International Journal of Psychophysiology 88 (2013) 55–63

Contents lists available at SciVerse ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

Clarifying the nature of startle habituation using latent curve modeling

Stephanie T. Lane *, Joseph C. Franklin, Patrick J. Curran

University of North Carolina at Chapel Hill, United States

ARTICLE INFO

Article history:
Received 17 October 2012
Received in revised form 17 January 2013
Accepted 21 January 2013
Available online 27 January 2013

Keywords:
Startle
Habituation
Latent curve modeling
Gender

ABSTRACT

Startle habituation is present in all startle studies, whether as a dependent variable, discarded habituation block, or ignored nuisance. However, there is still much that remains unknown about startle habituation, including the following: (1) what is the nature of the startle habituation curve?; (2) at what point does startle habituation approach an asymptote?; and (3) are there gender differences in startle habituation? The present study investigated these three questions in a sample of 94 undergraduates using both traditional means-based statistical methods and latent curve modeling. Results provided new information about the nature of the startle habituation curve, indicated that the optimal number of habituation trials with a 100 dB startle stimulus is 13, and showed that females display greater startle reactivity but habituate toward the same level as males.

1. Introduction

The acoustic startle eyeblink response is a defensive reflex that occurs in reaction to an intense and sudden stimulus (Blumenthal et al., 2005; Koch, 1999; Landis and Hunt, 1939; Yeomans et al., 2002). Thousands of studies have used startle modulation to investigate both basic and applied processes. For example, affective-valence modulation paradigms involve measuring startle reactivity in the context of an emotionally evocative foreground (e.g., a picture of food or a spider). This paradigm has provided unique insight into emotional abnormalities in internalizing, externalizing, psychotic, and autism spectrum disorders (Dichter et al., 2010; Patrick et al., 1993; Vaidyanathan et al., 2009a, 2009b; Yee et al., 2010). Similarly, prepulse inhibition of startle has been employed extensively to investigate information processing in a range of disorders (Braff et al., 1992; Swerdlow et al., 2008).

1.1. Group differences in startle habituation

Several studies have employed a third form of startle modulation, startle habituation. In learning and memory research, habituation has been defined as a "behavioral response decrement that results from repeated stimulation and that does not involve sensory adaptation/sensory fatigue or motor fatigue" (p. 136, Rankin et al., 2009; see also Thompson, 2009). Additionally, Thompson and colleagues described several specific characteristics that connote habituation (Rankin et al., 2009; Thompson, 2009). Despite decades of startle habituation research, we note that few of these habituation characteristics have been specifically examined in startle research and few studies have controlled for the potential contributions of sensory and motor fatigue to startle reactivity decrements across stimulus presentations. Nevertheless, startle habituation traditionally has been conceptualized as an index of sensorimotor gating in psychiatric populations (see Braff and Geyer, 1990). For example, studies have found diminished habituation in association with schizophrenia (Braff et al., 1992; Ludewig et al., 2003; Meincke et al., 2004), schizotypal personality disorder (Cadenhead et al., 1993), bipolar depressive disorder (Perry et al., 2001), posttraumatic stress disorder (Kozaric-Kovacic et al., 2011), panic disorder (Ludewig et al., 2005), and children with a parental history of alcoholism (Grillon et al., 1997). On the other end of the spectrum, LaRowe et al. (2006) found that faster habituation was associated with extraversion. In contrast to these studies, several investigations have failed to find diminished habituation in association with schizophrenia (Hasenkamp et al., 2010; Perry et al., 2002), attention-deficit hyperactivity disorder (Ornitz et al., 1997), suicidality and major depressive disorder (Quednow et al., 2006), bipolar disorder (Rich et al., 2005), or anxiety disorders (Ross et al., 1989; Hoenig et al., 2005).

One potential explanation for these discrepancies is the limitations of the methods used to quantify startle habituation. Many of these studies measured small blocks of startle-alone trials (i.e., between two and six trials) before and after large blocks of prepulse inhibition trials (i.e., 18 to 36 trials). Other studies measured startle-alone trials that were embedded in prepulse inhibition blocks (e.g., Cadenhead et al., 1993; Rich et al., 2005). A minority of studies examined only startle-alone reactivity over the course of several trials (LaRowe et al., 2006; Ornitz et al., 1996, 1997; Ross et al., 1989; Schicatano and Blumenthal, 1995, 1998). Regardless of the habituation method, most studies condensed several individual startle trials into blocks and a mean was calculated for each block. T-tests or analyses of variance (ANOVAs) were then performed to determine if

---

* Corresponding author at: CB #3270, Psychology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3270, United States. Tel.: +1 704 657 7128; fax: +1 919 962 2537.

E-mail address: slane@unc.edu (S.T. Lane).

0167-8760/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpsycho.2013.01.010

startle reactivity was significantly lower in later blocks compared to earlier blocks. If there was a significant difference, then startle habituation was said to have occurred.

These means-based analyses have three major limitations that make it difficult to describe or find significant differences in startle habituation. First, these methods often artificially condense startle habituation (e.g., the mean of the first six trials is compared to the mean of the second six trials). Important changes may happen during startle habituation from trial to trial; condensing several trials into a single block accordingly discards potentially important information (cf. MacCallum et al., 2002). Groups may differ on startle habituation in important but subtle ways that condensation into means may mask.

Second, a closely related limitation is that traditional methods for evaluating mean differences are not well-equipped to describe startle habituation in detail. Specifically, these methods may be able to detect that habituation has occurred, but they are less able to describe specific rates of habituation or how rates of habituation change across trials or individuals. The minority of studies that employ long blocks of startle habituation trials (e.g., Ornitz et al., 1996; Ross et al., 1989; Schicatano and Blumenthal, 1998) may be able to overcome this limitation with post hoc tests that can describe patterns of data. However, these contrasts often have restrictive assumptions (Maxwell and Delaney, 2004) that startle habituation data may not always meet. Moreover, although such contrasts can detect if a specific pattern exists (e.g., linear, quadratic), they are less able to describe the specific nature of that contrast (e.g., what type of quadratic pattern). For example, it could be possible that individuals high and low in trait fear would both show a quadratic startle habituation pattern; however, low fear individuals might display a substantial drop in startle reactivity across the first few trials, whereas high fear individuals might display a much more gradual (though still quadratic) pattern. An ANOVA would describe these patterns as essentially the same (i.e., significant habituation with a quadratic slope), but latent curve modeling (LCM) would be more likely to detect the important differences between these two groups. Thus, a more detailed account of startle habituation with a more appropriate statistical technique would provide more insight into the nature of group differences in startle habituation.

Third, means-based techniques that artificially group startle trials into blocks may confound differences in startle habituation (i.e., slope) with differences in initial startle reactivity (i.e., intercept). Individuals who display higher initial startle reactivity may have farther to fall to reach a startle reactivity asymptote. This would result in a steeper startle habituation curve that is an artifact of initial startle reactivity. More fine-grained statistical methods may be able to more effectively model this slope/intercept relationship and to avoid this potential confound.

