SMOKING-CUE MODULATION OF THE STARTLE REFLEX

AND THE RELATIONSHIP TO STAGES OF CHANGE

by

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ABSTRACT

The current study investigated the appetitive nature of smoking-related drug cues among 22 nicotine dependent males, taking into consideration participants' Stage of Change. Affective modulation of the startle reflex was measured in order to gauge affective response to positive, negative, neutral, and smoking images. Results revealed that smoking images were physiologically pleasant, as indicated by startle reflex attenuation, for participants (N = 11) in the Precontemplation stage. However, those participants in the contemplation stage showed atypical affective modulation, with responses to positive images similar to neutral images. Additionally, those participants in the Contemplation stage (N = 11) rated smoking images similar to neutral images, while those in the Precontemplation stage rated these images similar to pleasant images. Overall results support the notion that smoking cues are physiologically appetitive in nature for nicotine dependent individuals, after accounting for Stage of Change.

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CHAPTER 1
INTRODUCTION

In the addicted individual, drug-cues are known to increase motivation to consume the addictive substance (Carter & Tiffany, 1999). The process by which drug-cues become influential in drug-seeking motivation is thought to be due to a combination of the classical conditioning of stimuli present in the environment (e.g., images, sounds), as well as the addictive substance's interoceptive effects (see Glautier, 2004 for review). The reinforcing properties that facilitate an individual's motivation to consume the addictive substance in the presence of such cues are less certain and are at the center of an ongoing theoretical debate. Motivation to consume a substance of abuse may be due to the result of negative reinforcement, such that the facilitating process is a desire to eliminate an aversive withdrawal-like state brought about by the cues (Siegel, 1979). Conversely, motivation to consume the substance may also be the result of positive reinforcement, such that the positive state induced by the substance is primed through conditioned cues (Stewart, de Wit, & Eikelboom, 1984).

Research involving cue-reactivity among nicotine dependent individuals has been mixed, with both aversive (Elash et al., 1995) and appetitive (Geier et al., 2000) responses documented. In addition to the widely differing methodological techniques employed, this relatively new research area of affective reaction to smoking-cues has also relied heavily on the subjective reports of participants indicating their affective responses (e.g., McDermut & Haaga, 1998; Mucha, Geier, & Pauli, 1999). More recently, this line of research has begun to use more objective, psychophysiological measures of individuals' affective responses to smoking cues while viewing affect-laden photographic images. However, these recent studies have neglected to incorporate important individual differences among the participants, which may help to explain the mixed findings.

Specifically, not all nicotine-dependent individuals intend to continue smoking indefinitely and as a result may have different affective responses to cues associated with this behavior. Indeed, examination of the Stages of Change in a large-scale randomized survey found that 40% of smokers are not considering changing their habit (Precontemplation), 40% are considering quitting within 6 months (Contemplation), and 20% are planning on quitting within the next 30 days (Preparation; Velicer et al., 1995). Given the substantial difference observed in the Stages of Change among the general smoking population, it is possible that individual affective responses to smoking-cues also differ. In fact, the one identified study which assessed affective responses to smoking-cues, within the context of the Stages of Change, found differences between Contemplators and Precontemplators (McDermut & Haaga, 1998). Specifically, Contemplators reported greater feelings of guilt in response to smoking-cues, in comparison to Precontemplators. However, affective responses were assessed only subjectively via participant self-report. More objective methods of measuring cue-reactivity, in conjunction with Stages of Change, have yet to be utilized.

Modulation of the acoustic startle reflex is a technique through which affective responses to smoking cues may be gauged objectively. The acoustic startle reflex is a non-conscious behavioral reaction occurring in the presence of a sudden, intense noise.

Further, the startle reflex can be reliably modulated in the presence of an emotionally salient event. Simply stated, if a startle reflex occurs while an individual is experiencing a positive or pleasant event, the startle reflex is inhibited. Conversely, if a startle reflex occurs when an individual is experiencing a negative or unpleasant event, the startle reflex is magnified (Peter J. Lang et al., 1990). Perhaps the most common method of inducing differing affective experiences is via the presentation of an emotionally evocative picture, followed by a startle probe. Standardized images ranging from unpleasant to pleasant have been widely utilized in research incorporating affective modulation of the startle reflex (Center for the Study of Emotion and Attention, 1995). Given that affective modulation of startle is highly replicable, outside the control of the participant, and highly responsive to changes in stimuli (see Bradley, Cuthbert, & Lang, 1999 for review), it provides an excellent objective technique for assessing affective responses.

Following a thorough review of the literature, only two studies could be identified that specifically examined the potential influence of smoking cues on the affective modulation of the startle response. The first study (Geier, Mucha, & Pauli, 2000), examined the effect of smoking cues on the startle reflex via presentation of smoking cue pictures to smokers and non-smokers. Further, they presented non-smoking-cue affective pictures to allow for comparison with standardized pleasant, neutral, and unpleasant images. Results from this study revealed that smokers showed significant startle attenuation in the presence of smoking cues. Specifically, smokers' responses to smoking cues were shown to be significantly lower than those observed for unpleasant affective pictures and similar in magnitude to pleasant affective pictures. Conversely, non-smokers showed significant startle magnification when viewing smoking-cue stimuli. In particular, non-smokers' responses to smoking cues were significantly larger than those observed with the pleasant affective pictures, and similar to responses observed with unpleasant pictures. Additionally, this psychophysiological data corresponded nicely with participants' subjective ratings. Specifically, participants' self-report reactions indicated that smokers perceived smoking-cues as pleasant while non-smokers perceived smoking-cues as unpleasant. In all, these results suggest that smoking-cues may induce an appetitive motivation in the addicted individual (Stewart, de Wit, & Eikelboom, 1984) rather than engendering a negative withdrawal-like state that motivates consumption (Siegel, 1979).

The findings of Geier and colleagues (2000), however, are in contrast to a more recent study comparing the modulatory effect of smoking-cue pictures on the startle reflex among smokers and non-smokers (Orain-Pelissolo et al., 2004). This group failed to find significant differences between smokers and non-smokers with respect to any modulatory effect for smoking-cue pictures. Orain-Pelissolo et al. concluded therefore, that "…smoking cues have no effect on the positive reinforcement of nicotine consumption" (pg. 165). Given the discrepant findings across these two studies, it is possible differing methodologies led to the conflicting results. For example, different smoking cue images were used and participants differed greatly in level of nicotine dependence. Another important consideration is that both of these studies neglected to take into account the Stages of Change among their participants. However, Geier and colleagues do suggest that motivational factors may be influential and suggest the consideration of these factors in future research. Such motivational information may be useful in developing a better understanding of why disparate results have been documented in the psychophysiological literature. Indeed, previous research has documented self-report differences in smoking-cue reactivity among nicotine-dependent participants when considering Stage of Change (McDermut & Haaga, 1998).

The present study was designed to assess affective reaction to smoking-cues among nicotine dependent participants, through objective psychophysiological measurement, considering the Stage of Change of the participant. Given the substantial motivational differences between Precontemplators and Contemplators (i.e., not considering quitting vs. considering quitting), and also given that these two groups comprise the vast majority of the smoking population (approximately 80%; Velicer et al., 1995), smokers included in this study were from one of these two groups. Acoustic startle probes were administered while participants viewed standardized positive, negative, and neutral images from the International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1995) as well as while viewing standardized smoking-cue images utilized by Geier et al. (2000). The Self-Assessment Manikin (SAM) was also utilized to collect self-reported affective responses to the present images (Margaret M. Bradley & Lang, 1994).

The present study utilized only male dependent smokers given the well documented gender differences on several of the components of the study. Reliable gender differences have been documented in affective modulation of the startle reflex by picture category type (M. M. Bradley, Codispoti, Sabatinelli, & Lang, 2001), as well as other physiological and neuro-imaging reactions to emotional images (Sabatinelli, Flaisch, Bradley, Fitzsimmons, & Lang, 2004; Schupp et al., 1996; Wrase et al., 2003). Additionally, differential physiological reaction by gender has been documented in response to smoking cues among dependent smokers (Field & Duka, 2004). However, assessment of both genders is particularly important for this line of research and is discussed further at the end of this document.

Hypotheses

For the present study, it was expected that both Precontemplators and Contemplators would follow the expected affective modulation of startle patterns for the standardized positive, negative, and neutral images. That is, startle reflex magnitudes were expected to be increased when viewing negative images and decreased when viewing positive images (see Lang, et al., 1990 for review). Given that affective modulation of the startle reflex is robust and highly replicable (Dawson, Schell, & Boehmelt, 1999), the typical affective modulation pattern was not expected to differ in the current study. In fact, in a replication study, pleasantness ratings were found to have a Pearson correlation of .99 to IAPS standardized ratings (Cuthbert, Bradley, & Lang, 1996). However, several hypotheses regarding modulation to smoking-cue images and the Stage of Change were made.

Hypothesis 1: It was hypothesized that significant startle attenuation would be seen in response to smoking-cue images, for the Precontemplation group only. That is, for the Precontemplation group, startle reflex magnitudes while viewing smoking images would be significantly smaller in comparison to startle reflex magnitudes while viewing neutral images.

While inconsistencies exist, a wealth of research has documented positive self-reports among addicted individuals, in response to substance-cues. For example, smokers and alcoholics viewing substance-specific cues subjectively reported increases in craving, without reporting unpleasantness, and also “calming” feelings in response to the cues (Mucha et al., 1999). Additionally, among alcoholics, images of drinking-cues have been documented as having similar startle reflex magnitudes as those seen with the standardized pleasant images (Mucha et al., 2000). While the two primary studies investigating smoking-cue modulation of startle documented inconsistent results (Geier et al., 2000; Orain-Pelissolo et al., 2004), these inconsistencies are thought to reflect a neglected consideration of Stage of Change in their participants. Research has shown that as smokers transition from Precontemplation to Contemplation, they become more cognizant of the negative implications of smoking (Velicer, DiClemente, Prochaska, & Brandenburg, 1985). Smokers in the Precontemplation stage, therefore, being less cognizant of the negative implications, are hypothesized to show a stronger positive affective response to smoking-cues (i.e., significant startle attenuation).

Hypothesis 2: Given that nicotine-dependent smokers who are classified as Contemplators are believed to be more cognizant of the negative implications of their habit (Velicer, et al., 1985), yet are still addicted to the substance, it was hypothesized that smoking-cues would engender ambivalent approach-avoid motivations. Thus, it was hypothesized that Contemplators’ startle magnitude, when viewing smoking-cues, would be significantly larger than standardized pleasant image responses and significantly lower than standardized unpleasant images – being similar to standardized neutral image reactions.

Hypothesis 3: It was hypothesized that Contemplators' startle magnitudes would be significantly higher than Precontemplators' startle magnitude, when viewing smoking-cue images. That is, it was hypothesized that smokers in the Contemplation stage will have a less pleasant reaction to smoking-cues than their Precontemplator counterparts. This view is based on the findings of Velicer, DiClemente, Prochaska, and Brandenburg (1985), who found that Contemplators report a higher awareness of the negative aspects of smoking, compared to their Precontemplator counterparts. Additionally, McDermut and Haaga (1998), using subjective self-reported reactions to smoking-cue images, found that Contemplators responded with greater feelings of "guilt" than Precontemplators to smoking-cue images.

Hypothesis 4: it was hypothesized that self-reported emotional reactions (SAM) would coincide with the hypothesized physiological reactions. That is, it was hypothesized that self-report ratings results would be consistent with those hypothesized for physiological results (consistent with hypotheses 1 through 3). Thus, it was hypothesized that:

4a) Only Precontemplators would rate smoking images as significantly more pleasant than neutral images.

4b) Contemplator ratings of smoking images would be similar to their neutral image ratings, being rated significantly more pleasant than negative images and significantly less pleasant than positive images.

4c) Precontemplators would rate smoking images significantly more pleasant than Contemplators

CHAPTER II

METHOD

Participants

Twenty-six male nicotine-dependent smokers participated in the current study. Participants were identified through a screening survey conducted in Introductory Psychology classes and through on-campus advertisement. Four participants were excluded from the current study, as one participant disclosed current illicit drug use (subsequent to running experimental protocol), one fell asleep during the protocol, and while running two participants equipment failure occurred. This resulted in a total of 22 participants included for all subsequent analyses. Smoking status was biologically verified, with a required Carbon Monoxide (CO) expired breath reading of ≥ 10ppm and nicotine dependence was verified by self-report, with a required score of ≥ 4 on the Fagerström Test of Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991). The average CO reading was 22.95 (SD = 11.72, Range = 41), while the average Fagerström score was 5.45 (SD = .91, Range = 3), indicating a medium level of nicotine dependence (Fagerstrom, Heatherton, & Kozlowski, 1990).

Participant exclusion criteria included: (1) current or previous cardiovascular disorder, (2) known or suspected hearing difficulties, (3) ongoing illicit substance use, and (4) current use of psychotropic medications. The exclusionary criteria were assessed via a screening measure and also a demographic/participant variable measure completed prior to study. Participants recruited from Introductory Psychology courses were awarded class points contributing to their course grade (*n* = 7). Participants recruited from campus were entered in a raffle for one first place prize ($75.00) and two second place prizes ($50.00) as compensation for participation (*n* = 16).

Given that approximately 70% of dependent college smokers can also be classified as "binge drinkers" (Schorling, Gutgesell, Klas, & Smith, 1994), information regarding potential drinking problems was gathered. This information was gathered only to ensure that experimental groups did not significantly differ in their level of potential binge drinking, or time of last consumption. This is particularly important given that nicotine dependence is quite common in alcohol disorders, with some estimates as high as 87% of all alcohol dependent individuals being nicotine dependent as well (John, Hill, Rumpf, Hapke, & Meyer, 2003). Ensuring that the potential for alcohol disorders does not differ across groups decreases the chance of a cross-cue activation confound (e.g., smoking images increasing urge to drink), which has been documented (Drobes, 2002).

Participants were further selected based on their current Stage of Change, which included eleven Precontemplators and eleven Contemplators. The size of eleven per group was determined by conducting a power analysis utilizing previously reported affective modulation of startle data to standardized pleasant, unpleasant, and neutral images (Vrana, Spence, & Lang, 1988). The power analysis was conducted using the procedures and power chart recommended by Keppel (1991). The results indicated that a sample size of eleven per group, with an alpha level of .05, would result in power of approximately .80. The chosen group size of eleven also appears appropriate when considering the large effect size documented in several related studies. A meta-analysis of cue reactivity found large effect sizes for physiological measures of smokers in response to smoking-cues, in both heart rate (d = .21) and sweat gland activity (d = .44) (Carter & Tiffany, 1999). Additionally, affective modulation of startle with standardized images has been documented as having a substantially large effect size (d = .93) (Bradley, Cuthbert, & Lang, 1996).

In order to account for any demographic or other participant-specific variables, preliminary analyses were conducted. Both the Precontemplator and Contemplator groups were similar in ethnic background, being primarily Caucasian (73% in each group). The participants did not significantly differ in age [$F(1, 21) = 0.95$, $p > .05$], education [$F(1, 21) = 0.95$, $p > .05$], reimbursement type ($\chi^2 = .210$, $p > .05$), pre-experiment carbon monoxide level [$F(1, 21) = 0.2$, $p > .05$], Fagerström Test for Nicotine Dependence score [$F(1, 21) = 0.00$, $p > .05$], or score on Alcohol Use Disorders Identification Test [$F(1, 21) = .102$, $p > .05$]. Presentation order of experimental images was counter-balanced to control for potential order effects. Further information regarding participant variables can be found in Table 1 and Appendix B.

Materials

Carbon Monoxide Measurement

Carbon monoxide (CO) levels were collected using a Micro CO Meter (MicroDirect, Inc.). CO readings have been found to discriminate reliably between smokers and non-smokers and been found to highly correlate ($r = .97$) with Carboxyhaemoglobin levels, a blood screening for carbon monoxide level (Wald, Idle, Boreham, & Bailey, 1981). A CO reading of 10 parts per million (ppm) has been suggested as a level indicative of high nicotine dependence (Tonnesen, Norregaard, Mikkelsen, Jorgensen, & Nilsson, 1993; Wald et al., 1981), and a cut-off score of 5ppm showed sensitivity of 96% and specificity of 98% for identification of a regular smoker (Low, Ong, & Tan, 2004).

Stage of Change Measure

The Stage of Change theory is based on the Transtheoretical Model which postulates that behavior change occurs through a series of stages, rather than a singular event (Velicer, Prochaska, Fava, Norman, & Redding, 1998). The Smoking Stage of Change Form is a set of three questions which categorize smokers as being in either the Precontemplation stage, Contemplation stage, Preparation stage, Action stage, or Maintenance stage (C C DiClemente et al., 1991; W. F. Velicer et al., 1995). Smokers in different Stages of Change have been documented as having different affective responses to smoking-cues (McDermut & Haaga, 1998), and report different levels of pros and cons associated with smoking (Velicer et al., 1985). Previous research (Morera et al., 1998) has revealed that the Stages of Change model in smokers has sound psychometric qualities, having an adequate fit ($\chi^2 = 6.33$, $p < .79$), an adjusted goodness of fit = .98, stability ranging from .88 to .98, and reliability ranging from .69 to .76. See Appendix C for a copy of this measure.

Self-Assessment Manikin

The Self-Assessment Manikin (SAM) is a picture-oriented instrument devised as a non-verbal assessment scale of pleasure, arousal, and dominance associated with the perception of an object or image (see Bradley & Lang, 1994). These three domains are assessed by the rater placing an “x” on, or in between, a character displaying a linear progression of pleasure, arousal, and dominance, allowing for a 9-point rating scale for each dimension. A replication study comparing a smaller sample of participant SAM ratings to the International Affective Picture System ratings of standardized positive negative and neutral images (Center for the Study of Emotion and Attention, 1995) showed a Pearson correlation of .99 for pleasure and .93 for arousal ratings. Additionally, comparison of SAM ratings to a different type of rating scale (Semantic-Differential Scale) showed Pearson correlation ratings of .94 for pleasure, .94 for arousal, and .66 for dominance (Lang, 1985). In line with the current study's hypotheses, participants ranked images on only the pleasure scale. See Appendix D for a copy of this measure.

Fagerström Test of Nicotine Dependence

The Fagerström Test of Nicotine Dependence (FTND; Heatherton et al., 1991) is a 6-item self-report measure of nicotine dependence. Scores can range from 0 to 10, with increasing values suggesting higher levels of nicotine dependence. The FTND is an adaptation of the Fagerström Tolerance Questionnaire (FTQ; Fagerström, 1978) that has been shown to have improved internal consistency ($\alpha = .61$) to its' predecessor (Heatherton et al., 1991). Total score on the FTND has been shown to be related to prediction of continued smoking (F [2, 7992] = 163.2, $p < .001$), is significantly correlated with years of smoking ($r = .25$, $p < .001$), and a 6-week "reliability of recall" analysis (having participants recall their previous ratings following 6 weeks of abstinence) was high ($r = .87$, $p < .001$; Haddock, Lando, Klesges, Talcott, & Renaud, 1999). See Appendix E for a copy of this measure.

Alcohol Use Disorders Identification Test

The Alcohol Use Disorders Identification Test (AUDIT) is a 10-item self-report instrument designed to identify harmful drinking patterns and negative consequences related to past drinking. Responses can range from 0 to 4. The AUDIT was developed by researchers working for the World Health Organization (Saunders, Aasland, Babor, De La Fuente, & Grant, 1993). Psychometric properties for this instrument are sound, showing a test-retest reliability of .86, internal consistency coefficient alphas ranging from .75 to .94, and a cut-off score of 8 has been documented with sensitivity of .77 and specificity of .81 (See Measurement Excellence and Training Resource Information Center, 2006, for review of psychometric properties). However, the psychometric data regarding sensitivity and specificity noted above was based upon adult medical setting populations. A cut-off score of 9 has been shown to have sensitivity of .62 and specificity of .86 for past year alcohol problems in a college sample (Kokotailo et al., 2004). See Appendix F for a copy of this measure.

Startle Modulation Images and Presentation

The photographic images presented included the 64 images utilized by Geier et al., 2000. The images were presented on a 17 inch LCD computer monitor (Dell Ultrasharp 1703FPs), approximately 4 feet in front of the participant. The 64 images included 16 smoking images, 16 International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1995) neutral images (2840, 5534, 6150, 7000, 7002, 7006, 7010, 7034, 7050, 7090, 7150, 7217, 7025, 7030, 7040, 7233), 16 IAPS pleasant images (1710, 4660, 5621, 5700, 5910, 7270, 7502, 8030, 8080, 8200, 8370, 8380, 8190, 8420, 5480, 8470), and 16 IAPS unpleasant images (1300, 3060, 3102, 3170, 3530, 6212, 6230, 6313, 9410, 9570, 9910, 9921, 3000, 3150, 6560, 9252). Consistent with Geier et al. (2000) the images were presented in two image-type balanced sets of 32 pictures, in five pseudorandomized orders. Pictures were presented for varying times between 7-8 seconds, with inter-picture intervals (black screen) varying between 17-26 seconds. For two-thirds of the images, acoustic startle probes were presented approximately 4 seconds post presentation of the image. A probe latency of approximately 4 seconds was chosen as previous research has documented that at a latency of 3 seconds affective modulation magnitude reaches asymptote (M.M. Bradley, Drobes, & Lang, 1996). In order to control for expectancy effect in startle probe timing, the 4 second onset was used as an average, with a range of presentation being from 3 to 5 seconds. Acoustic probes were 95 dB (SPL A) white noise, 50 ms in duration, with instantaneous rise time (<1ms), and were administered binaurally via Optimus Pro headphones (model 135).

