Airpuff startle probes: an efficacious and less aversive alternative to white-noise

Shmuel Lissek[a,*], Johanna M.P. Baas[b], Daniel S. Pine[a],
Kaebah Orme[a], Sharone Dvir[a], Monique Nugent[a],
Emily Rosenberger[a], Elizabeth Rawson[a],
Christian Grillon[a]

[a]*Mood and Anxiety Disorders Program, National Institute of Mental Health (NIH),
DHHS, 15K North Drive, Bldg 15k, MSC 2670, Bethesda, MD 20892-2670, USA*
[b]*Helmholtz Institute, Psychological Laboratory, Utrecht University, P.O. Box 80082,
3508 TB Utrecht, The Netherlands*

Abstract

Fear-potentiated startle (FPS) is an increasingly popular psychophysiological method for the objective assessment of fear and anxiety. Studies applying this method often elicit the startle reflex with loud white-noise stimuli. Such intense stimuli may, however, alter psychological processes of interest by creating unintended emotional or attentional artifacts. Additionally, loud acoustic probes may be unsuitable for use with infants, children, the elderly, and those with hearing damage. Past studies have noted robust and reliable startle reflexes elicited by low intensity airpuffs. The current study compares the aversiveness of white-noise (102 dB) and airpuff (3 psi) probes and examines the sensitivity of each probe for the assessment of fear-potentiated startle. Results point to less physiological arousal and self-reported reactivity to airpuff versus white-noise probes. Additionally, both probes elicited equal startle magnitudes, response probabilities, and levels of fear-potentiated startle. Such results support the use of low intensity airpuffs as efficacious and relatively non-aversive startle probes.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Startle; Psychophysiology; Anxiety; Fear

* Corresponding author. Tel.: +1 301 402 7219; fax: +1 301 402 6353.
  E-mail address: lisseks@intra.nimh.nih.gov (S. Lissek).

1. Introduction

The human startle reflex has emerged as an important psychophysiological correlate of emotional activation. Startle magnitude is reliably potentiated by cues marking the imminent onset of aversive events such as electric shocks, blasts of air, and loud white-noises (Grillon et al., 1991, 1998a; Skolnick and Davidson, 2000). Additionally, the valence specificity of startle modulation is supported by evidence of enhanced startle magnitudes to equally arousing unpleasant versus pleasant visual stimuli (Cuthbert et al., 1996; Vrana et al., 1988). In such research, emotional states are probed by eliciting the startle response at a variety of points throughout the experimental task. While many stimuli engaging different sensory modalities evoke the startle response, most investigations of the emotional effects on startle employ high intensity broadband noises (40–50 ms, 95–116 dB) with a near instantaneous rise time to elicit the reflex. Although, in concept, such startle stimuli are passive probes of ongoing emotional functions, in reality they are loud, intrusive, and likely to distort psychologically relevant processes by either creating unintended emotional states, disrupting the emotional response of interest, or altering attentional focus. This seems particularly plausible given that loud white-noises are frequently used as unconditioned stimuli (USs) in aversive conditioning experiments (Ashcroft et al., 1991; Peri et al., 2000; Pliszka et al., 1993), and given that anticipation of white-noises increases self-reported anxiety (Grillon and Ameli, 1998) and potentiates the startle response (e.g., Patrick and Berthot, 1995; Skolnick and Davidson, 2000). Furthermore, participants tend to rate such white-noises as aversive whether used as USs (Miller et al., 1999; Patrick and Berthot, 1995) or startle probes (Haerich, 1994). However, white-noises used as USs tend to be longer in duration (100 ms–6 s) than those used as startle probes (40–50 ms). This difference is not trivial, as intense white-noises of longer duration are likely to elicit the defensive reflex along with the startle reflex. As such, responses to white-noise stimuli used as probes and USs are not entirely comparable.

In addition to the above problems, the white-noise probe may not be suitable for such human populations as infants, young children, the elderly, and those with hearing damage. Infants and young children are particularly sensitive to acoustic stimuli of high intensities and thus repeated exposure to high intensity acoustic stimuli is inadvisable (American Academy of Pediatrics Committee on Environmental Health, 1994). Children also seem to be extremely sensitive to white-noise probes and display strong dislike for such stimuli (Grillon, 2004, unpublished observation). Furthermore, testing the elderly with white-noise probes may be subject to complications produced by presbycusis (Haerich, 1998; Ludewig et al., 2003). Finally, the effectiveness of white-noise as a startle probe is likely to be compromised by hearing damage, a condition afflicting 3.4% of Americans (National Campaign for Hearing Health, 2004) and up to 42% of US combat veterans (Ben-Tovim et al., 1990) who have been and continue to be an important target of study in startle experiments (e.g., Grillon and Morgan, 1996; Grillon et al., 1998b; Morgan et al., 1996; Orr et al., 1997).

Eliciting startle blinks with a tactile airpuff directed towards the surface of the forehead may obviate some of the difficulties associated with the white-noise probe. Low intensity airpuffs to the surface of facial skin (1–5 psi) elicit robust blink reflexes with response probabilities approaching 1.0 (Haerich, 1998). Such airpuff startle probes have been rated

as relatively non-aversive (Miller et al., 1999) and hedonically neutral (Hawk and Cook, 1997) in past studies and only airpuffs with far greater intensities (60–80 psi) have been used to elicit anticipatory anxiety (e.g., Merikangas et al., 1999). Because low intensity airpuffs are effective and appear to be relatively non-aversive startle stimuli, airpuff stimulation may provide a method for eliciting startle with less interruption of ongoing psychological processes. Additionally, the use of airpuff probes may be safer for children and more effective for the elderly and other individuals with auditory complications.

