripts were common to both males and females, and all but one showed opposite directional differences in abundance (higher vs. lower levels of the same transcript in WMI vs. WLI) between the sexes.

Given that the microarray analyses were done in an earlier generation of adult male and female WMIs and WLIs only, and the RT-qPCR validation of selected transcripts from these microarray analysis did not show a relationship between fold change and P value (Andrus et al. 2012), we used a different selection criteria. We chose the transcripts based on strain and sex specificity, significance (P < 0.01) and FDR (0.05) of hippocampal expression level differences between the WMIs and WLIs, some of which are therefore not shown in Table S1. We further selected transcripts where primers could be designed in the region of the microarray probe and those that had human orthologs.

Solute carrier family 4, sodium bicarbonate cotransporter, member 5 (Slc4a5) and dehydrodolichyl diphosphate synthase (Dhdds) were selected for quantification by
RT-qPCR because they showed strain differences in hippocampal transcript levels in both sexes and, therefore, were good correlates for the strain-specific depression trait. Insulin growth factor 2 (Igf2), ubiquitin-specific peptidase 40 (Usp40), yip domain family, member 3 (Yipf3), proteasome (prosome, macropain) 26S subunit and ATPase, 6 (Psmc6) were selected because they showed strain differences in males only and, therefore, were good correlates for the male-specific anxiety trait. Finally, DEAH (Asp-Glu-Ala-His) box polypeptide 36 (Dhx36), upstream transcription factor 1 (Usf1), solute carrier family 29 (nucleoside transporters), member 3 (Slc29a3) and UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminytransferase-like 1 (Galnt16; new synonym is Galnt16) showed strain differences only in the female hippocampus and were selected as candidate transcripts contributing to the depression- and anxiety-like behaviors that are present in the adult female WMI.

Three major groups emerged, distinguished by their expression patterns from the hippocampal gene expression data. First, these four show sex-specific strain differences in adulthood without differences in early adolescence or sex-specific strain differences in early adolescence without differences in adulthood: Yipf3, Usf1, Dhx36 and Psmc6 (Fig. 3). Transcript levels of Yipf3 were significantly higher in early adolescent compared with adult hippocampus (Fig. 3a) \( (F_{1,29} = 52.8, P < 0.001) \). Yipf3 expression was significantly higher in adult WMI male compared with WLI hippocampus (Fig. 3a).

Among those transcripts selected for female specificity from the microarray data, Usf1 and Dhx36 maintained hippocampal abundance differences between the strains in the adult females (Sex: Usf1: \( F_{1,42} = 5.3, P < 0.05; \) Dhx36: \( F_{1,42} = 10.1, P < 0.001 \).)

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**Figure 1:** Immobility measured in the FST. (a) Males and (b) females. Number of floats measured in 5-second bins. Adults underwent a 2-day test, and floating was scored from all 5 min of the second day, resulting in a maximum number of 61 bins. Early adolescents underwent a 1-day, 6-min test, and floating was scored from the last 4 min, resulting in a maximum of 49 bins. *\( P < 0.05 \) and **\( P < 0.01 \) Bonferroni-adjusted post hoc test; ##\( P < 0.01 \) Student’s t-test between strains. Data are presented as mean ± SEM. \( N=16–37/\text{strain/sex/age.} \)

**Figure 2:** Time spent in the anxiety-provoking center of the arena in the OFT. (a) Males and (b) females. Expressed as a percentage of total time. Early adolescents were tested in the smaller open field for 5 min. Adults were tested in the larger open field for 10 min. **\( P < 0.01 \) Bonferroni-adjusted post hoc test; #\( P < 0.05 \) Student’s t-test between strains. Data are presented as mean ± SEM. \( N=11–32/\text{strain/sex/age.} \)
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Figure 3: Hippocampal transcript abundance of Yipf3, Usf1, Dhx36 and Psmc6 differs between WMI and WLI in adulthood. 
(a) Yipf3, (b) Usf1, (c) Dhx36 and (d) Psmc6. Histograms represent relative quantities of genes measured with quantitative real-time PCR and calculated using the −ΔΔCt method. All expression levels were normalized to 18S. *P < 0.05 Bonferroni-adjusted post-hoc test; #P < 0.05 and ##P < 0.01 Student’s t-test between strains. Data are presented as mean ± SEM. N = 3–8/strain/sex/age.

\[ F_{1.40} = 3.9, P = 0.05 \]. Additionally, hippocampal expression of Usf1 decreased from early adolescence to adulthood in male WLIs with no changes in WMIs (Age × Strain: \[ F_{1.42} = 5.4, P < 0.05 \]) (Fig. 3b). In contrast, Usf1 expression increased from early adolescence to adulthood in female WMIs resulting in a strain difference in adulthood (Sex × Strain: \[ F_{1.42} = 6.2, P < 0.05 \]; Age × Sex: \[ F_{1.42} = 4.6, P < 0.05 \]).