1.2. Latent curve modeling

To address the limitations of means-based analyses, we will utilize a more recently developed method of analysis known as latent curve modeling. This is an advanced structural equation modeling approach based on the assumption that there is an underlying (i.e., latent) trajectory for a variable and that repeated measurements allow for the estimation of that trajectory (Bollen and Curran, 2006; Meredith and Tisak, 1984, 1990). For startle habituation data, this technique would establish a latent habituation trajectory that allows for a continuous model of startle habituation. Latent curve modeling would also provide statistical information on the nature of habituation (e.g., rate of change throughout the trajectory) and how exogenous variables affect habituation (e.g., gender). In sum, latent curve modeling overcomes the limitations of means-based techniques noted above. Moreover, latent curve modeling has additional advantages over means-based methods, including the following: (a) allowing continuous exogenous variables; (b) separating intercept and various slope factors; (c) permitting the prediction of the habituation trajectory with exogenous variables; and (d) including participants who are missing data on one or more habituation trials.

1.3. Startle habituation blocks

The majority of startle studies present a startle habituation block designed to have participants approach a startle reactivity asymptote before the experimental portion of the study (e.g., affective-valence startle modulation or prepulse inhibition blocks). The major goal of these blocks is to reduce variance in the data caused by startle habituation rather than the independent variables (cf. Blumenthal, 1997). There is a wide range in the number of trials in startle habituation blocks across studies. Some studies do not report a habituation block, but others have reported habituation blocks consisting of one (Braff et al., 1992; Cadenhead et al., 1993; Hoenig et al., 2005; Grillon et al., 1997), three (e.g., Csomor et al., 2006, 2008; Franklin et al., 2009a, 2010), four (e.g., Glenn et al., 2011), six (e.g., Schachinger et al., 2008), nine (e.g., Grillon and Charney, 2011), or ten (e.g., Grillon and Morgan, 1999) startle stimuli. The wide range of startle habituation block trials demonstrates a lack of empirically-based number of trials for habituation blocks. It is possible that one trial is sufficient to reach an asymptote, but it is also possible that a block of ten trials is insufficient to reach an asymptote. In the present study we will examine this question with a variety of statistical techniques. Our specific focus will be demonstrating the strengths of the latent curve model, as we believe the latent curve model better corresponds the process of habituation.

1.4. The present study

We pursued three major goals in the present study. The first was to utilize latent curve modeling to provide new information about the nature of startle habituation. We aimed to address basic questions such as: How does initial startle reactivity (i.e., intercept) covary with the trajectory (i.e., slope) of startle habituation? Is there a substantial amount of individual variability in startle habituation or does everyone follow a very similar pattern? What exactly does startle habituation look like from trial to trial? The second goal was to employ both means-based and latent curve modeling techniques to establish an empirical basis for the number of trials that should be used to have most individuals reach a startle reactivity asymptote.

Our final goal was to examine potential gender differences in startle habituation. There is some evidence that females display higher startle reactivity compared to males (e.g., Blumenthal and Gescheider, 1987; Della Casa et al., 1998; Kofler et al., 2001 [though note that this study did not find differences for reactivity measured from the orbicularis oculi]); however, many studies have not found gender differences for startle reactivity or startle habituation (e.g., Ludewig et al., 2003; Quednow et al., 2006; Swerdlow et al., 1993). It is possible that there are no gender differences in startle reactivity, but it is also possible that these differences have been too subtle for traditional methods to detect. Overall, the present study has the potential to provide new insights into a phenomenon present in all startle studies — startle habituation.

2. Method

2.1. Participants

The participants were 96 undergraduate introductory psychology students who participated in order to partially fulfill a class research requirement. Data from two participants were discarded because their average startle magnitude level was more than three standard deviations above the mean. The final sample size was 94, with 51 males and 43 females. The age range was 18–21 years, with a mean of 18.74 years. Ethnically, 69.6% of the participants self-identified as

Caucasian, 17.4% as African-American, 5.4% as Latino, 1.1% as Asian, and 6.5% as Other.

2.2. Procedure

All procedures were approved by the institutional review board of the University of North Carolina at Chapel Hill. The participants were seated alone in a sound-attenuated room. The participants signed consent forms and filled out a questionnaire assessing demographics, hearing difficulties, and medication status (see below). Following recommended procedures for startle eyeblink (see Blumenthal et al., 2005), the participants were then prepared for psychophysiological data collection. The area of skin underneath the left eye and the left temple was cleaned with an alcohol swab. Two electromagnetically-shielded In Vivo Metric (Ag/AgCl, 11 mm outer diameter, 4 mm inner diameter contact surface) surface recording electrodes were affixed to the skin overlaying the orbicularis oculi muscle. The first electrode was placed in line with the participant's pupil, below the eyelid; the second electrode was placed approximately 15 mm to the right of the first, and slightly above. The ground electrode was placed on the left temple. The participants then had Sennheiser PX200 headphones placed over their ears.

2.2.1. Stimulus presentation

Similar to many prepulse inhibition studies (which account for the majority of startle habituation studies, see above), the participants were presented with background noise for three minutes in the absence of other stimuli and this noise remained on throughout the session (cf. Blumenthal et al., 2006). The participants were then administered 21 startle stimuli. Intertrial intervals varied randomly between 13 s and 25 s.

2.2.2. Stimuli

Background noise was 70 dB SPL(A) broadband (20 Hz–20 kHz) noise. Startle stimuli were 50 ms 100 dB SPL(A) broadband noises with a rise/fall time of <1 ms. Stimulus intensities were calibrated with steady-state signals presented through headphones and measured with a sound pressure level meter. All of the stimuli were generated in Adobe Audition 3.0 and presented with SuperLab Pro 4.0.

2.2.3. Data collection and scoring

The raw EMG signals were measured by the surface recording electrodes. These signals were amplified with a Biopac EMG amplifier and sampled at 1000 Hz with a Biopac MP150 workstation, filtered online with a passband of 28–500 Hz, rectified, and then smoothed with a five-sample boxcar filter. Data analyses were based on the smoothed EMG data. Trials on which no response was found were assigned a value of zero. Trials with intrusive spontaneous blinks (2.68%) were omitted from analysis.

2.3. Data analytic plan

2.3.1. Startle eyeblink magnitude

Startle eyeblink magnitude was quantified by the difference between the peak and the onset voltage of the smoothed EMG within a window of 20–150 ms after the onset of the stimulus. Consistent with the scoring methods of the lab of Dr. Terry Blumenthal, response onset was determined by trained researchers. Startle magnitude was calculated for each trial for each participant. Across the participants, magnitude was averaged for each trial, resulting in 21 conditions.