One potential pitfall of using a low intensity airpuff probe lies in the possibility that affective modulation of startle is best elicited when using startle stimuli that are relatively aversive. According to the emotional priming hypothesis, when the aversive motivational system is primed by an aversive foreground, defensive and startle reflexes are potentiated (for a review, see Lang et al., 1998). One assumption of this model is that startle stimuli engage the same aversive motivational system primed by the aversive foreground. As such, successful fear-potentiation of startle may require a startle probe that is sufficiently aversive. Although past studies have demonstrated fear-potentiated startle (FPS) with low intensity airpuff probes (Grillon and Ameli, 1998; Miller et al., 1999), it remains unclear whether the level of potentiation is equivalent to that elicited by intense white-noise probes.

The present study was designed to compare the characteristics of the startle reflex elicited using each probe within a fear-potentiated startle experiment (Threat Study). The fear-potentiated paradigm employed was previously developed in our lab to assess anxious arousal to the threat of predictable and unpredictable aversive events (Grillon et al., 2004). We also conducted a Pilot Study to confirm the impression that air-puff startle probes are less aversive than white-noise probes. It was expected that airpuff startle probes would elicit less physiological and self-reported arousal, would produce as many responses (probability), and would be as sensitive to threat as white-noise startle probes.

2. Pilot Study

2.1. Methods

2.1.1. Participants

Participants were 19 healthy volunteers (8 males, 11 females) with mean age of 26.7 (S.D. = 8.7). A description of the study was given prior to participation and participants gave written informed consent that had been approved by the NIMH human Investigation Review Board. Inclusion criteria included (1) no past or current psychiatric disorders as per Structured Clinical Interview for DSM-IV (SCID: First et al., 2001); (2) no medical condition that interfered with the objectives of the study; and (3) no current use of drugs or psychoactive medications as per self-report. Additionally, participants were asked not to consume caffeinated beverages on the day of testing.

2.1.2. Experimental design

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments). The physiological measures included eyeblink EMG (startle reflex) and skin conductance. The airpuff startle probe was a 40 ms, 3 psi puff (20.64 kPa; measured at the

level of the regulator) of compressed room air delivered to the center of the forehead through a poly-ethylene tube (2.0 ft long, 1/8 in. inside diameter) affixed 1 cm from the skin by way of a headpiece worn by the participant. Using this headpiece allowed for head movements of the participant while maintaining the constant placement of the airpuff. A visor was positioned between the poly-ethylene tube and participants' eyes to prevent the puff from reaching the cornea. A solenoid valve with an AC switch controlled the delivery of the airpuff. This airpuff probe setup was the same as the setup shown to work effectively in a previous fear-potentiated startle study in our lab (Grillon and Ameli, 1998) with the exception that the startle probe intensity was reduced from 15 to 3 psi in the current study. The 3 psi airpuff probe was chosen to minimize the intrusiveness and unpleasantness of the probe and because pilot data demonstrated similar blink magnitudes when using the 3 psi airpuff and a 102 dB white-noise probe.

The airpuff startle reflex has been found to have an acoustic component (Flaten and Blumenthal, 1999) produced by the flow of air itself, the vibrations resulting from the physical impact of the puff against the skin, and the audible clicks from the solenoid. The first two sources of acoustic artifact can be effectively reduced by using a low intensity airpuff (Haerich, 1998) such as the 3 psi probe used in the current study. Acoustic artifacts from solenoid clicking have been reduced by delivering a constant broadband noise to participants (Miller et al., 1999). Such background noise may, however, alter baseline blink reflexes (Ison and Russo, 1990), and as such, no continuous masking noise was used in this study. Participants did, however, wear sound attenuating headphones (Sennheiser HD 25-1) that provided 32 dB of background noise reduction. Given that the solenoid produced 65 dB clicks, wearing the headset was likely to reduce the audible click to a sound level that approximated the 50 dB baseline sound level of the room.

The acoustic startle stimulus was a 40-ms duration, 102 dB (A) burst of white-noise with a near instantaneous rise time presented binaurally through headphones. The eyeblink reflex was recorded with two 6-mm tin cup electrodes placed under the right eye. Amplifier band width was set to 30–200 Hz. The left palmar skin conductance was recorded from the index and middle finger of the left hand according to published recommendations (Prokasy and Ebel, 1967).

2.1.3. Procedure

Participants underwent a screening session that consisted of a SCID, a physical exam and the completion of the Spielberger State and Trait Anxiety Inventory (Spielberger et al., 1983), after which recording electrodes (EMG, SCR), headphones, visor, and the puff delivery headpiece were placed. In order to simulate the attentional demands of the typical psychophysiology experiment, participants were given the task of circling all letter *e*'s in a three page text. While searching for the letter *e*, eight white-noise and eight airpuff probes were delivered to participants (probe inter-trial interval of 18–25 s) in a quasi-random order where no more than two probes of the same type were delivered consecutively. Participants then rated the white-noise and airpuff stimuli on 10-point scales reflecting the "intensity", "distractability", "intrusiveness", and "unpleasantness" of each probe type. Additionally participants indicated the amount of anxiety provoked by the probes and the type of probe (white-noise versus airpuff) they would prefer to receive if given the choice.

2.1.4. Data analysis

Startle EMG was rectified and then smoothed (20-ms moving window average). The onset latency window for the blink reflex was 20–100 ms and the peak magnitude following the onset up to 120 ms was determined. Additionally, the average baseline EMG level for the 50 ms immediately preceding delivery of the startle stimulus was subtracted from the peak magnitude. Skin conductance responses (SCR) to the probes were required to have an onset within a 1–5 s latency window of probe delivery. Probe SCRs were calculated by subtracting the skin conductance level at onset from the peak skin conductance level of the response wave. EMG and SCR data were transformed to normalize data and to reduce the influence of between subjects variability unrelated to psychological processes. SCR scores underwent square root transformation and range correction (Lykken, 1972). EMG magnitudes were standardized using within subject t-score conversions. Eyeblink and SCR data were averaged separately for white-noise and airpuff probes. Paired samples t-tests were then used to analyze EMG, SCR, and self-report differences across probe types. Alpha was set at .05 for all statistical tests.

