A Twin Study of the Genetics of Fear Conditioning

John M. Hettema, MD, PhD; Peter Annas, PhD; Michael C. Neale, PhD; Kenneth S. Kendler, MD; Mats Fredrikson, PhD, Dr Med Sci

Background: Fear conditioning is a traditional model for the acquisition of fears and phobias. Studies of the genetic architecture of fear conditioning may inform gene-finding strategies for anxiety disorders. The objective of this study was to determine the genetic and environmental sources of individual differences in fear conditioning by means of a twin sample.

Methods: Classic fear conditioning data were experimentally obtained from 173 same-sex twin pairs (90 monozygotic and 83 dizygotic). Sequences of evolutionary fear-relevant (snakes and spiders) and fear-irrelevant (circles and triangles) pictorial stimuli served as conditioned stimuli paired with a mild electric shock serving as the unconditioned stimulus. The outcome measure was the electrodermal skin conductance response. We applied structural equation modeling methods to the 3 conditioning phases of habituation, acquisition, and extinction to determine the extent to which genetic and environmental factors underlie individual variation in associative and nonassociative learning.

Results: All components of the fear conditioning process in humans demonstrated moderate heritability, in the range of 35% to 45%. Best-fitting multivariate models suggest that 2 sets of genes may underlie the trait of fear conditioning: one that most strongly affects nonassociative processes of habituation that also is shared with acquisition and extinction, and a second that appears related to associative fear conditioning processes. In addition, these data provide tentative evidence of differences in heritability based on the fear relevance of the stimuli.

Conclusion: Genes represent a significant source of individual variation in the habituation, acquisition, and extinction of fears, and genetic effects specific to fear conditioning are involved.

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between the underlying sources of liability for the 3 phases (habituation, acquisition, and extinction) of the fear conditioning process?

**METHODS**

**SUBJECTS**

Subjects were twin pairs recruited by mailed inquiry from the Swedish Twin Registry after approval from the Swedish Twin Board. Twins who agreed to participate were contacted by telephone and informed of the nature and procedures used in the study as well as potential risks and benefits. The Ethical Committee of Karolinska Hospital, Stockholm, Sweden, approved this study. Strict confidentiality was maintained by stripping any identifying information in a locked safe.

The sample consisted of 90 monozygotic (MZ) and 83 dizygotic (DZ) same-sex twin pairs, including 62 male-male and 111 female-female pairs. The sample size varied slightly between analyses because of exclusion of extreme values (see “Statistical Analysis” section). A narrow age range (23-38 years) was chosen to minimize known age-dependent effects on the skin conductance response. Zygosity determination was based on questions of physical similarity (eg, “During childhood were you and your twin as similar as 2 peas in a pod?”), a method that is proved serologically to be accurate in 96% of cases.10

**EXPERIMENTAL PROCEDURE**

After informed consent was obtained, the subjects were seated in an armchair 2 m from a projection screen. Sequences of either evolutionary fear-relevant (snakes and spiders) or fear-irrelevant (circles and triangles) pictorial stimuli serving as CS were shown to the subjects for 8-second intervals in habituation, acquisition, and extinction phases (Figure 1). Approximately half (54%) of the subjects viewed fear-relevant and the remainder fear-irrelevant stimuli throughout the protocol. For the habituation phase, 2 slides were displayed 2 times each. During the acquisition phase, there were 8 presentations of each slide, where one conditioned stimulus (CS+) but not the other (CS−) was paired with a mild 18-Hz alternating electric current applied to each subject’s right index and middle fingers for a duration of 0.5 second, serving as the unconditioned stimulus (UCS). This was followed by the extinction phase, in which both slides were presented again 8 times each, but without pairing with the UCS.