2.3.2. Means-based analyses

To examine how startle reactivity looks across 21 trials we plotted the means and standard errors of all 21 trials. We then investigated the point at which startle habituation reaches an asymptote by testing whether a given trial is significantly different from all following trials (e.g., trial 13 compared individually to trials 14 through 21). The asymptotic point was quantified as the point at which a given trial is no longer significantly different from any later trials. We then employed a within-participants analysis of variance (ANOVA) to detect and provide information about startle habituation. Because this analysis was specifically designed to examine habituation, it only included trials during which significant habituation occurred (as determined by the prior asymptote analysis). Consistent with recommendations (Jennings, 1987), we employed Greenhouse–Geisser degrees of freedom in order to diminish the effect of any violations of sphericity and to reduce the chance of Type I error.

2.3.3. Latent curve modeling

All latent curve analyses were conducted with Mplus version 6.1. First, we employed a freed loading latent curve model to examine the degree of total change across the 21 trials. With this analysis, we employed an alternative method of determining the point at which startle habituation approaches an asymptote. Second, we fit increasingly complex latent curve models to startle habituation data until we arrived at the model that best reproduced the underlying growth trajectory of habituation. Increasingly complex models add additional factors to the model. For example, a linear model includes factors for intercept and linear slope whereas a quadratic model includes factors for intercept, linear slope, and quadratic slope. Similar to the means-based analyses, these analyses only included trials through which startle habituation occurs (i.e., until habituation approaches asymptote). Based on a visual inspection of startle mean patterns across trials, we began by fitting a linear model and then progressed to a quadratic model. From these analyses, we examined the association between intercept and slope, and examined individual variability in intercept and slope. These latter analyses informed whether the model would benefit from including exogenous variables, such as gender.

It should also be noted that, in contrast to means-based analyses, latent curve models do not have to drop an entire participant if they are missing data on one or more trials. This is because these models use data from each trial to estimate the latent trajectory of habituation and this process does not require data from all trials (though more data points permit a more effective estimation). Provided that the missing data are random, direct maximum likelihood techniques are automatically employed to minimize the difference between the observed and the reproduced covariance structures. In order to evaluate the model fit, we employ two standard indices of fit from the literature of structural equation models. The first is the comparative fit index (CFI), which compares the present model to the baseline model where all variances are free parameters and all covariances are zero (Bentler and Bonett, 1980). For this index, fit levels above .90 are considered acceptable (Hu and Bentler, 1995). The second is the root mean square error of approximation (RMSEA), which is an absolute fit index that measures discrepancy per degrees of freedom (Steiger, 1990). For this index, fit levels below .10 are considered acceptable (Browne and Cudeck, 1993).

2.3.4. Gender

We investigated the effect of gender on startle habituation with both means-based and latent curve methods. First, we conducted a mixed ANOVA (gender × time) to test for interaction and main effects of gender on startle habituation. Pending significant effects, post hoc tests explored the nature of the effects. Second, we regressed the growth factors on gender in the best-fitting latent curve model of startle habituation. This allowed for a test of the effects of gender on startle habituation intercept and slope. Unlike means-based analyses, latent curve modeling analyzed intercept and slope effects independently.

3. Results

3.1. Means-based analyses

3.1.1. Plot of startle habituation

As shown in Fig. 1, startle reactivity increased from trial 1 to trial 2 (cf. Swerdlow and Talledo, 2009). Thereafter, reactivity decreased precipitously for about 10 trials and then appeared to level off.

3.1.2. Asymptote detection

A within-participants ANOVA across the 21 trials revealed that trial 13 was the last trial that demonstrated a significant difference from the previous trial, $F(1, 63) = 4.86$, $p = .03$. Further analyses across trials 13–21 showed that no trial after 13 was significantly different from the magnitude value of trial 13. Accordingly, these analyses indicated that startle habituation only occurred from trials 2 to 13 (note: startle reactivity increased from trials 1 to 2). Consequently, habituation analyses below were only conducted on trials 2 to 13.

3.1.3. Account of habituation

A within-participants ANOVA across trials 2 to 13 revealed a significant effect of trial on startle reactivity, $F(7.94, 587.839) = 12.94$, $p < .001$. A follow-up contrast effect indicated that habituation fits a quadratic pattern, $F(1, 74) = 11.92$, $p = .001$. It should be noted that this analysis dropped 19 participants due to the ANOVA requirement that all participants have data for each trial to be included in the analyses.

3.1.4. Effect of gender

A mixed ANOVA (trial × gender) revealed a significant interaction effect of trial and gender on startle reactivity, $F(8.12, 576.40) = 2.06$, $p = .04$. Females start with higher startle reactivity than males, but the groups begin to converge after several trials. After employing the Benjamini–Hochberg correction for multiple comparisons (Benjamini and Hochberg, 1995), there were no gender differences following the significant difference observed at trial 7, $t(90.305) = 3.12$, $p = .02$. Importantly, the between-subjects effect of gender was not significant, $p = .20$. Similar to the above analyses, this analysis also dropped 19 participants.

3.2. Latent curve modeling

3.2.1. Asymptote detection

A freed loading latent curve model indicated that 0% of total habituation accrued by the second trial, 6.1% by the third trial, 61.7% by the ninth trial, and 87.5% by the thirteenth trial. That is, of the total change that occurred during the process of habituation, 87.5% of the total change had occurred by the 13th trial. Thereafter, additional trials provided only minor increases in habituation (see Table 1). This result is an additional clarification on the previous finding that no trial after 13 was significantly different from 13. Similar to means-based analyses, this model suggested that 13 trials may be the optimal length of a startle habituation block.

3.2.2. Account of habituation — linear model

The linear model fit the data poorly, $\chi^2(73) = 148.62$, $p < .001$, CFI = .92, RMSEA = .11. The CFI value indicates moderate model fit (Hu and Bentler, 1995), and the RMSEA indicates poor fit (Browne and Cudeck, 1993). This model revealed a significant fixed effect of both the intercept and slope factors, indicating that the starting point and rate of linear change over time are both significantly different from zero. In other words, this model quantifies that startle reactivity began above zero and that habituation did occur across trials. The fixed effect of the slope indicated that startle reactivity decreased by .23 mV each trial. This model also indicated significant individual variability in the intercept and slope factors, meaning that individuals varied in both the starting point of their trajectory of habituation and the rate of change over time. Additionally, unlike the means-based analyses, this model did not drop any participants from the analyses.

