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Genetic relationship between anxiety- and fear -related behaviors in BXD recombinant inbred mice

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Abstract

Mood and anxiety disorders and rodent phenotypic measures modeling these disorders have a strong genetic component. Various assays are used to study the neurobiological basis of fear- and anxiety-related behaviors, phenotype genetically modified mice, and elucidate pharmacological modulation of these behaviors for medication development. However, previous work suggests that different trait measures are mediated by partly overlapping but ultimately distinct genetic factors. In the present study, we assessed a novel panel of 23 C57BL/6J × DBA/2J (BXD) recombinant inbred strains on various trait measures of Pavlovian fear conditioning and anxiety-like behavior (novel open field, elevated plus-maze), as well as sensory (acoustic startle, prepulse inhibition of startle) and motor (baseline coordination and learning on accelerating rotarod) function. Results showed that traits were continuously distributed across strains and had modest to strong R^2 values. Principal components analysis resolved the data into 5 factors: factor 1 loaded fear-related traits, factor 2 loaded elevated plus-maze measures as well as context fear, factor 3 loaded novel open field measures and plus-maze closed arm entries, factor 4 loaded rotarod motor function, and factor 5 loaded acoustic startle and prepulse inhibition. These data add to evidence that murine measures of fear- and anxiety-like traits reflect distinct constructs mediated by dissociable gene variants.

Keywords

mouse; gene; learning; genetics; open field; plus-maze; rotarod; startle; QTL

Introduction

Individual differences in risk for mood and anxiety disorders likely stem from multiple predisposing factors, including genetic variants that shape the developmental formation and/or adult functional integrity of the neural circuitry subserving emotion (Caspi and Moffitt 2006; Hariri and Holmes 2006; Uher and McGuffin 2008). In preclinical models of these disorders, inbred mouse strains exhibit marked differences in anxiety- and fear-related phenotypes. Of note, the C57BL/6J strain typically shows lesser anxiety- and stress-related behavior but greater conditioned fear behavior relative to certain other strains including BALB/

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cJ and in some cases DBA/2J (reviewed in (Belzung and Griebel 2001; Jacobson and Cryan 2007; Millstein and Holmes 2006; Tannenbaum and Anisman 2003)).

Over the last thirty years, the C57BL/6J and DBA/2J strains have been intercrossed to produce a large and still growing panel of recombinant inbred (BXD RI) strains (Peirce et al., 2004; Taylor 1978). These strains have proved to be a valuable experimental tool to study the genetic underpinnings of mouse anxiety- and fear-related behaviors. For example, BXD RI strain panels have identified candidate chromosomal loci associated with variation in fear-related traits (Caldarone et al., 1997; Owen et al., 1997a; Radcliffe et al., 2000; Wehner et al., 1997). BXD RI strains also offer an approach to estimating the relative genetic contribution to specific traits, as well as the genetic interrelatedness of traits. This latter issue is of particular relevance to the issue of whether different trait measures of rodent anxiety- and fear-related behaviors are driven by common or distinct mechanisms (Cryan and Holmes 2005; Fernandez-Teruel et al., 2002; File 1995; Milner and Crabbe 2008; Ponder et al., 2007a; Ponder et al., 2007b; Ramos and Mormede 1998; Talbot et al., 2003; Trullas and Skolnick 1993; Turri et al., 2001). Demonstrating that the different assays recruit different underlying mechanisms would be a step towards better linking individual preclinical assays to discrete forms of pathophysiology found in specific mood and anxiety disorders. In order to address this issue in the present study, we assessed a panel of twenty-three (mostly recently generated) BXD RI strains on a battery of commonly used anxiety- and fear-related behaviors and performed principal components analysis (PCA) to identify correlations between the various trait measures obtained.