The outcome measures were electrodermal skin conductance response (SCR) amplitudes recorded at discrete intervals during the presentation sequences. The SCR was measured with a Hagfors-type constant voltage circuit11 using 8-mm-diameter silver-silver chloride electrodes (Beckman-Offner; Siemens-Elema, Stockholm) attached to the subject’s left index and middle fingers with a paste made of isotonic 0.5% sodium chloride and 100 mL of water. The maximum SCR measured in microsiemens starting 1 to 4 seconds after the stimulus onset was recorded.12 This measure has been shown to provide the best internal consistency and stability to assess individual differences in conditioning.13

**STATISTICAL ANALYSIS**

For our analyses, we used the measured SCR from each subject corresponding to the 3 phases over the applicable pairing conditions: habituation, acquisition CS−, acquisition CS+, extinction CS−, and extinction CS+. Several preliminary steps were undertaken to suitably process the data before entering it into the main analyses. First, the outliers in the upper tail of the distribution were 3 SDs greater than the mean were removed, as they were likely experimental artifacts (coughing, etc). Second, as the distribution of each of these variables was

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*Figure 1. Experimental protocol. Each subject viewed either a sequence of fear-irrelevant slides (circles and triangles; top) or fear-relevant slides (spiders and snakes; bottom). For the habituation phase, 2 slides were displayed 2 times each. During the acquisition phase, there were 8 presentations of each slide, where one conditioned stimulus (CS+) but not the other (CS−) was paired with a mild 18-Hz alternating electric current applied to each subject’s right index and middle fingers for a duration of 0.5 second, serving as the unconditioned stimulus (UCS). This was followed by the extinction phase, in which both slides were presented again 8 times each, but without pairing with the UCS.*
rightward skewed, a square-root transformation was applied to produce more normalized measures. Third, the effects of sex on SCR magnitude were regressed out, since they were significant (males> females, P < .001), allowing us to combine data from male and female pairs. We would not have the statistical power in our twin models to detect significant sex differences in variance components. Fourth, for the purpose of this analysis, we averaged the responses over successive stimulus presentations by phase and pairing. For habituation, all 4 responses were combined, since no pairing had yet occurred to distinguish CS− from CS+ stimuli. Finally, a standard procedure in analysis of the SCR is range-correcting each subject’s responses by dividing by the maximal response for that individual, the effects of which we explored via our analyses.

Twin resemblance for the measures was determined by the intraclass correlation separately by zygosity. We performed structural equation modeling in twin pairs to estimate the proportion of variance contributed by genes and environment for each of the measures. Under the basic twin model, individual differences of variance contributed by genes and environment for each of the measures.15

Table 1. Twin Pair Intraclass Correlations by Zygosity for the 3 Phases by Pairing Conditions and Stimulus

<table>
<thead>
<tr>
<th>Measure</th>
<th>Stimulus</th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitation</td>
<td>Fear-relevant</td>
<td>0.47</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Fear-irrelevant</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Acquisition CS+</td>
<td>Fear-relevant</td>
<td>0.54</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Fear-irrelevant</td>
<td>0.28</td>
<td>0.13</td>
</tr>
<tr>
<td>Acquisition CS−</td>
<td>Fear-relevant</td>
<td>0.46</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Fear-irrelevant</td>
<td>0.33</td>
<td>0.13</td>
</tr>
<tr>
<td>Extinction CS+</td>
<td>Fear-relevant</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Fear-irrelevant</td>
<td>0.42</td>
<td>0.33</td>
</tr>
<tr>
<td>Extinction CS−</td>
<td>Fear-relevant</td>
<td>0.26</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Fear-irrelevant</td>
<td>0.39</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Abbreviations: CS, conditioned stimulus; CS+ and CS−, CS was paired with or without, respectively, the unconditioned stimulus; DZ, dizygotic; MZ, monozygotic.

Conditioning Measures

During the habituation phase, habituation was demonstrated by a decremental SCR with stimulus repetition from the first 2 presentations (mean, 0.38 microsiemens) to the last 2 presentations (mean, 0.23 microsiemens) (t(342) = 15.48, P < .001). Fear conditioning was confirmed by significantly higher average response to the reinforced (CS+) than to the nonreinforced (CS−) stimuli (t(342) = 16.66, P < .001). Supporting extinction, response discrimination between the reinforced (CS+) and nonreinforced (CS−) stimuli persisted (t(342) = 12.54, P < .001), but to a lesser extent compared with acquisition. The MZ twins did not differ from DZ twins in average response magnitudes for any of the experimental measures. In a separate sample, temporal stability in 28 subjects and internal consistencies in 223 subjects had been investigated.13 For the 5 measures of habituation, acquisition CS−, acquisition CS+, extinction CS−, and extinction CS+, the measured internal consistencies were 0.93, 0.91, 0.96, 0.90, and 0.93 and 1-month test-retest reliabilities were 0.72, 0.57, 0.85, 0.37, and 0.62, respectively.