Of the 19 participants tested in the Pilot Experiment, there was one SCR and one EMG nonresponder. One additional participant could not be included in analyses of self-report data because several items were left blank. As such, data for 18 subjects were included in SCR, EMG, and self-report comparisons.

2.1.5. Results

Table 1 displays means, standard deviations, and t-test results for blink EMG, SCR, and self-report measures across probes. White-noise and airpuff groups did not differ in terms of startle magnitude, $t(17) = .03$, $p > .96$, or probability of a blink, $t(17) = 1.01$, $p > .32$.

Table 1

Means (standard deviations) and within subject inferential statistics for blink EMG, SCR, and subjective ratings across white-noise and airpuff startle probes

| | Probe type | | | | |
	N	White-noise	Airpuff	Statistic[a]	p
Blink EMG					
Magnitude[b]	18	50.04 (4.98)	49.96 (4.98)	$t = .03$	>.97
Probability	18	94% (14%)	88% (20%)	$t = 1.01$	>.32
SCR magnitude[c]	18	.46 (.16)	.26 (.15)	$t = 5.01$	<.001
Subjective ratings[d]					
Intense	18	6.17 (2.28)	2.83 (1.62)	$t = 4.96$	<.001
Distracting	18	5.72 (1.77)	3.31 (1.45)	$t = 5.64$	<.001
Intrusive	18	5.89 (2.05)	3.14 (1.80)	$t = 4.12$	<.001
Unpleasant	18	5.75 (2.13)	2.39 (1.54)	$t = 5.22$	<.001
Anxiety provoking	18	5.00 (1.92)	2.72 (1.36)	$t = 5.02$	<.001
Preference[e]	18	6%	94%	$\chi^2 = 14.22$	<.001

[a] All are t-tests except "Preference" which is a nonparametric chi-square.

[b] t-Score units.

[c] MicroS square rooted and range corrected.

[d] Rated on a 10-point scale where 1 is the minimum and 10 is the maximum of the characteristic.

[e] Percent of participants who prefer white-noise vs. airpuff startle probes.

Additionally, white-noise probes evoked larger SCRs than did airpuff probes, $t(17) = 5.01$, $p < .001$.

As can be seen in Table 1, airpuff compared to white-noise probes were rated as less intense, distracting, intrusive, unpleasant, and anxiety provoking (all $p < .002$). Additionally, 17 of the 18 subjects (94%) said they would choose to receive the airpuffs if given a choice, while only 1 of the 18 (6%) chose white-noises resulting in greater overall preference for airpuff probes, $\chi^2(1) = 14.22$, $p < .001$.

Results of the Pilot Study demonstrate less physiological arousal and self-reported aversiveness for airpuff probes. Additionally airpuff and white-noise probes were equally effective startle-stimuli as reflected by equal startle magnitudes and response probabilities across probe types.

3. Threat Study

3.1. Methods

3.1.1. Participants

Participants were 35 healthy volunteers divided into a white-noise group ($n = 18$, mean age = 27.9, 50% males) and an airpuff group ($n = 17$, mean age = 25.9, 53% males) with equivalent mean age ($p > .87$) and gender distribution ($p > .66$). Additionally, state and trait anxiety scores (Spielberger et al., 1983) for the white-noise (state = 26.9, trait = 29.4) and airpuff group (state = 27.8, trait = 30.8) were approximately equal (both $p > .28$). A complete description of the study was given prior to participation and participants gave written informed consent that had been approved by the NIMH Human Investigation Review Board. Inclusion criteria were the same as those applied to participants in the Pilot Study and participants were again asked to abstain from drinking caffeinated beverages on the day of testing.

3.1.2. Experimental design

Participants were randomly assigned to either the white-noise or airpuff group. With the exception of different startle probe modalities, both groups underwent identical experimental procedures. The design was created to test the hypothesis that unpredictable, rather than predictable, aversive events evoke anxious states most analogous to pathological anxiety (for a review, see Grillon, 2002). Because our laboratory often uses this design to test effects of psychopharmacology and psychopathology on fear-potentiated startle, it was most important for us to identify airpuff versus white-noise differences in this paradigm.

In the current study, aversive stimuli, referred to as unpleasant events, consisted of four different 3-s duration, 95 dB noises: (1) a white-noise; (2) a high pitch tone (2 kHz); (3) a pulsating smoke alarm sound used by Pizzagalli et al. (2003); and (4) a human female scream (the human scream was accompanied by a picture of a fearful woman). The primary experiment consisted of three conditions: neutral (N), predictable (P), and unpredictable (U), lasting 2 min each. In the N condition, no unpleasant events were delivered. In the P condition, unpleasant events were administered predictably, that is, only

in the presence of a threat cue. In the U condition, unpleasant events could be delivered at any time. In each condition, an 8-s duration cue was presented twice. The cues were different colored geometric shapes (e.g., green circle, blue triangle, etc.). The cues signaled the possibility of receiving an aversive stimulus only in the P condition, but they had no signal-value in the N and U conditions. For the duration of each 2 min N, P, and U condition, a computer monitor apprised participants of the current condition by displaying the following information: ''no unpleasant event'' (N), ''unpleasant event only during shape'' (P), or ''unpleasant event at any time'' (U). During each predictable and unpredictable condition, two different unpleasant events were administered and each of the four unpleasant events were given equally often in the P and U conditions. The unpleasant events were delivered at cue offset in the predictable conditions and in the absence of the cues in the unpredictable conditions. Acoustic or puff startle stimuli were delivered (1) 4–6 s following the onset of each cue and (2) during inter-trial intervals (ITI) between cues every 20–40 s. The startle magnitudes elicited in the three conditions in the absence of cues (i.e., during ITIs) were measured to assess anxious arousal during the N, P, and U contexts. Throughout this paper, *condition* refers to the N, P, and U conditions regardless of the presence or absence of the cue. *Context*, on the other hand, refers to the period of time in each condition when no cue is present.