3.2.3. Account of habituation — quadratic model

The inclusion of the quadratic growth factor significantly improved model fit (see Fig. 2). Specifically, the likelihood ratio test indicated a significant decrement in χ^2 moving from the linear to the quadratic model, $\Delta\chi^2(1) = 12.69$, $p < .001$. The indices of model fit also improved moving to the quadratic model, $\chi^2(72) = 135.94$, $p < .001$, CFI = .94, RMSEA = .097. The CFI improved, and the RMSEA now indicates moderate model fit. In addition to the significant fixed effect of the intercept and linear slope, this model revealed a significant fixed effect of the

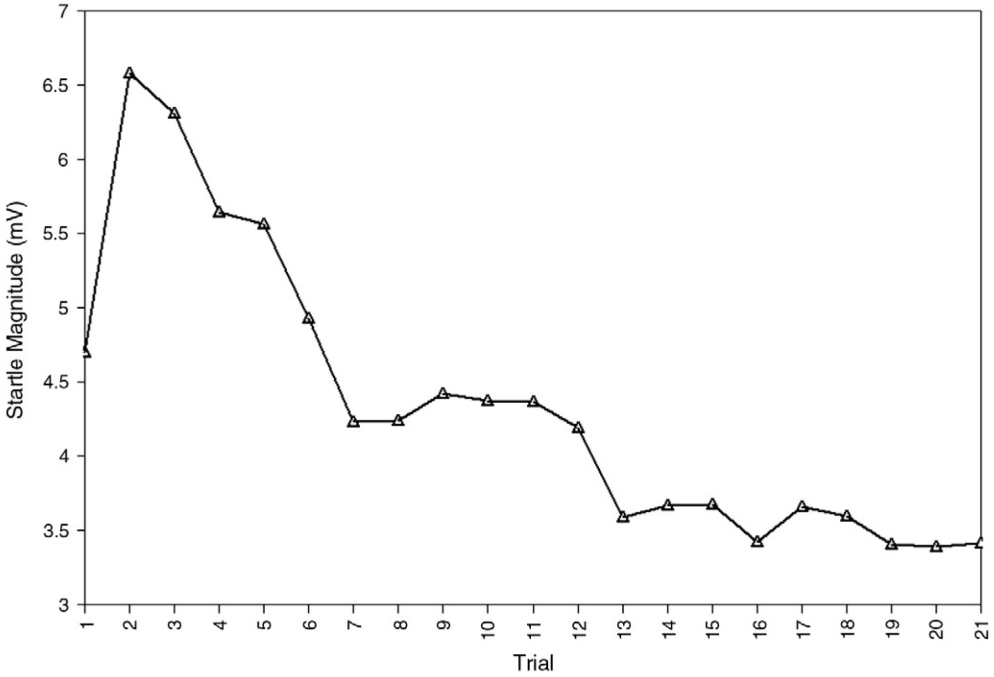

Fig. 1. Startle magnitude across 21 trials.

Table 1
Proportion of total change for each trial.

Trial	Proportion of change	*p* value
2	0	–
3	.061	.520
4	.268	.004
5	.292	.001
6	.505	<.001
7	.640	<.001
8	.675	<.001
9	.617	<.001
10	.567	<.001
11	.691	<.001
12	.783	<.001
13	.875	<.001
14	.848	<.001
15	.921	<.001
16	.963	<.001
17	.934	<.001
18	.968	<.001
19	1	<.001
20	1	<.001
21	1	<.001

quadratic factor, which explained the trajectory curvature. More specifically, the inclusion of the quadratic factor allows us to more precisely quantify the nature of change over time present in habituation. That is, the initial slope value is −.48, indicating that the slope of the tangent line to the curve at time = 0 is negative, or the curve is decreasing.

The inclusion of the quadratic factor allows us to assess the change in this rate of change over time. Therefore, the positive quadratic value of .02 tells us that the slope is less steep at each successive time point; in other words, the curve is beginning to flatten. Thus, where the linear model provides a picture of change as monotonically decreasing, the quadratic model clarifies this by providing information regarding how the change slows over time. The level of curvature did not vary significantly across individuals, though significant individual variability was still observed in the intercept and linear slope factors (see Fig. 3). This indicates that the variance in the quadratic trajectory across participants does not deviate sufficiently to provide room for exogenous variables (e.g., predictor variables, group variables) to account for variance in the quadratic factor underlying the trajectory. The R^2 values indicate that the quadratic model provides a good fit to the data, with the underlying growth factors explaining 60% to 74% of the variability in the trajectory of startle habituation. In contrast to means-based analyses, this analysis did not drop any participants from the analyses. We do not pursue a cubic model because, as a symmetric polynomial form, it would not be well-suited for capturing the dramatic initial change and the slower monotonic decrease present in the full trial-by-trial course of habituation.

3.2.4. Intercept and slope association

There was a strong negative association between intercept and slope, $r = -.70$, $p < .001$, with higher intercepts being associated with steeper slopes (see Fig. 4). In other words, higher initial startle reactivity was associated with a more dramatic habituation curve.

3.2.5. Effect of gender

As shown in Fig. 5, analyses revealed a significant effect of gender on the intercept, with females displaying a higher intercept ($z = -2.20$, $p = .028$). The linear slope factor demonstrated a significant amount of individual variability, but the effect of gender on the slope factor only trended toward significance ($z = 1.79$, $p = .07$). Given the lack of variability in the quadratic slope factor, we could not examine whether gender had a significant effect on the level of curvature in the trajectory of habituation.

4. Discussion

Startle habituation is a phenomenon present in all startle modulation studies. Some studies examine it as a dependent variable, others use startle habituation blocks in an attempt to prevent it from adding noise to prepulse inhibition or affective valence startle modulation blocks, and many others ignore it. The present study provides new information about startle habituation that has the potential to advance the study of it as a dependent variable and to establish empirical guidelines for the length of habituation blocks. Specifically, the present results advance knowledge about the nature of the startle habituation curve, suggest that 13 trials are the optimal length of startle habituation blocks, and clarify that females display greater initial startle reactivity but habituate