Pair Similarity and Model Fitting

Table 1 shows the twin pair intraclass correlation coefficients for the 5 measures separately by zygosity. In all cases, the intraclass correlation coefficient was significantly greater in MZ than DZ twins, indicating a sub-
stantial genetic component. Furthermore, the intraclass correlation coefficient for MZ pairs was at least twice that for DZ pairs for all of the fear-relevant and some of the fear-irrelevant stimuli.

**Table 2** provides the results of univariate modeling of the 5 measures. In each case, the full ACE model (model I) provided an adequate fit of the data but did not, for several of the measures, provide significant parameter estimates for \( a^2 \) or \( c^2 \) because of lack of power.\(^8\) The fit of the AE submodels (model II) was nearly identical to that of the full models by \( \chi^2 \) difference tests and provided improved fit by the Akaike information criterion statistic. In addition, the point estimates for \( c^2 \) were zero for all of the measures except 2. Consequently, CE models (model III) fit slightly worse by these criteria. E-only models (model IV) that did not include sources of twin similarity provided significantly worse fit to the data, supporting familial resemblance. Model Ila was constructed by starting with the best-fitting submodel (model II) and constraining the parameter estimates for the fear-relevant and fear-irrelevant stimuli groups to be equal, testing for significant differences between them. Although the full models suggested a trend for the heritability (\( a^2 \)) of fear-relevant stimuli to differ from that of fear-irrelevant stimuli, only for acquisition CS− did the best-fit model support this with 2-tailed tests at the \( P = .05 \) level of significance.

**Table 3** provides results for the multivariate modeling of the 5 measures. Again, the models provide the most parsimonious fit when only additive genetic and individual-specific environmental sources of variance are included. Constraining the parameter estimates to be equal between the fear-relevant and fear-irrelevant groups (model Ila) produced a near-significant degradation in fit (\( \Delta \chi^2 = 36.51, P = .08 \)). This result suggests that there may be quantitative differences in the genetic effects on fear conditioning by stimuli type (see the “Comment” section). Model IIB differed from model Ila in that the third genetic factor (A₃) was removed by constraining it to zero. We lacked the statistical power to discriminate between these 2 models (\( \Delta \chi^2 = 1.74, P = .42 \)), although model IIB possessed a lower Akaike information criterion, making it best fitting by that criterion. We could definitively reject model IIC, which included only one genetic source of variance (A₁), by \( \chi^2 \) difference tests (\( P < .001 \)). We present the model-fitting results for models Ila and IIB in **Figure 2** and **Figure 3**, respectively.

In summary, our 3-factor model collapsed into a best-fit model containing 2 additive genetic and no familial environmental sources of variance, with the remainder of the variance explained by individual-specific environment. The distribution of the total genetic variance across these 2 factors is calculated in **Table 4** for the best-fit...
The goal of this study was to investigate the potential genetic and environmental sources of individual differences underlying human fear conditioning by means of the twin method. We used the skin conductance response as the indicator variable for fear conditioning in a differential conditioning paradigm. Standard univariate and multivariate structural equation modeling methods were used to analyze the data. We also examined the impact of the fear relevance of the stimuli on our results.

As indicated in Table 1, there was substantial twin similarity for all of the MZ and at least some of the DZ measures, supporting the familial aggregation of fear conditioning. Univariate structural equation modeling of the 5 phase-pairing conditions confirmed this, since E-only models could be rejected for lack of fit to the data. In addition, higher correlations in MZ compared with DZ twin pairs suggested substantial genetic influences, with additive genetic effects accounting for between 34% and 43% of the total variance in our best-fit models. Thus, fear conditioning is a moderately heritable trait in humans. In fact, these values are likely underestimates of the heritability due to confounding of occasion-specific measurement error with the effects of individual-specific environment, reducing the estimate of the additive genetic variance. This may be a substantial effect, given that the 1-month test-retest reliability for by Genetic Factors in the Best-Fit Model