The threat experiment consisted of two recording blocks with a 5–10 min rest between blocks. Each block consisted of three N, two P, and two U conditions in one of the following two orders: P N U N U N P or U N P N P N U. Each participant was presented with the two orders, with half the participants starting with the P condition.

3.1.3. Procedure

Participants underwent a screening session that consisted of a SCID, a physical exam and the completion of Spielberger's State and Trait Anxiety Inventory (Spielberger et al., 1983). Within two weeks of screening, participants returned for the testing session. This session started with the presentation of nine startle stimuli delivered every 18–25 s to assess the baseline startle reflex prior to the Threat Study. Participants were then given an explanation of the study including explicit instructions regarding the conditions under which they would and would not receive an unpleasant event. Following this instruction, the threat experiment was run.

At the end of the experiment, participants were asked to rate the overall level of subjective anxiety elicited by the cue and context in the N, P, and U conditions on an analog scale ranging from 1 (not at all) to 10 (extremely). Participants used a similar 10-point scale to report ''how intense'', ''how unpleasant'', and ''how anxiety provoking'' the aversive events were during the experiment. Finally, participants indicated the degree to which they would like further exposure to the aversive stimuli using a 10-point scale (1, definitely; 10, definitely not).

3.1.4. Apparatus and physiological responses

As in the Pilot Study, stimulation and recording were controlled by a commercial system by Contact Precision Instruments and the physiological measures included eyeblink EMG and skin conductance responses. SCRs to the context were required to have an onset within a 1–5 s latency window of the start of the N, P or U condition while SCRs to the cues were required to have the same latency of onset following the presentation of the N, P, or U cue.

The airpuff and white-noise probes as well as the apparatus for their production was identical to that used in the Pilot Study. The unpleasant sounds used as aversive stimuli were presented through the headphones that were also used to deliver the white-noise probes.

3.1.5. Data analysis

The methods for identifying, quantifying, and transforming blink EMG and SCR magnitudes were identical to those applied to Pilot Study data. For each physiological variable, the data were averaged for context and cue for each condition across blocks. The magnitude of the eyeblink was analyzed raw, as well as after standardization within-subjects using t-scores. Because similar results were obtained with the raw scores and with the t-scores for within-subjects comparisons, only inferential analyses of the t-scored data are presented. The data were analyzed with a Condition (N, P, or U) × Cue (Cue on or off) × Group (White-noise or Puff) MANOVA with repeated measures. MANOVAs were computed using Wilk's Lambda and were followed when necessary by paired samples t-tests. Although only one dependent variable was included in each analysis, MANOVA was chosen because it affords protection against sphericity without performing the univariate correction (Tabachnick and Fidell, 1996). Alpha was set at 0.05 for all statistical tests.

No EMG activity was detectable for one participant in the white-noise group and was dropped from analyses. Additionally, no detectable SCR was present for four participants (three white-noise; one airpuff) leaving data for 15 white-noise and 16 airpuff participants for SCR analyses.

4. Results

4.1. Self-report data

4.1.1. Anxiety to cue and context

Mean levels of reported anxiety are displayed by group in Fig. 1. Significant main effects were found for condition, $F(2, 31) = 52.71$, $p < .001$, and cue, $F(1, 32) = 16.24$,
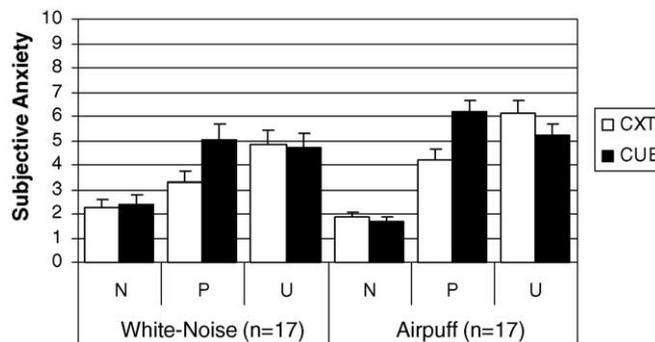

Fig. 1. Average levels of reported anxiety in the presence of neutral (N), predictable (P) and unpredictable (U) contexts (CXT) and cues (CUE) by group. Error bars display standard errors of the mean.

$p < .001$, and the Condition $\times$ Cue interaction was significant, $F(2, 31) = 30.06$, $p < .001$. Follow-up comparisons revealed greater anxiety to the cue versus context in the predictable condition, $t(30) = 7.32$, $p < .001$, greater anxiety to the context versus cue in the unpredictable condition, $t(30) = 2.56$, $p < .02$, and no difference of reported anxiety to cue and context in the neutral condition ($p > .81$). Relative to the neutral context, reported anxiety was increased by both the predictable, $t(30) = 6.45$, $p < .001$, and unpredictable contexts, $t(30) = 8.62$, $p < .001$. Additionally, the unpredictable context evoked more reported anxiety than the predictable context, $t(30) = 6.00$, $p < .001$. Such results suggest that the Threat Study was able to elicit anxious responding to both predictable and unpredictable threat of aversive events.