Materials and methods

Mice were bred at University of Tennessee Health Science Center and transported to the NIH and housed 1–4/cage in same-BXD strain groupings in a temperature- and humidity-controlled vivarium under a 12 h light/dark cycle (lights on 0600 h). There were a total of 124 male and female mice from 23 BXD strains, plus the progenitor strains and an F1 intercross (see figure legend for mice/strain). With the exception of one BXD line (BXD45 only males tested) males and females were approximately equally represented for all lines. Four of the BXD strains belong the original BXD generated by Taylor and colleagues (Taylor 1978) (BXD8, 9, 14, and 32), whereas the remaining 19 strains (BXD45 through BXD100) belonging to the new set generated by Peirce and colleagues (Peirce et al., 2004).

Mice were tested (putatively more stressful assays later in the test sequence) on novel open field test, elevated plus-maze, and Pavlovian fear conditioning. In addition, to provide control measures of sensory and motor functions, mice were then tested for the acoustic startle response and prepulse inhibition of startle, followed by 10 trials on accelerating rotarod. There was at least 1 week between assays. Mice were acclimated to the test room for 1 hr prior to testing. Where appropriate, apparatuses were cleaned with 70% ethanol and dried between subjects. All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee and strictly followed the NIH guidelines ‘Using Animals in Intramural Research.’

Tests were conducted using the same methods previously described in our laboratory and will not be reiterated here: novel open field (Weidholz et al., 2007), elevated plus-maze (Hefner and Holmes 2007), Pavlovian fear conditioning (Yang et al., 2008), acoustic startle and prepulse inhibition (Millstein et al., 2006), and accelerating rotarod (Boyce-Rustay and Holmes 2005). To simplify the PCA, we focused on 11 major trait measures. The effect of strain on these 11 traits was assessed using analysis of variance (ANOVA) (statistical significance set at $p < 0.05$). ANOVA results were used to calculate R^2 ($=SS_{\text{STRAIN}}/SS_{\text{TOTAL}}$), which is the proportion of the total variance in each trait due to strain and provides a simple estimate of broad sense heritability. Relationships among traits were examined via PCA (varimax rotation).

Factor selection was based upon examination of the Scree plot and a criterion of eigenvalues >1 . The factor structure was solved with an orthogonal rotation (Holmes and Rodgers 1998; Ramos et al., 1998) using the Statistica (Tulsa, OK) software program.

Results

There was a significant effect of strain on total distance traveled ($F_{25,96}=2.24$, $p<0.01$, $R^2=37$, Figure 1A), and duration of time spent in the center of a novel open field ($F_{25,96}=1.68$, $p<0.05$, $R^2=30$, Figure 1B). There was also significant effect of strain on time spent in the open arms ($F_{25,96}=3.99$, $p<0.01$, $R^2=51$, Figure 1C) and entries into the closed arm ($F_{25,96}=5.02$, $p<0.01$, $R^2=57$, Figure 1D) of the elevated plus-maze.

For Pavlovian fear conditioning, baseline freezing prior to conditioning was negligible (maximum $<2\%$) and ANOVA found no effect of strain (data not shown). On the other hand, freezing to the final tone during conditioning was significantly affected by strain ($F_{24,90}=2.85$, $p<0.01$, $R^2=34$, Figure 1E), as was freezing to the conditioned cue ($F_{24,91}=4.48$, $p<0.01$, $R^2=55$, Figure 1F) and freezing to the conditioned context ($F_{24,91}=5.05$, $p<0.01$, $R^2=57$, Figure 1G).

There was a significant effect of strain on the acoustic startle response ($F_{24,92}=5.05$, $p<0.01$, $R^2=41$, Figure 1H), and prepulse inhibition of startle ($F_{24,92}=1.82$, $p<0.05$, $R^2=32$, 1I). By contrast, there was no significant effect of strain for latency to fall on trial 1 of the accelerating rotarod ($R^2=22$, Figure 1J). The delta change in latency to fall over 10 training trials was significantly affected by strain ($F_{25,95}=1.84$, $p<0.05$, $R^2=32$, Figure 1K). All these trait data can be viewed and analyzed at www.Genenetwork.org by searching the *Mouse BXD Phenotypes* database using the key word “Brigman” (GN Record ID numbers 11004 through 11015).