<table>
<thead>
<tr>
<th>Measure</th>
<th>Total Genetic Variance</th>
<th>Proportion of Genetic Variance Accounted for by Factor A1</th>
<th>Proportion of Genetic Variance Accounted for by Factor A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habituation CS+</td>
<td>0.34</td>
<td>1.00</td>
<td>NA</td>
</tr>
<tr>
<td>Acquisition CS−</td>
<td>0.40</td>
<td>0.34</td>
<td>0.66</td>
</tr>
<tr>
<td>Acquisition CS+</td>
<td>0.41</td>
<td>0.69</td>
<td>0.31</td>
</tr>
<tr>
<td>Extinction CS−</td>
<td>0.36</td>
<td>0.24</td>
<td>0.76</td>
</tr>
<tr>
<td>Extinction CS+</td>
<td>0.33</td>
<td>0.25</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Abbreviations: CS, conditioned stimulus; CS+ and CS−, CS was paired with or without, respectively, the unconditioned stimulus; NA, not applicable.
ties ranged from 0.37 to 0.85 depending on the variable. A crude correction for measurement error may be made by including the measured reliability in the calculation of the total variance. For example, the estimated heritability for acquisition CS+, which had the highest reliability (0.85), increases from 0.42 to 0.49. In future analyses, we plan to examine this more quantitatively by modeling the effects of measurement error in the repeated presentation sequences rather than by taking their average.

The finding that experimental measures of fear conditioning areheritable and possess heritability estimates that are in the same range as those found in twin studies of anxiety disorders has potentially important implications. There have been suggestions that clinical phenotypes measured by self-report may not reflect the underlying pathophysiological processes involved in the etiology of psychiatric disorders and, therefore, may not provide optimum targets for gene-finding strategies. One approach to overcome this limitation is to investigate potential endophenotypes that more robustly reflect processes more proximal to gene expression and are involved in the development of pathologic anxiety states. One putative endophenotype already being explored is the increased sensitivity to panicogenic carbon dioxide inhalation in patients with panic disorder.

Fear conditioning paradigms, if substantially correlated with clinical measures of anxiety, provide another potential endophenotype. We plan to examine the sources of shared liability between the experimentally derived measures of fear conditioning and self-report anxiety measures in this sample.

Our data suggest that the heritability of fear conditioning with the use of evolutionary fear-relevant stimuli, such as snakes and spiders, may differ from that with neutral stimuli, such as circles and triangles. Although we did not have the statistical power to confirm this unequivocally, one of the univariate models does support this, and our multivariate model is close to significance (2-tailed \( P = .08 \)). Such a conclusion, although tentative, is consistent with the concept of prepotency in which a species is biologically “primed” to automatically and selectively attend to evolutionary fear-relevant stimuli for survival purposes, making this category of stimuli highly conditionable and less easily extinguished.

The finding of MZ twin pair intraclass correlations that were more than twice those of the DZ pairs, particularly for fear-relevant stimuli, suggested the possibility of dominant genetic influences, but our sample did not possess the statistical power to substantiate this. 

Modeling attempts reflect this: ADE models provided a reasonable fit to the data without statistical power to reject the simpler AE model. Nonadditive genetic effects have been detected in animal studies of classic conditioning. 

Nonadditive genetic effects have been detected in animal studies of classic conditioning. If Fisher's arguments about the evolution of dominance are correct, traits under natural selection should have relatively low broad heritability of which much of the variance is due to nonadditive (dominance and epistatic) factors. This type of genetic architecture is expected to evolve regardless of the direction of selection, although bidirectional or “stabilizing” selection would be required to maintain genetic variation in the population and would lead to relatively less dominance variation. We might expect fear levels to be under stabilizing selection, since fear levels that are either too high or too low would interfere with the effective functioning (and ultimately reproductive fitness) of the animal.