Similarly, developmental changes of Dhx36 expression showed a sex difference (Age: \[ F_{1.40} = 5.6, P < 0.05 \]; Age × Sex: \[ F_{1.40} = 6.4, P < 0.05 \]) (Fig. 3c). Female WLIs expressed greater levels of hippocampal Dhx36 than WMIs, regardless of age. In contrast, the low levels of Dhx36 in early adolescent male WMIs increased by adulthood, abolishing the existing strain differences (Sex × Strain: \[ F_{1.40} = 4.6, P < 0.05 \]).

The male-specific adult strain differences of hippocampal Psmc6 expression were significant but in the opposite direction from the earlier microarray data (Strain: \[ F_{1.38} = 6.0, P < 0.05 \]; Sex: \[ F_{1.38} = 33.3, P < 0.001 \]) (Fig. 3d). Developmental changes of Psmc6 hippocampal expression showed a significant sex difference (Age: \[ F_{1.38} = 30.8, P < 0.001 \]; Age × Sex: \[ F_{1.38} = 26.7, P < 0.001 \]). Specifically, Psmc6 levels were extremely elevated during early adolescence in the WMI female but reduced by adulthood (Age × Sex × Strain: \[ F_{1.38} = 8.2, P < 0.01 \]) resulting in no strain differences in adult females.

Next, four genes showed hippocampal expression differences between the strains in early adolescence but not adulthood: Slc29a3 (Fig. 4a), Galntl1 (Fig. 4b), Dhdds (Fig. 4c) and Igf2 (Fig. 4d). Early adolescent female WLIs showed particularly high hippocampal transcript abundance of all four genes compared with all other groups: Slc29a3 (Strain: \[ F_{1.41} = 14.4, P < 0.001 \]; Sex: \[ F_{1.41} = 10.0, P < 0.01 \]; Age: \[ F_{1.41} = 47.6, P < 0.001 \]; Age × Strain: \[ F_{1.41} = 14.5, P < 0.001 \]; Strain × Sex: \[ F_{1.41} = 4.8, P < 0.05 \]; Age × Sex: \[ F_{1.41} = 7.9, P < 0.01 \]; Strain × Age × Sex: \[ F_{1.41} = 5.4, P < 0.05 \]); Galntl1 (Strain: \[ F_{1.43} = 8.1, P < 0.01 \]; Sex: \[ F_{1.43} = 5.7, P < 0.05 \]; Age: \[ F_{1.43} = 5.9, P < 0.05 \]; Age × Strain: \[ F_{1.43} = 17.9, P < 0.001 \]; Dhdds (Strain: \[ F_{1.35} = 4.4, P < 0.05 \]; Sex: \[ F_{1.35} = 5.6, P < 0.05 \]; Age: \[ F_{1.35} = 17.5, P < 0.001 \]; Age × Sex: \[ F_{1.35} = 10.1, P < 0.01 \]; Strain × Age: \[ F_{1.35} = 7.2, P < 0.01 \]; Sex × Strain: \[ F_{1.35} = 6.5, P < 0.05 \]) and Igf2 (Age: \[ F_{1.33} = 7.6, P < 0.01 \]). Early adolescent male WLIs also expressed higher levels of Slc29a3 compared with WMIs, but to a lesser degree. A similar pattern emerged in hippocampal levels of Galntl1. The elevated levels of these four genes in early adolescent female WLIs decreased by adulthood, and adult males and females of both strains showed similar expression.

Lastly, two genes, Usp40 (Fig. 5a) and Slc4a5 (Fig. 5b), showed higher expression in early adolescent females of both strains that decreased by adulthood (Fig. 5a) (Usp40: Sex: \[ F_{1.39} = 10.6, P < 0.01 \]; Age: \[ F_{1.39} = 36.6, P < 0.001 \]; Age × Sex: \[ F_{1.39} = 13.1, P < 0.001 \]; Slc4a5: Age: \[ F_{1.34} = 13.8, P = 0.001 \]; Sex: \[ F_{1.34} = 20.4, P < 0.001 \]; Age × Sex: \[ F_{1.34} = 5.4, P < 0.05 \]).

The comparison of the microarray results and of the current RT-qPCR data indicates a partial agreement in the hippocampal gene expression profile between the 13th generation and the current 23rd–26th generation. Table S2 indicates transcripts that show significant and non-significant same directional strain effects between the two methods.