Procedure

Participants were screened for eligibility by completing the Stage of Change form and Fagerström Test of Nicotine Dependence. Participant screening from Introductory Psychology courses completed the measures as part of a "mass survey," which included screening measures from other studies being conducted. Students recruited from campus were screened via telephone to verify inclusion criterion. Eligible participants were then scheduled to participate in the study. Participants were instructed to smoke at their usual rate throughout the day of testing but were informed that they would be asked to smoke one cigarette just prior to the experimental session. After arriving for the experiment the participants reviewed and signed the consent form and completed initial measures (e.g., demographic questionnaire, list of current medications). The experimenter escorted the participants outside the laboratory for the pre-session cigarette in order to verify consumption and to standardize the time of last cigarette across participants. Upon returning to the laboratory, participants were seated in a chair facing a computer monitor. For safety and consistency purposes, acoustic startle probe intensity was verified to be at 95dB, prior to each experimental session, via a Radioshack Digital Sound Level Meter (model 33-2055). Electrodes were then attached, verified for signal integrity, and a preprogrammed experimental session was administered using Coulbourn Instruments LabLinc V modular instrument system, in conjunction with Coulbourn Instruments Human Startle Software (version 5.100-00). Consistent with Geier et al. (2000), prior to administration of the experimental session, a habituation trial was conducted, which included 10 startle probes. The habituation probes were presented while viewing one smoking, pleasant, unpleasant, and neutral image, and 6 black-screen images (in randomized order). The participants were instructed to view each picture and ignore any sounds coming from the headphones. Subsequent to administration of habituation probes, the 2-block preprogrammed experimental session was initiated. Between blocks, participants were instructed to rest for 3 minutes in their chair. Following the completion of the experimental sessions, participants were shown paper versions of the 64 images viewed and completed SAM ratings for each image.

Physiological Data Acquisition

Probe locations were chosen based on previous research as well as standardization recommendations by the Society for Psychophysiological Research (Blumenthal et al., 2005; Dawson et al., 1999). Prior to probe placement, the sites were cleansed with an alcohol wipe, and abraded with Nuprep skin prepping gel (Weaver & Company). Ag-AgCl 4 mm surface electrodes (In Vivo Metric, E220-LS) were then filled with Microlyte Gel (Coulbourn Instruments) and placed in a bipolar configuration on the left *orbicularis oculi* (see Appendix G for representation). A reference (ground) electrode was also placed on the left *mastoid process* (bony region located behind ear left ear). Once placed, electrodes were verified to have an impedance level below 10 KOhms via an electrode impedance meter (UFI Checktrode, model 1089mkIII). The raw electromyography (EMG) signal was collected using an amplification setting of 50,000 on a Coulbourn V75-05 Bioamplifier. The signal was then full-wave rectified using a bandpass filter setting of 8-150 Hz, and a time-constant of 10ms, on a Coulbourn V76-23 contour-following integrator.

The integrated EMG signal was then manually-scored by the investigator using Coulbourn Instruments Human Startle Software. Consistent with recent guidelines published by the Society for Psychophysiological Research (Blumenthal et al., 2005), eyeblink reflex magnitudes were calculated by taking the difference between baseline (data point just prior to response onset) and peak integrated EMG signal between 21 to 120 ms post probe onset. Trial rejection criterion include: a) excessive noise ($\geq$ 20 μV) during baseline and/or b) natural (non-startle) blink activity occurring at 0 to 21 ms post probe onset.

Data Analysis

Given that tonic startle levels typically differ from person to person, EMG data was standardized within subject, and averaged by picture type, a procedure commonly utilized by affective modulation of startle researchers (Blumenthal et al., 2005). Given that the SAM data are on the same scale across participants, scores were averaged overall by picture category. Primary analyses included Profile Analyses, or Repeated Measures Multivariate Analysis of Variance. Family-wise error variance was utilized for all analyses. No alpha corrections were used for tests of *a priori* hypotheses. For non-planned, or exploratory analyses, a modified Bonferroni correction was utilized to control for alpha (Keppel, 1991).

CHAPTER III
RESULTS

Affective Modulation of Startle

Preliminary Manipulation Check

A 2X4 (group, picture type) Profile Analysis (repeated-measures MANOVA) was conducted on EMG-recorded *orbicularis oculi* startle reflex magnitude while viewing experimental images. This analysis was conducted to multivariately test for overall differences in Stage of Change groups, differences between experimental picture type, and potential interactions. Test of flatness was significant, $F(3, 18) = 13.20$, $p < .001$, Pillai's Trace, indicating a difference between picture types. Results for level and parallelism were non-significant [$F(1, 20) = .208$, $p > .05$, $F(3, 18) = 1.68$, $p > .05$, respectively], indicating that analyses failed to show an overall difference between Stage of Change groups or an interaction between groups and pictures. Mean and standard deviation scores of EMG by picture and group are listed in Table 2.

Subsequent to the significant flatness in the profile analysis, simple multivariate analyses were conducted to determine if the same pattern existed for groups separately. For both the Precontemplation and Contemplation groups, results were significant for both tests of flatness [$F(3, 8) = 10.67$, $p = .001$, Pillai's Trace, $F(3, 8) = 4.82$, $p = . 034$, Pillai's Trace, respectively], indicating significant differences between picture types for each group independently.

In order to determine which combinations of picture types specifically differed in the overall and simple multivariate analyses, contrast coefficients were conducted. First, contrasts were conducted for picture type overall, collapsing between groups. A modified Bonferroni adjustment was computed in order to control for alpha. Results indicated that startle reflex magnitude while viewing negative images was significantly larger than while viewing positive, neutral and smoking images. See Table 3 for statistical data regarding EMG values for overall picture-type contrasts. Of the non-significant contrasts, one pair was particularly notable given the significance prior to modified Bonferroni alpha adjustment and trend towards significance post alpha adjustment. Specifically, EMG startle reflex showed a trend towards significance for smaller startle values while viewing smoking images, in comparison to startle reflexes while viewing neutral images $F(1, 21) = 5.611$, $p = .056$ (modified Bonferroni applied).

Contrast coefficients were then conducted separately for each group. For the Precontemplation group, EMG startle magnitude while viewing negative images was significantly larger than while viewing positive, neutral, and smoking images. EMG startle magnitude while viewing smoking images was significantly smaller than while viewing neutral images. For the Contemplation group, only one set of pair wise comparisons reached significance, with EMG startle magnitude while viewing negative images being larger than while viewing smoking images. For statistical data on picture-type contrasts by group, see Table 4. Additionally, see Figure 1 for mean ratings of images, by group, in graphical form.

Test of Hypothesis 1

For this hypothesis it was predicted that significant startle attenuation would be seen for smoking-cues, for the Precontemplation group only. As expected, the

Precontemplation group showed significantly lower EMG startle magnitude while viewing smoking images compared to when they viewed neutral images, $F(1, 10) = 4.37$, $p = .032$, one-tailed. Also as expected, this same test for the Contemplation group failed to reach significance, $F(1, 10) = 2.21$, $p > .05$.

Test of Hypothesis 2

For hypothesis 2 it was predicted that Contemplators' startle magnitudes when viewing smoking images would be significantly smaller than while viewing negative images, and significantly larger than while viewing positive images. As expected, the Contemplation group EMG startle magnitude while viewing smoking images was significantly lower than while viewing negative images [$F(1, 10) = 12.77$, $p = .003$, one-tailed]. However, smoking image EMG startle magnitude was not found to be significantly larger than positive images [$F(1, 10) = 4.87$, $p > .05$, one-tailed]. In fact, contrary to what was hypothesized, smoking image startle magnitudes were substantially smaller than for positive images, with an uncorrected critical value showing trend towards significance in the opposite direction ($p = .052$).

Test of Hypothesis 3

For hypothesis 3, it was predicted that Contemplators' startle magnitude while viewing smoking images would be significantly larger than Precontemplators' startle magnitude while viewing smoking images. A one-way ANOVA was conducted to determine if Precontemplator EMG startle magnitude while viewing smoking images was significantly smaller than their Contemplator counterparts. This hypothesis was not supported as test results did not reach statistical significance, $F(1, 21) = .37$, $p > .05$, one-tailed.

Self-Report SAM Data

Preliminary Manipulation Check

A 2X4 (group, picture type) Profile Analysis (repeated-measures Multivariate Analysis of Variance) was conducted on Self-Assessment Manikin (SAM) ratings of experimental images to assess for overall differences by group and picture type. Test of flatness was significant [$F(3, 18) = 39.82$, $p < .001$, Pillai's Trace], indicating that picture types significantly differed in self report. The test of levels was non-significant [$F(1, 20) = .01$, $p > .05$], suggesting that groups did not significantly differ overall. Finally, the test of parallelism was also non-significant [$F(3, 18) = .77$, $p > .05$], suggesting a lack of interaction between group and picture type. Means and standard deviation scores of SAM ratings by picture and group are listed in Table 5.

To further understand the multivariate significant difference in SAM picture-type ratings, subsequent simple multivariate analyses were conducted, for groups separately, to assess for continuity of picture-type differences at an independent group level. For the Precontemplation group, results indicated a significant effect for pictures, $F(3, 8) = 13.24$, $p = .002$, Pillai's Trace. For the Contemplation group, the results also indicated a significant effect for picture type, $F(3, 8) = 34.15$, $p < .001$, Pillai's Trace. These results indicate that picture-type differences are significant multivariately, and for group when analyses are conducted independently.

Given that differences were found for picture type in both overall and simple multivariate analyses, contrast coefficients were then conducted to determine the specific differences between pictures. Contrasts were first conducted for picture-type overall, collapsing between groups. A modified Bonferroni adjustment was calculated in order to control for alpha on all contrasts that were not *a priori* hypotheses. Results indicated that all combinations of contrasts were significant, even when the more conservative adjusted alpha value was used. All results were in the hypothesized or expected direction, with positive pictures being rated significantly more pleasant than smoking, neutral, and negative images. Smoking images were rated significantly more pleasant than neutral and negative images. Lastly, neutral images were rated significantly more pleasant than negative images. See Table 6 for statistical data regarding overall picture-type contrasts.

Contrast coefficients were also conducted independently within groups. For the Precontemplation group, positive images were rated significantly more pleasant than negative images, and neutral images. Smoking images were rated significantly more pleasant than negative and neutral images, and neutral images were rated as significantly more pleasant than negative images. For the Contemplation group, the same pattern of significant differences were found, except for smoking and neutral images, which did not significantly differ. For statistical data on picture-type contrasts by group, see Table 7. Additionally, see Figure 2 for mean ratings of images by group.

Test of Hypothesis 4

Hypothesis 4a: For hypothesis 4a, it was hypothesized that Precontemplators, but not Contemplators, would rate smoking images significantly more pleasant than neutral images. As expected, for the Precontemplation group, smoking images were rated significantly more pleasant than neutral images [$F(1, 10) = 12.16$, $p = .003$, one-tailed]. Also as expected, this same test for the Contemplation group failed to reach significance, [$F(1, 10) = 1.76$, $p > .05$].

Hypothesis 4b: For hypothesis 4b, it was hypothesized that Contemplators would rate smoking images similar to neutral images, being significantly less pleasant than positive images and rated significantly more pleasant than negative images. As expected, the Contemplation group rated smoking images significantly more pleasant than negative images [$F(1, 10) = 56.34$, $p < .001$, one-tailed] and significantly less pleasant than positive images [$F(1, 10) = 5.68$, $p = .019$, one-tailed].

Hypothesis 4c: For hypothesis 4c, it was hypothesized that Precontemplators would rate smoking images significantly more pleasant than Contemplators. A one-way analysis of variance was conducted and the results did not support this hypothesis, $F(1, 21) = .015$, $p > .05$, one-tailed.

The relationship between self-ratings of EMG startle reflex to experimental images was further assessed through additional, non-proposed analytic procedures. These procedures are beyond the scope of this manuscript and therefore are included in Appendix H.

CHAPTER IV
DISCUSSION

The present study was conducted to physiologically assess the affective reactions of nicotine-dependent, male participants to smoking cues, taking into consideration the Stage of Change classification of the participant. The current study replicated past research (Geier, Mucha, & Pauli, 2000), showing that physiological reactions to smoking cues are appetitive in nature for smokers, after taking into consideration the participants stage of change. It is important to note, however, that these findings are in contrast to a more recent study, which found no modulation of the startle reflex to smoking cues by smokers (Orain-Pelissolo, Grillon, Perez-Diaz, & Jouvent, 2004). As noted earlier, part of the difficulty in disentangling the disparate results across these studies are the differences in the stimuli, methodologies employed, and participant type. The present study utilized the same smoking-cue images and similar procedures as Geier and colleagues, with successful laboratory-independent replication of appetitive affective modulation to smoking cues.

It is also important to note that in addition to potential stimulus differences (i.e., different smoking images) which may have contributed to the null results found by Orain-Pelissolo and colleagues, participants in that study reported an average Fagerström score of 3, indicating "low nicotine dependence" (Fagerstrom, Heatherton, & Kozlowski, 1990). In the present study, as well as in the study by Geier and colleagues, the participants reported FTND scores of 5.45 and 6.27, respectively, indicative of "medium" to "high" levels of nicotine dependence (Fagerstrom et al., 1990). While one of the preliminary aims of the current study was to better understand the conflicting results of the two previously mentioned studies, through replication, additional specific hypotheses were also made regarding the Stage of Change of the smoking participant (W. F. Velicer et al., 1995).

One unique aspect of the present study was that smoking groups were defined based upon their Stage of Change, including the Precontemplation group which were not considering quitting smoking at the time of the study and the Contemplation group which were planning on quitting smoking within 6 months of the study. For hypothesis 1, it was hypothesized that smoking images would significantly attenuate the startle reflex (suggesting that these images would be seen as pleasant), for the Precontemplation group only. This hypothesis was supported both physiologically (hypothesis 1), and via self-report (hypothesis 4a). Specifically, the Precontemplation group's startle magnitude was significantly smaller when viewing smoking images as compared to neutral image. Further, the average self-report rating while viewing smoking images was rated significantly more pleasant than neutral images. As anticipated, both of these tests failed to reach statistical significance for the Contemplation group. Previous research has reported that smokers become more cognizant of the negative implications of their smoking habit as they transition from Precontemplation to Contemplation (Velicer, DiClemente, Prochaska, & Brandenburg, 1985). The current findings add further support to this notion, suggesting that physiological assessment can be utilized to detect differences in Stage of Change. However, it is important to note that the Contemplation group showed atypical affective modulation, with responses to positive images being similar to neutral images. Thus, extrapolation should be tempered given these findings.

Given that Contemplation participants were theorized as experiencing approach-avoid motivations when viewing smoking cues, hypothesis 2 predicted that the startle magnitude for smoking images would be similar to neutral images, being both smaller in magnitude than the unpleasant images and larger than the positive images. That is, smoking images would be perceived as less pleasant then positive images and more pleasant then negative images among this group. Hypothesis 2 was only partially supported as smoking cue startle reflex magnitudes were significantly smaller than negative images but startle reflex to positive images did not significantly differ from smoking images. In fact, positive image startle magnitudes were actually higher than smoking image startle magnitudes, albeit not significantly. Smoking image startle reflex magnitudes were also not significantly smaller than neutral images. Overall, therefore, it appears that smoking images are perceived more as a positive stimulus than a neutral one, when examining the physiological data. As for the self-report aspect of this hypothesis (hypothesis 4b) results were fully supported, with pleasantness ratings for smoking images being significantly higher than negative images and lower than positive images. As hypothesized, this pattern was found for the Contemplation group only. In sum, self-reported ratings for the smoking images were similar to positive images for the Precontemplation group and similar to neutral images for the Contemplation group. It is unclear as to why this hypothesis was fully supported for self-report but not for physiological assessment. Affective assessment through startle modulation occurs at a non-conscious level. In fact, motivational priming has been documented as occurring as early as 150ms post image presentation (Globisch, Hamm, Esteves, & Ohman, 1999). No introspection is requested of the subject. In contrast to the physiological assessment, where the subject is asked to remain still and merely watch the images, in the self-report assessment the subject is asked to consider their emotional reaction to each image and then give a rating on a likert scale. Perhaps the negative reactions associated with the Contemplation Stage of Change (Velicer et al., 1985) are weaker with passive associations (i.e., cues). Future studies could assess this possibility by asking the smoker to consider "how they feel" about each image they see during physiological assessment.

Lastly, for hypothesis 3, it was also hypothesized that the Precontemplation group would have significantly smaller startle magnitudes to smoking images in comparison to the Contemplation group. That is, the Precontemplation group would experience greater pleasant reactions to smoking images in comparison to the Contemplation group. This hypothesis was not confirmed as both physiological and self-report measures (hypothesis 4c) failed to reach significance. This could, in part, be due to an atypical affective modulation pattern in the Contemplation group, which is discussed in detail later.

Affective Modulation of Startle Reflex by Group

In the current study, the Contemplation group showed an atypical pattern of affective modulation, with few significant differences among picture-type startle magnitudes. This likely contributed to the lower correlation discussed previously. In comparison to the Precontemplation group, the Contemplation group startle magnitude when viewing negative images appeared slightly blunted, but in the appropriate direction.

Positive image startle reflex however, did not serve as a startle inhibitor, in fact showing a slightly increased startle reflex in comparison to neutral images. Positive images among this group appeared to actually serve as an aversive stimulus, slightly increasing startle magnitude, albeit not significantly. However smoking images in the Contemplation group did show some attenuating properties on the startle reflex, being significantly lower than negative images and not significantly lower than neutral images. In contrast, the Precontemplation group showed robust and significant affective modulation in all expected directions. It is unclear as to why positive images in the Contemplation group did not serve as startle inhibitors. Perhaps the experimental environment, in conjunction with the acknowledgement of a desire to quit smoking, altered the mood of the participants during the trials. A study by Postma and colleagues (2001) found that startle reflex magnitude (independent of any cue modulation) was predictive of successful cessation one month out in a smoking cessation program, with higher startle magnitude values for those who later relapsed. This was independent of nicotine dependence level. The authors suggest that this may be related to motivation to quit smoking or Stage of Change. The current study may contribute to this theory. The Precontemplation Stage of Change group showed strong and consistent affective modulation of startle reflex, while those seeking to quit smoking (Contemplation Stage of Change group) show an atypical pattern of modulation response. Future studies should be conducted in order to better understand the relationship between motivation to change (i.e., current study) and ability to change (i.e., Postma, et al., 2001) with nicotine dependence and startle reflex research.

Theoretical and Methodological Implications

The current study lends support to the theory that drug cues engender positive affect, which is related to drug-seeking behavior (Stewart, de Wit, & Eikelboom, 1984). The current results also suggest that the paradigm of affective modulation of the startle reflex can indeed be utilized to assess the appetitive nature of nicotine-cues in the addicted individual, and perhaps other drug-cues as well. It is unclear as to why Orain-Pelissolo et al. (2004) did not find significant results in their study. Taking into consideration findings from the present study on Stage of Change, and the much more salient results with Precontemplation addicted smokers, it is possible that Orain-Pelissolo – not assessing for Stage of Change – had an overabundance of Contemplation smokers, which demonstrated atypical modulation in the current study. From a probabilistic perspective, this would not be too unlikely given that 40% of all smokers are considering quitting, or are in the Contemplation stage (W. F. Velicer et al., 1995). However, given that the current study, in part, replicated the procedures of Geier and colleagues (2000), the lack of significant results by Orain-Pelissolo could also be due to many methodological differences such as smoking-cue stimuli, and weak dependence among participants. At any rate, the replication of previous results, documenting the ability to physiologically assess the appetitive nature of nicotine cues, support the use of startle modulation as a viable tool in the research of nicotine addiction, and potentially addition in general.

Consideration of the Stage of Change model with subjects utilized in nicotine-startle research has been suggested in several publications (e.g., Bradley & Lang, 2000;

Postma, Kumari, Sharma, Hines, & Gray, 2001). No research, however, could be found investigating the role of Stage of Change on the affective modulation of the startle reflex. The current study suggests that consideration of the Stage of Change model increases salience of results, and also may help explain previous inconsistent results (e.g., Geier & colleagues, 2000, vs. Orain-Pelissolo and colleagues, 2004). Future studies should consider collecting information regarding Stage of Change for analysis in their studies of nicotine and startle.

Clinical Implications

From a clinical perspective, affective modulation of startle to smoking cues could potentially serve as an object assessment of "readiness to change." The number of quit attempts prior to successful smoking cessation can be extremely high, with documented ranges from 1 to 20 attempts prior to success (West, McNeill, & Raw, 2000). While some practitioners may incorporate the maxim "relapse is part of recovery" into treatment, research suggests that as the number of relapses increase, the chance of failed cessation also increases (Murray et al., 2000). Future studies could investigate the viability of drug-cue modulation of startle as an assessment of readiness to attempt cessation, thus potentially decreasing the number of relapses prior to successful cessation. For example, those with high physiological pleasure reactions to smoking cues could be placed in pre-cessation treatment programs incorporating motivational interviewing techniques (Miller & Rollnick, 2002), to help increase ambivalence regarding their smoking habit. In the United States alone, the annual cost of treatment and lost work productivity related to smoking is approximately $97.2 billion (American Lung

Association, 2006). Deciding when the patient should initiate cessation, and deciding which treatment option is best for each particular patient could help reduce the number of relapses and thus decrease associated costs with smoking treatment.

