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Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence

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Abstract

Rationale—Chronic cocaine use results in long-lasting neurochemical changes that persist beyond the acute withdrawal period. Previous work from our group reported a profound reduction in the acoustic startle response (ASR) in chronic cocaine-dependent subjects in early abstinence compared to healthy controls that may be related to long-lasting neuroadaptations following withdrawal from chronic cocaine use.

Objectives—This study aims to investigate the persistence and time course of the decrements in the ASR of cocaine-dependent subjects during prolonged abstinence.

Methods—Seventy-six cocaine-dependent (COC) subjects and 30 controls (CONT) were tested, the former after a period of heavy cocaine dependence. COC subjects were retested sequentially for 1 year of abstinence or until relapse. ASR testing was conducted at 3-dB levels and the eye-blink component of the startle response was quantified with electromyographic recording of the orbicularis oculi muscle.

Results—While there was no difference in startle magnitude between CONT and COC in early abstinence, by day 40 of abstinence COC subjects exhibited a statistically significant decline ($p=0.0057$) in ASR magnitude as compared with CONT and this decrement persisted for up to 1 year of abstinence ($p=0.0165$). In addition, startle latency was slower in COC subjects as compared with CONT at all stages of abstinence.

Conclusions—These results replicate and expand upon the earlier finding that chronic cocaine use impairs the ASR in a manner that persists beyond the acute withdrawal period. This phenomenon may represent a biological measure of long-term neural changes accompanying cocaine dependence and subsequent withdrawal.

Keywords

Acoustic startle response; Cocaine; Addiction; Withdrawal

Introduction

Cocaine dependence is a chronic relapsing disorder in which addicts often cycle between periods of use, withdrawal, abstinence, and relapsing back to heavy use. Chronic cocaine dependence causes physiological and neurochemical alterations in brain function, that persist long after the cessation of active use. While cocaine interacts with the serotonin (5-HT) and norepinephrine (NE) systems, it is the interaction with the dopamine (DA) system via blockade of the dopamine transporter (DAT) that is believed to be the major action responsible for the intensely reinforcing effects of cocaine (Johanson and Fischman 1989; Koob and Bloom 1988; Wise and Bozarth 1987). Cocaine binds to and blocks re-uptake of DA at the DAT, ultimately potentiating the synaptic actions of DA by increasing its concentration in the synapse (Woolverton and Johnson 1992). In contrast to the DA activation associated with active cocaine use, the long-term effects of chronic cocaine acting on the DA system may result in homeostatic adaptations that ultimately lead to a decrease in DA during periods of cocaine abstinence (Kuhar and Pilotte 1996). The neuroadaptations associated with abstinence following chronic cocaine use are characterized by long-lasting decreases in cerebral glucose metabolism, decreases in drug-induced DA release, as well as alterations in DA receptor availability and binding, all consistent with a hypofunctional DA system that has been well established in abstinent cocaine addicts (Dackis and Gold 1985; Kuhar and Pilotte 1996; Volkow and Fowler 2000; Volkow et al. 1999, 1992).

Chronic cocaine use and subsequent abstinence leads to alterations in brain stress circuits and is characterized by dysphoria, depression, anxiety, and irritability (Gawin and Kleber 1986). Acute withdrawal states are associated with elevations in corticotropin releasing factor (CRF) and hypothalamic-pituitary-adrenal (HPA) axis hormones (Kreek and Koob 1998; Mello and Mendelson 1997). Atypical responses to stress have been well documented in former addicts, suggesting that alterations to the stress systems persist well beyond the acute withdrawal stage (Kreek 1987; Gawin and Ellinwood 1988; Kreek and Koob 1998).