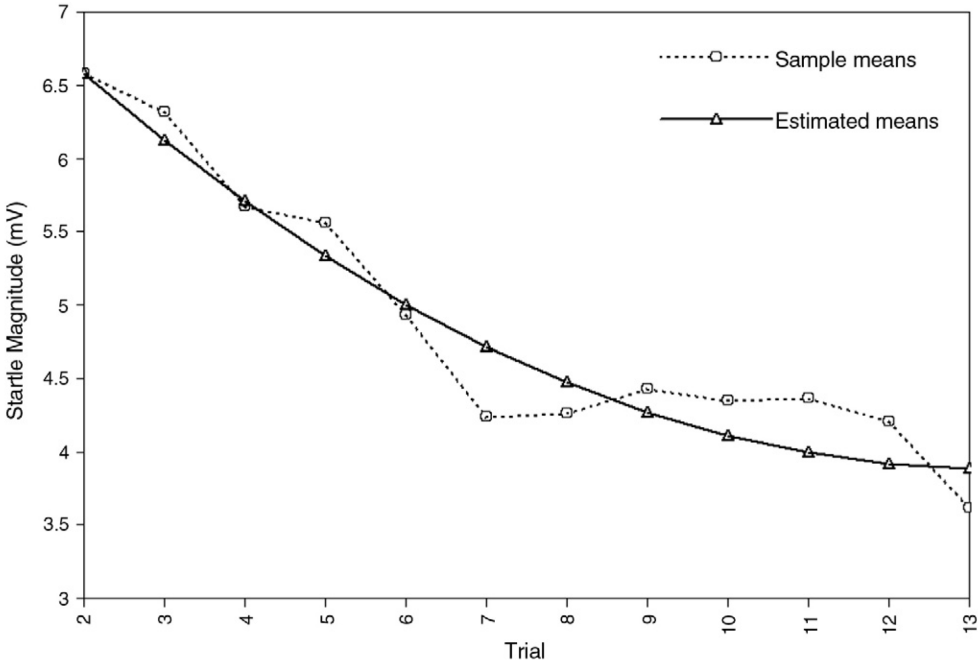

Fig. 2. Sample and estimated means across trials 2–13.

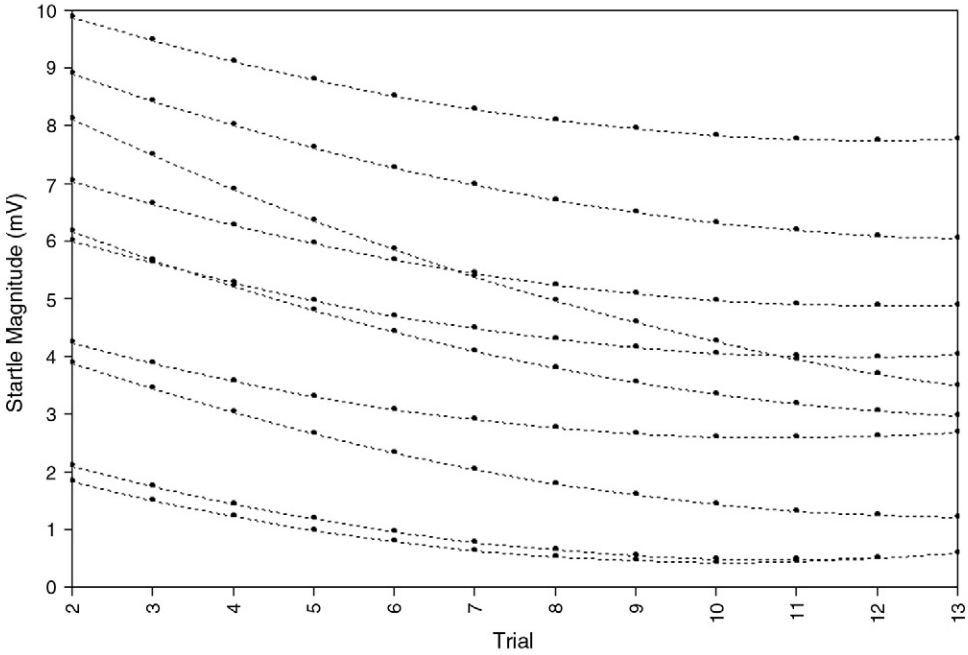

Fig. 3. Estimated individual curves for 10 random individuals.

similarly to males. In general, compared to means-based methods, latent curve models provided more flexible analyses, revealed finer-grained information about habituation, and retained all participants in each analysis. Findings are discussed in greater detail below.

Fig. 1 provides a view of startle habituation across 21 trials. Startle habituation *increased* from trial 1 to trial 2, consistent with the early work in habituation (Groves and Thompson, 1970). Strikingly, startle reactivity did not recede to trial 1 level until trial 6. The common technique of condensing startle habituation trials into blocks may have made it more difficult for most prior studies to detect this effect; however, we note that at least one prior study has obtained a similar finding (Swerdlow and Talledo, 2009). The explanation for this effect remains unclear; however, it may be that participants anticipate and attend more to the second stimulus after experiencing the first stimulus.

It is also clear from Fig. 1 that startle reactivity appears to approach an asymptote around trial 13. Both means-based and latent curve analyses indicated that the vast majority of habituation occurred between trials 2 and 13 (87.5%; see Table 1). This suggests that the optimal length of startle habituation blocks is around 13 trials. This number is in stark contrast to the typical number of trials included in these blocks — three or fewer. In the present study, only 6.1% of habituation occurred by the third trial. The present results also indicate that studies that use less than 6 trials (i.e., most studies) may actually *sensitize* participants to startle stimuli because reactivity was greater on trials 2–5 than on trial 1. Overall, these analyses indicated that

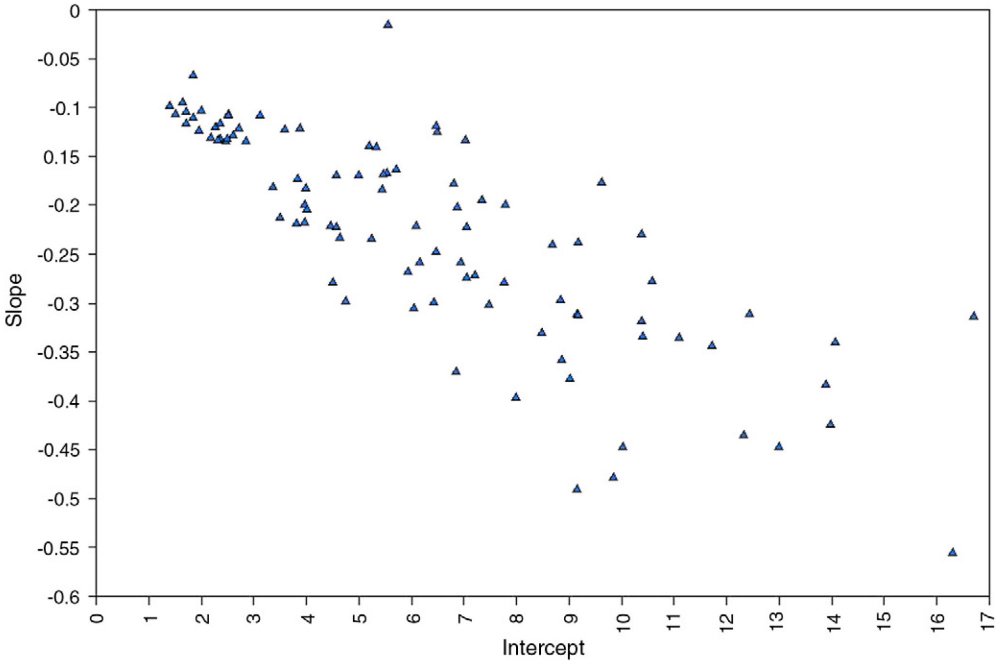

Fig. 4. Scatterplot of intercept and linear slope values.