Limitations of Current Study

One of the major limitations of the current study is inclusion of only male participants. The two primary studies related to the current study included female participants (Geier et al., 2000, 33%, Orain-Pelissolo, et al., 2004, 56%). While neither of these studies reported analyses regarding gender differences specific to the assessment of smoking cues inhibiting the startle reflex, such analyses could provide important information regarding gender differences. This is particularly important given the well documented gender differences observed in physiological reaction to emotionally salient images (Bradley et al., 2001; Kumari, Gray, Gupta, Luscher, & Sharma, 2003; Schupp et al., 1996). Additionally, inclusion of female participants is particularly important for a number of reasons. As reviewed by Perkins, Donny, and Caggiula (1999), over the past several decades the ratio of female smokers has increased dramatically, now approximately matching that of males. Additionally, clinical treatment of smoking dependence among women is often less efficacious in comparison to males and self-quitting success rates are often lower. Perkins and colleagues also point to several lines of research that suggest women are at an even greater risk for the deleterious health effects of smoking, and that death from lung cancer among women now exceeds that of breast cancer.

Another limitation is the lack of biological verification of abstinence from illicit substance use. Illicit substance use was an exclusionary criterion, but information was collected via self-report only. Given that smoking is often ubiquitous among drug users (e.g., Frosch, Shoptaw, Nahom, & Jarvik, 2000), it is possible that smoking images may prime the participant to another substance as well. Such a study has been conducted investigating cross-cue activation of nicotine and alcohol. It was found that among alcoholic smokers, smoking images increased urges to drink and drinking images increased the urge to smoke (Drobes, 2002).

Future Research

In the current study, Contemplation smokers demonstrated an atypical pattern of affective modulation of startle, which may have contributed to the lack of consistent findings between groups. Future studies should be conducted to determine if this pattern is a reliable phenomena or merely a sampling anomaly. Future studies should also assess whether passive reaction vs. requested introspection to experimental images differentially influence startle modulation. Lastly, it may be advantageous for future studies researching nicotine and startle to collect information regarding Stage of Change, as Stage of Change had a substantial influence on the results of the present study.

Conclusion

The current study supports the theory that drug-cues engender positive affect, which is associated with drug-seeking behavior, after taking into consideration the participants' stage of change. The current study also replicated contended findings of previous research, supporting that smoking-cues do indeed serve as a startle attenuator in the dependent smoker. Stage of Change in the smoker was found to play a substantial role with both physiological and self-report data, regarding affective reactions to experimental stimuli. Those participants in the Precontemplation Stage of Change (not considering quitting smoking) showed robust affective modulation of the startle reflex and responses to smoking images, similar to positive images. Those in the Contemplation stage (considering quitting) demonstrated an atypical pattern of affective positive picture-type modulation, being similar to neutral images. Smoking images, however, were still physiologically perceived as pleasant. Overall, the current study demonstrates that affective modulation of the startle reflex is a viable paradigm for investigating drug addiction, while also suggesting that researchers should be mindful of how the addicted individual perceives their addiction, as well as their intentions for continuing in their drug use.

Table 1: Demographic and Screening Variables of Participants

		Precontemplator			Comptemplator		
Ethnicity							
	Asian	1			2		
	Caucasian	8			8		
	Hispanic	1			1		
	Other	1			0		
		Mean	**SD**	**Range**	**Mean**	**SD**	**Range**
Age		21.91	2.91	9.00	24.82	5.46	18.00
Education		14.64	2.01	7.00	15.55	2.34	7.00
Carbon Monoxide		22.00	10.54	32.00	23.91	13.24	41.00
Fagerström		5.45	0.93	2.00	5.45	0.93	3.00
AUDIT		6.82	5.06	13.00	7.45	4.25	16.00

Fagerström is the Fagerström Test for Nicotine Dependence. AUDIT is the Alcohol Use Disorders Identification Test.

Table 2: Standardized Electromyographic Startle Reflex Magnitudes

	Precontemplator	Comptemplator	Total
Positive (SD)	-0.23 (0.28)	0.02 (0.26)	-0.11 (0.30)
Negative (SD)	0.38 (0.21)	0.18 (0.30)	0.28 (0.27)
Neutral (SD)	0.005 (0.13)	-0.003 (0.35)	0.001 (0.25)
Smoking (SD)	-0.15 (0.21)	-0.20 (0.17)	-0.17 (0.19)

EMG scores are presented in standardized Z-score form. Scores were standardized with-in participant.

Table 3: Contrast Analyses for EMG Startle Magnitude

	df	**$Mean_1 – Mean_2$**	**F**
Positive - Smoking	1, 21	-0.106 – -0.175	0.60
Positive - Neutral	1, 21	-0.106 – 0.001	1.24
Positive - Negative	1, 21	-0.106 – 0.275	12.92*
Smoking - Neutral	1, 21	-0.175 – 0.001	5.61
Smoking - Negative	1, 21	-0.175 – 0.275	41.38***
Neutral - Negative	1, 21	0.001 – 0.275	8.67*

*EMG values in Z-score form, standardized within participant. Larger values equal greater relative startle. Modified Bonferroni Critical $F(1, 21)$: 5.83 * $p < .05$, 9.83 ** $p < .01$, 16.57 *** $p < .001$.*

Table 4: Contrast Analyses for EMG Startle Magnitude by Group

		df	$Mean_1, Mean_2$	F
Precontemplator				
	Positive - Smoking	1, 10	-0.23, -0.15	0.39
	Positive - Neutral	1, 10	-0.23, 0.005	5.47
	Positive - Negative	1, 10	-0.23, 0.38	**20.35****
	Smoking - Neutral	1, 10	-0.15, 0.005	**4.37**†
	Smoking - Negative	1, 10	-0.15, 0.38	**32.75*****
	Neutral - Negative	1, 10	0.005, 0.38	**22.93****
Contemplator				
	Positive - Smoking	1, 10	0.02, -0.20	4.87
	Positive - Neutral	1, 10	0.02, -0.003	0.02
	Positive - Negative	1, 10	0.02, 0.18	1.27
	Smoking - Neutral	1, 10	-0.20, -0.003	2.21
	Smoking - Negative	1, 10	-0.20, 0.18	**12.77**††
	Neutral - Negative	1, 10	-0.003, 0.18	1.11

*Range of scores 1-9. A score of 5 indicates neutral affect, lower scores indicating negative affect and higher scores indicating positive affect. Modified Bonferroni Critical F(1, 10): 6.94 * p < .05, 12.83 ** p < .01, 24.85 *** p < .001. For directional a priori hypotheses, one-tailed standard critical F values were used:* $^{\dagger}p < .05$, $^{\dagger\dagger}p < .01$, $^{\dagger\dagger\dagger}p < .001$

Table 5: Self-Assessment Manikin Ratings

	Precontemplator	**Comptemplator**	**Total**
Positive (SD)	6.33 (0.98)	6.54 (0.85)	6.43 (0.90)
Negative (SD)	2.87 (1.09)	2.36 (0.77)	2.62 (0.95)
Neutral (SD)	4.77 (0.81)	5.05 (0.41)	4.91 (0.64)
Smoking (SD)	5.54 (0.71)	5.49 (1.01)	5.52 (0.85)

Range of scores 1-9. A score of 5 indicates neutral affect, lower scores indicating negative affect and higher scores indicating positive affect.

Table 6: Contrast Analyses for SAM Ratings of Pictures

	df	$Mean_1 – Mean_2$	F
Positive - Smoking	1, 21	6.43 – 5.52	11.99**
Positive - Neutral	1, 21	6.43 – 4.91	56.18***
Positive - Negative	1, 21	6.43 – 2.62	120.34***
Smoking - Neutral	1, 21	5.52 – 4.91	9.25*
Smoking - Negative	1, 21	5.52 – 2.62	92.21***
Neutral - Negative	1, 21	4.91 – 2.62	72.31***

*Range of scores 1-9. A score of 5 indicates neutral affect, lower scores indicating negative affect and higher scores indicating positive affect. Modified Bonferroni Critical F(1, 21): 5.83 * p < .05, 9.83 ** p < .01, 16.57 *** p < .001.*

Table 7: Contrast Analyses for SAM Ratings of Pictures by Group

		df	Mean$_1$ – Mean$_2$		F
Precontemplator					
	Positive - Smoking	1, 10	6.33 – 5.54		6.28
	Positive - Neutral	1, 10	6.33 – 4.77		**21.90****
	Positive - Negative	1, 10	6.33 – 2.87		**36.94*****
	Smoking - Neutral	1, 10	5.54 – 4.77		**12.16**††
	Smoking - Negative	1, 10	5.54 – 2.87		**35.90*****
	Neutral - Negative	1, 10	4.77 – 2.87		**17.11****
Contemplator					
	Positive - Smoking	1, 10	6.54 – 5.49		**5.68**†
	Positive - Neutral	1, 10	6.54 – 5.05		**35.39*****
	Positive - Negative	1, 10	6.54 – 2.36		**109.68*****
	Smoking - Neutral	1, 10	5.49 – 5.05		1.76
	Smoking - Negative	1, 10	5.49 – 2.36		**56.34**†††
	Neutral - Negative	1, 10	5.05 – 2.36		**113.72*****

*Range of scores 1-9. A score of 5 indicates neutral affect, lower scores indicating negative affect and higher scores indicating positive affect. Modified Bonferroni Critical F(1, 10): 6.94 * p < .05, 12.83 ** p < .01, 24.85 *** p < .001. For directional a priori hypotheses, one-tailed standard critical F values were used:* $^{†}p < .05$, $^{††}p < .01$, $^{†††}p < .001$

Table 8: Correlation between SAM and EMG Data

	EMG– Positive	**EMG – Negative**	**EMG – Neutral**	**EMG – Smoking**
SAM – Positive	-.367 (.093)	.255 (.253)	.175 (.437)	.031 (.890)
SAM – Negative	.080 (.723)	-.137 (.542)	.195 (.384)	-.161 (.474)
SAM – Neutral	-.058 (.797)	-.195 (.384)	.147 (.513)	.254 (.255)
SAM – Smoking	.065 (.773)	-.144 (.523)	.023 (.918)	.076 (.737)

EMG values in Z-score form, standardized within participant. SAM ratings yoked to EMG data (see results for details). Significance level included in parentheses.

Table 9: Correlation between SAM and EMG Data by Group

	EMG– Positive	EMG – Negative	EMG – Neutral	EMG – Smoking
Precontemplation				
SAM – Positive	-.283 (.399)	.519 (.102)	-.122 (.720)	-.044 (.899)
SAM – Negative	.226 (.505)	-.503 (.115)	**.624 (.040)**	-.132 (.699)
SAM – Neutral	-.309 (.355)	-.185 (.586)	.281 (.402)	.522 (.099)
SAM – Smoking	-.110 (.748)	-.130 (.702)	-.132 (.699)	.317 (.342)
Contemplation				
SAM – Positive	**-.695 (.018)**	.200 (.556)	.328 (.324)	.174 (.609)
SAM – Negative	.211 (.533)	-.014 (.969)	.020 (.953)	-.361 (.276)
SAM – Neutral	.119 (.727)	-.093 (.786)	.167 (.623)	-.300 (.371)
SAM – Smoking	.180 (.596)	-.148 (.664)	.028 (.934)	-.108 (.751)

EMG values in Z-score form, standardized within participant. SAM ratings yoked to EMG data (see results for details). Significance level included in parentheses

Figure 1. EMG Startle Reflex Magnitude

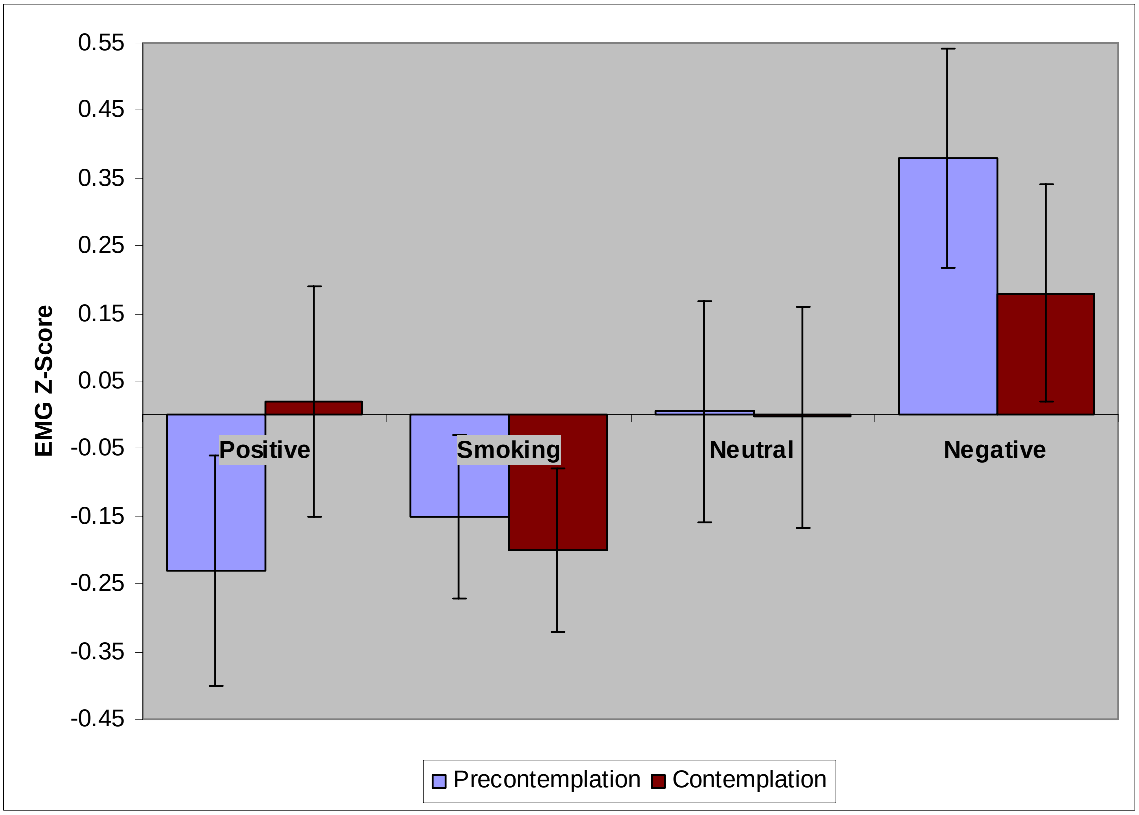


Figure 2: Standardized EMG startle magnitude, by picture type, with 95% confidence interval T-bars.

Figure 2. SAM Pleasantness Ratings

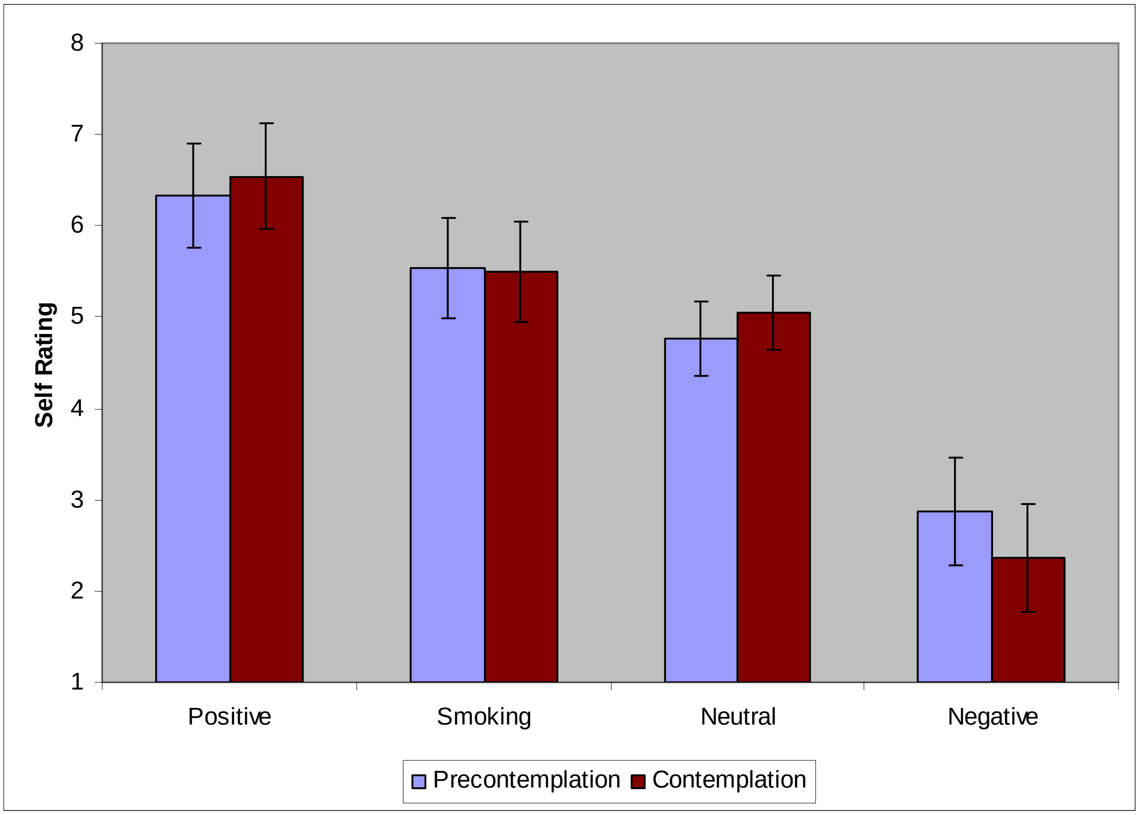


Figure 1: Ratings on a 1-9 scale, with higher values related to equaling more pleasant ratings and lower values equaling unpleasant ratings. A value of 5 indicates neutral affect rating. T-bars represent the 95% confidence interval for each individual score.

Figure 3. Covariation of Self-Ratings and Physiological Response

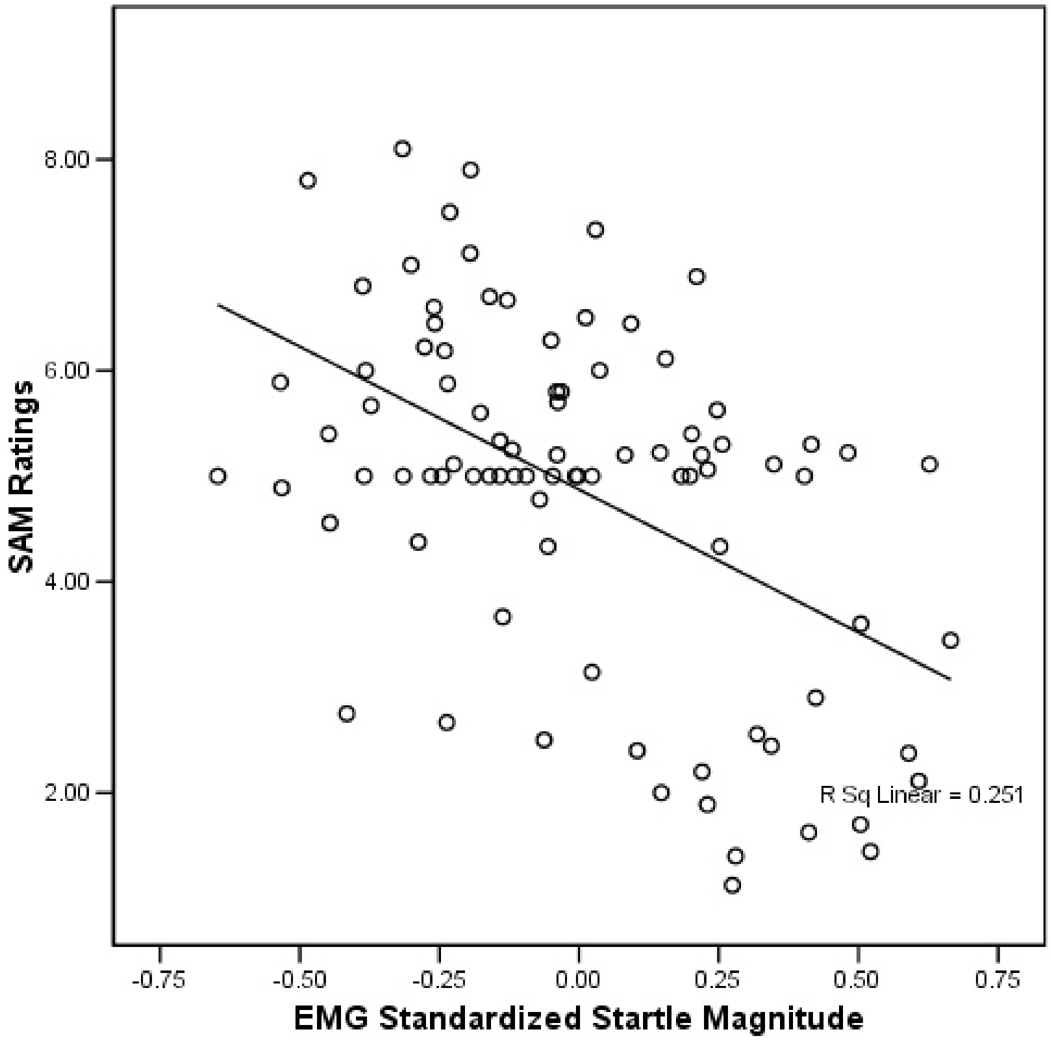


Figure 3: Correlation between SAM ratings and standardized EMG startle values r = -.501, p < .001. Data points reflect average value per picture category by participant (i.e., 4 data points per participant).

REFERENCES

Acri, J. B. (1994). Nicotine modulates effects of stress on acoustic startle reflexes in rats: dependence on dose, stressor and initial reactivity. *Psychopharmacology, 116*(3), 255-265.

Acri, J. B., Brown, K. J., Saah, M. I., & Grunberg, N. E. (1995). Strain and age differences in acoustic startle responses and effects of nicotine in rats. *Pharmacology, biochemistry, and behavior, 50*(2), 191-198.