An earlier study from our group reported that the acoustic startle response (ASR) was profoundly reduced in 15 cocaine-dependent subjects after 1–2 weeks of abstinence as compared to a group of healthy controls (Efferen et al. 2000). The ASR is a reflexive contraction of skeletal musculature in response to a sudden, intense auditory stimulus that occurs in all mammals (Davis 1984; Landis and Hunt 1939). In humans, the eyeblink component of the startle reflex can be reliably measured in a laboratory setting using electromyographic (EMG) quantification of the contraction of the orbicularis oculi muscle (Graham 1975; Hoffman and Searle 1968). The ASR is mediated by a simple three-synapse subcortical circuit (Davis 1997; Davis et al. 1982; Koch 1999; Lee et al. 1996) and is sensitive to mediation and modulation by DA, 5-HT, glutamate, NE, and CRF. The ASR is

robustly increased by drugs that increase DA (Davis 1980; Johansson et al. 1995). Conversely, DA antagonists reduce ASR amplitude, although to a lesser degree than DA agonists increase ASR (Johansson et al. 1995; Mansbach et al. 1988). It is thought that DA modulates startle rather than having a tonic excitatory effect (Davis 1980). D1 receptors are particularly important in regulating ASR, but this process is dependent on a complex cooperative interaction between D1 and D2 receptors (Meloni and Davis 1999). Acute increases in 5-HT levels or 5-HT agonists can also alter startle activity (Davis et al. 1980b; Fechter 1974; Geyer et al. 1975; Johansson et al. 1995). 5-HT activity in the forebrain inhibits startle, whereas 5-HT acting on motor neurons in the spinal cord markedly increases startle (Davis et al. 1980a, b, 1986; Astrachan and Davis 1981; Commissaris and Davis 1982). The ASR is increased by acute NE stimulation via actions at alpha-1 adrenergic receptors on motor neurons in the facial motor nucleus and spinal cord (Davis 1980; Astrachan et al. 1983; Fendt et al. 1994). CRF has a very potent enhancing effect on startle (Liang et al. 1992). There is significant modulation of the ASR from several brain areas: the hippocampus, the ventral tegmental area that provides DAergic inputs by way of the amygdala, the nucleus accumbens-subpallidal projection, and the pedunculopontine nucleus (Davis et al. 1982). Additionally, the striatum, an area particularly important in drug dependence, is known to modulate startle by an input to the basic startle circuit via the substantia nigra and deep layers of the superior colliculus/mesencephalic reticular formation (Davis et al. 1982; Koch 1999; Meloni and Davis 2000; Swerdlow et al. 1993).

Based on the earlier finding of reduced ASR in abstinent cocaine-dependent subjects and the significant overlap in underlying neurochemistry and neurocircuitry of the ASR with the actions of cocaine, there is a compelling interest to study the ASR as a potential biomarker for assessing the time course of cocaine withdrawal as it relates to the neurochemical alterations produced by chronic cocaine use and potential normalization with prolonged periods of abstinence. The present study was conducted to extend the earlier findings of reduced ASR in abstinent cocaine-dependent subjects by investigating the persistence and time course of the decrements in the ASR in cocaine-dependent subjects during prolonged abstinence.

Materials and methods

Subjects

Seventy-six cocaine-dependent (COC) subjects (74 males and two females) and 30 healthy control (CONT) subjects (26 males and four females) participated in the study after signing an informed consent form approved by the Emory University Institutional Review Board and the Atlanta VAMC Research and Development Committee. COC and CONT subjects were matched on age, race, and sex.

Adequate hearing acuity, as demonstrated by tone detection at 40 dB [A] SPL at frequencies ranging from 250 to 4,000 Hz (assessed with pure threshold audiometer, Grason-Stadler, Model GS1710) was required for inclusion. Subjects were excluded from participation in either group (COC or CONT) for history of neurological illness, a history of head trauma with loss of consciousness 5 min or longer, known positive HIV status, and positive urine toxicology for drugs of abuse (cocaine, amphetamine, methamphetamine, benzodiazepines, morphine, and THC) at the time of startle testing. However, COC subjects were permitted to have urine toxicology positive for cocaine only at the first session. All subjects had a Structured Clinical Interview for DSM-IV (First et al. 1996) to rule out current or past axis I pathology other than cocaine-related diagnoses in the COC group. Additionally, COC subjects were excluded for abuse of or dependence on any substance other than cocaine, with the exception of alcohol abuse and nicotine abuse or dependence. Those with current or past alcohol dependence were excluded. Subjects were allowed to smoke their normal