Results of multivariate modeling were limited by lack of power to discriminate between 2- and 3-factor models for underlying genetic sources of variance. Note that our choice of a Cholesky multivariate structure made intuitive sense for the conditioning protocol, but is only one of several potential model types. Our best-fit models produced results suggesting that 2 sources of genetic variance make significant contributions to the conditioning process: the first most strongly affects nonassociative processes involved in habituation, but also extends to acquisition and extinction, while the second appears related to associative aspects of the fear conditioning process. This finding of a genetic source of variance specific to fear conditioning is reflected in a recent candidate gene association study in a selected sample of these subjects, in which the cases and controls were dichotomized into good and poor conditioners, indexed by the difference between CS+ and CS− measures. The results of that study suggest a role for the serotonin transporter gene promoter and possibly dopamine type-4 receptor genes and platelet monoamine oxidase activity in human fear conditioning.

Our results are consistent with 2 previous twin studies of human habituation and motor conditioning. Lykken and colleagues studied the SCR during habituation in 121 twin pairs with the use of loud tones. They reported estimates of additive genetic variance in SCR of around 50% to 60% depending on whether range correction was performed (their Table 2), with some suggestion of nonadditivity. Merrill and colleagues examined conditioning of the eye-blink response in 47 sibling pairs that included 44 twin pairs, finding significantly higher MZ than DZ correlations.

Analyses using the raw SCR before range correction and square-root transformation did not significantly change our results. Interestingly, analyses of both the baseline SCR (prestimulus) and maximal SCR (which usually occurred in response to the unconditioned electrical stimulus) produced best-fit models containing only common familial (C) and individual-specific (E) environmental sources of variance, although we did not have sufficient statistical power to differentiate this model from one that included only additive genetic effects (A) instead. We hypothesize that this may represent a source of systematic error caused by a temporal correlation in measurement, as both members of the same twin pair underwent the conditioning protocol on the same day. This could potentially inflate twin pair correlations independent of zygosity, resembling a source of common environmental variance. This may well have produced the greater resemblance in intraclass correlations for MZ and DZ pairs seen in Table 1 for extinction-phase fear-irrelevant stimuli, as these SCRs were of the smallest magnitude and therefore closer to the SCR baseline. Thus, there is no evidence of a genetic component of baseline or maximal SCR that biases our results toward a genetic etiology-based model. In fact, it may have reduced our power to detect additive genetic effects in favor of those
caused by common environment, as was seen in modeling the extinction-phase responses, for which the full ACE model produced nonzero point estimates of c².

One might speculate as to whether these findings reflect the genetics of conditioning or anxiety in general. These are likely to be confounded for several reasons. First, as discussed above, there may be a substantial genetic correlation between fear conditioning and anxiety. Second, some of the literature suggests that an individual’s response to the conditioning protocol may depend on personality characteristics related to anxiety. There is a genetic component to habituation, and patients with anxiety disorders tend to show retarded habituation, particularly to anxiogenic but also to neutral stimuli, and slow habituation correlates with increased conditionability. Also, while some studies indicate that highly anxious individuals condition better, other studies do not support this.

The results of this study are subject to several potential limitations. First, the sample size is relatively small compared with recent epidemiologic twin studies because of the immense effort and expense required for this experimental protocol, limiting the power to significantly distinguish potential sources of variance. Second, the maximum likelihood method used by Mx in the twin models assumes normally distributed variables, which we were able only to approximate via transformation procedures. Third, the validity of interpreting increased similarity of MZ over DZ twins as representing genetic effects is predicated on the equal environment assumption, which states that MZ and DZ twins are equally correlated for environmental experiences of relevance to the trait under study. If this is not the case, higher MZ similarity could potentially result from increased MZ environmental similarity instead of higher genetic similarity. Although there have been several twin studies designed to detect such violations, with negative findings, we do not have any explicit means of establishing the assumption’s validity in this sample. Fourth, if liability to participate in the study is related to the conditioning variables studied, this could bias results toward a lower estimate of familial resemblance. Finally, the sample was limited to white subjects, and as such, the results may not be generalizable to other ethnic groups.

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Corresponding author and reprints: John M. Hettema, MD, PhD, Virginia Institute for Psychiatric and Behavioral Genetics, PO Box 980126, Richmond, VA 23298 (e-mail: jhettema@hsc.vcu.edu).

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