Acri, J. B., Grunberg, N. E., & Morse, D. E. (1991). Effects of nicotine on the acoustic startle reflex amplitude in rats. *Psychopharmacology, 104*(2), 244-248.

Acri, J. B., Morse, D. E., Popke, E. J., & Grunberg, N. E. (1994). Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. *Psychopharmacology, 114*(2), 369-374.

American Lung Association. (2006). *Quit Smoking*. Retrieved April 16, 2006, from http://www.lungusa.org

Andreassi, J. L. (2000). Muscle activity and behavior. In *Psychophysiology: Human behavior and physiological response (4th ed.).* (pp. 164-190): Lawrence Erlbaum Associates, Publishers.

Andrews, S. E., Blumenthal, T. D., & Flaten, M. A. (1998). Effects of caffeine and caffeine-associated stimuli on the human startle eyeblink reflex. *Pharmacology, Biochemistry and Behavior, 59*(1), 39-44.

Anthony, B. J., & Graham, F. K. (1985). Blink reflex modification by selective attention: Evidence for the modulation of "automatic" processing. *Biological Psychology, 21*(1), 43-59.

Anthony, B. J., & Putnam, L. E. (1985). Cardiac and blink reflex concomitants of attentional selectivity: A comparison of adults and young children. *Psychophysiology, 22*(5), 508-516.

Berg, K. M. (1974). *Elicitation of acoustic startle in the human.*, Univ Microfilms International, Cited in Berg & Balaban (1999).

Berg, W. K., & Balaban, M. T. (1999). Startle Elicitation: Stimulus parameters, recording techniques, and quantification. In M. E. Dawson & A. M. Schell (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science.* (pp. 21-50): Cambridge University Press.

Blaszczyk, J. W. (2003). Startle response to short acoustic stimuli in rats. *Acta Neurobiologiae Experimentalis, 63*(1), 25-30.

Blumenthal, T. D. (1988). The startle response to acoustic stimuli near startle threshold: Effects of stimulus rise and fall time, duration, and intensity. *Psychophysiology, 25*(5), 607-611.

Blumenthal, T. D. (1998). Quantifying human startle response magnitude: Effects of filter passband and integrator time constant on eyeblink EMG response peak and area. *Journal of Psychophysiology, 12*(2), 159-171.

Blumenthal, T. D. (1999). Short lead interval startle modification. In M. E. Dawson & A. M. Schell (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science.* (pp. 51-71): Cambridge University Press.

Blumenthal, T. D., & Berg, W. K. (1986). Stimulus rise time, intensity, and bandwidth effects on acoustic startle amplitude and probability. *Psychophysiology, 23*(6), 635-641.

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies., *Psychophysiology* (Vol. 42, pp. 1-15): Blackwell Publishing Limited.

Blumenthal, T.D., & Goode, C.T. (1991). The startle eyeblink response to low intensity acoustic stimuli. *Psychophysiology, 28*(3), 296-306.

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: defensive and appetitive reactions in picture processing. *Emotion, 1*(3), 276-298.

Bradley, M. M., Codispoti, M., Sabatinelli, D., & Lang, P. J. (2001). Emotion and motivation II: sex differences in picture processing. *Emotion, 1*(3), 300-319.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1990). Startle reflex modification: Emotion or attention? *Psychophysiology, 27*(5), 513-522.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1993). Pictures as prepulse: Attention and emotion in startle modification. *Psychophysiology, 30*(5), 541-545.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1996). Lateralized startle probes in the study of emotion. *Psychophysiology, 33*(2), 156-161.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1996). Picture media and emotion: Effects of a sustained affective content. *Psychophysiology, 33*(6), 662-670.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1999). Affect and the startle reflex. In M. E. Dawson & A. M. Schell (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science.* (pp. 157-183): Cambridge University Press.

Bradley, M. M., Drobes, D., & Lang, P. J. (1996). A probe for all reasons: Reflex and RT measures in perception. *Psychophysiology, 33*, S25

Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: The Self-Assessment Manikin and the semantic differential. *Journal of Behavior Therapy & Experimental Psychiatry, 25*(1), 49-59.

Bradley, M. M., & Lang, P. J. (2000). Affective reactions to acoustic stimuli. *Psychophysiology, 37*(2), 204-215.

Bradley, M. M., Lang, P. J. & Cuthbert, B. N. (1993). Emotion, novelty, and the startle reflex: habituation in humans. *Behavioral neuroscience, 107*(6), 970-980.

Burke, J., & Hackley, S. A. (1997). Prepulse effects on the photic eyeblink reflex: evidence for startle-dazzle theory. *Psychophysiology, 34*(3), 276-284.

Caballería, J. (2003). Current concepts in alcohol metabolism. *Annals Of Hepatology: Official Journal Of The Mexican Association Of Hepatology, 2*(2 (Print)), 60-68.

Carlson, L. E., Taenzer, P., Koopmans, J., & Casebeer, A. (2003). Predictive value of aspects of the Transtheoretical Model on smoking cessation in a community-based, large-group cognitive behavioral program. *Addictive behaviors, 28*(4), 725-740.

Carter, B. L., & Tiffany, S. T. (1999). Meta-analysis of cue-reactivity in addiction research., *Addiction* (Vol. 94, pp. 327-340): Blackwell Publishing Limited.

Center for the Study of Emotion and Attention. (1995). *The international affective picture system: Photographic slides*. Gainsville, FL: The Center for Research in Psychophysiology, University of Florida.

Code of Federal Regulations. (1996). OSHA Standards 1910, *Title 29*. Washington DC: Government Printing Office.

Codispoti, M., Bradley, M. M., & Lang, P. J. (2001). Affective reactions to briefly presented pictures. *Psychophysiology, 38*(3), 474-478.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, N.J.: L. Erlbaum Associates.

Committee to Assess the Science Base for Tobacco Harm Reduction. (2001). Nictoine Pharmacology. In K. Stratton, P. Shetty, R. Wallace & S. Bondurant (Eds.), *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction*. Washington, D.C.: NATIONAL ACADEMY PRESS.

Coulbourn Instruments. (2002a). Lab Linc V: Hardware user's guide, *Physiological data aqusition system: System and module operation and specifications* (pp. 87).

Coulbourn Instruments. (2002b). Lab Linc V: Hardware user's guide, *Physiological data aqusition system: System and module operation and specifications* (pp. 12).

Curtin, J. J., Patrick, C. J., Lang, A. R., Cacioppo, J. T., & Birbaume, N. (2001). Alcohol affects emotion through cognition. *Psychological Science: A Journal Of The American Psychological Society / APS, 12*(6 (Print)), 527-531.

Cuthbert, B. N., Bradley, M. M. & Lang, P. J. (1996). Probing picture perception: Activation and emotion. *Psychophysiology, 33*(2), 103-111.

Davis, M., Walker, D., & Lee, Y. (1999). Neurophysiology and Neuropharmacology. In M. E. Dawson, A. M. Schell & A. H. Boehmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science*. New York, NY, US: Cambridge University Press.

Dawson, M. E., Schell, A. M., & Boehmelt, A. H. (1999a). *Startle modification: Implications for neuroscience, cognitive science, and clinical science.* New York, NY, US: Cambridge University Press.

Dawson, M. E., Schell, A. M., & Boehmelt, A. H. (1999b). Startle modification: Introduction and overview. In A. H. Boehmelt (Ed.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science*. New York, NY, US: Cambridge University Press.

Della Casa, V., Hofer, I., Weiner, I., & Feldon, J. (1998). The effects of smoking on acoustic prepulse inhibition in healthy men and women., *Psychopharmacology* (Vol. 137, pp. 362): Springer - Verlag New York, Inc.

DiClemente, C. C. (2003). *Addiction and change: How addictions develop and addicted people recover.*: Guilford Press.

DiClemente, C. C., Prochaska, J. O., Fairhurst, S. K., Velicer, W. F., Velasquez, M. M., & Rossi, J. S. (1991). The process of smoking cessation: an analysis of precontemplation, Contemplation, and preparation Stages of Change. *Journal of consulting and clinical psychology, 59*(2), 295-304.

DiClemente, C. C., Schlundt, D., & Gemmell, L. (2004). Readiness and Stages of Change in addiction treatment. *American journal on addictions, 13*(2), 103-119.

Drobes, D. J. (2002). Cue reactivity in alcohol and tobacco dependence. *Alcoholism: Clinical & Experimental Research, 26*(12), 1928-1929.

Duncan, E., Madonick, S., Chakravorty, S., Parwani, A., Szilagyi, S., Efferen, T., et al. (2001). Effects of smoking on acoustic startle and prepulse inhibition in humans. *Psychopharmacology (Berl), 156*(2-3), 266-272.

Elash, C. A., Tiffany, S. T., & Vrana, S. R. (1995). Manipulation of smoking urges and affect through a brief-imagery procedure: Self-report, psychophysiological, and startle probe responses. *Experimental & Clinical Psychopharmacology, 3*(2), 156-162.

Essex, M. J., Goldsmith, H. H., Smider, N. A., Dolski, I., Sutton, S. K., & Davidson, R. J. (2003). Comparison of video- and EMG-based evaluations of the magnitude of children's emotion-modulated startle response. *Behavior Research Methods, Instruments & Computers, 35*(4), 590-598.

Fagerstrom, K. O., Heatherton, T. F., & Kozlowski, L. T. (1990). Nicotine addiction and its assessment. *Ear, Nose, & Throat Journal, 69*(11 (Print)), 763-765.

Fagerström, K. O., & Schneider, N. G., (1989). Measuring nicotine dependence: a review of the Fagerström Tolerance Questionnaire. *Journal of behavioral medicine, 12*(2), 159-182.

Fagerström, K. O., (1978). Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addictive Behaviors, 3*(3), 235-241.

Field, M., & Duka, T. (2004). Cue reactivity in smokers: the effects of perceived cigarette availability and gender. *Pharmacology, biochemistry, and behavior, 78*(3), 647-652.

Flaten, M. A., Aasli, O., & Blumenthal, T. D. (2003). Expectations and placebo responses to caffeine-associated stimuli. *Psychopharmacology, 169*(2), 198-204.

Flaten, M. A., & Blumenthal, T. D. (1999). Caffeine-associated stimuli elicit conditioned responses: An experimental model of the placebo effect. *Psychopharmacology, 145*(1), 105-112.

Flaten, M. A., Simonsen, T., Zahlsen, K., Aamo, T., Sager, G., & Olsen, H. (2004). Stimulant and relaxant drugs combined with stimulant and relaxant information: A study of active placebo. *Psychopharmacology, 176*(3), 426-434.

Fridlund, A. J., & Cacioppo, J. T. (1986). Guidelines for human electromyographic research. *Psychophysiology, 23*(5), 567-589.

Frosch, D. L., Shoptaw, S., Nahom, D., & Jarvik, M. E. (2000). Associations between tobacco smoking and illicit drug use among methadone-maintained opiate-dependent individuals. *Experimental and Clinical Psychopharmacology, 8*(1), 97-103.

Funayama, E. S., Grillon, C., Davis, M., & Phelps, E. A. (2001). A Double Dissociation in the Affective Modulation of Startle in Humans: Effects of Unilateral Temporal Lobectomy., *Journal of Cognitive Neuroscience* (Vol. 13, pp. 721-729): MIT Press.

Geier, A., Pauli, P., &. Mucha, R. F. (2000). Appetitive nature of drug cues confirmed with physiological measures in a model using pictures of smoking. *Psychopharmacology, 150*(3), 283-291.

Ginsberg, G., Hattis, D., Russ, A., & Sonawane, B. (2004). Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. *Journal Of Toxicology And Environmental Health. Part A, 67*(4 (Print)), 297-329.

Glautier, S. (2004). Measures and models of nicotine dependence: Positive reinforcement. *Addiction, 99*, 30-50.

Globisch, J., Hamm, A. O., Esteves, F., & Ohman, A. (1999). Fear appears fast: temporal course of startle reflex potentiation in animal fearful subjects. *Psychophysiology, 36*(1), 66-75.

Greene, W. A., Turetsky, B., & Kohler, C. (2000). General laboratory safety. In J. T. Cacioppo & L. G. Tassinary (Eds.), *Handbook of psychophysiology (2nd ed.).* (pp. 951-977): Cambridge University Press.

Hackley, S. A., & Boelhouwer, A. J. (1997). The more or less startling effects of weak prestimulation - revisited: Prepulse modulation of multicomponent blink reflexes. In P. J. Lang & R. F. Simons (Eds.), *Attention and orienting: Sensory and motivational processes.*: Lawrence Erlbaum Associates, Publishers.

Haddock, C. K., Lando, H., Klesges, R. C., Talcott, G. W., & Renaud, E. A. (1999). A study of the psychometric and predictive properties of the Fagerström Test for Nicotine Dependence in a population of young smokers. *Nicotine & Tobacco Research, 1*(1), 59.

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerström, K. O. (1991). The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire., *British Journal of Addiction* (Vol. 86, pp. 1119-1127): Blackwell Publishing Limited.

Helton, D. R., Modlin, D. L., Tizzano, J. P., & Rasmussen, K. (1993). Nicotine withdrawal: A behavioral assessment using schedule controlled responding, locomotor activity, and sensorimotor reactivity. *Psychopharmacology, 113*(2), 205-210.

Hutchison, K. E., Niaura, R., & Swift, R. (1999). Smoking cues decrease prepulse inhibition of the startle response and increase subjective craving in humans. *Experimental and clinical psychopharmacology, 7*(3), 250-256.

Hutchison, K. E., Niaura, R., & Swift, R. (2000). The effects of smoking high nicotine cigarettes on prepulse inhibition, startle latency, and subjective responses. *Psychopharmacology, 150*(3), 244.

John, U., Hill, A., Rumpf, H.-J., Hapke, U., & Meyer, C. (2003). Alcohol high risk drinking, abuse and dependence among tobacco smoking medical care patients and the general population. *Drug And Alcohol Dependence, 69*(2 (Print)), 189-195.

Junghoefer, M., Bradley, M. M., Elbert, T. R., & Lang, P. J. (2001). Fleeting images: A new look at early emotion discrimination. *Psychophysiology, 38*(2), 175-178.

Keppel, G. (1991). Correction for cumulative type I error. In G. Keppel (Ed.), *Design and analysis: A researcher's handbook* (3rd ed., pp. 163-186): Prentice-Hall, Inc.

Keppel, G. (1991). *Design and analysis: A researcher's handbook (3rd ed.).* Prentice-Hall, Inc.

Koch, M. (1999). The neurobiology of startle. *Progress in neurobiology, 59*(2), 107-128.

Kokotailo, P. K., Egan, J., Gangnon, R., Brown, D., Mundt, M., & Fleming, M. (2004). Validity of the alcohol use disorders identification test in college students. *Alcoholism, Clinical And Experimental Research, 28*(6 (Print)), 914-920.

Kumari, V., Checkley, S. A., & Gray, J. A. (1996). Effect of Cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology, 128*, 54-60.

Kumari, V., Cotter, P., Checkley, S. A., & Gray, J. A. (1997). Effects of acute subcutaneous nicotine on prepulse inhibition of the acoustic startle reflex in health male non-smokers. *Psychopharmacology, 132*, 389-395.

Kumari, V., Gray, J. A., Gupta, P., Luscher, S., & Sharma, T. (2003). Sex differences in prepulse inhibition of the acoustic startle response. *Personality & Individual Differences, 35*(4), 733-742.

Landis, C., & Hunt, W. (1939). *The startle pattern.* Oxford, England: Farrar & Rinehart.

Lang, P. J. (1985). The cognitive psychophysiology of emotion: Fear and anxiety. In A. H. Tuma & J. D. Maser (Eds.), *Anxiety and the anxiety disorders.* (pp. 131-170): Lawrence Erlbaum Associates, Inc.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review, 97*(3), 377-395.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). Motivated attention: Affect, activation, and action. In R. F. Simons (Ed.), *Attention and orienting: Sensory and motivational processes.* (pp. 97-135): Lawrence Erlbaum Associates, Publishers.

Lang, P. J., Bradley, M. M., Drobes, D., & Cuthbert, B. N. (1995). Emotional perception: Fearful beasts, scary people, sex, sports, disgust, and disasters. *Psychophysiology, 32*, S48.

Lang, P. J., Bradley, M. M., Fitzsimmons, J. R., Cuthbert, B. N., Scott, J. D., Moulder, B., et al. (1998). Emotional arousal and activation of the visual cortex: An fMRI analysis. *Psychophysiology, 35*(2), 199-210.

Lang, P. J., Greenwald, M. K., Bradley, M. M., & Hamm, A. O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology, 30*(3), 261-273.

Leahy, R. L., & Holland, S. J. (2000). Appendix A. In *Treatment plans and interventions for depression and anxiety disorders.* (pp. 8): Guilford Press.

Lewis, M. C., & Gould, T. J. (2003). Nicotine and ethanol enhancements of acoustic startle reflex are mediated in part by dopamine in C57BL/6J mice. *Pharmacology, biochemistry, and behavior, 76*(1), 179-186.

Lovallo, W. R. (2005). Personal Communication. Oklahoma City, OK: Behavioral Sciences Laboratories - Oklahoma City VA Medical Center.

Low, E. C. T., Ong, M. C. C., & Tan, M. (2004). Breath carbon monoxide as an indication of smoking habit in the military setting. *Singapore Medical Journal, 45*(12 (Print)), 578-582.

Marks, M. J., Stitzel, J. A., & Collins, A. C. (1989). Genetic influences on nicotine responses. *Pharmacology, Biochemistry & Behavior, 33*(3), 667-678.

McDermut, W., & Haaga, D. A. (1998). Effect of Stage of Change on cue reactivity in continuing smokers. *Experimental and clinical psychopharmacology, 6*(3), 316-324.

McDougal, D., Van Lieshout, D., & Harting, J. (2004). *OCULOMOTOR NUCLEAR COMPLEX*. Retrieved June 5, 2004, from http://128.104.8.64/virtualbrain/Index.html

Measurement Excellence and Training Resource Information Center. (2006). *Critical review of Alcohol Use Disorders Identification Test (AUDIT).* Retrieved March 19, 2006, from http://www.measurementexperts.org//instrument/instrument_reviews.asp?detail=22

Mikalsen, A., Bertelsen, B., & Flaten, M. A. (2001). Effects of caffeine, caffeine-associated stimuli, and caffeine-related information on physiological and psychological arousal. *Psychopharmacology, 157*(4), 373-380.

Miller, W. R., & Rollnick, S. (2002). *Motivational interviewing : preparing people for change* (2nd ed.). New York: Guilford Press.

Miranda, R., Jr, Meyerson, L. A., Buchanan, T. W., & Lovallo, W. R. (2002). Altered emotion-modulated startle in young adults with a family history of alcoholism. *Alcoholism, clinical and experimental research., 26*(4), 441-448.

Morecraft, R. J. (1999, September 17 - 19). *Brain Control of Facial Muscles.* Paper presented at the Benign Essential Blepharospasm Research Foundation Conference, Asilomar, California.

Morera, O. F., Johnson, T. P., Freels, S., Parsons, J., Crittenden, K. S., Flay, B. R., et al. (1998). The measure of stage of readiness to change: Some psychometric considerations. *Psychological Assessment, 10*(2), 182-186.

Mucha, R. F., Geier, A., & Pauli, P. (1999). Modulation of craving by cues having differential overlap with pharmacological effect: evidence for cue approach in smokers and social drinkers., *Psychopharmacology* (Vol. 147, pp. 306): Springer - Verlag New York, Inc.

Mucha, R. F., Geier, A., Stuhlinger, M., & Mundle, G. (2000). Appetitive effects of drug cues modelled by pictures of the intake ritual: generality of cue-modulated startle examined with inpatient alcoholics. *Psychopharmacology, 151*(4), 428.

Murray, R. P., Gerald, L. B., Lindgren, P. G., Connett, J. E., Rand, C. S., & Anthonisen, N. R. (2000). Characteristics of participants who stop smoking and sustain abstinence for 1 and 5 years in the Lung Health Study. *Preventive Medicine, 30*(5 (Print), 392-400.

Orain-Pelissolo, S., Perez-Diaz, F., Jouvent, R., & Grillon, C. (2004). Lack of startle modulation by smoking cues in smokers. *Psychopharmacology, 173*(1/2), 160-166.

Patrick, C. J., Bradley, M. M., & Lang, P. J. (1993). Emotion in the criminal psychopath: startle reflex modulation. *Journal of abnormal psychology., 102*(1), 82-92.

Perkins, K. A., Donny, E., & Caggiula, A. R. (1999). Sex differences in nicotine effects and self-administration: Review of human and animal evidence. *Nicotine & Tobacco Research, 1*(4), 301-315.

Postma, P., Kumari, V., Sharma, T., Hines, M., & Gray, J. A. (2001). Startle response during smoking and 24 h after withdrawal predicts successful smoking cessation. *Psychopharmacology, 156*(2-3), 360-367.

Prochaska, J. O., Velicer, W. F., Rossi, J. S., Goldstein, M. G., Marcus, B. H., Rakowski, W., et al. (1994). Stages of Change and decisional balance for 12 problem behaviors. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association, 13*(1), 39-46.

Ruiz-Padial, E., Sollers III, J. J., Vila, J., & Thayer, J. F. (2003). The rhythm of the heart in the blink of an eye: Emotion-modulated startle magnitude covaries with heart rate variability. *Psychophysiology, 40*(2), 306-313.

Sabatinelli, D., Flaisch, T., Bradley, M. M., Fitzsimmons, J. R., & Lang, P. J. (2004). Affective picture perception: gender differences in visual cortex? *Neuroreport, 15*(7), 1109-1112.