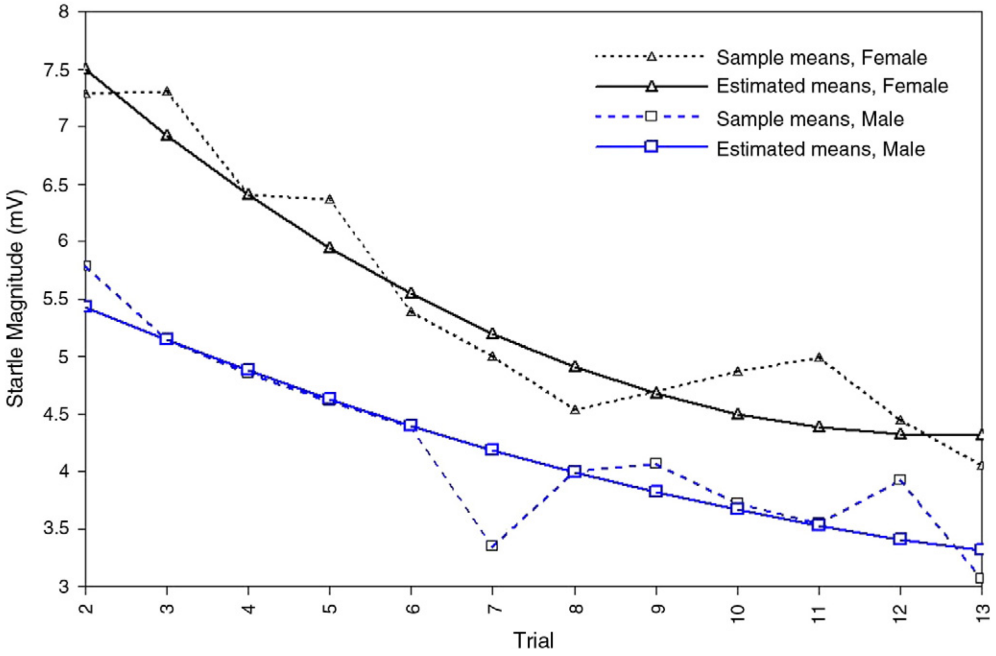

Fig. 5. Sample and estimated means by gender.

even the longest startle habituation blocks that some studies have employed (e.g., 10 in Grillon and Morgan, 1999) fall short of the optimal length. The inclusion of more trials in startle habituation blocks may substantially reduce the amount of noise in startle modulation studies, helping to clarify prepulse inhibition or affective valence modulation effects.

The latent curve analyses provided new information on several basic aspects of startle habituation. First, these analyses empirically demonstrated that a quadratic curve best fits startle habituation data. This information goes beyond means-based quadratic contrast effect analyses. Specifically, these analyses did not simply connect the dots trial-by-trial (as in means-based analyses); rather they estimated a continuous latent trajectory of habituation. The linear growth factor revealed the average decrease in startle reactivity across each trial between trials 2 and 13 (i.e., .23 mV per trial). The quadratic growth factor went beyond this information to quantify the nature of the habituation curve (i.e., an initial slope of the tangent line to the curve of −.48 that flattens out at the rate of .02 per trial). To our knowledge, this is the first quantitative description of the startle habituation curve. Studies examining group differences in startle habituation may utilize this finer-grained information to more effectively describe and find differences between groups. In terms of between group differences, the ANOVA did not detect a between-subjects effect of gender. Though the ANOVA did detect an interaction effect of gender and trial, the effect of gender on the intercept factor within the LCM precisely clarified the nature of this interaction. Latent curve modeling also had the advantage of including every participant in each analysis whereas means-based analyses often dropped a substantial number of participants (e.g., 19). This approach provided a more complete and detailed account of startle habituation (Table 2).

Second, these analyses quantitatively demonstrated that there was little individual variation in the quadratic growth factor (though there was significant variability in the intercept and linear growth factors). This indicated that most individuals followed a quadratic habituation pattern so closely that there was little room to bring in exogenous variables to account for variations in this pattern. Third, these analyses showed that there is a strong negative correlation between startle habituation intercept and slope (see Fig. 4). This relationship indicated that higher initial startle reactivity was associated with a steeper slope of startle habituation. This result raises the possibility that group differences in startle habituation rates (i.e., slope) may sometimes be an artifact of group differences in initial startle reactivity (i.e., intercept). Individuals with certain traits or states that tend to be associated with higher startle reactivity may display steeper habituation slopes; however, these individuals may not possess any of the neurological or psychological abnormalities that are sometimes associated with abnormal startle habituation (e.g., psychosis). Instead, they may simply have “farther to fall” in terms of startle reactivity; this potential highlights the strength of the LCM's ability to control for initial reactivity. Means-based analyses were unable to parse intercept and slope differences, precluding the investigation of intercept and slope associations. Overall, latent curve analyses proved to be effective for obtaining unique and valuable information about startle habituation with its specific parameterization of change over time.

As noted above, findings have been mixed regarding the effect of gender on startle reactivity and habituation. The present means-based analyses showed that there was a significant interaction effect of gender and trial on startle reactivity (see Fig. 5). Specifically, females appeared to have higher initial startle reactivity, but gradually approached the reactivity level of males. Latent curve analyses clarified this finding by parsing intercept and slope effects. Results indicated that females tend to have higher initial startle reactivity, but habituate toward the same level as males (see Fig. 5). This finding may help to explain some of the mixed findings on gender differences in startle. The present results indicate that studies that assess gender differences over the first few trials will be more likely to obtain significant differences. Including a large number of trials in such an investigation would obscure these initial differences as females gradually approach the startle reactivity levels of males. These results suggest that studies

Table 2
Advantages of applying LCM to startle habituation data.

Continuous modeling of habituation over time
Detailed quantitative information about trajectory of habituation
Dissociation of intercept and slope growth factors underlying habituation
Incorporation of polynomial growth factor for nonlinear trajectory of habituation
Directly test fit of competing models for identifying optimal functional form
Direct prediction of growth factors using covariates
Robust against missing data caused by missing trials

hoping to avoid a gender effect on startle reactivity would benefit from including startle habituation blocks that are around 13 trials long.

Although the present investigation advanced knowledge on startle habituation, these results should be interpreted in light of the limitations of the present study. First, it is unclear how well the present results would generalize beyond the present startle stimulus characteristics (i.e., 100 dB, white noise, 50 ms duration, instantaneous rise/fall time, etc.). Given that more intense startle stimuli tend to induce greater initial startle reactivity, we speculate that more intense stimuli may be associated with steeper habituation slope. A preliminary investigation using both 100 and 105 dB stimuli supported this pattern and showed that startle reactivity habituated to a similar asymptote in both stimulus conditions (Franklin et al., 2009b). Stimuli that tend to evoke less startle reactivity (e.g., less intense, more restricted spectral composition, slower rise/fall times) may generate flatter slopes and necessitate fewer trials to reach habituation; future studies are needed to test these possibilities.