Additionally, the main effect of Group was nonsignificant, $F(1, 25) = 1.02$, $p > .32$, as was the Group $\times$ Condition $\times$ Cue interaction, $F(2, 31) = 1.49$, $p > .23$, indicating that the pattern of self-reported anxiety to contexts and cues was not different across groups.

4.1.2. Reactions to unpleasant events

On a scale of 1–10, unpleasant events received intensity, unpleasantness, anxiety, and avoidance ratings of 7.06, 7.19, 5.96, and 8.30, respectively. Additionally, groups did not differ in their ratings of aversive stimulus intensity, $t(32) = 1.10$, $p > .27$, or unpleasantness, $t(32) = .84$, $p > .40$, and groups reported similar levels of anxious reactivity to such stimuli, $t(32) = 1.44$, $p > .15$. Finally, groups reported approximately equal preferences to avoid additional exposure to the aversive stimuli $t(32) = .54$, $p > .58$. Such results suggest that the loud sounds were equally aversive to white-noise and airpuff participants.

4.1.3. Startle reflex

Fig. 2 displays average blink EMGs for *t*-scored data across groups, contexts, and cues. Analyses of *t*-scored data revealed significant main effects of both condition, $F(2, 31) = 36.48$, $p < .001$, and cue, $F(1, 32) = 76.99$, $p < .001$, as well as a condition by cue interaction, $F(2, 31) = 11.80$, $p < .001$. Follow up comparisons revealed significant potentiation to the cue relative to context in the predictable, $t(1, 33) = 6.66$, $p < .001$, and
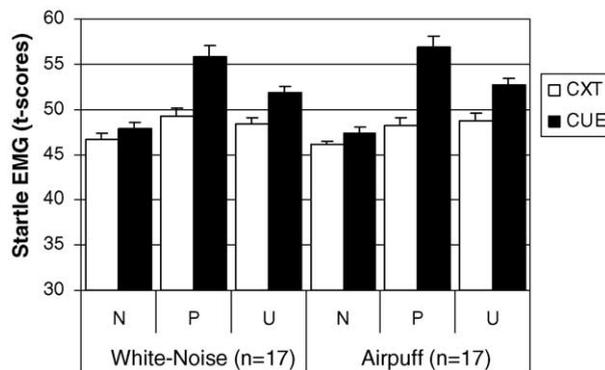

Fig. 2. Average standardized eyeblink EMG (*t*-scores) across neutral (N), predictable (P) and unpredictable (U) contexts (CXT) and cues (CUE) by group. Error bars display standard errors of the mean.

unpredictable conditions, $t(1, 33) = 5.20$, $p < .001$, and a trend for potentiation to the cue versus context in the neutral condition, $t(2, 31) = 1.82$, $p > .07$. Relative to the magnitude of startle elicited during the neutral context, startle was potentiated during the predictable, $t(1, 33) = 3.30$, $p < .003$, and unpredictable contexts, $t(1, 33) = 2.71$, $p < .02$, but no difference in startle was found across predictable versus unpredictable contexts, $t(1, 33) = .20$, $p > .84$.

All interactions between group and other independent variables were nonsignificant (all $p > .26$) indicating that the pattern of startle magnitudes across conditions did not differ by the type of startle probe used. Importantly, enhanced startle to the predictable cue versus context was significant in both white-noise, $t(1, 16) = 4.16$, $p < .002$, Hedge's $g = .96$ (95% CI = .25–1.67), and airpuff groups, $t(1, 16) = 5.21$, $p < .001$), Hedge's $g = .1.20$ (95% CI = .47–1.93), and no group difference in such fear-potentiated startle was found whether computing FPS as a predictable cue minus context difference score, $t(32) = .90$, $p > .37$, Hedge's $g = .30$ (95% CI = −.37 to .98), or cue over context proportion, $t(32) = .98$, $p > .32$, Hedge's $g = .33$ (95% CI = −.35 to 1.00).

With regards to contextual anxiety, startle magnitudes during the unpredictable context did not exceed those during the predictable context in either the white-noise, $t(1, 16) = .85$, $p > .40$, or airpuff group, $t(1, 16) = .40$, $p > .70$. Thus, effects of unpredictability where not evident in either group.

Given reports of greater startle probability when using airpuff as opposed to white-noise probes (Haerich, 1998), overall blink probabilities were assessed for each group. White-noise and airpuff probes elicited startle responses 83% and 79% of the time, respectively, and the 4% difference between groups was nonsignificant, $t(1, 32) = .42$, $p > .67$. In addition to the lack of group difference in response probability, white-noise and airpuff probes elicited blinks with approximately equal raw EMG magnitudes, $t(32) = .43$, $p > .66$.

4.1.4. Skin conductance response

Mean SCR across contexts and cues are displayed in Fig. 3. SCR varied by condition, $F(2, 28) = 3.20$, $p = .05$, but not by cue, $F(1, 29) = .65$, $p > .42$, and a Condition × Cue interaction was present, $F(2, 28) = 6.63$, $p < .005$. Follow up comparisons revealed larger
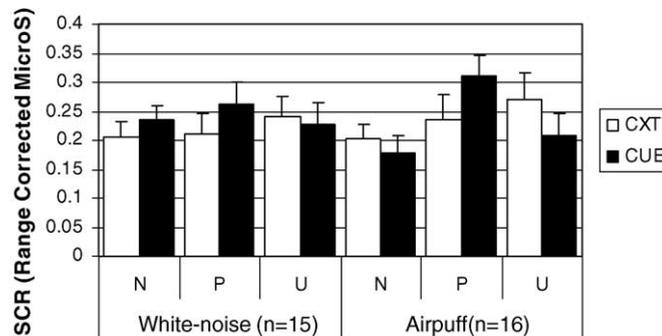

Fig. 3. Range corrected skin conductance responses to the neutral (N), predictable (P) and unpredictable (U) contexts (CXT) and cues (CUE) by group. Error bars display standard errors of the mean.