amount up to the time they presented at the laboratory for testing. Date of last cocaine use was determined from COC subject self-report and was verified with clinical records from the Atlanta VAMC. Days clean on each test day were calculated from the date of last cocaine use. If a COC subject had a urine toxicology positive for cocaine at the first session, days clean were set at zero. Abstinent periods for COC subjects were confirmed by at least weekly urine toxicologies and close follow-up in a substance abuse treatment program through the Atlanta VAMC. CONT subjects were excluded for any current or past substance dependence. COC subjects were retested up to seven times over the course of a year or until relapse. They were tested at their baseline (first test) day and 7, 30, 84, 182, 266, and 365 days after the first test day or until relapse. CONT subjects were tested three times over a 3-month period (baseline, days 30 and 90).

Acoustic startle testing

Methodology for measuring the acoustic startle reflex was similar to that of Braff et al. (1992) and to that used previously in our laboratory (Duncan et al. 2001; Efferen et al. 2000). The eyeblink component of the acoustic startle response was measured via EMG of the right orbicularis oculi muscle. Two 5-mm silver/silver chloride electrodes filled with electrolyte gel were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus and a ground electrode was placed behind the right ear over the mastoid according to methods of Blumenthal et al. (2005). All resistances were less than 6 k ohms. EMG activity was amplified and digitized using a computerized EMG startle response monitoring system (SR-LAB, San Diego Instruments). Amplifier gain was held constant at 1.0 for all subjects. The EMG signal was filtered with low- and high-frequency cutoffs at 30 and 1,000 Hz, respectively. The system was set to record 250 1-ms readings starting at the onset of the startle stimulus.

Subjects were seated in a chair in a sound-attenuating audiology booth and asked to look straight ahead at a neutral picture and to keep their eyes open during the test session. All acoustic stimuli were delivered binaurally through headphones (Maico, TDH-39-P). The startle session began with a 60-s acclimation period consisting of 70-dB white noise, which continued as the background noise throughout the session. The startle stimulus was a 40-ms burst of white noise with near-instantaneous rise time. Startle stimuli at three decibel levels (104, 112, and 116 dB) were presented to generate a “dose-response curve” of ASR magnitude to differing intensities of stimuli. The session consisted of four blocks of 12 trials each (four trials at each of the three decibel levels presented in random order) for a total of 48 startle stimuli. Inter-trial intervals were 14–45 s in duration. ASR magnitudes are reported in microvolts (μV) and the digital signals were smoothed by an averaging routine that calculates a rolling average of the digital signals, where the number of digital signals averaged is ten.

Baseline measures were calculated by taking the average of the minimum and maximum values recorded during the first 20 ms after the startle stimulus. Trials were discarded if excessive EMG activity was observed during this baseline measure (i.e., if there was a high baseline prior to the blink response). Less than 1% of all trials were discarded from the current study based on these criteria. Onset latency was defined as the time (ms) between the startle stimulus and a shift of $7.33 \mu\text{V}$ (six machine units) from the baseline value. To be considered a valid blink response, the blink onset had to occur between 21 and 120 ms after the stimulus. On trials in which a valid blink response could not be scored, ASR magnitude was recorded as zero and there was no startle latency value.

Statistical analyses

ASR magnitudes were averaged across all blocks to yield a session mean at each decibel level. The outcome of ASR magnitude was log 10+1 transformed in order to improve the symmetry of the dependent variable. The plus 1 was added in order to keep those trials with zero ASR magnitude values. Startle latency measures were averaged across block 1 only to yield a mean for each decibel level. In later blocks, there are an increasing number of trials in which the ASR magnitude is equal to 0 and thus there is no startle latency value for those trials in which no blink occurred. In order to minimize the number of missing latency values, only trials from block 1 were analyzed.