PCA yielded a 5-factor structure accounting for 68% of the total variance. Factor 1 (20% of the variance) had high (i.e., >0.4) loadings for all 3 measures of fear, with higher scores for freezing to cue than to context (Table 1). Factor 2 (15% variance) comprised a high negative loading for plus-maze open arm time a lesser negative loading for closed arm entries and a positive loading for freezing to context. Factor 3 (12% variance) had high loadings for open field distance travelled and center time and a modest loading for plus-maze closed entries. Factor 4 (11% variance) had a high negative loading for baseline rotarod coordination and a high positive loading for change in rotarod performance with training. Lastly, factor 5 (10% variance) loaded acoustic startle amplitude and prepulse inhibition of startle.

Discussion

The present study provides one of the first systematic collections of phenotypes related to measures of anxiety- and fear-related behaviors in a new panel of advanced recombinant inbred strains of BXD mice (Peirce et al., 2004). As anticipated, given substantial work on the initial set of twenty-six BXD strains (Belknap et al., 1992; Crabbe et al., 1994), there was a statistically significant degree of variability across strains for all traits with the exception of baseline rotarod performance, within the panel of strains tested. The relative contribution of strain, and by proxy genetic factors, to this variance was reflected in R^2 values which ranged from 30, for time in center of open field, to a high as 57 for freezing to conditioned context.

Consistent with previous studies, DBA/2J tended to show higher anxiety-like behaviors and poorer fear conditioning than C57BL/6J (Belzung and Griebel 2001; Jacobson and Cryan 2007; Millstein and Holmes 2006; Radcliffe et al., 2000; Tannenbaum and Anisman 2003). However, it was noteworthy that trait variability was often high even in those cases in which the progenitor strains did not represent extreme phenotypes. This observation is entirely in

keeping with the fact that the combination of alleles at multiple loci inherited from the progenitors can lead to the enhancement or diminution of a behavior that is not manifestly different between the progenitors themselves (Mozhui et al., 2007; Neumann et al., 1993). Another important point is that all traits were continuously distributed. This is again consistent with previous data in other BXD panels and is demonstrative of trait variation that is driven by multiple genes, each of modest effect size. Such polygenic complexity is typical of most mouse 'emotion-related' traits as well as human mood and anxiety disorders (Caspi and Moffitt 2006; Holmes and Hariri 2003; Uher and McGuffin 2008).

We exploited the large within-subjects dataset test to examine relations among all eleven major phenotypic measures obtained, using PCA. The results of this analysis were revealing. The main conclusion was that measures of anxiety- and fear-related behaviors were weakly related (i.e., loaded on separate factors), and therefore likely to be driven by different networks of genetic factors. All three measures of fear (freezing cue to during conditioning and recall, and freezing to context) loaded together on one factor. Interestingly however, the contextual freezing co-loading was modest and this trait also loaded (inversely) on a separate factor with plus-maze open time and closed entries. A relatively weak statistical relation between cued and contextual conditioning is consistent with earlier work indicating that these measures of fear are dissociated at the anatomical, molecular and genetic levels (LeDoux 2000; Mozhui et al., 2007; Owen et al., 1997b; Talbot et al., 1999; Yang et al., 2008). In addition, the co-loading of context fear with plus-maze anxiety-like behavior indicates a common genetic influence.