Saunders, J. B., Aasland, O. G., Babor, T. F., De La Fuente, J. R., & Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction, 88*(6), 791-804.

Scharf, B., & Houtsma, A. J. M. (1986). Audition II: Loudness, pitch, localization, aural distortion, pathology. In K. R. Boff, L. Kaufman & J. P. Thomas (Eds.), *Handbook of perception and human performance* (Vol. 1, pp. 15-11 - 15-60). New York: Wiley.

Schicatano, E. J. (2005). Effects of Caffeine on the Trigeminal Blink Reflex. *Perceptual and Motor Skills, 100*(2), 493-496.

Schicatano, E. J., & Blumenthal, T. D. (1994). Caffeine delays habituation of the human acoustic startle reflex. *Psychobiology, 22*(2), 117-122.

Schorling, J. B., Gutgesell, M., Klas, P., & Smith, D. (1994). Tobacco, alcohol and other drug use among college students. *Journal of Substance Abuse, 6*(1), 105-115.

Schupp, H. T., Cuthbert, B. N., Hillman, C., Raymann, R., Bradley, M. M., & Lang, P. J. (1996). ERPs and blinks: Sex differences in response to erotic and violent picture content. *Psychophysiology, 33*(Suppl. 1), S75.

Siddle, D. A. T., & Lipp, O. V. (1997). Orienting, habituation, and information processing: The effects of omission, the role of expectancy, and the problem of dishabituation. In P. J. Lang & R. F. Simons (Eds.), *Attention and orienting: Sensory and motivational processes.* (pp. 23-40): Lawrence Erlbaum Associates, Publishers.

Siegel, S. (1979). The role of conditioning in drug tolerance and addiction. In J. D. Keehn (Ed.), *Psychopathology in animals: Research and clinical implications.* (pp. 143-168): Academic Press, Inc.

Simons, R. F., & Zelson, M. F. (1985). Engaging visual stimuli and reflex blink modification. *Psychophysiology, 22*(1), 44-49.

Stewart, J., de Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological review, 91*(2), 251-268.

Swerdlow, N. R., Eastvold, A., Gerbranda, T., Uyan, K. M., Hartman, P., Doan, Q., et al. (2000). Effects of caffeine on sensorimotor gating of the startle reflex in normal control subjects: Impact of caffeine intake and withdrawal. *Psychopharmacology, 151*(4), 368-378.

Tassinary, L. G., & Cacioppo, J. T. (2000). The skeletomotor system: Surface electromyography. In J. T. Cacioppo & L. G. Tassinary (Eds.), *Handbook of psychophysiology (2nd ed.).* (pp. 163-199): Cambridge University Press.

Tonnesen, P., Norregaard, J., Mikkelsen, K., Jorgensen, S., & Nilsson, F. (1993). A double-blind trial of a nicotine inhaler for smoking cessation. *JAMA: The Journal Of The American Medical Association, 269*(10 (Print)), 1268-1271.

Turpin, G., Schaefer, F., & Boucsein, W. (1999). Effects of stimulus intensity, risetime, and duration on somatic and behavioral responding: implications for the differentiation of orienting, startle, and defense responses. *Psychophysiology, 36*(4), 453-463.

van Boxtel, A., Boelhouwer, A. J., & Bos, A. R. (1998). Optimal EMG signal bandwidth and interelectrode distance for the recording of acoustic, electrocutaneous, and photic blink reflexes. *Psychophysiology, 35*(6), 690-697.

van Boxtel, A., Goudswaard, P., & Schomaker, L. R. (1984). Amplitude and bandwidth of the frontalis surface EMG: Effects of electrode parameters. *Psychophysiology, 21*(6), 699-707.

VanderWerf, F., Brassinga, P., Reits, D., Aramideh, M., & Ongerboer de Visser, B. (2003). Eyelid movements: behavioral studies of blinking in humans under different stimulus conditions. *Journal of neurophysiology, 89*(5), 2784-2796.

Vanman, E. J., Mejia, V. Y., Dawson, M. E., Schell, A. M., & Raine, A. (2003). Modification of the startle reflex in a community sample: Do one or two dimensions of psychopathy underlie emotional processing? *Personality & Individual Differences, 35*(8), 2007-2021.

Velicer, W. F., DiClemente, C. C., Prochaska, J. O., & Brandenburg N. (1985). Decisional balance measure for assessing and predicting smoking status. *Journal of personality and social psychology, 48*(5), 1279-1289.

Velicer, W. F., Prochaska, J. O., Fava, J. L., Norman, G. L., & Redding, C. A., (1998). Smoking cessation and stress management: Applications of the transtheoretical model of behavior change. *Homeostasis in Health & Disease, 38*(5), 216-233.

Velicer, W. F., Fava, J. L., Prochaska, J. O., Abrams, D. B., Emmons, K. M., & Pierce, J. P. (1995). Distribution of smokers by stage in three representative samples. *Preventive medicine, 24*(4), 401-411.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: a new measure of emotion? *Journal of abnormal psychology, 97*(4), 487-491.

Wald, N. J., Idle, M., Boreham, J., & Bailey, A. (1981). Carbon monoxide in breath in relation to smoking and carboxyhaemoglobin levels. *Thorax., 36*(5), 366-369.

West, R., McNeill, A., & Raw, M. (2000). Smoking cessation guidelines for health professionals: an update. Health Education Authority. *Thorax, 55*(12 (Print)), 987-999.

Wrase, J., Klein, S., Gruesser, S. M., Hermann, D., Flor, H., Mann, K., et al. (2003). Gender differences in the processing of standardized emotional visual stimuli in humans: a functional magnetic resonance imaging study. *Neuroscience letters, 348*(1), 41-45.

Wynn, J. K., Dawson, M. E., & Schell, A. M. (2000). Discrete and continuous prepulses have differential effects on startle prepulse inhibition and skin conductance orienting. *Psychophysiology, 37*(2), 224-230.

Yates, S. K., & Brown, W. F. (1981). Light-stimulus-evoked blink reflex: methods, normal values, relation to other blink reflexes, and observations in multiple sclerosis. *Neurology, 31*(3), 272-281.

Yeomans, J. S., & Frankland, P. W. (1996). The acoustic startle reflex: neurons and connections. *Brain research. Brain research reviews, 21*(3), 301-314.

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), 1-11.

Zeigler, B. L., Graham, F. K., & Hackley, S. A. (2001). Cross-modal warning effects on reflexive and voluntary reactions. *Psychophysiology, 38*(6), 903-911.

APPENDIX A
EXTENDED LITERATURE REVIEW

The eye-blink startle reflex is a somatic, uncontrolled action, seen across species, resulting from an abrupt and intense sensory stimulus (e.g., a loud noise). The study of the startle reflex has proven valuable across several fields of research, including studies of emotions, cognitive processes, neurological disorders, and psychiatric disorders (Dawson et al., 1999a). The startle reflex can be reliably modulated by the presence of emotionally salient stimuli, with increases in startle occurring when accompanied by unpleasant stimuli and decreases in startle occurring when accompanied by pleasant stimuli (Peter J. Lang et al., 1990). While most research in this area has traditionally utilized emotionally salient pictures as the means to modulate the startle reflex, recent research has begun to investigate how drug cues may modulate startle. The following extended literature review will provide an overview of the physiological measurement of the startle reflex for research purposes. Additionally, research documenting the phenomenon of affective stimuli modulating the startle reflex will also be reviewed. As for the new area of drug-cues modulating the startle reflex, this review will focus specifically on how smoking-cues have been documented as influencing startle, as well as how nicotine in general interacts with the startle reflex. Given that Stages of Change in smoking has been documented as an influential variable in nicotine dependence research (Carlson, Taenzer, Koopmans, & Casebeer, 2003; Carlo C. DiClemente, 2003; Carlo C DiClemente, Schlundt, & Gemmell, 2004; Prochaska et al., 1994), this model will also be briefly reviewed.

Physiological Measurement of Startle Reflex

The physiological measurement of startle can be acquired through several different means. The methodology varies and is guided by the type of research being conducted. Several startle reflex elicitation methods and physiological measurement sources have been utilized in the past. However, the most commonly used method utilizes an acoustic startle probe, measuring eye-blink magnitude with electromyography (EMG) (Dawson, Schell, & Boehmelt, 1999b). Documentation regarding this commonly used technique, including known influential factors, will be fully explicated during the preceding segment.

Startle Elicitation Methods

The startle reflex is common to both animals and humans. As early as 1939, scientists reported that abrupt sensory data elicits rapid flexor movements throughout the body, in most mammals (Landis & Hunt, 1939). It has been hypothesized that "abrupt data" can elicit a startle reflex through all sensory systems (W. K. Berg & Balaban, 1999). However, identifying reliable stimuli to produce a startle reflex through the olfactory and gustatory systems have proven difficult, and as such have been researched only minimally. The majority of research being conducted today utilizes either auditory, cutaneous, or visual probes, as methods of eliciting a startle reflex, with acoustic probes most commonly in use. The following section detail the acoustic probe technique as it has been used in research investigating startle.

Acoustic Startle Probe

There are several reasons that may explain why acoustic probes have been widely utilized in research examining startle. The most significant reasons are that this technique is less invasive than other elicitation methods (e.g., electrical stimulation startle), it reliably induces startle, and rarely interferes with foreground tasks. The most common startle probe is a sudden white-noise, 50 milliseconds in duration, and an intensity of approximately 90 decibels (Blaszczyk, 2003; Peter J. Lang, Bradley, & Cuthbert, 1997). However, four different aspects of the acoustic probe have been identified as having influential effects on the startle reflex. These aspects are the bandwidth, duration, intensity, and rise-time of the acoustic probe.

The bandwidth of the acoustic probe can be thought of as the spectrum of noise; with a single-tone probe at one extreme, and a wideband multi-tone probe at the other. In comparing wideband white-noise versus single-tone probes, white-noise probes have been shown to more reliably induce startle and produce startle reflex magnitudes of larger amplitude (T. D. Blumenthal & Berg, 1986). Research on prepulse inhibition of startle using acoustic probes has also shown that white noise is more effective than a single tone (Wynn, Dawson, & Schell, 2000). One possible explanation that may explain why wideband noises produce more startle is that they are perceived as more intense (louder) than single tone noises. Subjective ratings of “loudness” have been shown to increase, as the bandwidth of the noise increases, even when the actual decibel level stays the same (Scharf & Houtsma, 1986).

Duration of the acoustic probe concerns the actual amount of exposure time to the stimulus (i.e., how long the startling sound is played). A recent study investigating acoustic probe duration in rats found that longer durations were related to increased magnitude in startle (Blaszczyk, 2003). However, this linear relationship was found only for a specific period of exposure duration, 2 to 8 milliseconds. This linear relationship asymptotes at approximately 8 milliseconds, with maximum level of amplitude maintained for any probe longer than this point. Berg and Balaban (1999) discuss duration of acoustic startle probes in humans and report that most are 10-50 milliseconds in duration. They further report that any probe lasting longer than this span may be inappropriate given that the actual startle reflex occurs at approximately 30-50 milliseconds post onset of the probe. Naturally, probes extending past the actual startle reflex can not influence that startle reflex.

Intensity of the startle probe is the decibel level or loudness. Overall, increased intensity is related to increased startle amplitude. Turpin, Schaefer, and Boucsein (1999) compared 100 dB and 60 dB white noise startle probes and found significantly more head movements, eye-blinks, and body movements in response to the 100 dB probes. Berg and Balaban (1999) cite dissertation research investigating characteristics of acoustic startle probes and found a decibel threshold level of 84-87 dB in adult humans (K. M. Berg, 1974). However, more recent research has shown that startle can be reliably induced with decibel levels as low as 50 to 60 dB (Blumenthal & Goode, 1991; Turpin et al., 1999). This research demonstrated that the differential effects on startle reflex seen commonly at higher intensities (via manipulation of probe characteristics) can also be ascertained at low levels of probe intensities. However, higher intensity probes have been shown to increase the probability of a startle reflex (Blumenthal & Goode, 1991).

Rise-time of the startle probe is the latency of onset to peak amplitude in signal. In other words, rise-time concerns how long the probe takes to reach its maximum "loudness". Faster rise-times have consistently been shown to produce greater and more reliable startle responses (e.g., Blumenthal & Goode, 1991; T. D. Blumenthal, 1988). Turpin, Schaefer, and Boucsein (1999) compared rise-times of 5 and 200 milliseconds, finding that the former was associated with larger electrodermal responses, more eye-blinks, more head and body movements, and larger heart-rate acceleration. Furthermore, they found that the slower rise-time startle probe was associated with greater habituation to probes across trials. Given the solid documentation of this relationship, it is understandable that the majority of current research using acoustic startle probe uses "near instantaneous" rise-time (defined as <1 millisecond) (Orain-Pelissolo et al., 2004; Ruiz-Padial, Sollers III, Vila, & Thayer, 2003; Vanman, Mejia, Dawson, Schell, & Raine, 2003).

Another important consideration when utilizing sound stimuli in human research is safety. As mentioned previously, the commonly used decibel level in acoustic startle probes is 90 dB. To put this intensity level in perspective, normal speech is approximately 60 dB, heavy traffic approximately 90 dB, and large jet engine noise is approximately 100 dB. In the United States, the Occupational Safety and Health Administration (OSHA) sets standards of safety that research laboratories must comply with. OSHA's standards dictate the durations and intensities at which protective hearing devices are mandated. For example, protective devices must be worn for consistent sounds of 90 dB lasting longer than 8 hours. For sounds of 115 dB, protective devices must be worn for durations lasting longer than 15 minutes (Code of Federal Regulations, 1996). Greene, Turetsky, and Kohler (2000) report that noises over 140 dB can cause permanent hearing loss, and recommend that no greater than 115 dB be used in research.

In sum, characteristics of the acoustic probe are important, with 90-100 dB white-noise sound, lasting 30 milliseconds, and having a near instantaneous rise-time, producing the best results. The following step after identifying a reliable method for inducing a startle reflex concerns choosing the source (or location) in which the startle will be registered.

Measurement Sources of Startle

Initially, Landis and Hunt (1939) used high-speed motion pictures to investigate the human startle reflex to loud noise. They detailed several common responses, including blinking of eyes, moving head forward, flexion of fingers, and tensing of abdomen. Of the many automatic responses documented, they reported blinking of eyes to be the first reaction, and additionally reported that it was the least susceptible to habituation. Today, eye-blink is the most common source of measurement in startle research (Dawson et al., 1999b). Since the reports of Landis and Hunt, technological advances have allowed researchers to document additional autonomic reflexes, including changes in heart-rate, and skin conductance. However, these sources have primarily been researched as associated features of startle, with eye-blink continuing to be utilized as the primary source for the objective measurement of startle.

Eyeblink

At a physiological level, the eyeblink is controlled by two primary muscles, the orbicularis oculi and the levator palpebrae (Tassinary & Cacioppo, 2000; VanderWerf, Brassinga, Reits, Aramideh, & Ongerboer de Visser, 2003). The orbicularis oculi is an out-layer sphincter muscle that surrounds the eye. The levator palpebra is a thin triangular shaped muscle, posterior to the orbicularis, attached to the inner eyelid. These two muscles work in an antagonistic fashion. As a blink begins, the levator palpebrae relax, followed by tensing of the orbicularis oculi, resulting in a rapid lowering of the upper eyelids. It has been reported that while passive tensing of the orbicularis oculi can cause a mild blink (especially if the levator palpebrae are relaxed), reflexive blink velocities of lid lowering can reach 3,000 degrees per second (Hackley & Boelhouwer, 1997). The levator palpebrae are innervated by the superior oculomotor nerve, extending from the oculomotor nuclear complex in the brain stem (McDougal, Van Lieshout, & Harting, 2004), whereas the orbicularis oculi are innervated by the intermediate subnuclei of the facial nuclei (Morecraft, 1999). Objective measurement of blink intensity and duration is primarily recorded by EMG, with electrodes measuring activity in the orbicularis oculi. However, a recent study showed that video-based subjective ratings of eyeblink magnitude was highly correlated with EMG measurement (Essex et al., 2003). The authors of this study conceded that video-ratings did not allow for measurements of latency and duration, suggesting that this method only be used in situations where EMG is not available or feasible.

The end result of a startling stimulus is a somatic behavioral response (e.g., eyeblink, abdomen tension). However, these reflexes are directed by neural mechanisms, associated with specific areas in the brain. The reticular formation is a group of nerve cells near the base of the brainstem. This area is commonly known to be involved in sleep, arousal and attention. However, research has also identified areas in the reticular formation which are directly involved in the startle reflex. Yeomans et al. (2002) discuss several studies showing that lesions of the ventrocaudal pontine reticular formation (PnC) completely eliminate startle reflex to acoustic and air-puff startle stimuli. They further report that PnC activation has been identified in responses elicited by cutaneous and vestibular nerve startle stimuli. Additionally, they report that the largest neurons in the entire reticular formation have been consistently documented as being activated by acoustic startle probes. Yeomans and Frankland (1996) report that activation of the PnC neurons occurs approximately 3-8 milliseconds post probe, and that 20-60 "giant" PnC neurons are likely responsible for most of the startle reflex in rat models. Yeomans et al. (2002) suggest that these neurons are most likely large in diameter, with myelinated axons, given several studies showing very short refractory periods in response to electrical stimulation. Koch (1999) reports that the PnC can also be conceptualized as a sensorimotor interface to the facial and somatic reactions, given that PnC neurons project directly to facial, cranial, and spinal motor neurons. Looking specifically at acoustic startle probes, Davis, Walker, and Lee (1999) suggest that the neural pathway of startle is much simpler than previously thought. They surmise the path to be 3 ordered neural sections: the cochlear root neurons, to the PnC, and lastly to motoneurons (facial and spinal cord).

Measurement Method of Startle: Electromyography

Discussions thus far have concerned techniques for eliciting a startle reflex, and physiological reactions in response to startling stimuli (i.e., eyeblink). The following section details the methodology and technique of recording physiological responses to stimuli that elicit startle. Given that the majority of current startle research in human participants utilizes electromyography (EMG) recordings of orbicularis oculi (eyeblink) (W. K. Berg & Balaban, 1999), this technique will be the exclusive focus for review. EMG is a technique that measures electrical potentials which are associated with musculature contractions. These electrical impulses can be measured directly from the muscle, through inserted needle electrodes, or from the skin surface above the muscle using surface electrodes. Surface EMG measurements are possible due to the fact that part of the action potential produced in muscle fibers reaches the skin (Andreassi, 2000). Although psychophysiological research most commonly employs bipolar (i.e., paired electrode) surface EMG, other less common configurations are employed. The interested reader is referred to Fridlund and Cacioppo (1986) for a review of the various EMG techniques. However, such a review is beyond the scope of this paper. The following several sections will discuss factors related to bipolar electrode site preparation, placement of electrodes, acquisition of data, and quantification of startle probe eyeblink EMG data.

Site Preparation

Prior to attaching electrodes, the skin site must be prepared in order to minimize noise in the EMG signal. Preparation of site often includes cleansing and abrading. Techniques utilized for cleaning the site include using alcohol wipes (Fridlund & Cacioppo, 1986), however, extreme care has been recommended given the potential for irritation of the eye with certain substances. Abrading the skin area is also important, as it lowers skin resistance, thus reducing noise (Berg & Balaban, 1999). However, recently published research guidelines for human startle eyeblink EMG studies do not recommend the use of abrasion (Blumenthal et al., 2005). Caution is given due to the sensitive nature of this area of the face and the likely interference in startle if irritation is caused. Earlier publication guidelines surveyed top psychophysiological researchers concerning the use of abrasion and other methodologies employed (Fridlund & Cacioppo, 1986). There were several techniques reported for abrading the skin site, including fine sandpaper, scratching site with a sterilized needle, and specialized abrading substances. However, little consensus was found among researchers concerning the use of abrasion, which contributed to advances in recording equipment technology that reduce the need. A recent manual for EMG equipment was reviewed for suggestions concerning abrasion of skin site. Coulbourn Instruments, in their Lab Linc V manual, recommend the use of an abrasive prep pad or abrasive hand soap when using their EMG equipment (Coulbourn Instruments, 2002). Given previous disagreements concerning the necessity of abrading skin sites, a conservative approach would be to cleanse the area well and abrade if impedance levels are found to be above 5000 Ω (see Blumenthal et al., 2005, and Fridlund & Cacioppo, 1986 for details on impedance levels in EMG research).

Electrode Placement

Prior to attaching EMG surface electrodes, conductive gel must first be inserted in the electrode cavity. A conductive substance in the electrode stabilizes hydration and conductivity on the skin site, and reduces the chance of artifactual signals from electrode movement (Fridlund & Cacioppo, 1986). Conductive gel is filled to the rim of the electrode and care should be given to minimize large air-bubbles which can reduce conductivity (Coulbourn Instruments, 2002a). In EMG eyeblink startle research the orbital section of the orbicularis oculi is targeted. Berg and Balaban (1999) suggest centering based on geographical markers to insure consistent placement. The first electrode is centered underneath the eye, with placement on, or just below, the lower bony orbit. The second electrode is placed one centimeter lateral to the first. It has been reported that a consistent distance between electrodes is imperative given that variations can influence EMG levels in this particular site (van Boxtel, Goudswaard, & Schomaker, 1984 – cited in Berg and Balaban 1999).