SCRs to the cue versus context in the predictable condition, $t(1, 30) = 2.49$, $p < .02$, and a trend for larger SCRs to the context versus cue in the unpredictable condition, $t(1, 30) = 1.71$, $p > .09$. No cue versus context difference was found for the neutral condition ($p > .94$). With regards to context effects, a trend for larger SCRs in the unpredictable versus neutral context was found, $t(1, 30) = 1.63$, $p > .10$, and no SCR differences were found between the predictable and neutral or predictable and unpredictable contexts (both $p > .22$).

The main effect of group was nonsignificant, $F(1, 27) = .27$, $p > .62$. Additionally, group did not interact with condition ($p > .49$) or cue ($p > .14$) and the Group $\times$ Condition $\times$ Cue interaction was nonsignificant ($p > .38$). Such nonsignificant interactions with group indicate that white-noise and airpuff participants displayed a similar pattern of SCR across the various experimental conditions.

4.1.5. SCR to startle probes

Consistent with results from the Pilot Study, white-noise probes evoked larger SCR's relative to airpuff probes $t(29) = 1.90$, $p < .04$ (one-tailed), indicating that the white-noise versus airpuff probe elicited more physiological arousal.

5. Discussion

White-noise and airpuff probes elicited blinks of equal magnitude with comparable response probabilities, yet the airpuff probes evoked smaller skin conductance responses and were rated as being the less intense, distracting, intrusive, unpleasant, and anxiety provoking of the two probes. Additionally, both probe groups displayed equal levels of fear-potentiated startle to cues predicting imminent onset of unpleasant noises and images. Although past studies have demonstrated successful FPS using low intensity airpuff startle-probes (Grillon and Ameli, 1998; Miller et al., 1999), the present study is the first to contrast levels of potentiation elicited using airpuff versus white-noise probes within a single experimental paradigm. While the conclusion that airpuff probes are at least as efficacious as white-noise probes for eliciting FPS rests on a null finding, this conclusion is not thought to be the spurious product of inadequate statistical power for a several reasons. For one, the results of a power analysis (Hedge's $g = .33$, alpha = .05) reveal that as many as 476 subjects would need to be added to the existing 34 subjects before group differences in FPS to the predictable cue would reach significance. Such a large number of additional subjects weakens the notion that the null between-group finding is a product of insufficient power. Additionally, the direction of the probe effect points to greater FPS elicited by airpuffs (an increase of 8.69 versus 6.63 t-score units for airpuff and white-noise groups, respectively). Thus adding power may well reveal greater FPS using airpuff versus white-noise probes which would further support the use of airpuff probes for eliciting FPS.

Airpuff probes were rated as relatively non-aversive (on a scale of 10: intensity = 2.8, unpleasant = 3.3, anxiety provoking = 2.7) and were able to capture a level of startle potentiation equivalent to that evoked when using a significantly more aversive white-noise stimulus (on a scale of 10: intensity = 6.2, unpleasant = 5.6, anxiety provoking = 5.0) suggesting that startle probe aversiveness does not influence levels of fear-potentiated

startle. This conclusion may be at odds with the motivational priming model in which probe aversiveness is implicated as a potentially important factor for eliciting affective modulation of startle. According to this model, emotional enhancement of startle occurs because of a hedonic match between a primed aversive motivational state and a subsequently elicited reflex. One assumption of this model is that startle stimuli engage the same aversive motivational system primed by the aversive foreground. As such, one might expect successful fear-potentiation of startle to require a startle probe that is sufficiently aversive. This expectation was not confirmed in the present study nor was it confirmed by a past study reporting equal emotional modulation when using acoustic startle probes of small, moderate, and high intensity (Cuthbert et al., 1996). Although null relations between probe aversiveness and startle modulation may have further implications for the motivational priming model, a full discussion of this issue is beyond the scope of the present paper.

From an empirical standpoint, it might be suggested that a low intensity startle probe would be best achieved by simply reducing the intensity of the white-noise probe. Because the 3 psi airpuff and 102 dB white-noise elicit blinks with the same reliability, a reduced intensity white-noise probe would likely yield a response probability falling below that of the 3 psi airpuff, rendering the 3 psi probe a better option. It should also be noted that alterations in the direction of the airpuff may substantially improve startle response probabilities for the airpuff probe. More specifically, airpuffs directed lateral to the outer canthus of the eye have been found to produce blink response probabilities approaching 1.0 (Haerich, 1998). Thus, the puff probe may have the potential to surpass the response probability of the acoustic probe.

Participants in the airpuff condition received probes and unpleasant events through different sensory modalities (i.e., tactile and auditory), whereas white-noise participants received probes and unpleasant events of the same modality (auditory). Thus, during predictable cues and unpredictable contexts when participants were anticipating an aversive acoustic stimulus, white-noise but not airpuff subjects may have focused their attention on the sensory modality of the startle probe. Because some find startle facilitation when the sensory modality of the startle stimulus matches the modality of the stimulus to which attention is being directed (Bohlin and Graham, 1977; Bohlin et al., 1981; Hackley and Graham, 1983), one might argue that startle potentiations among white-noise but not airpuff subjects were enhanced by attentional effects and are thus incomparable. This is not thought to be the case for several reasons. For one, the assumption that attentional modification of startle is modality specific has been challenged by findings of startle enhancement when attention is directed to lead stimuli in sensory modalities that differ from the startle probe modality (Lipp et al., 1998). This suggests that fear-potentiated startle effects are influenced by attention to lead stimuli of sensory modalities that match or mismatch the modality of the startle probes. Additionally, it is unclear whether participants attend to the acoustic modality during anticipation of aversive noises. Although the current study does not provide a means for testing the direction of participants' attention, it is plausible that participants avert their attention from the acoustic modality as part of a passive avoidance response to the aversive acoustic stimuli. Finally, it is likely that the startle potentiation in the white-noise and airpuff groups were mostly a function of anticipatory anxiety rather than attention given that attentional effects on FPS in threat

studies have been found to be less robust than emotional effects (Böcker et al., in press; Bradley et al., 1990).