Second, it is unclear if these results would generalize to habituation assessed pre-post or during experimental blocks of prepulse inhibition or affective valence startle modulation. Indeed, Blumenthal (1997) showed that habituation may vary based on some of these factors. Future studies may benefit from investigating the nature of habituation across a variety of methods. Third, we examined gender as an exogenous factor that may contribute to startle habituation differences, but there are many other potentially important factors. Future studies may benefit from employing latent curve analyses to examine associations between startle habituation and factors such as fear, attention, psychotic disorders, and anxiety disorders.

The present study advanced knowledge on several aspects of startle habituation. Results clarified the nature of the startle habituation curve, indicated that 13 trials are the optimal length of startle habituation blocks, demonstrated that there is a strong relationship between initial startle reactivity and startle habituation slope, and showed that gender differences in startle reactivity appeared to be confined higher initial startle reactivity in females. Analyses also revealed that latent curve modeling provided a more detailed, powerful, and flexible method of investigating startle habituation. Future studies may benefit from building on the present results to further clarify the nature of startle habituation and its relevance to factors such as psychopathology.

Conflict of interest

The authors declare no conflicts of interest.

References

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society 57, 289–300.

Bentler, P.M., Bonett, D.G., 1980. Significance tests and goodness of fit in the analysis of covariance structures. Psychological Bulletin 88, 588–606.

Blumenthal, T.D., 1997. Prepulse inhibition decreases as startle reactivity habituates. Psychophysiology 34, 446–450.

Blumenthal, T.D., Gescheider, G.A., 1987. Modification of the acoustic startle reflex by a tactile prepulse: the effects of stimulus onset asynchrony and prepulse intensity. Psychophysiology 24, 320–327.

Blumenthal, T.D., Cuthbert, B., Filion, D., Hackley, S., Lipp, O., van Boxtel, A., 2005. Committee report: guidelines for human startle eyeblink electromyographic studies. Psychophysiology 42, 1–15.

Blumenthal, T.D., Noto, J.V., Fox, M.A., Franklin, J.C., 2006. Background noise decreases both prepulse elicitation and inhibition of acoustic startle blink responding. Biological Psychology 72, 173–179.

Bollen, K.A., Curran, P.J., 2006. Latent Curve Models: A Structural Equation Approach. Wiley Series on Probability and Mathematical Statistics. John Wiley & Sons, New Jersey.

Braff, D.L., Geyer, M.A., 1990. Sensorimotor gating and schizophrenia: human and animal models. Archives of General Psychiatry 47, 181–188.

Braff, D.L., Grillon, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. Archives of General Psychiatry 49, 206–215.

Browne, M.W., Cudeck, R., 1993. Alternative ways of assessing model fit. In: Bollen, K.A., Long, J.S. (Eds.), Testing Structural Equation Models. Sage, Newbury Park, CA.

Cadenhead, K.S., Geyer, M.A., Braff, D.L., 1993. Impaired startle prepulse inhibition and habituation in schizotypal personality disordered patients. American Journal of Psychiatry 150, 1862–1867.

Csomor, P.A., Yee, B.K., Quednow, B.B., Stadler, R.R., Feldon, R., Vollenweider, F.X., 2006. The monotonic dependency of prepulse inhibition of the acoustic startle reflex on the intensity of the startle-eliciting stimulus. Behavioural Brain Research 174, 143–150.

Csomor, P.A., Yee, B.K., Vollenweider, F.X., Nicolet, T., Quednow, B.B., 2008. On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behavioral Neuroscience 122, 885–900.

Della Casa, V., Hofer, I., Weiner, I., Feldon, J., 1998. The effects of smoking on acoustic prepulse inhibition in healthy men and women. Psychopharmacology 137, 362–368.

Dichter, G.S., Benning, S.D., Holtzclaw, T.N., Bodfish, J.W., 2010. Affective modulation of the startle eyeblink and postauricular reflexes in autism spectrum disorder. Journal of Autism and Developmental Disorders 40, 858–869.

Franklin, J.C., Bowker, K.B., Blumenthal, T.D., 2009a. The association between anxiety and prepulse inhibition of acoustic startle in a normative sample: the importance of signal-to-noise ratio. Personality and Individual Differences 46, 369–373. http://dx.doi.org/10.1016/j.paid.2008.11.004.

Franklin, J.C., Mathiowetz, R.E., Wheaton, M.G., Heilbron, N., Blumenthal, T.D., 2009b. Latent growth curve modeling provides a more precise account of startle habituation. Psychophysiology 46, S71.

Franklin, J.C., Hessel, E.T., Aaron, R.V., Arthur, M.S., Heilbron, N., Prinstein, M., 2010. The functions of nonsuicidal self-injury: support for cognitive–affective regulation and opponent processes from a novel psychophysiological paradigm. Journal of Abnormal Psychology 119, 850–862.

Glenn, C.R., Blumenthal, T.D., Klonsky, E.D., Hajcak, G., 2011. Emotional reactivity in nonsuicidal self-injury: divergence between self-report and startle measures. International Journal of Psychophysiology 80, 166–170.

Grillon, C., Charney, D.R., 2011. In the face of fear: anxiety sensitizes defensive responses to fearful faces. Psychophysiology 48, 1745–1752.

Grillon, C., Morgan, C.A., 1999. Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. Journal of Abnormal Psychology 108, 134–142.

Grillon, C., Dierker, L., Merikangas, K.R., 1997. Startle modulation in children at risk for anxiety disorders and/or alcoholism. Journal of the American Academy of Psychoanalysis 36, 925–932.

Groves, P.M., Thompson, R.F., 1970. Habituation: a dual-process theory. Psychology Review 77, 419–450.

Hasenkamp, W., Epstein, M.P., Green, A., Wilcox, L., Boshoven, W., 2010. Heritability of acoustic startle magnitude, prepulse inhibition, and startle latency in schizophrenia and control families. Psychiatric Research 178, 236–243. http://dx.doi.org/10.1016/j.psychres.2009.11.012.

Hoenig, K., Hochrein, A., Quednow, B.B., Maier, W., Wagner, M., 2005. Impaired prepulse inhibition of acoustic startle in obsessive–compulsive disorder. Biological Psychiatry 57, 1153–1158.

Hu, L., Bentler, P.M., 1995. Evaluating model fit. In: Hoyle, R. (Ed.), Structural Equation Modeling: Issues, Concepts, and Applications. Sage, Newbury Park, CA, pp. 76–99.

Jennings, J.R., 1987. Editorial policy on analyses of variance with repeated measures. Journal of Motor Behavior 15, 235–254.