Quantification of EMG data

An important aspect of EMG data acquisition is signal bandwidth filtration. While the target signal is the electrical impulse from the orbicularis oculi, other signals, both biological and mechanical can also be collected, creating artifacts. For example, many fluorescent lighting systems cause an electrical spike every 1/60th of a second, which can create noise in EMG readings (Coulbourn Instruments, 2002b). Many different bandwidth techniques are employed, all of which are differing combinations of high-pass filtering and low-pass filtering. High-pass filtering removes low frequency components and low-pass filtering removes high frequency components. Commonly, these filters are combined creating a passband range, which captures the range of the targeted signal. An appropriately set passband range will filter out nearby biological noise, or mechanical noise, which falls outside this range. Unfortunately, EMG eyeblink startle research has varied tremendously in passband ranges employed; with many researchers limited to preset ranges designated by EMG equipment manufacturers (van Boxtel, Boelhouwer, & Bos, 1998). Given this fact, van Boxtel and colleagues set out to determine what bandpass range is optimal for EMG orbicularis oculi acoustic probe startle measurements. Optimal high-pass filter was determined to be 28 Hz, and optimal low-pass to be 500 Hz (i.e., a passband range of 28 to 500 Hz). However, with a strong startle reflex being seen, they report that having a higher high-pass filter setting would not be problematic. Additionally, they report that if participant movement is creating artifacts, a higher high-pass filter may actually be beneficial. As for the optimal low-pass filter level, they found 500 Hz to be an optimal level for all conditions researched. In reviewing EMG equipment utilized in this area of research, van Boxtel and colleagues find that optimal bandpass ranges are not provided by the most commonly used manufacturing companies. They advise that researchers take this into consideration when using such equipment (e.g., realizing that low intensity orbicularis oculi activity will be filtered out when using high-pass filter presets significantly above 28 Hz).

Once the appropriate filters have been established, thus narrowing-in on the target signal, the captured signal must be "smoothed" through rectification and integration, transforming this signal into a form more appropriate for analysis. Basically, a raw EMG signal is a bipolar alternating current, with sharp up-down peaks. Rectifying involves taking any part of this bipolar signal that falls below baseline (below zero) and inverting it, combining with the signal above the baseline (W. K. Berg & Balaban, 1999). The next step involves integrating the now rectified signal. The most commonly used method of rectifying an EMG signal is through *contour following* (Tassinary & Cacioppo, 2000). This technique yields an output signal which provides a fluctuating average of the rectified EMG signal strength, based on a preset time-constant. Shorter time-constants show more fluctuations in the signal, whereas longer time-constants smooth-out rapid changes in signal strength. Berg and Balaban (1999) report that most eyeblink startle research utilizes a time-constant of 100 milliseconds. However, Blumenthal (1998) investigated differing time-constants of 10 and 100 milliseconds finding that the former was associated with larger peak amplitudes and total area (duration) of startle reflex. After the contour following process, the signal is smoothed and ready for analysis.

Analysis traditionally includes magnitude and latency of the eyeblink startle reflex, to the acoustic probe. However, several other measurements can also be collected (e.g., duration of response). An adequate baseline measurement is imperative in completing these analyses, given that many algorithms utilize this baseline in their calculations. For example, the magnitude of EMG startle reflex is calculated by subtracting the baseline level from peak level of response. Commonly in psychophysiological research, baseline is a measurement taken during a specified period of time prior to probe onset (Tassinary & Cacioppo, 2000). However, baseline definitions can differ. Baseline can also be the first 20 milliseconds after probe onset. Although seemingly counterintuitive, given that the latency of startle reflex to acoustic startle probe is longer than 20 milliseconds, this time period (i.e., onset of probe to 20 milliseconds) can be used as an appropriate baseline (W. K. Berg & Balaban, 1999). Given the potential noise artifacts included in the EMG signal collected, and the potential for low magnitude blink reflexes, it is also important to have an algorithmic standard for determining a "true" response. Berg and Balaban (1999) report methods including: preset threshold levels in slope or amplitude, and also a statistical threshold in which change must surpass a preset standard deviation level. The obvious goal of response detection is to correctly identify true responses, eliminating EMG fluctuations due to artifactual noise.

In summary, the previous section discussed important aspects related to the physiological measurement of the startle reflex. While the startle reflex can be elicited through various probe methodologies, acoustic startle probes are the most commonly used and preferable method of elicitation. It was discussed that while the startle reflex is characterized by numerous physiological responses, EMG measurement of the obicularis oculi is the current method of choice for research involving human participants due to numerous methodological advantages (see Dawson et al., 1999c). Following this overview of the physiological measurement of the startle reflex will now be a discussion of how startle can be reliably modulated by the affective content of foreground stimuli.

Affective Modulation of Startle Reflex

The eye-blink startle reflex, a somatic reaction to a sudden intense stimulus, can be reliably modulated when occurring in the presence of an emotionally salient event. Simply stated, if a startle reflex occurs while an individual is experiencing a positive or pleasant event, the startle reflex will be inhibited. If a startle reflex occurs when an individual is experiencing a negative or unpleasant event, the startle reflex will be magnified. A common method of inducing differing affective experiences, for research purposes, is presenting an emotionally evocative picture, followed by a startle probe. In general, studies have found that pictures which induce the most startle inhibition are erotic in content (opposite-sex erotica, erotic couples), and pictures that induce the most startle magnification include scenes of threat (attacking people/animals) (Margaret M. Bradley et al., 1999). However, it is important to note that affective startle modulation is not a direct measure of emotion, but more a measure of motivational priming at a non-conscious level (Peter J. Lang et al., 1990). Also, this phenomenon is not a simple process, with several features of the startle probe and the affective foreground (i.e., emotionally evocative pictures) playing a role in the modulation. These features will be reviewed in the following sections, beginning with a review of the major theories explaining the affective modulation phenomenon.

Theories of the Affective Modulation of Startle

Crossmodal Attentional differences hypothesis

One of the first hypotheses attempting to explain affective modulation of the startle reflex was the crossmodal attentional differences hypothesis. This theory proposed that startle inhibition occurs in the presence of positive pictures because attention is drawn to the visual mode, and thus away from (or having fewer resources available for) the differing-mode startle probe. When presenting a negative picture, the stimulus is presumed to be rejected and attention is not given to the visual mode, therefore not inhibiting the startle reflex to acoustic probe (see Bradley, Cuthbert, and Lang, 1999; Lang, 1990, for reviews). This early hypothesis was based on research showing that directing attention towards or away from the sensory mode of the startle probe can modulate the startle reflex. For example, Anthony and Putnam (1985) presented participants with a reaction time test involving a tactile vibration signal of several seconds in which a response was requested of the participants following the end of the signal. The authors randomly administered an acoustic startle probe both between trials (i.e., no vibrating signal occurring), and during random times while the vibrating signal was being administered. They found that eyeblink startle reflex magnitudes were significantly lower in amplitude when the startle probe was administered during a reaction time trial (i.e., vibrating signal occurring) in comparison to administration between trials. They concluded that this relative inhibition is explained by the crossmodal attentional differences hypothesis, with attention being drawn to the tactile sensory system and away from the acoustic system of the startle probe.

Anthony and Graham (1985) tested the crossmodal attentional hypothesis more directly by having participants attend to either visual or acoustic foreground stimuli and administered mode matching, or non-matching startle probes. The foreground stimuli were also “interesting” or “non-engaging”. They found that startle magnitudes were larger for matching-mode startle probes and in comparison to dull slides, interesting slides produced larger startle magnitudes, when the startle probe was mode-matching. These findings suggest that the more that is attention drawn to the startle probe mode (i.e., “interesting” same-mode foreground stimuli), the greater the startle magnitude. At about the same time, Simons and Zelson (1985) used varying picture presentation as a way of manipulating attention to the foreground, followed by an acoustic startle probe. The “interesting” foreground stimuli, presumed to increase attention, were pictures of attractive nudes, and the “non-interesting” were pictures of a wicker basket. They found significantly lower startle reflex magnitudes for the interesting foreground stimuli, in comparison to the non-interesting. They concluded that their results supported the crossmodal attentional hypothesis. Specifically, with more resources being applied to the interesting visual foreground stimuli, fewer resources were available to participants to process the acoustic probe.

Motivational Priming Theory

Lang, Bradley, and Cuthbert (1990) suggested a different interpretation of the above mentioned studies of crossmodal attentional differences. Specifically, they hypothesized that the modulation of startle represented motivational priming. They believed that affective modulation would occur in the presence of emotionally evocative foreground stimuli, regardless of whether the startle probe matched or did not match in modality. Hence, their motivational priming theory, posits that emotions activate neural structures and pathways in the motivational system which are either appetitive or aversive (see Lang, Bradley, and Cuthbert, 1997, pg. 107, for a review of these neural pathways).

Specifically, if the foreground stimulus elicits a positive emotion, the appetitive motivational system is activated. Conversely, if the foreground stimulus elicits a negative emotion, the aversive motivational system is activated. The startle reflex, being a defensive reflex, is then either magnified during an aversive motivational state, or attenuated during an appetitive motivational state. Lang and colleagues hypothesized that this same pattern of either magnification or attenuation would be seen regardless of whether the foreground stimulus and probe stimulus are of the same or differing sensory modes.

Bradley, Cuthbert, and Lang (1990) conducted a study to determine which theory best explained startle modulation by foreground pictorial stimuli; crossmodal attentional differences theory, or motivational priming theory. Participants were shown sets of pictures in three categories: pleasant, neutral, and unpleasant. While viewing these pictures, visual and acoustic startle probes were independently and randomly administered. The critical feature of this study is the visual startle probe results, which puts both hypotheses at theoretical odds. That is, the theory of motivational priming predicts a startle magnification for unpleasant pictures and attenuation for pleasant pictures, during the visual startle probe. However, the theory of crossmodal attentional differences predicts just the opposite. Taking into consideration that the startle probe is of the same mode as the foreground pictorial stimuli, pleasant pictures should direct more "resources" or attention to the visual mode and therefore elicit a relatively magnified startle reflex. Conversely, negative pictures should show the opposite results, directing attention away from the visual mode, attenuating the startle reflex. Results of this study supported the theory of motivational priming and provided evidence against the theory of crossmodal attentional differences. The pleasant pictures significantly attenuated the startle reflex while the unpleasant pictures significantly magnified the startle reflex, regardless of the sensory mode of the startle probe.

Research in the area of affective modulation of startle reflex burgeoned from this point, with many significant details concerning the startle probe and foreground stimuli (i.e., emotionally evocative pictures) being identified. The next several pages will discuss these findings, detailing the relevant factors associated with startle probe and foreground stimuli characteristics and presentation.

Startle Probe Factors

There are several factors of the startle probe that have been shown to influence affective modulation of the startle reflex. The following will discuss these factors, which include: the intensity and aversiveness of the probe, unilateral lobe presentation (e.g., right ear only versus left ear only presentation of acoustic probe), and the latency of the probe onset post affective picture presentation.

Probe Intensity and Aversiveness

As discussed earlier, increased intensity of the startle probe has been shown to be related to an increased startle reflex (Turpin et al., 1999; Yates & Brown, 1981). Additionally, an early affective startle modulation study compared subjective reports of aversiveness of the same intensity visual probe, finding that those participants who rated aversiveness as high, showed significantly higher startle reflex (Margaret M. Bradley et al., 1990). Due to the possibility that differing levels of probe intensity may be perceived differently in aversiveness, Cuthbert, Bradley, and Lang (1996) investigated how this may influence affective modulation of startle reflex. Specifically, they examined acoustic startle probes of three different intensity levels (80, 95, and 105 dBs) while participants viewed pleasant, neutral, and unpleasant pictures. Increased probe intensity was found to be related to increased startle magnitude. Also, increased startle probe intensity level was also subjectively rated as more aversive. However, there was no interaction with picture valence (pleasantness) or arousal level. These findings suggest that the same pattern of affective modulation is seen at all levels of probe intensity, even though there is a linear increase in startle magnitude by probe intensity level.

Unilateral Hemisphere Probe Presentation

In 1996, Bradley, Cuthbert, and Lang set out to confirm their earlier research suggesting a hemispheric specialization in the affective modulation of startle (e.g., Bradley, Cuthbert, & Lang, 1991 – cited in Bradley, Cuthbert, and Lang, 1996). Specifically, they set out to confirm previous evidence that acoustic startle probes presented monaurally to the left ear (right lobe) exhibited a strong affective modulation of startle, while probes presented monaurally to the right ear (left lobe) showed no affective modulation. Two experiments were conducted, with a blocked presentation to either ear, and also a mixed presentation of monaural probe presentation. The first experiment was conducted to replicate the methodology of previous studies, while the second was conducted, in part, to rule-out any effect of blocked presentation. For both experiments results were consistent with previous findings. There was an affective modulation effect for left ear probe presentation of startle, but not for right ear presentation. Interestingly, also consistent with previous studies, there was *not* a significant interaction observed between probe presentation ear and foreground picture valence. The authors suggest that there may be a small modulatory effect for right ear presentation, despite the non-significant findings. The authors further note that this "small effect," if actually present, would still support the notion of unilateral hemispheric involvement for affective modulation, given that auditory signals sent from the right ear have approximately one-third of their afferent fibers extending ipsilaterally to the right lobe.

More recent studies provide additional evidence for a unilateral emphasis in the affective modulation of startle. Studies examining neurological correlates of affective picture processing including functional magnetic resonance imaging (Peter J. Lang et al., 1998) and event related potentials (Junghoefer, Bradley, Elbert, & Lang, 2001) find greater activation in the right hemisphere when participants view emotionally evocative pictures. Funayama, Grillon, Davis, and Phelps (2001) extended upon previous neuroimaging and monaural acoustic probe presentation studies by investigating the hemispheric unilaterality of startle modulation. They took an extirpative approach, investigating startle reflex modulation by emotionally evocative pictures, with a clinical sample of participants post unilateral temporal lobectomy. A temporal lobectomy is a protocolled surgical procedure, in which 70-80% of the amygdala and all of the hippocampus is removed; utilized primarily for treatment of epileptic seizures. Funayama and colleagues compared individuals with left-only temporal lobectomy (LTL), right-only temporal lobectomy (RTL), and an age/education matched control group. Consistent with previous studies of right-lobe unilateral emphasis with startle modulation, the RTL group showed no startle potentiation in the presence of negative pictures, whereas both the LTL and control group did. Additionally, within group analyses in the LTL and control group revealed significant differences in startle, for picture type, in the expected direction (i.e., attenuation for positive pictures, and potentiation for negative). However, the RTL group did not show significant differences in startle magnitude by picture type, with positive and negative pictures showing nearly identical eyeblink EMG amplitude. The authors cite several lines of research which coincide with their findings, concluding that the amygdala is most likely responsible for modulation of emotional responses, as measured by EMG eyeblink of the acoustic startle reflex. Overall, there appears to be substantial evidence in support of the notion that emotional modulation of startle reflex primarily, if not entirely, occurs in the right hemisphere of the brain.

Latency of Probe Presentation

The latency in which the startle probe is presented, in comparison to the presentation of the affective foreground stimulus, has also been found to be a critical factor in affective modulation. Bradley, Cuthbert, and Lang (1993) systematically investigated varying picture to startle probe latency times, assessing the changes in affective modulation. They were particularly interested in whether picture presentation could act as a prepulse inhibitor, given a small enough latency to startle probe. Prepulse inhibition (PPI) is a well-documented phenomenon, concerning the reliable attenuation of the startle reflex when the startling probe is preceded immediately by a non-startling, less-intense stimulus (prepulse) (e.g., non-startling sound, Blumenthal, 1988; low-intensity flash of light, Burke & Hackley, 1997). While a detailed discussion is outside the scope of this review, this inhibition is thought to reflect a neuro-protective process in which the first stimulus (prepulse) is protected during initial processing by giving less processing resources to an immediately following stimulus (i.e., startle probe) (see Blumenthal, 1999, for review). Bradley, Cuthbert, and Lang presented positive, negative, and neutral pictures for 6 seconds, with acoustic startle probes being presented at either 300, 800, 1300, or 3800 milliseconds after picture onset. They also presented acoustic startle probes at structured times post picture offset (no image) to assess the duration of the affective modulation. The results of the study showed that the magnitude of startle reflex was significantly affected by latency of startle probe to picture onset. Furthermore, they found that picture presentation acted as a prepulse inhibitor, significantly attenuating startle reflex, if the startle probe is presented 300 to 800 milliseconds after picture onset. Affective modulation from the emotionally laden picture was reliably found only after 1300 milliseconds post picture presentation. Results also revealed that the affective modulation terminates rather quickly after picture offset, with non-significant results found at 300 milliseconds and later, post picture off-set. A few years after this study, Bradley, Drobes, and Lang (1996) presented a replication study and noted consistent results. They also included additional latency points in this replication study, finding that while affective modulation can be seen at shorter latency points, startle magnitude increases linearly with increased latency time, reaching asymptote at approximately 3 seconds (data presented in Bradley et al., 1999).

These studies suggest that the latency of startle probe to picture onset is critical. If the probe is presented too early (e.g., 300-800 ms) then affective modulation will most likely not be seen and the startle reflex magnitude will be significantly attenuated. Furthermore, based on previous studies, it appears that a latency of 3 seconds post picture presentation is the most appropriate point to elicit a startle reflex, when studying affective modulation.

Foreground Stimuli Factors

This section will review previous research on foreground stimuli (affective pictures), and its relationship to the affective modulation of startle. Several issues related to the presentation of the affective pictures will be discussed, including how duration of picture presentation, and also repetitive presentation, can influence affective modulation. A review of the literature regarding the specific characteristics of the affective picture will follow, including a discussion of how the arousal level of the picture content, and the category or type of picture, can influence affective modulation.

Foreground Presentation

As discussed earlier, it has been well documented that affective foreground can modulate the startle reflex. It has also been discussed how the latency in which the startle probe is presented after the picture can be influential. Research has also been conducted looking more specifically at the foreground stimulus, and in particular how the duration of presentation can influence affective modulation. Codispoti, Bradley, and Lang (2001) investigated how briefly presented affective pictures modulate the startle reflex. Neutral, pleasant and unpleasant pictures were presented for 500 milliseconds, with startle probe times being presented at times ranging from 300 to 4800 milliseconds post onset of affective picture. Interestingly, even though the majority of probes were presented after offset of the affective picture, the pattern of affective modulation obtained was very similar to results obtained with pictures being present. In other words, even though the picture presentation had stopped at 500 milliseconds, the affective influence of that picture was still present at startle probe points past this time. However, some differences were seen. For example, although startle reflex magnitude for unpleasant pictures were significantly greater than for pleasant pictures, they were not significantly larger than neutral pictures, as seen traditionally in affective modulation. The results of this study may initially seem to be contrary to an earlier discussed article by Bradley, Cuthbert, and Lang (1993), in which no modulation was found post offset of picture. It is important to remember, however, that in this previously discussed article, picture offset was after 6 seconds of viewing, whereas with Codispoti, Bradley, and Lang picture offset was after 500 milliseconds of viewing. Bradley, Cuthbert and Lang (1999) suggest that this type of difference may be due to the fact that picture processing is complete by 6 seconds of viewing, while processing may continue until some point before 6 seconds.

The duration of affective picture presentation was also investigated by Globisch, Hamm, Esteves, and Ohman (1999). Affective pictures were presented for a mere 150 milliseconds, with startle probes at several set points after onset. In this study, all of the unpleasant pictures were of snakes and spiders. In addition to including a "normal" participant group, this study also utilized a group of individuals with reported high fear of snakes and spiders. In both groups, as expected, prepulse inhibition was seen for probes presented soon after picture presentation. Interestingly, in the “normal” (non-fear) group, no affective modulation was seen; whereas in the high-fear group there was a significant potentiation of startle reflex for the unpleasant, feared pictures. These results suggest that a picture presentation time of 150 milliseconds is generally insufficient for inducing affective modulation of startle, unless this stimulus is particularly salient to the participant.

Another factor in the presentation of affective foreground is repetition. The startle reflex per se has long been known to habituate to the repeated presentation of a startling stimulus (see Siddle & Lipp, 1997); but what about affective modulation of startle? Does the phenomenon of affective modulation of startle continue despite repetitive presentation of the same stimuli, or does it habituate? Bradley, Lang, and Cuthbert (1993) sought to answer this question directly. The authors presented 2 pleasant, 2 neutral, and 2 unpleasant pictures repetitively for a total of 72 trials. Each affective picture was seen a total of 12 times, with a startle probe being presented on 9 of these 12 presentations randomly. As expected, they found that the overall magnitude of startle reflex showed a linear decrease across trials for all picture types. However, despite of this overall decrease in magnitude, the pictures continued to show affective modulation throughout the entire habituation series. Surprisingly, the results actually suggested that affective modulation may be even stronger after some habituation of startle has occurred. Bradley, Lang and Cuthbert further assessed the role a novel foreground stimulus plays in affective modulation of startle by presenting an unseen affective picture immediately following the habituation trials. The results suggested that affective modulation is insensitive to the novelty of the affective foreground, with the startle magnitude and modulation of the novel picture being the same as the last habituation trial.

Bradley, Lang, and Cuthbert (1993) report that the results from their affective picture repetition study "strongly support" the dual startle circuit hypothesis proposed by Davis and File (1984 - cited in Bradley, Lang, and Cuthbert, 1993). This hypothesis proposes that the neural path of the startle reflex is composed of two circuits, the basic and modulatory. The overall decrease in startle magnitude is seen as a habituation of the "basic" startle reflex, while the modulatory startle reflex – as assessed by affective modulation – does not appear to habituate. The results of Bradley, Lang, and Cuthbert (1993) also support an earlier hypothesis put forth by Lang, Bradley, and Cuthbert (1990). Specifically, the authors hypothesize that startle augmentation to unpleasant pictures, and attenuation to pleasant pictures, is part of a single process, not two different phenomena. The fact that affective modulation, in both directions, is maintained despite an overall habituation of startle magnitude, suggests the possibility of a singular phenomenon.