In terms of relations among trait measures of anxiety-like behavior, those obtained from the elevated plus-maze and novel open field were generally poorly correlated. This is not unexpected, as previous studies have reported poor correlations between measures of anxiety-like behaviors in these and other test assays in rats and mice (File 1995; Holmes et al., 2003; Ramos et al., 1998; Trullas and Skolnick 1993) (but see (Milner and Crabbe 2008)). It is worth noting that plus-maze closed arm entries did co-load with open field measures consistent with this being a good measure of exploratory activity (File, 1995; Rodgers, 1997; Holmes, 2000). Finally, the present dataset is one of the few to systematically explore the potential relation between fear- and anxiety-like behaviors with sensory and motor functions (herein assayed via acoustic startle and prepulse inhibition of startle, and rotarod motor coordination and motor learning). The PCA clearly showed that these measures were statistically independent of the fear- and anxiety-related traits, and are therefore likely to have separate genetic origins at least in our BXD panel (see also (Fernandez-Teruel et al., 2002)).

Putting the present data in the context of previous work, Ponder and colleagues selectively bred for high and low fear conditioning in a F2 C57BL/6J $\times$ DBA/2J population and found that high fear predicted relatively high anxiety-like behavior on the open field and elevated zero-maze but not light/dark exploration tests (Ponder et al., 2007a). Studies in selected (Roman avoidance) lines of rats also found that elevated plus-maze and open field anxiety-related measures mapped to the same quantitative trait locus as conditioned fear, while acoustic startle associated with an independent locus (Fernandez-Teruel et al., 2002). These data suggest a degree of genetic commonality between fear and anxiety. However, using a heterogeneous stock of mice derived from intercrossing multiple strains, Talbot et al. found that while cued fear, context fear and open field anxiety-like behavior were both associated to the same locus, these traits mapped to different loci within this region (Talbot et al., 2003). Similarly, using an intercross of the DeFries strains Turri and coworkers identified some loci common to multiple anxiety-related measures but others that were trait-specific (Turri et al., 2001). Thus, taken together with the present data, these findings support the conclusion that rodent measures of anxiety and fear recruit partially overlapping but ultimately dissociable genetic variants. This has implications for the choice of test assays in mutant phenotyping and drug discovery.

In summary, using a novel panel of BXD RI strains, the present study provides further evidence that while murine measures of fear- and anxiety-like traits have a strong genetic component, the gene variants driving specific traits are to a large degree genetically independent. With further expansion, this dataset could be valuable for future genetic studies aimed at identifying the specific chromosomal regions and, ultimately, the specific sequence variants that contribute to these constructs.

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References

- Belknap JK, Crabbe JC, Plomin R, McClearn GE, Sampson KE, O'Toole LA, Gora-Maslak G. Single-locus control of saccharin intake in BXD/Ty recombinant inbred (RI) mice: Some methodological implications for RI strain analysis. *Behav Genet* 1992;22:81–100. [PubMed: 1590732]
- Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* 2001;125:141–149. [PubMed: 11682105]
- Boyce-Rustay JM, Holmes A. Functional roles of NMDA receptor NR2A and NR2B subunits in the acute intoxicating effects of ethanol in mice. *Synapse* 2005;56:222–225. [PubMed: 15803501]
- Caldarone B, Saavedra C, Tartaglia K, Wehner JM, Dudek BC, Flaherty L. Quantitative trait loci analysis affecting contextual conditioning in mice. *Nat Genet* 1997;17:335–337. [PubMed: 9354801]
- Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* 2006;7:583–590. [PubMed: 16791147]
- Crabbe JC, Belknap JK, Buck KJ, Metten P. Use of recombinant inbred strains for studying genetic determinants of responses to alcohol. *Alcohol Alcohol Suppl* 1994;2:67–71. [PubMed: 8974318]
- Cryan JF, Holmes A. The ascent of mouse: Advances in modelling human depression and anxiety. *Nat Rev Drug Discov* 2005;4:775–790. [PubMed: 16138108]
- Fernandez-Teruel A, Escorihuela RM, Gray JA, Aguilar R, Gil L, Gimenez-Llort L, Tobena A, Bhomra A, Nicod A, Mott R, Driscoll P, Dawson GR, Flint J. A quantitative trait locus influencing anxiety in the laboratory rat. *Genome Res* 2002;12:618–626. [PubMed: 11932246]
- File SE. Animal models of different anxiety states. *Adv Biochem Psychopharmacol* 1995;48:93–113. [PubMed: 7653328]
- Hariri AR, Holmes A. Genetics of emotional regulation: The role of the serotonin transporter in neural function. *Trends Cogn Sci* 2006;10:182–191. [PubMed: 16530463]
- Hefner K, Holmes A. Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. *Behav Brain Res* 2007;176:210–215. [PubMed: 17098297]
- Holmes A, Hariri AR. The serotonin transporter gene-linked polymorphism and negative emotionality: placing single gene effects in the context of genetic background and environment. *Genes Brain Behav* 2003;2:332–335. [PubMed: 14653304]
- Holmes A, Parmigiani S, Ferrari PF, Palanza P, Rodgers RJ. Behavioral profile of wild mice in the elevated plus-maze test for anxiety. *Phys & Behav* 2000;71:509–516.
- Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L, Vishwanath J, Saavedra MC, Innerfield CE, Jacoby AS, Shine J, Iismaa TP, Crawley JN. Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. *Neuropsychopharmacology* 2003;28:1031–1044. [PubMed: 12700679]
- Holmes A, Rodgers RJ. Responses of Swiss-Webster mice to repeated plus-maze experience: Further evidence for a qualitative shift in emotional state? *Pharmacol Biochem Behav* 1998;60:473–488. [PubMed: 9632231]
- Jacobson LH, Cryan JF. Feeling strained? Influence of genetic background on depression-related behavior in mice: A review. *Behav Genet* 2007;37:171–213. [PubMed: 17029009]
- LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155–184. [PubMed: 10845062]