Practically, applying low intensity airpuff probes to the study of differential aversive conditioning may be particularly useful. In such conditioning, startle evoked during a cue associated with an aversive event (CS+ or threat cue) is compared to startle evoked during a cue associated with the absence of an aversive event (CS− or safety cue). Ostensibly, the CS− provides a period free of anticipatory anxiety during which the startle response can be measured and compared to startle responses during the CS+. Delivering loud white-noise probes during the CS− is, however, likely to engender anticipatory anxiety to the CS−. In other words, when the onset of the CS− is generally followed by the white-noise probe, the CS− is likely to evoke anxiety associated with the anticipation of the white-noise probe. Future studies might compare startle potentiation to the CS− (relative to ITI) when using white-noise versus low intensity airpuff probes to test the degree to which each probe interferes with the safety signal-value of the CS−.

The application of low intensity airpuff probes may benefit future startle studies in several additional ways. To start, such probes may be less unpleasant for infants and children and more efficacious for both the elderly and those with hearing damage. In addition, airpuff probes provide a useful alternative when studying startle modulation during anticipation or presentation of aversive sounds. Using white-noise, but not airpuff probes in this context might introduce attentional artifacts due to the unimodality of probes and aversive stimuli. Eliciting startle with airpuff probes may also be useful in the fMRI context where loud noise from the gradient switching of the imaging system is likely to interfere with the processing of acoustic stimuli (Mathews, 2001). Furthermore, the airpuff probe may allow researchers to optimize response probabilities on a subject by subject basis. A 3 psi probe eliciting blinks 80% of the time in a given individual could be raised anywhere up to 60 psi in an effort to improve response probability. This technique could not be used in the case of a 102 dB white-noise probe with an 80% response probability because the 102 dB intensity is already approaching the limit of what is considered safe.

The fear-potentiated startle paradigm employed in the current study was created in our laboratory to assess anxious arousal during threat of aversive stimuli and was particularly designed to test the notion that "unpredictability" is a stimulus characteristic that increases the anxiogenic quality of aversive events (Maier, 1991; Mineka and Kihlstrom, 1978; Staub et al., 1971). To this end, anxious arousal associated with both predictable and unpredictable threat was measured. Although both probe groups displayed enhanced startle magnitudes during the predictable threat condition, neither group displayed larger startle magnitudes to the unpredictable relative to predictable context (although subjects reported more anxiety to the unpredictable versus predictable context). Such results are not seen as evidence against the anxiogenic properties of "unpredictability", but may rather result from the use of an aversive stimulus that is insufficiently anxiogenic. This interpretation is consistent with past results demonstrating unpredictability effects on startle only when using sufficiently aversive unconditioned stimuli (Grillon et al., 2004).

In summary, airpuff stimuli rated as relatively non-aversive elicited the fear-potentiated and unmodulated startle response with the same efficacy as the more common and more aversive white-noise probe. Fear-potentiated startle experiments may benefit from the use of the airpuff probe by allowing for startle elicitation with less disruption of ongoing

emotional and attentional processes. Additionally, use of the airpuff probe may improve the viability of studying startle phenomena in infants, children, the elderly, and individuals with hearing damage. Finally, application of the airpuff probe may prove particularly useful for maximizing response probabilities, eliciting blinks in the fMRI environment, and testing emotional reactions to acoustic stimuli.

References

Amercian Academy of Pediatrics Committee on Environmental Health, 1994. Noise pollution: neonatal aspects. Pediatrics 54, 476–479.

Ashcroft, K.R., Guimaraes, F.S., Wang, M., Deakin, J.F.W., 1991. Evaluation of a psychophysiological model of classical fear conditioning in anxious patients. Psychopharmacology 104, 215–219.

Ben-Tovim, D.I., Potts, N.L., Dortmans, R.J., Weiss, A.M., Potter, R., Jones, E.M., White, A.J., 1990. Acquired hearing impairment and psychiatric disorders amongst Australian Vietnam veterans. Lancet 336 (8710), 324.

Böcker, K.B.E., Baas, J.M., Kenemans, J.L., Verbaten, M.N., in press. Differences in startle modulation during instructed threat and selective attention. Biological Psychology.

Bohlin, G., Graham, F.K., 1977. Cardiac deceleration and reflex blink facilitation. Psychophysiology 14, 423–430.

Bohlin, G., Graham, F.K., Silverstein, L.D., Hackley, S.A., 1981. Cardiac orienting and startle blink modification in novel and signal situations. Psychophysiology 18, 603–611.

Bradley, M.M., Cuthbert, B.N., Lang, P.J., 1990. Startle reflex modification: emotion or attention. Psychophysiology 27, 513–522.

Cuthbert, B.N., Bradley, M.M., Lang, P.J., 1996. Probing picture perception: activation and emotion. Psychophysiology 33, 103–111.

First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B.W., 2001. Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (Research Version). New York State Psychiatric Institute, New York.

Flaten, M.A., Blumenthal, T.D., 1999. A parametric study of the separate contributions of the tactile and acoustic components of airpuffs to the blink reflex. Biological Psychology 48, 227–234.

Grillon, C., 2002. Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. Biological Psychiatry 52, 958–975.

Grillon, C., Ameli, R., 1998. Effects of threat and safety signals on startle during anticipation of aversive shocks, sounds, and airblasts. Journal of Psychophysiology 12, 329–337.