In summary, several important factors of presentation have been identified. As for the duration of picture presentation, even briefly presented pictures can induce affective modulation, post offset, just as if the stimuli were actually present. However, at very brief presentation times (e.g., 150 milliseconds), the picture decreases in salience, showing affective modulation only for those participants in which the stimulus is particularly relevant (e.g., specific phobias). As for repetition of presentation, it appears the phenomenon of affective modulation is immune to habituation, as well as the novelty, of affective foreground. Previous research suggests that startle is most likely comprised of two different and independent neural circuits, a basic and a modulatory (Bradley, Cuthbert, & Lang, 1993). The basic startle circuit shows habituation, decreasing in magnitude with repetitive presentation. However, the modulatory circuit, as assessed via affective modulation, persists regardless of overall habituation of magnitude.

Foreground Characteristics

Picture characteristics have also been documented as influencing the affective modulation of the startle reflex. As discussed earlier, pleasant pictures have been documented as attenuating, and unpleasant pictures as enhancing, the startle reflex. However, emotionally evocative pictures can differ in their level of intensity (i.e., level of arousal). Cuthbert, Bradley, and Lang (1996) sought to determine the relationship between differing arousal level of pleasant, unpleasant, and neutral pictures on affective modulation of startle. The authors utilized pictures from the International Affective Picture System (IAPS) which had previously been rated by a large sample of participants for level of valence (pleasant, unpleasant, and neutral) and arousal (Center for the Study of Emotion and Attention, 1995). Pictures were selected in order to derive a range of arousal level for each picture type. However, gathering a complete range of arousal for each type of picture was not possible given the unfeasibility of finding very arousing neutral pictures, or very pleasant/unpleasant pictures that were very low in arousal. Although previously being rated on valence and arousal, the participants in the study also provided their own ratings on the presented pictures after the completion of the study.

Additionally, autonomic measurements (skin conductance) were taken during the study as an objective means of determining arousal level of picture. On a parenthetical note, the replication in rated pleasantness and arousal in the current study showed an astounding .99 and .93 Pearson correlation, respectively, with the IAPS normative ratings. This suggests it may be feasible for future studies to utilize the IAPS ratings directly, forgoing replicative participant ratings, when using an average (i.e., non-clinical) population.

The results of the study by Cuthbert, Bradley, and Lang (1996) found that affective modulation of the startle reflex is highly dependent on the arousal level of the affective foreground stimulus. This finding was consistent with both subjective (e.g., participant rated) and objective (e.g., skin conductance response) measures. Startle augmentation for unpleasant, and attenuation for pleasant pictures was seen only for those pictures high in arousal. For pictures which are moderate in arousal level, both unpleasant and pleasant pictures show startle attenuation. The authors hypothesize that this reflects more of an "orienting-interest" reaction, rather than affective modulation, due to the moderate arousal of content. Pictures low in arousal showed little systematic variation for any picture type. The authors conclude that arousal level of the affective foreground is an important moderating variable in affective modulation of startle, and that researchers should take this into consideration when studying this phenomenon.

Arousal content of affective foreground was investigated more recently, utilizing sounds instead of pictures (Bradley & Lang, 2000). The participants rated the sounds for valence (level of pleasantness) and arousal, and showed the best recall for sounds rated higher in arousal content. Affective modulation was also found, however there was not a significant effect of rated arousal level as seen with pictures. Interestingly, physiological correlates with subjective reports for arousal level were particularly low ($r = .26$) in comparison to that seen traditionally with pictures (e.g., $r = .81$; Lang, Greenwald, Bradley, & Hamm, 1993). It is important to note that Bradley and Lang utilized a visual startle probe (flash of light) in their study, which makes direct comparison with the acoustic startle probe studies less feasible. It is quite possible that arousal level of acoustic affective foreground does moderate affective startle modulation, but is not seen given a less intense startle probe (flash of light). As discussed in previous sections, direct comparisons of visual, cutaneous, and acoustic startle probes have shown that visual probes produce the weakest startle reflex, and the longest latency to response (Zeigler, Graham, & Hackley, 2001).

Another characteristic of affective foreground stimuli that has been shown to be influential in affective modulation of startle is picture category, or type of scene depicted. In 1995, Lang, Bradley, Drobes, and Cuthbert conducted a preliminary investigation of categorical differences of pleasant and unpleasant pictures in affective modulation. Of the many different types of pleasant pictures utilized, it was found that erotic scenes produced the greatest startle inhibition. Another preliminary study by Schupp et al. (1996) included additional categories of erotica (erotic couples, romantic courtship, and attractive opposite sex nudes), as well as neutral and violent/aggressive pictures. Strong blink inhibition was seen with erotic pictures, with relatively more inhibition seen in males for pictures of opposite sex nudes, and females for pictures of romantic courtship.

Both males and females, however, showed similar startle augmentation for violent pictures.

The role of picture category was investigated further by Bradley, Codispoti, Cuthbert, and Lang, in 2001. The authors proposed that, from an evolutionary perspective, emotions are guided by two motivational systems: appetitive and defensive. From this standpoint, the defensive system has evolved to activate in threatening situations, and the appetitive system in the presence of situations involving the promotion of survival - including situations involving "sustenance, procreation, and nurturance" (pg. 276). Thus, attenuation of startle in the presence of pleasant stimuli is thought to reflect activation of the appetitive motivational system, whereas augmentation of startle, in the presence of unpleasant stimuli, is thought to result from activation of the defensive motivational system. In this study, Bradley and colleagues (2001) examined several categories of pleasant and unpleasant pictures. The authors hypothesized that pictures which represented events most threatening to survival would show larger startle augmentation in comparison to other unpleasant non-threatening pictures. Pleasant pictures which depicted fundamental primary reinforcers (e.g., copulation, or erotica) were hypothesized to show greater startle attenuation than other pleasant pictures (e.g., sports, families, nature). Results were consistent with their hypotheses as it was found that pictures of attacking animals and attacking humans showed the greatest startle augmentation. They also found that pictures of mutilated bodies showed a significant startle increase, whereas negative pictures of pollution or loss produced little to no startle augmentation. As for pleasant pictures, again as hypothesized, those involving erotic scenes (couples, and opposite sex) showed the greatest startle attenuation. The pleasant picture categories of adventure, nature, and food produced only a minimal amount of startle attenuation, whereas the pleasant categories of sports and families did not show any startle attenuation. Interestingly, while participants rated erotic same-sex pictures (i.e., same sex as participant) as unpleasant and low in arousal, somatic and autonomic reactions were consistent with other opposite sex and couple erotic pictures (i.e., attenuated startle, augmented skin conductance response). The authors hypothesize that pictures of same sex individuals in erotic poses may activate appetitive motivations through cueing memories of erotic behavior in general. They suggest that the mismatch of subjective reports and somatic measurements may be a cultural product, with western heterosexual culture typically frowning upon increased sexual arousal by members of the same sex. However, sexual orientation and sexual conservativeness was not assessed for in this study; therefore extrapolation may best be left for future studies.

In summary, both the arousal level and categorical type of the affective foreground have been shown to be influential in the affective modulation of startle. Startle augmentation for unpleasant foreground stimuli, and attenuation for pleasant foreground stimuli, has been shown to occur, but only with stimuli high in arousal. When utilizing pictures as the affective foreground, the category of the picture has also been found to be important. Specifically, pictures involving threat, via images of attacking humans or animals, show the greatest startle augmentation, and pictures of erotica show the greatest startle attenuation. As a whole, the research reviewed suggests that arousal level and category should be taken into consideration when selecting foreground stimuli in future affective modulation research.

The previous two sections have reviewed both the fundamental aspects of eliciting and collecting startle reflex data. The phenomenon of affective modulation of startle was also reviewed, including how different features of the startle probe and foreground stimulus can influence this modulation. The following section will build upon both these two previously reviewed sections, looking specifically at startle methodology utilized in nicotine research.

Effects of Nicotine on the Startle Reflex

In smokeless tobacco, cigarettes, and other tobacco products, there are thousands of chemicals ingested when used. However, nicotine is widely believed to be the principal chemical involved in addiction to these products (Committee to Assess the Science Base for Tobacco Harm Reduction, 2001). The features of nicotine dependence include tolerance to the substance, and withdrawal symptoms following a period of abstinence or reduced use. While a vast body of literature exists regarding the negative effects and consequences of smoking, the following will discuss how nicotine specifically influences the startle reflex. Literature regarding nicotine's influence on the startle reflex in the animal model will first be discussed. This will be followed by a discussion of the current body of knowledge regarding nicotine, smoking, and the startle reflex.

Nicotine's Influence on Startle: Animal Models

Two of the main features of the startle reflex that are analyzed in research examining this response include the amplitude of reflex and the amount of prepulse inhibition (PPI). (As noted previously, PPI is a decreased amount of startle reflex when startling noise is preceded directly by a non-startling noise.) In animal research, nicotine has been shown to influence both of these features, increasing both startle amplitude and amount of PPI (Acri, Grunberg, & Morse, 1991; Acri, Morse, Popke, & Grunberg, 1994). Furthermore, amplitude and PPI have been shown to be independently influenced by nicotine, with very minute administrations of nicotine (.01mg/kg) showing no influence on startle amplitude but significantly increasing PPI (Acri et al., 1994). In addition to influences of nicotine administration, influences of nicotine withdrawal have also been investigated. Rats that have been administered nicotine for several days, and then deprived of nicotine, show increased startle amplitude, in comparison to controls (Helton, Modlin, Tizzano, & Rasmussen, 1993). This effect reportedly to lasts up to four days post cessation before returning to control levels.

Nicotine's influences on the startle reflex have been extended beyond a simple one-to-one relationship, with researchers investigating how third variables may play a role. Acri (1994) investigated nicotine's influence on the startle reflex in rats, taking into consideration differing levels of nicotine and induced stress. The intent of the study was to help clarify the contradictory phenomenon of individuals utilizing nicotine (a stimulant) to reduce stress. The author utilized three stress level groups: no-stress control, moderate (subject in presence of restrained rat), and high (subject restrained). The different levels of induced stress were verified biologically via plasma corticosterone concentration. Three different levels of nicotine administration were also utilized (0mg, 6mg, and 12mg; per kilogram of weight). Acri found that both stress and nicotine independently increased the amplitude of startle reflex and amount of pre-pulse inhibition. However, one of the most notable results in this study was the significant interaction between nicotine and stress. At low doses of nicotine (6mg/kg), there was a linear increase in the amplitude of the startle reflex, with increased stress. At high levels of nicotine (12mg), there was a linear decrease in amplitude of startle reflex, with increased stress level. In other words, Acri found that low levels of nicotine increases the level of stress experienced, whereas high doses of nicotine decreases the level of stress experienced (as assessed by startle reflex). A similar interaction, nicotine by stress, was found for level of prepulse inhibition. When looking at the no-stress condition, high levels of nicotine increase PPI (i.e., very little startle reflex), and low levels of nicotine show no effect. However, as level of stress increases, high levels of nicotine decrease PPI, and low levels of nicotine increase PPI. Taken as a whole, this study suggests that nicotine has the ability to increase and decrease experienced distress, depending on the level of stress and amount of nicotine administered.

The neural mechanism through which nicotine influences startle has also been investigated. As discussed in the beginning of this section, maintaining the use of, and dependence to, nicotine has been tied to nicotine's relationship to the neurotransmitter dopamine. Given this, Lewis and Gould (2003) investigated whether mecamylamine (nicotinic receptor antagonist) and haloperidol (dopamine receptor antagonist) would block startle magnitude augmentation from nicotine administration in mice. As expected, the authors found significant augmentation of startle with administration of nicotine. The results also revealed that mecamylamine prevented the augmentation of startle reflex, suggesting that the activation of nicotinic receptors mediate the enhancement of startle from nicotine. Haloperidol was also found to prevent startle enhancement from nicotine, suggesting that dopamine is also critical in the process of startle augmentation via nicotine. Given that nicotinic receptor stimulation leads to the release of dopamine, it appears that Lewis and Gould most likely prevented nicotine startle augmentation through chemical blocks at two different points along the same path. This process, however, appears to be the neurological means through which nicotine enhances the startle reflex.

Complicating a more simple explanation of the relationship between nicotine and startle, genetic studies in mice show great variation in the influence of nicotine, by strain of mouse (Marks, Stitzel, & Collins, 1989). Specifically, Marks and colleagues tested 19 different strains of mice finding that approximately 1/3 of the mice strains showed an increase in startle when administered nicotine, 1/3 showed a decrease, and 1/3 showed no difference from baseline. Moreover, baseline measure differences in each strain could not explain the differential influence of nicotine (i.e., significantly higher or lower baseline level not related to specific directional influence by nicotine). Acri, Brown, Saah, and Grunberg (1995) also investigated genetic differences in acoustic startle. The authors noted a significant difference by strain interaction for startle magnitude and PPI in general, however, the different strains tested showed no differential reactivity to nicotine through startle reflex magnitude. It is important to note that the authors utilized only three different strains in their study, which could account for the inconsistencies with Marks, Stitzel, and Collins (1989). In addition to their genetic findings, Acri, Brown, Saah, and Grunberg (1995) note that age is another important variable to consider when looking at nicotine's influence on startle in mice. The authors found that nicotine significantly enhanced both startle magnitude and PPI in older mice, but did not find any significant influence of nicotine among younger mice.

In sum, animal studies of nicotine and startle have shown that nicotine can increase the intensity of the startle reflex, and the amount of prepulse inhibition. Research has also indicated that this increase can be blocked chemically by nicotinic and dopaminergic receptor antagonists. However, as with many phenomena in science, the relationship between nicotine and startle is anything but simple. The amount of stress being experienced, genetics, and even age, has been shown to interact with nicotine's influence on startle in the animal model. The following section will now review the research conducted on nicotine and startle in the human model.

Nicotine's Influence on Startle: Human Models

The following section reviews research investigating nicotine's influence on the startle reflex in humans. The phenomena of increased prepulse inhibition (PPI) and changes in startle amplitude due to nicotine administration will be reviewed. Additionally, the influence of smoking-cues on the startle reflex will be discussed. Finally, a discussion of a recent study suggesting that startle data may be utilized to predict successful smoking cessation will be detailed.

Prepulse Inhibition

Kumari, Checkley, and Gray (1996) produced the first publication documenting the effects of nicotine on prepulse inhibition (PPI) of the startle reflex, in healthy participants. In this study, a group of smokers were asked to abstain from smoking over night and arrive for testing the following morning. The startle reflex was assessed while participants were still in a deprived state and then again towards the end of testing, 25 minutes later. At the later startle probe, participants were either allowed to smoke just prior to startle assessment, or continued to be deprived of nicotine. It was found that those smokers who were allowed to smoke prior to the second startle assessment showed significant PPI enhancement, in comparison to their first deprived-state assessment. However, those smokers who continued in a nicotine deprived state showed no difference in level of PPI. Although finding significant results, the authors concede that the study does not provide conclusive evidence that nicotine is the key chemical responsible for the increased PPI observed. Indeed, as discussed in earlier sections, cigarettes contain thousands of chemicals, many of which could potentially interfere with the startle reflex. Additionally, the significant improvement with those who smoked after deprivation could have reflected a return to baseline after a deprival-state decrement. This quandary was resolved a short while later by a follow-up study assessing nicotine's effect on startle directly, through intravenous injections of nicotine, among healthy non-smokers (Kumari, Cotter, Checkley, & Gray, 1997). It was found that prepulse inhibition was significantly enhanced after administration of 12 milligrams of nicotine, in comparison to a placebo (saline) injection. These results, in conjunction with the animal studies discussed earlier, provide strong evidence that nicotine is indeed the chemical responsible for increased PPI from smoking. Also, given that non-smokers were utilized in this latter study, withdrawal effects have been ruled out as the sole impetus for increase PPI.

In order to further clarify the effects of nicotine and withdrawal on PPI of the startle reflex, Duncan et al. (2001) conducted a study utilizing both nicotine dependent smokers and non-smokers. The results showed that smokers who abstained from cigarettes overnight showed similar PPI as non-smokers. However, when the nicotine dependent participants smoked after a night of abstinence, PPI was significantly enhanced, and was also significantly greater than non-smoker PPI. A particularly notable finding in this study was the lack of increased PPI in nicotine dependent participants when they were allowed to smoke ad lib the previous night and morning. In this condition, smoking just prior to the second testing produced no increased PPI. Therefore, while previous studies show that nicotine increases PPI, there also appears to be a "withdrawal effect" from nicotine which can also independently contribute to PPI variance. Also notable, in this study and others (e.g., Postma, Kumari, Sharma, Hines, & Gray, 2001), is that there has been a non-significant trend suggesting that withdrawal state inhibits PPI below levels of non-smoking controls. Overall, there appears to be strong evidence that nicotine mediates the effect of increased PPI following smoking, and for nicotine dependent smokers withdrawal state can also influence PPI.

Startle Amplitude

Several studies have shown that nicotine, in and of itself, has little effect on the amplitude of the startle reflex in the human model. One of the purest examples is with a sample of non-smokers subcutaneously injected with differing levels of nicotine, followed by an assessment of startle reflex changes (Kumari et al., 1997). This study showed that neither a placebo, 6mg of nicotine, or 12mg of nicotine injection significantly differed in their influence on the amplitude of startle reflex. An additional study (K. E. Hutchison, Niaura, & Swift, 2000) assessed the differences in startle factors between low and high nicotine content cigarettes. Differing levels of nicotine in cigarettes failed to show any significant difference in startle magnitude. Della Casa, Hofer, Weiner, and Feldon (1998) also assessed startle amplitude in both male and female nicotine-dependent smokers finding no significant differences in deprived and non-deprived conditions. Although rare, a few studies have documented changes in startle amplitude following nicotine administration, but involve several other factors. For example, in smokers who have been deprived overnight, the amplitude of the startle reflex was shown to significantly decrease after participants were allowed to smoke (Kumari et al., 1996). However, this finding has been inconsistently found in other studies utilizing similar deprivation procedures (Della Casa et al., 1998; Duncan et al., 2001). Methodological choices, however, could have played a role in the Duncan et al. (2001) study's inconsistent findings. Specifically, the authors of this study utilized "friends and family" as participants, and even more surprising used the "honesty system" to insure compliance with abstinence (i.e., no biological measures were utilized to verify smoking abstinence). Overall, it appears that nicotine does not have a direct effect on the amplitude of the startle reflex. However, it remains to be seen whether certain third variables (e.g., withdrawal state) interact to influence startle amplitude.

Smoking Cues

In previous sections, affective modulation of startle was discussed. The phenomenon of affective modulation of startle basically refers to the ability of foreground stimuli to influence the magnitude of startle, based on their affective content – startle augmentation for negative affective content, and attenuation for pleasant affective content. Elash, Tiffany, and Vrana (1995) investigated how imaginal smoking cues influenced the startle reflex. The authors dictated positive, negative and neutral sentences containing cues of smoking to nicotine-dependent participants. Additionally, the participants also imagined positive, negative and neutral sentences which did not contain smoking cues. Although subjective ratings by participants showed increased urges to smoke for smoke-related sentences, and appropriate ratings for affective directions of sentences, startle modulation did not follow the traditional pattern seen in affective modulation. All imaginal smoking cues, regardless of affective valence, showed significant startle augmentation in comparison to control (non-smoking cue) imagined sentences. The results from this study suggest that imaginal smoking cues are likely perceived as negative by dependent smokers, given the augmented startle reflex.

Although imaginal material has been shown to be effective in modulating the startle reflex, it is difficult to standardize imagined data given that vividness and specific imagined content therein most likely varies from person to person. For example, when conducting systematic desensitization with imaginal exposure material, patients can vary in their level of imagined vividness or even engage in distraction activities; thus reducing the effectiveness of treatment (Leahy & Holland, 2000).

In 1999, Hutchison, Niaura, and Swift assessed the effect of smoking cues on the startle reflex, utilizing *in-vivo* exposure (e.g., having participants hold and light a cigarette, then staring at it for several seconds). Subjective ratings measures showed that smoking cues induced negative affect when tested in a deprived state. Furthermore, the authors found that smoking cues produced a significant decrease in level of prepulse inhibition, which the authors hypothesize was the result of smoking cues eliciting dopaminergic activity from cued association. However, *in-vivo* smoking cues, in comparison to control (e.g., holding and looking at pencil), failed to produce a significant difference in level of startle magnitude. The results of this study are difficult to extrapolate. Taking into consideration that the authors utilized an *in-vivo* smoking cue, several cues were present, including tactile cues from holding the cigarette, visual cues from seeing the cigarette, and olfactory cues from a lit cigarette. The challenge for studies after this point was isolating these cues and determining their independent effects.

Geier, Mucha, and Pauli (2000) further examined the effect of smoking cues on the startle reflex via presentation of smoking cue pictures, among smokers and non-smokers. The authors also presented standardized non-smoking-cue affective pictures (e.g., pleasant, neutral, and unpleasant). They found that smokers showed significant startle attenuation in the presence of smoking cues, with responses being significantly lower than unpleasant affective pictures and a similar magnitude to pleasant affective pictures. Interestingly, no differences were seen between non-deprived smokers, deprived smokers, and deprived smokers who were informed that they would smoke soon after testing. In other words, all smokers perceived smoking cues as positive (via objective physiological measures, and subjective ratings). As for non-smokers, a significant startle magnification was seen with smoking-cue stimuli, being significantly larger than pleasant affective pictures, and similar to responses to unpleasant pictures.