- Millstein RA, Holmes A. Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neurosci Biobehav Rev*. 2006
- Millstein RA, Ralph RJ, Yang RJ, Holmes A. Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. *Genes Brain Behav* 2006;5:346–354. [PubMed: 16716204]
- Milner LC, Crabbe JC. Three murine anxiety models: Results from multiple inbred strain comparisons. *Genes Brain Behav*. 2008
- Mozhui K, Hamre KM, Holmes A, Lu L, Williams RW. Genetic and structural analysis of the basolateral amygdala complex in BXD recombinant inbred mice. *Behav Genet* 2007;37:223–243. [PubMed: 17131200]
- Neumann PE, Garretson JD, Skabardonis GP, Mueller GG. Genetic analysis of cerebellar folial pattern in crosses of C57BL/6J and DBA/2J inbred mice. *Brain Res* 1993;619:81–88. [PubMed: 8374795]
- Owen EH, Christensen SC, Paylor R, Wehner JM. Identification of quantitative trait loci involved in contextual and auditory-cued fear conditioning in BXD recombinant inbred strains. *Behav Neurosci* 1997a;111:292–300. [PubMed: 9106670]
- Owen EH, Logue SF, Rasmussen DL, Wehner JM. Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: Implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neuroscience* 1997b;80:1087–1099. [PubMed: 9284062]
- Peirce JL, Lu L, Gu J, Silver LM, Williams RW. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet* 2004;5:7. [PubMed: 15117419]
- Ponder CA, Kliethermes CL, Drew MR, Muller J, Das K, Risbrough VB, Crabbe JC, Gilliam TC, Palmer AA. Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. *Genes Brain Behav* 2007a;6:736–749. [PubMed: 17309658]
- Ponder CA, Munoz M, Gilliam TC, Palmer AA. Genetic architecture of fear conditioning in chromosome substitution strains: Relationship to measures of innate (unlearned) anxiety-like behavior. *Mamm Genome* 2007b;18:221–228. [PubMed: 17492333]
- Radcliffe RA, Lowe MV, Wehner JM. Confirmation of contextual fear conditioning QTLs by short-term selection. *Behav Genet* 2000;30:183–191. [PubMed: 11105392]
- Ramos A, Mellerin Y, Mormede P, Chaouloff F. A genetic and multifactorial analysis of anxiety-related behaviours in Lewis and SHR intercrosses. *Behav Brain Res* 1998;96:195–205. [PubMed: 9821556]
- Ramos A, Mormede P. Stress and emotionality: A multidimensional and genetic approach. *Neurosci Biobehav Rev* 1998;22:33–57. [PubMed: 9491939]
- Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: An ethological perspective. *Braz J Med Biol Res* 1997;30:289–304. [PubMed: 9246227]
- Talbot CJ, Nicod A, Cherny SS, Fulker DW, Collins AC, Flint J. High-resolution mapping of quantitative trait loci in outbred mice. *Nat Genet* 1999;21:305–308. [PubMed: 10080185]
- Talbot CJ, Radcliffe RA, Fullerton J, Hitzemann R, Wehner JM, Flint J. Fine scale mapping of a genetic locus for conditioned fear. *Mamm Genome* 2003;14:223–230. [PubMed: 12682774]
- Tannenbaum B, Anisman H. Impact of chronic intermittent challenges in stressor-susceptible and resilient strains of mice. *Biol Psychiatry* 2003;53:292–303. [PubMed: 12586448]
- Taylor, BA. Recombinant inbred strains: Use in gene mapping. In: Morse, HC., editor. *Origins of Inbred Mice*. New York: Academic Press; 1978. p. 423–438.
- Trullas R, Skolnick P. Differences in fear motivated behaviors among inbred mouse strains. *Psychopharmacology (Berl)* 1993;111:323–331. [PubMed: 7870970]
- Turri MG, Datta SR, DeFries J, Henderson ND, Flint J. QTL analysis identifies multiple behavioral dimensions in ethological tests of anxiety in laboratory mice. *Curr Biol* 2001;11:725–734. [PubMed: 11378382]
- Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: Review and methodological analysis. *Mol Psychiatry* 2008;13:131–146. [PubMed: 17700575]
- Wehner JM, Radcliffe RA, Rosmann ST, Christensen SC, Rasmussen DL, Fulker DW, Wiles M. Quantitative trait locus analysis of contextual fear conditioning in mice. *Nat Genet* 1997;17:331–334. [PubMed: 9354800]