Grillon, C., Ameli, R., Woods, S.W., Merikangas, K., Davis, M., 1991. Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. Psychophysiology 28, 588–595.

Grillon, C., Dierker, L., Merikangas, K.R., 1998a. Fear-potentiated startle in adolescent offspring of parents with anxiety disorders. Biological Psychiatry 44, 990–997.

Grillon, C., Morgan, C.A., 1996. Fear-potentiated startle in Vietnam veterans with PTSD. Biological Psychiatry 39, 555.

Grillon, C., Morgan, C.A., Davis, M., Southwick, S.M., 1998b. Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. Biological Psychiatry 44, 1027–1036.

Grillon, C., Baas, J.M., Lissek, S., Smith, K., Milstein, J., 2004. Anxious responses to predictable and unpredictable aversive events. Behavioral Neuroscience 118 (5), 916–924.

Hackley, S.A., Graham, F.K., 1983. Early selective attention effects on cutaneous and acoustic blink reflexes. Physiological Psychology 11, 235–242.

Haerich, P., 1994. Startle reflex modification: effects of attention vary with emotional valence. Psychological Science 5, 407–410.

Haerich, P., 1998. Using airpuffs to elicit the human blink reflex. Behavior Research Methods, Instruments, and Computers 30, 661–666.

Hawk, L.W., Cook, E.W., 1997. Affective modulation of tactile startle. Psychophysiology 34, 23–31.

Ison, J.R., Russo, J.M., 1990. Enhancement and depression of tactile and acoustic startle reflexes with variation in background noise level. Psychobiology 18, 96–100.

Lang, P.J., Bradley, M.M., Cuthbert, B.N., 1998. Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. Biological Psychiatry 44 (12), 1248–1263.

Lipp, O., Siddle, D., Dall, P., 1998. Effects of stimulus modality and task condition on blink startle modification and on electrodermal responses. Psychophysiology 35, 452–461.

Ludewig, K., Ludewig, S., Seitz, A., Obrist, M., Geyer, M.A., Vollenweider, F.X., 2003. The acoustic startle reflex and its modulation: effects of age and gender in humans. Biological Psychology 63, 311–323.

Lykken, D.T., 1972. Range correction applied to heart rate and GSR data. Psychophysiology 9, 373–379.

Maier, S.F., 1991. Stressor controllability, cognition and fear. In: Madden, J.I. (Ed.), Neurobiology of Learning, Emotion and Affect. Raven Press Ltd., New York, pp. 159–193.

Mathews, P.M., 2001. An introduction to functional magnetic resonance imaging of the brain. In: Jezzard, P., Matthews, P.M., Smith, S.M. (Eds.), Functional MRI: An Introduction to Methods. Oxford University Press, New York.

Merikangas, K.R., Avenevoli, S., Dierker, L., Grillon, C., 1999. Vulnerability factors among children at risk for anxiety disorders. Biological Psychiatry 46 (11), 1523–1535.

Miller, M.W., Curtin, J.J., Patrick, C.J., 1999. A startle-probe methodology for investigating the effects of active avoidance on negative emotional reactivity. Biological Psychology 50 (3), 235–257.

Mineka, S., Kihlstrom, J.F., 1978. Unpredictable and uncontrollable events: a new perspective on experimental neurosis. Journal of Abnormal Psychology 87, 256–271.

Morgan III, C.A., Grillon, C., Southwick, S.M., Charney, D.S., 1996. Exaggerated acoustic startle reflex in Gulf war veterans with posttraumatic stress disorder. American Journal of Psychiatry 153, 64–68.

National Campaign for Hearing Health, 2004. Retrieved January 30, 2004, from http://www.hearinghealth.net/cms/index.cfm?displayarticle=16.

Orr, S.P., Solomon, Z., Peri, T., Pitman, R.K., Shalev, A.Y., 1997. Physiologic responses to loud tones in Israeli veterans of the 1973 Yom Kippur War. Biological Psychiatry 41 (3), 319–326.

Patrick, C.J., Berthot, B.D., 1995. Startle potentiation during anticipation of a noxious stimulus: active versus passive response sets. Psychophysiology 32, 72–80.

Peri, T., Ben-Shakhar, G., Orr, S.P., Shalev, A.Y., 2000. Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. Biological Psychiatry 47 (6), 512–519.

Pizzagalli, D.A., Greischar, L.L., Davidson, R.J., 2003. Spatio-temporal dynamics of brain mechanisms in aversive classical conditioning: high-density event-related potential and brain electrical tomography analyses. Neuropsychologia 41 (2), 184–194.

Pliszka, S.R., Hatch, J.P., Borcherding, S.H., Rogeness, G.A., 1993. Classical conditioning in children with attention deficit disorder (ADHD) and anxiety disorders: a test of Quay's model. Journal of Abnormal Child Psychology 21, 411–423.

Prokasy, W.F., Ebel, H.C., 1967. Three components of the classically conditioned GSR in human subjects. Journal of Experimental Psychology 73, 247–256.

Skolnick, A.J., Davidson, R.J., 2000. Affective modulation of eyeblink startle with reward and threat. Psychophysiology 39, 835–850.

Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P., Jacobs, G.A., 1983. Manual for the State-Trait Anxiety Inventory. Consulting Psychologist Press, Palo Alto, CA.

Staub, E., Tursky, B., Schwartz, G., 1971. Self-control and predictability: their effects on reactions to aversive stimulation. Journal of Personality and Social Psychology 18, 157–162.

Tabachnick, B.G., Fidell, L.S., 1996. Using Multivariate Statistics, third ed. Harper Collins College Publisher, New York.

Vrana, S.R., Spence, E.L., Lang, P.J., 1988. The startle probe response: a new measure of emotion. Journal of Abnormal Psychology 97, 487–491.