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Figure 4: Hippocampal transcript abundance of Slc29a3, Galt1, Dhdds and Igf2 differs between WMI and WLI in early adolescence but not in adulthood. (a) Slc29a3, (b) Galt1, (c) Dhdds and (d) Igf2. Histograms represent relative quantities of genes measured with quantitative real-time PCR and calculated using the $-\Delta\Delta Ct$ method. All expression levels were normalized to 18S. *P < 0.05 and **P < 0.01 Bonferroni-adjusted post hoc; #P < 0.05 Student’s t-test between strains. Data are presented as mean ± SEM. N = 4–8/strain/sex/age.

Discussion

Here, we show that in a genetic animal model of depression the co-occurrence of depression- and anxiety-like behaviors differs between males and females. Specifically, adult male WMIs displayed elevated depression-, but low anxiety-like behaviors, whereas adult female WMIs showed both elevated depression-, and anxiety-like behaviors. While the behaviors of the male WMI were consistent from early adolescence to adulthood, the elevated depression- and anxiety-like behaviors of the adult female WMIs appeared only after early adolescence. These developmental and sex differences in behavior were paralleled by hippocampal expression differences in a set of a priori chosen genes.

Comorbid anxiety disorders are very common in patients with MDD (Gorwood 2004; Kessler et al. 2003; Lamers et al. 2011; Zimmerman & Chelminski 2003; Zimmerman et al. 2002), particularly in females (Marcus et al. 2005, 2008). Similarly, most, but not all, animal models of depression exhibit both depression- and anxiety-like behaviors, though few studies compare these behaviors between the sexes. The WKY, the parental strain of the WMI, shows both depression- and anxiety-like behaviors in both males and females (Pare 1994; Pare & Redei 1993a, 1993b; Tizabi et al. 2010). However, while the segregation of the depression- and anxiety-like behaviors of the male WMIs/WLIs occurred progressively throughout the generations, female WMIs retained their high anxiety-like behavior. Specifically, third-generation WMIs showed high anxiety-like behaviors (Will et al. 2003), but by the 11th generation, WMI and WLI males displayed similar levels of anxiety-like behaviors (Andrus et al. 2012). These behaviors further segregated between the strains in males through this study where WMIs displayed lower levels of anxiety-like behavior compared with WLIs. It is interesting to

Figure 5: Greater transcript abundance of Usp40 and Slc4a5 in early adolescent female WMIs and WLIs is decreased by adulthood. (a) Usp40 and (b) Slc4a5. Histograms represent relative quantities of genes measured with quantitative real-time PCR and calculated using the $-\Delta\Delta Ct$ method. All expression levels were normalized to 18S. Data are presented as mean ± SEM. N = 3–9/strain/sex/age.
point out that as the parental WKY was at their 17th generation of inbreeding at the time of distribution, and the animals used in this study are from the 23rd to 26th generation of inbreeding, these strains are highly inbred by now.

Among other selectively bred or genetic models, rats selected for anxiety-like behaviors or for low avoidance have increased depression- and anxiety-like behaviors (Landgraf 2003; Piras et al. 2010). However, three other models, selectively bred for low swim behavior, congenital helplessness or sensitivity to a cholinesterase inhibitor [Flinder’s sensitive line (FSL)], show increased depression-, but decreased anxiety-like behaviors (Overstreet et al. 1995, 2005; Shumake et al. 2005; Weiss et al. 1998).

Chronic stress models also provide varying results regarding concomitant depression- and anxiety-like behaviors as well as sex differences. For example, male rodents have either high or low anxiety behavior concomitant with anhedonia in response to chronic mild stress (D’Aquila et al. 1994; Li et al. 2010). Interestingly, chronic mild stress during adolescence leads to increased anhedonia, but decreased anxiety in female, but not male, rats (Pohl et al. 2007). Prenatal stress models of depression (Holmes et al. 2005) also result in sex-specific deficits with enhanced depressive- and anxiety-like behaviors in male, but not female offspring (Van den Hove et al. in press). Taken together, the preponderance of these different genetic and stress models shows both depression- and anxiety-like behaviors, suggesting that the molecular mechanisms underlying these behaviors are partially overlapping (Eley & Stevenson 1999; Kendler et al. 1992; Roy et al. 1995).

Previous developmental studies reported that depression- and anxiety-like behaviors are either increased, decreased or the same in early adolescents compared with adult male and female animals (Doremus et al. 2006; Doremus-Fitzwater et al. 2009; Hefner & Holmes 2007; Imhof et al. 1993; Klausz et al. 2011; Martinez-Mota et al. 2011; Slawecki 2005). Depression-like behaviors have been demonstrated in the pre-pubertal male WKY and FSL rat (Malkesman & Weller 2009; Malkesman et al. 2005, 2006). Pre-pubescent FSL males, like adult FSL males, do not exhibit anxiety-like behaviors (Malkesman et al. 2005). However, like adult WKY males, pre-pubescent WKY males display greater anxiety-like behaviors in the OFT compared with Wistars (Malkesman et al. 2005). The depression- and anxiety-like behaviors of males from these genetic models of depression are consistent from pre-pubescence to adulthood, similar to the behaviors of the WMI male in this study. Within environmental models, adult male rats, but not adolescents, show anhedonia in response to chronic mild stress (Toth et al. 2008). Similarly, adults, but not adolescent males and females, show increased anxiety after prenatal stress (Fride et al. 1986; Vallee et al. 1997; Weinstock et al. 1992; Zagron & Weinstock 2006).