- Weidholz LM, Owen AM, Horton RE, Feyder M, Karlsson RM, Hefner K, Sprengel R, Ceikel T, Daws LC, Holmes A. Mice lacking the AMPA GluR1 receptor exhibit striatal hyperdopaminergia and 'schizophrenia-related' behaviors. *Mol Psychiatry*. 2007in press
- Yang RJ, Mozhui K, Karlsson RM, Cameron HA, Williams RW, Holmes A. Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacology*. 2008in press

Figure 1.

Variation in anxiety- and fear-related behaviors across BXD RI strains. Total distance travelled (A) and time spent in the center (B) of a novel open field. Percent time spent in the open arms (C) and closed arm entries (D) in the elevated plus-maze. Variation in anxiety- and fear-related behaviors across BXD RI strains Freezing to the final tone presentation during conditioning (E), to the conditioned cue (F) and to the conditioned context (G) in the Pavlovian fear conditioning paradigm. Startle responses to loud acoustic stimulus (H) and prepulse inhibition of the acoustic startle response (I), and baseline (trial 1) latency to fall in the accelerating rotarod (J) and rotarod motor learning (trial 1-trial 10 delta) (K). B6D2F1 n=5; C57BL/6J n=3; DBA/2J n=4; BXD n=4; BXD9 n=4; BXD14 n=4; BXD32 n=9; BXD45 n=4; BXD51 n=6; BXD55

n=8; BXD60 n=5; BXD61 n=5; BXD66 n=4; BXD69 n=4; BXD70 n=4; BXD75 n=11; BXD81 n=4; BXD86 n=3; BXD87 n=4; BXD90 n=4; BXD95 n=3; BXD96 n=6; BXD97 n=3; BXD99 n=4; BXD100 n=4. Data are means $\pm$ SEM.

Figure 2.