Psychophysiological measures coincided with subjective ratings, showing that non-smokers perceived smoking cues as unpleasant. These findings are in contrast to a recent study by Orain-Pelissolo, Perez-Diaz, Jouvent, and Grillon (2004). The authors of this recent study compared the modulatory effect of smoking-cue pictures on the startle reflex among smokers and non-smokers. They found no significant differences between smokers and non-smokers, and found no modulatory effect for smoking-cue pictures. Orain-Pelissolo et al. conclude that "…smoking cues have no effect on the positive reinforcement of nicotine consumption". However, it should be noted that the authors covaried out baseline differences prior to conducting their analyses, a statistical procedure not typically conducted in human startle analysis. This covariation procedure was conducted despite the fact that previous research has shown that differences in baseline among participants are independent of the effects of modulation by affective foreground pictures (Bradley et al., 1993). It is also interesting to note that the baseline differences in Orain-Pelissolo et al. were at higher levels in non-smokers, and slightly (albeit non-significantly) so during smoking-cue testing; suggesting that covariance pulled the results towards non-significance. Also of interest were the skin-conductance results. No covariate was utilized in this analysis and the results indicated that smokers had significantly larger skin conductance responses, in comparison to non-smokers, for smoking-cue related pictures.

Overall, this relatively new direction in research – smoking-cue modulation of startle – is in dire need of replication with consistency in stimuli presentation modality. One study used imaginal stimuli and suggested that smoking-cues are perceived as aversive (Elash et al., 1995). Another study used *in-vivo* smoking-cue presentation and showed a decrease in PPI but no affective modulation (Hutchison et al., 1999). Yet another study has used picture presentation smoking-cues and has found that smokers view smoking-cues as pleasant, and non-smokers view these cues as unpleasant (Geier et al., 2000). In addition to consistency in cue presentation modality, it may also be of benefit for future studies to begin utilizing a standardized set of smoking-cue pictures, similar to the affective pictures standardized by The Center for the Study of Emotion and Attention (1995) and utilized widely in startle research today.

Cessation Relapse Prediction

While many studies have investigated the relationship between nicotine and the startle reflex, only one study to date has investigated nicotine cessation as related to the startle reflex. Postma, Kumari, Sharma, Hines, and Gray (2001) collected startle data on dependent smokers identified as wishing to quit smoking. Startle data was collected prior to cessation, 24 hours post cessation, and at 1 month follow-up. The results showed that startle reflex facets predicted successful cessation at one month follow-up. Baseline (pre-cessation) startle amplitude was significantly higher for the successful cessation group, in comparison to the later-relapse group. Furthermore, the successful cessation group showed a significant drop in their level of startle amplitude from pre-cessation to 24-hour post cessation, whereas the later-relapse group showed a non-significant increase for this same time-period. Subsequent analyses revealed that the two groups also significantly differed in their level of nicotine dependence, as assessed by the Fagerström Tolerance Questionnaire (FTQ) (Fagerström & Schneider, 1989), with the later-relapse group having higher scores. The authors report that while higher scores have been shown to be related to increased risk of relapse, FTQ scores in this study were not significantly correlated with startle reflex measurements, suggesting independence in predictability. The authors contrasted their findings with that of McDermut and Haaga (1998) who found that an increased level of commitment to quit smoking was related to increased heart rate reactivity to smoking cues. In accordance with McDermut and Haaga (1998), Postma et al. (2001) suggest that the initial higher amplitude of startle reflex seen in successful cessation group may reflect their higher level of commitment to quit smoking (perceiving the first testing event as more aversive – and therefore magnifying startle – given their commitment to quit). However, Postma et al. (2001) did not assess the "Stage of Change" or level of commitment to change in this study, therefore support for this theory awaits future studies.

Stages of Change Theory

The Transtheoretical model explains behavior change through "Stages of Change." The concept of Stages of Change has been successfully incorporated into many treatments of addiction as well as many other health and psychological issues (see DiClemente et al., 2004). In looking at Stages of Change, the Transtheoretical model describes five specific stages. For the purpose of this review, these stages will be described in terms of smoking behavior. In the Precontemplation stage, the smoker has little or no current interest in quitting smoking. In the Contemplation stage, the benefit versus cost of the behavior begins to be assessed by the individual. This stage is defined as the individual considering quitting smoking within the next 6 months. In the

Preparation stage, the smoker begins active planning and is more fully committed to changing this unhealthy behavior. This stage is defined as the individual planning on quitting within the next 30 days. In the fourth stage, the Action stage, the individual has stopped smoking for a duration less than 6 months. The Action stage does not include merely a reduction in the number of cigarettes, but a complete cessation. However, in considering other health behaviors, partial modification does qualify as the Action stage (Velicer et al., 1998). In the final stage, the Maintenance stage, the individual has continued cessation for a period longer than 6 months. In this stage, the individual is working to prevent relapse, they are less tempted to relapse, and are more confident in their behavior change (Velicer et al., 1998).

In looking at continuing smokers, a fairly dispersed distribution in Stage of Change has been documented. In a large-scale randomized survey, it was found that 40% of smokers are not considering changing their habit (Precontemplation), 40% are considering quitting within 6 months (Contemplation), and 20% are planning on quitting within the next 30 days (Preparation; Velicer et al., 1995). Substantial differences have been documented between continuing smokers in different Stages of Change. For example, research has shown that as smokers transition from Precontemplation to Contemplation, they become more cognizant of the negative implications of smoking (Velicer et al., 1985). Research has also documented the possibility of different stages being related to differences in affective response to smoking cues. In one identified study which assessed affective responses to smoking-cues, within the context of the Stages of Change, differences were found between Contemplators and Precontemplators

(McDermut & Haaga, 1998). It was found that Contemplators reported more feelings of guilt while viewing smoking images, in comparison to the Precontemplators.

The pros and cons associated with the behavior of smoking has also been investigated, taking into consideration the Stages of Change. The Decisional Balance measure (Velicer et al., 1985) identified an expected pattern of pro and con feelings in Stages of Change. In the Precontemplation stage, the pros of smoking were significantly higher than the ratings of the cons of smoking. In the Contemplation stage, as expected by Velicer et al. (1985), the pros and cons were approximately equal. In the Preparation stage, the cons associated with the behavior of smoking are greater than the pros. As the smoker continues through the remaining Stages of Change the cons continue to outweigh the pros of continuing the behavior.

The Transtheoretical Model, in which the Stage of Change theory is based upon, postulates that behavior change occurs through a series of stages, rather than a singular event (Velicer et al., 1998). Assessment of Stage of Change can be accomplished though a set of three simple questions (C C DiClemente et al., 1991; W. F. Velicer et al., 1995). Considering the Stages of Change in addiction treatment has been shown to be facilitative of successful treatment (Carlo C DiClemente et al., 2004). Additionally, looking at a range of unhealthy behaviors, considering Stage of Change provides unique information, which differs by behavior (Prochaska et al., 1994). Thus, considering Stages of Change can be accomplished relatively easily and provide data which may be informative to research in unhealthy behaviors, as well as the treatment of such behaviors.

Conclusion

The use of startle reflex methodology in scientific research has substantial implications. Stated simply, "the study of startle modification offers the potential to expose and clarify a number of important issues across diverse areas of psychology, psychiatry, and neuroscience" (Dawson, Schell, & Boehmelt, 1999; pg. xiii). As reviewed, the startle reflex is a ubiquitous phenomenon, seen across species, outside the control of the subject, and highly malleable to the presence of other stimuli (e.g., affective foreground, stress, chemicals, etc.). More specifically, it offers the potential to better understand motivations to consume addictive substances and also the process through which individuals overcome addiction. Startle research provides an objective glimpse into many cognitive processes, emotional factors, and neurological mechanisms, and as Dawson et al. (1999) suggest, may provide the unique opportunity to unify research in several branches of research including medicine, behavioral science, and cognitive science.

APPENDIX B
PARTICIPANT MEDICATION ALCOHOL CAFFEINE USE

Included in the demographic questionnaire used for this study was information regarding use of over-the-counter medications, alcohol, and caffeine use. Among the Precontemplator group, one participant reported daily use of minocycline (oral medication for treatment of facial acne), and one participant listed Humalog (insulin lispro, subcutaneous medication for treatment of diabetes). Among the Contemplation group, one participant listed daily use of Clariton (loratadine, oral allergic rhinitis medication), one participant listed daily use of Zyrtec (cetirizine, oral allergic rhinitis medication), Liquibid-D (guaifenesin-phenylephrine, oral decongestant/antitussive medication), Tazorac (tazarotene, topical psoriasis medication), and BenzaClin (benzoyl peroxide/clindamycin, topical acne medication).

As stated in the Participant section of this manuscript, participants did not significantly differ in score on Alcohol Use Disorders Identification Test, $F(1, 21) = .102$, $p > .05$, suggesting that the potential for an alcohol use disorder was similar across groups. Also included in the demographic questionnaires was a question regarding time of last consumption of alcohol. Responses varied greatly with 3 participants reporting that the question was "not applicable," and other participants listing times ranging from years to 12 hours. An analysis was conducted to determine if participants significantly differed by group in time of last consumption. Given the rather large skew in data, an upper limit was set at 48 hours. This resulted in a sample size of 4 Precontemplators and

5 Contemplators. Results were non-significant for difference by group [$F(1, 7) = .221$, $p > .05$, Precontemplator $M = 25.25$ (15.95), Contemplator $M = 30.40$ (16.64)].

Two participants listed a time of consumption of 12 hours prior to study, one Precontemplator, one Contemplator. Limited research could be found regarding affective modulation in an intoxicated state and no research could be located looking specifically at time since last consumption and affective modulation. However, concerning alcohol intoxication concurrent with affective modulation, research indicates that there appears to be a reduction in negative modulation (i.e., increased startle to negative stimulation), which appears to be mediated by alcohols reduction in attentional abilities (Curtin, Patrick, Lang, Cacioppo, & Birbaume, 2001). In considering alcohol metabolism per se, many factors may influence elimination of ethanol from the system, and thus any potential confounding influence on research. Caballería (2003) reviewed the factors related to alcohol metabolism in humans and the potential influential variables, which include gastric function, food intake, hepatic function, ethnicity, gender, body weight, and body-water amount. In considering research related specifically to the current study, a previous study looked specifically at male humans, with standardized laboratory alcohol administration, assessing arterio blood alcohol and venous blood alcohol concentration. The results found that blood alcohol content should reach zero (or full metabolism) at approximately 391 to 420 minutes (6-1/2 to 7 hours). Given that the most recently reported time of alcohol consumption in the current study was 12 hours, it is likely that all alcohol was fully metabolized and did not significantly influence the results.

Use of caffeine (i.e., amount of time since last use and the type of caffeinated products consumed on the day of the study) was also assessed given the potential stimulant influence on the startle reflex and thus the need for equal distribution across groups. The amount of caffeine was converted to milligrams based upon disclosed amounts by manufacturer websites (e.g., Coca-Cola 8oz = 34.5mg caffeine). This figure was then converted to estimated non-metabolized amounts based upon the 5 hour half-life of caffeine in the average human (Ginsberg, Hattis, Russ, & Sonawane, 2004). Results indicated that participant groups did not significantly differ in mg of caffeine, $F(1, 21) = .093$, $p = .763$ (Precontemplation $M = 42.33$ $SD = 72.17$, Range = 235.00; Contemplation $M = 49.96$, $SD = 40.66$, Range = 125.96). Given that the question regarding caffeine type asked for products consumed for the day of the study, and the extensive half-life of caffeine, several subjects did not list the type of caffeinated product consumed but indicated consumption the night prior (via hours since last caffeine use). Given this, an analysis was also conducted for these individuals, with the conservative estimate of 1 cup of coffee (factoring metabolization by time period). This analysis, again, did not show a group difference in mg of caffeine, $F(1, 21) = .001$, $p = .976$ (Precontemplation $M = 49.22$, $SD = 68.38$, Range = 234.83; Contemplation $M = 49.96$, $SD = 40.66$, Range = 125.96). While it appears that no previous research exists regarding the potential influence of caffeine on the affective modulation of the startle reflex, it has been documented that large quantities of caffeine can decrease habituation of startle (Mikalsen, Bertelsen, & Flaten, 2001; Schicatano & Blumenthal, 1994; Swerdlow et al., 2000), increase startle amplitude (Andrews, Blumenthal, & Flaten, 1998; Flaten, Aasli, &

Blumenthal, 2003; Flaten & Blumenthal, 1999; Flaten et al., 2004) and decrease startle peak latency (Andrews et al., 1998; Schicatano, 2005). However, given that the current study showed no differences between groups and that data was standardized within each participant (see Procedure section) it is unlikely that caffeine consumption influenced the findings of this particular study.

APPENDIX C

SMOKING: STAGE OF CHANGE (SHORT FORM)

Please read each of the following questions and either circle or fill in the appropriate answer.

---

1. Are you currently a smoker?

- ☐ Yes, I currently smoke
- ☐ No, I quit within the last 6 months
- ☐ No, I quit more than 6 months ago
- ☐ No, I have never smoked (stop here)

---

2. In the last year, how many times have you quit smoking for at least 24 hours?

    ____

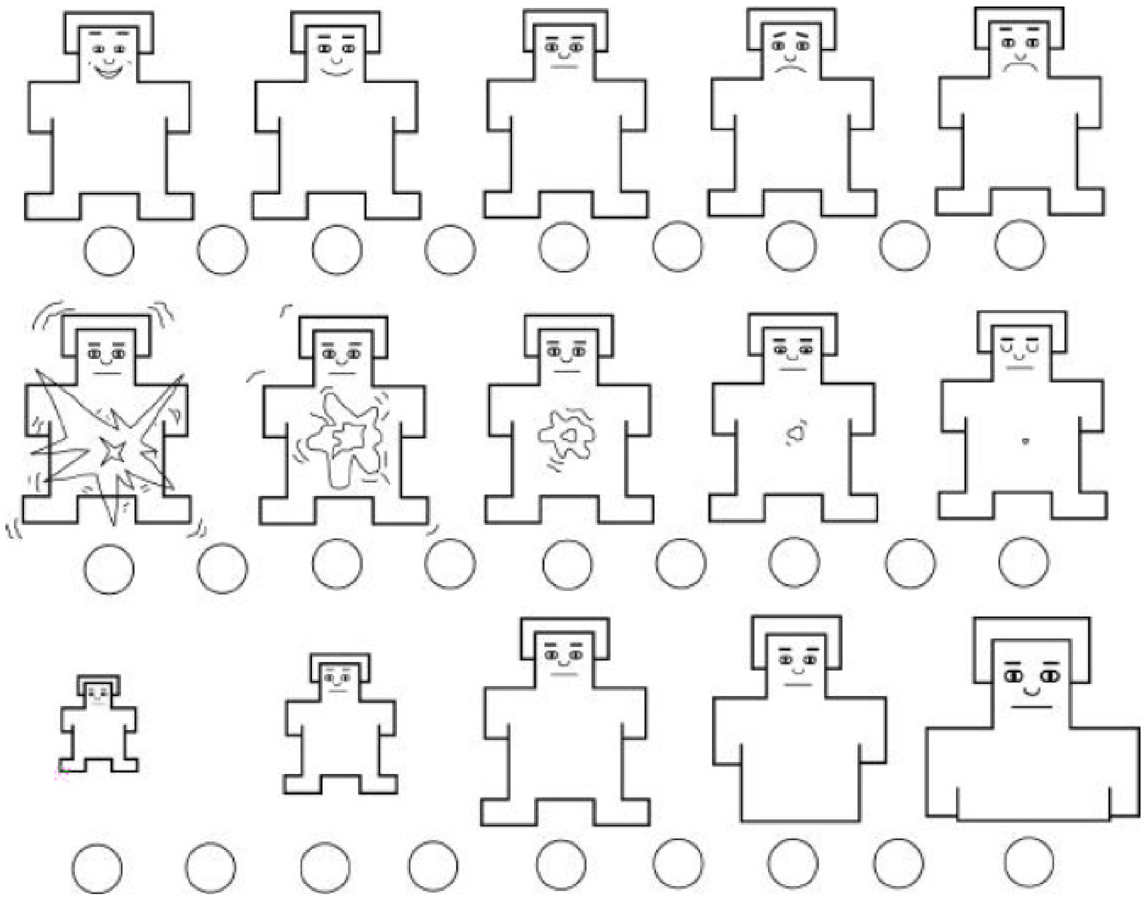
---

3. Are you seriously thinking of quitting smoking?

- ☐ Yes, within the next 30 days
- ☐ Yes, within the next 6 months
- ☐ No, not thinking of quitting

APPENDIX D
SELF-ASSESSMENT MANIKIN (SAM)

IMAGE #___________



APPENDIX E

FAGERSTRÖM TEST FOR NICOTINE DEPENDENCE

Please read each of the following questions and either circle or fill in the appropriate answer.

---

1. How soon after you wake up do you smoke your first cigarette?

   - ☐ After 60 minutes
   - ☐ 31-60 minutes
   - ☐ 6-30 minutes
   - ☐ Within 5 minutes

---

2. Do you find it difficult to refrain from smoking in places where it is forbidden?

   - ☐ No
   - ☐ Yes

---

3. Which cigarette would you hate most to give up?

   - ☐ The first in the morning
   - ☐ Any other

---

4. How many cigarettes per day do you smoke?

   - ☐ 10 or less
   - ☐ 11-20
   - ☐ 21-30
   - ☐ 31 or more

---

5. Do you smoke more frequently during the first hours after awakening than during the rest of the day?

☐ No

☐ Yes

---

6. Do you smoke even if you are so ill that you are in bed most of the day?

☐ No

☐ Yes

APPENDIX F

ALCOHOL USE DISORDERS IDENTIFICATION TEST

1. How often do you have a drink containing alcohol?
□ Never *stop* (0)
□ Monthly or less (1)
□ Two to four times a month (2)
□ Two to three times a week (3)
□ Four or more times a week (4)

2. How many drinks containing alcohol do you have on a typical day when you are drinking?
□ 1 or 2 (0)
□ 3 or 4 (1)
□ 5 or 6 (2)
□ 7 to 9 (3)
□ 10 or more (4)

3. How often do you have six or more drinks on one occasion?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

4. How often during the last year have you found that you were not able to stop drinking once you had started?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

5. How often during the last year have you failed to do what was normally expected from you because of drinking?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

7. How often during the last year have you had a feeling of guilt or remorse after drinking?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

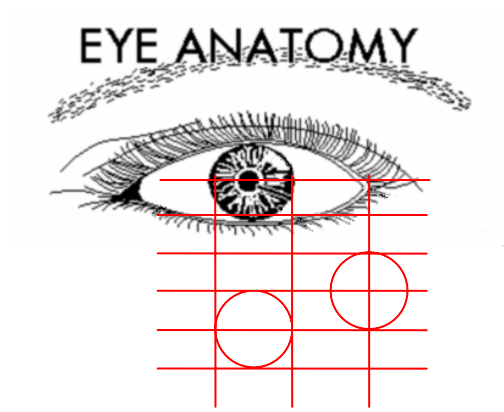
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
□ Never (0)
□ Less than monthly (1)
□ Monthly (2)
□ Weekly (3)
□ Daily or almost daily (4)

9. Have you or someone else been injured as a result of your drinking?
□ No (0)
□ Yes, but not in the last year (2)
□ Yes, during the last year (4)

10. Has a relative or friend, or a doctor or other health worker been concerned about your drinking, or suggested you cut down?
□ No (0)
□ Yes, but not in the last year (2)
□ Yes, during the last year (4)

APPENDIX G

LEFT EYE PLACEMENT OF ELECTRODES



Note: The referenced sections (circles) reflect bipolar electrode placement on the orbicularis oculi. The reference electrode on the mastoid process is not shown.

APPENDIX H

SELF-REPORT AND PSYCHOPHYSIOLOGICAL COVARIATION

The relationship between SAM rating would physiological EMG data was explored as a non-planned analysis. In exploring this relationship, the covariation between data points was assessed. All SAM ratings that had corresponding physiological data were included. That is, rated SAM images that did not include startle probes during EMG acquisition were not included. Also, those SAM rated images, per participant, that were excluded during EMG acquisition (based on exclusionary criteria) were not included in the covariation analyses.

In order to assess the overall relationship between SAM ratings and EMG startle, a bivariate correlational analysis was run, with all picture types (i.e., positive, negative, neutral, smoking) included as one variable, and matched all-picture-type EMG values included as another variable. Results indicated a large significant negative correlation between subjective ratings (SAM) and objective physiological responses to experimental images, $r = -.501$, $p < .001$. Figure 3 displays the overall correlation in scatterplot. Bivariate correlational analyses were also run for Precontemplation and Contemplation groups separately. Again, results showed significant negative correlations with Precontemplation $r = -.672$, $p < .001$, and Contemplation $r = -.341$, $p < .024$. Correlations were also conducted for picture categories independently, both overall and by group. At the picture level, correlations at both overall and group level, for the most part, did not reach significance. The one exception for expected correlation was for the Contemplation group, with SAM positive ratings significantly correlating with EMG positive startle magnitudes, $r = -.695$, $p = .018$. See Table 8 for overall picture category correlations and Table 9 for group-specific picture category correlations.

Overall, a strong negative correlation was found (-.501) between startle reflex magnitudes and picture-matched self-rated scores, being consistent with the theory of affective modulation of startle. As startle magnitude values increased (i.e., perceived more negatively) ratings for the image decreased (rated more negatively). This strength of correlation was consistent with a previous study conducting similar analyses (-.53; Bradley, & Lang, 2000). Of note in the current study are the results when separating smokers into Stage of Change group. In comparison to the overall analysis, an even stronger negative correlation is found in the Precontemplation group alone (-.672), and the strength reduced to a medium negative correlation for the Contemplation group (-.341; size categorization based on Cohen, 1988).