Koch, M., 1999. The neurobiology of startle. Progress in Neurobiology 59, 107–128.

Kofler, M., Müller, J., Reggiani, L., Valls-Solé, J., 2001. Influence of gender on auditory startle responses. Brain Research 921, 206–210.

Kozaric-Kovacic, D., Jambrosic-Sakoman, A., Jovanic, T., 2011. Startle reactivity in acute stress disorder and posttraumatic stress disorder. Studies in Health Technology and Informatics 167, 194–198.

Landis, C., Hunt, W.A., 1939. The Startle Pattern. Farrar & Rinehart, New York.

LaRowe, S.D., Patrick, C.J., Curtin, J.J., Kline, J.P., 2006. Personality correlates of startle habituation. Biological Psychology 72, 257–264.

Ludewig, K., Paulus, M.P., Vollenweider, F.X., 2003. Behavioural dysregulation of decision-making in deficit but not nondeficit schizophrenia patients. Psychiatric Research 119, 293–306.

Ludewig, S., Geyer, M.A., Ramseier, M., Vollenweider, F.X., Rechsteiner, E., Ludewig, K., 2005. Information processing deficits and cognitive dysfunctions in panic disorder. Journal of Psychiatry & Neuroscience 30, 37–43.

MacCallum, R.C., Zhang, S., Preacher, K.J., Rucker, D.D., 2002. On the practice of dichotomization of quantitative variables. Psychological Methods 7, 19–40.

Maxwell, S.E., Delaney, H.D., 2004. Designing Experiments and Analyzing Data: a Model Comparison Perspective, 2nd ed. Lawrence Erlbaum Associates, Mahwah, NJ.

Meincke, U., Mörth, D., Voß, T., Thelen, B., Geyer, M.A., Gouzoulis-Mayfrank, E., 2004. Prepulse inhibition of the acoustically evoked startle reflex in patients with an acute schizophrenic psychosis — a longitudinal study. European Archives of Psychiatry and Clinical Neuroscience 254, 415–421.

Meredith, W., Tisak, J., 1984. Tuckerizing curves. Paper Presented at the Annual Meeting of the Psychometric Society. Santa Barbara, CA.

Meredith, W., Tisak, J., 1990. Latent curve analysis. Psychometrika 55, 107–122.

Ornitz, D.M., Xu, J.S., Colvin, J.S., McEwen, D.G., MacArthur, C.A., Coulier, F., Gao, G.X., Goldfarb, M., 1996. Receptor specificity of the fibroblast growth-factor family. Journal of Biological Chemistry 271, 15292–15297.

Ornitz, E.M., Gabikian, P., Russel, A.T., Guthrie, D., Hirano, C., Gehricke, J.G., 1997. Affective valence and arousal in ADHD and normal boys during a startle habituation experiment. Journal of the American Academy of Child Psychiatry 36, 1698–1705.

Patrick, C.J., Bradley, M.M., Lang, P.J., 1993. Emotion in the criminal psychopath: startle reflex modulation. Journal of Abnormal Psychology 102, 82–92. http://dx.doi.org/10.1037/0021-843X.102.1.82.

Perry, W., Heaton, R.K., Potterat, E., Roebuck, T., Minassian, A., Braff, D.L., 2001. Working memory in schizophrenia: transient “online” storage versus executive functioning. Schizophrenia Bulletin 27, 157–176.

Perry, W., Feifel, D., Minassian, A., Bhattacharjie, I., Braff, D.L., 2002. Information processing deficits in acutely psychotic schizophrenia patients medicated and unmedicated at the time of admission. American Journal of Psychiatry 159, 1375–1381.

Quednow, B.B., Westheide, J., Kuhn, K.U., Werner, P., Wagner, M., 2006. Normal prepulse inhibition and habituation of acoustic startle response in suicidal depressive patients without psychotic symptoms. Journal of Affective Disorders 92, 299–303.

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiology of Learning and Memory 92, 135–138.

Rich, B.A., Bhangoo, R.K., Vinton, D.T., Berghorst, L.H., Dickstein, D.P., Grillon, C., et al., 2005. Using affect-modulated startle to study phenotypes of pediatric bipolar disorder. Bipolar Disorders 7, 536–545.

Ross, R.J., Ball, W.A., Cohen, M.E., Silver, S.M., Morrison, A.R., Dinge, D.F., 1989. Habituation of the startle reflex in posttraumatic stress disorder. Journal of Neuropsychiatry and Clinical Neurosciences 1, 305–307.

Schachinger, H., Blumenthal, T.D., Richter, S., Savaskan, E., Wirz-Justice, A., Kräuchi, K., 2008. Melatonin reduces arousal and startle responsiveness without influencing startle habituation or affective startle modulation in young women. Hormones and Behavior 54, 258–262.

Schicatano, E.J., Blumenthal, T.D., 1995. The effects of different doses of caffeine on habituation of the acoustic startle reflex. Pharmacology Biochemistry and Behavior 52, 231–236.

Schicatano, E.J., Blumenthal, T.D., 1998. The effects of caffeine and directed attention on acoustic startle habituation. Pharmacology Biochemistry and Behavior 59, 145–150.

Steiger, J.H., 1990. Structural model evaluation and modification: an interval estimation approach. Multivariate Behavioral Research 25, 173–180.

Swerdlow, N.R., Talledo, J.A., 2009. Effects of the first prepulse on the blink response to a startling noise. Behavioral Neuroscience 123, 607–613.

Swerdlow, N.R., Benbow, C.H., Zisook, S., Geyer, M.A., Braff, D.L., 1993. A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder (OCD). Biological Psychiatry 33, 298–301.

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., Braff, D.L., 2008. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology 199, 331–388.

Thompson, R.F., 2009. Habituation: a history. Neurobiology of Learning and Memory 92, 127–134.

Vaidyanathan, U., Patrick, C.J., Bernat, E.M., 2009a. Startle reflex potentiation during aversive picture viewing as an indicator of trait fear. Psychophysiology 46, 75–85.

Vaidyanathan, U., Patrick, C.J., Cuthbert, B.N., 2009b. Linking dimensional models of internalizing psychopathology to neurobiological systems: affect-modulated startle as an indicator of fear and distress disorders and affiliated traits. Psychological Bulletin 135, 909–942.

Yee, C.M., Mathis, K.I., Sun, J.C., Sholty, G.L., Lang, P.J., Nuechterlein, K.H., 2010. Integrity of emotional and motivational states during the prodromal, first-episode, and chronic phases of schizophrenia. Journal of Abnormal Psychology 119, 71–82.

Yeomans, J.S., Li, L., Scott, B.W., Frankland, P.W., 2002. Tactile, acoustic and vestibular systems sum to elicit the startle reflex. Neuroscience and Biobehavioral Reviews 26, 1–11. http://dx.doi.org/10.1016/S0149-7634(01)00057-4.