Although first episodes of both MDD and anxiety tend to occur before adulthood, anxiety onset usually precedes MDD onset (Kessler et al. 2005; Woodward & Ferguson 2001). Whether anxiety predisposes individuals to depression (Breslau et al. 1995; Fava et al. 2000; Kovacs et al. 1989) or the two disorders create mutual vulnerabilities, they likely share genetic and environmental etiologies (Eaves et al. 2003). Women show greater prevalence of MDD (Bebbington 1998; Kovacs et al. 1989; Merikangas et al. 2010; Weissman et al. 1993), anxiety (Angst & Dobler-Mikola 1985; Bruce et al. 2005; Kessler et al. 1994) and anxiety comorbidity (Breslau et al. 1995; Marcus et al. 2005, 2008; Simonds & Whiffen 2003). In contrast, men show greater severity or a decreased ability to cope with depression as indicated by a much higher suicide rate than women (Blair-West et al. 1999). The differences in presentation of MDD between men and women indicate that both overlapping and separate genes contribute to depression and anxiety and also that overlapping and disparate genes affect MDD in a sexually dimorphic manner. Findings from both human and animal genetic studies support this (Aragam et al. 2011; Kendler & Prescott 1999; Silberg et al. 1999; Solberg et al. 2004). Additionally, external challenges or internal hormonal environments may affect the genes contributing to depression and anxiety differently in males and females.

Our current results are consistent with the above assumptions. As male and female WMIIs are genetically identical (with the exception of sex chromosomes), and they were selected for depression-like behaviors, the genes mediating their depression-like behaviors must overlap. However, prior to the increase in sex-specific gonadal hormones that occurs during puberty, depression-like behaviors showed a sex difference between WMIIs and WLIs, suggesting that sex-specific genes may contribute to the development of depression-like behavior in the WI male and females. The adult sex differences in the extent of depression-like behavior in the WMIIs further imply the differential effects of sex hormones on this behavior. In contrast, anxiety-like behaviors did not show a sex difference between the strains in early adolescence, suggesting that both shared and separate sequence variations between WMIIs and WLIs mediate depression- and anxiety-like behaviors. While the depression- and anxiety-like behaviors of the adult WMI female are not yet present in early adolescents, these behaviors remain relatively consistent between the early adolescent and adult male WMI, implying that the female hormonal environment during puberty induces transcriptional changes in female-specific genes to precipitate depression- and anxiety-like behaviors in the adult females.

Similar to the sexually dimorphic behavioral patterns, hippocampal transcriptional differences between male and female WMIIs and WLIs had been found in our early microarray study and also in our study. Selected transcripts showed sex-specific strain differences in hippocampal expression in adulthood and/or in early adolescence. Other than being highly conserved across species, very little is known about YipF3, one of the transcripts with strain differences in adulthood. The functions of Dlx36 and Psmc6 with female- and male-specific strain differences, respectively, have not yet been investigated in the brain. Usf1, which showed female-specific strain differences in adulthood, has been shown to regulate angiotensinogen expression with consequences in females only (Park et al. 2012). Furthermore, polymorphisms in Usf1 have been associated with neuropathological lesions in Alzheimer’s disease (Isotalo et al. 2012). Female-specific expression differences of Dlx36 and Usf1 did not change from the 13th to 26th generation, suggesting that they contribute to...


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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher’s web-site:

**Table S1.** Differentially expressed transcripts in males and females from the microarray analysis. Data shown are fold change > 1.3, \( P < 0.01 \). When fold change < 1, WLI > WMI; when fold change > 1, WMI > WLI.

**Table S2.** Comparison of gene expression results between microarray and RT-qPCR. When fold change < 1, WLI > WMI; when fold change > 1, WMI > WLI.

**Figure S1.** Distance traveled in the anxiety-provoking open arms of the elevated plus maze test. (a) Males and (b) females. Distance traveled in the open arm measured in centimeters. Animals were tested for 5 min. * \( P < 0.05 \) and ** \( P < 0.01 \). Student’s \( t \)-test between strains. Data are presented as mean ± SEM. \( N = 6–9 \) strain/sex.