A generalized estimating equation (GEE) was used to model the repeated measures data. There were two repeated measures variables, decibel level and time. All GEE models assumed an identity link and an exchangeable working correlation structure. Three sets of models were fit for each of the acoustic startle testing outcomes. The first set of models was limited to the COC group. The repeated measures variable time for COC subjects was measured as the number of days clean. Exploratory analyses indicated that the number of days clean did not have a linear relationship with ASR, therefore this variable was categorized into the following levels or bins: 0–4, 5–10, 11–18, 19–39, 40–93, 94–190, 191–278, >279 days clean. The covariates in these GEE models included this categorized version of days clean, decibel level, and smoking status. This model allowed us to assess whether, over time, (1) there were any significant differences between the ASR outcomes at the different decibel levels and (2) whether these differences between decibel levels remained constant over time or whether there was some interaction between time and decibel level. The second set of models was limited to the CONT group. Covariates in these GEE models for CONT subjects included decibel level, smoking status, and time as indicated by the number of days since baseline test session. Separate COC-only and CONT-only analyses were necessary due to the different intrinsic meaning of time between the two groups. Finally, a third set of models combined the data from the COC and CONT groups. A categorical variable was constructed such that the first level indicated whether a subject belonged to the CONT group at any test session, and the subsequent levels simultaneously indicated a subject belonged to the COC group and categorized the number of days clean as 0–4, 5–10, 11–18, 19–39, 40–93, 94–190, 191–278, >279 days. This model allowed for the comparison of the average of the three test sessions for the CONT group to various category levels of days clean amongst the COC group, while controlling for decibel level. These models were used to assess significant differences annotated in Figs. 1 and 3, as well as to test specific contrasts reported in the results section. The interaction between time and decibel level was examined in each of the three sets of models, but was not found to be significant (Wald chi-square p value > 0.10) and thus was not included in the final models. An additional version of the third model, which included only nonsmoking COC and CONT subjects, was also run in order to further explore potential effects of smoking status on the ASR. All inferences from the GEE models were based on the empirical standard errors from the sandwich estimator and Wald chi-square tests. There are no adjustments for multiple comparisons and the significance level of each hypothesis test was 0.05.

Time to relapse was calculated as the last day +1 that a subject presented for testing with urine toxicology negative for COC and other drugs of abuse. If a subject completed all seven acoustic startle test sessions, they were considered censored at their maximum days abstinent value calculated at their final test session. The log-rank test was used to assess the bivariate effect of possible predictors of time to relapse and a Cox proportional hazards model was used to estimate the hazard ratio. An analysis was performed separately for each decibel level.

Results

Demographics

Demographic data are summarized in Table 1. COC and CONT groups did not differ significantly in age, race, and gender. However, COC and CONT groups did differ significantly in years of education (between group difference by t test: $t=5.22$, $p<0.0001$). The groups also differed in the proportion of smokers to non-smokers (chi-square test of independence $p<0.0001$). At the time of the initial test session, the mean number of years of cocaine use reported by COC subjects was 17.4 ± 8.5 years. COC subjects also reported spending a mean of $\$854\pm957$ per month on cocaine.

Startle magnitude

The ASR was tested repeatedly during continued abstinence in a group of COC subjects and matched CONT subjects. Both groups exhibited statistically significant differences in the ASR magnitudes across the three decibel levels (Wald chi-square p value <0.0001 , for each group) with ASR magnitude increasing with increasing stimulus intensity (Figs. 1 and 2, Table 2). For the COC group, number of days clean was significantly associated with ASR magnitude (Wald chi-square p value <0.0001). Table 2 shows that after controlling for decibel level, there are statistically significant differences from baseline after approximately 19 days clean within the COC group. There was no significant main effect of smoking status in the COC group, but there was a significant interaction of smoking status and days clean (Wald chi-square p value <0.0001) that appeared to be driven by a small number of non-smoking COC subjects in the 0–4 days clean bin. In contrast, after controlling for decibel level, ASR magnitude remained stable in CONT subjects tested three times over a period of 3 months (Wald chi-square p value $=0.540$; Fig. 2). There was no significant main effect of smoking status and no significant interactions in CONT subjects.

ASR magnitude in CONT and COC subjects was equivalent in early abstinence, but COC subjects exhibited a statistically significant decline in ASR magnitude over time. By day 40 of abstinence, ASR magnitude in COC subjects was significantly lower than that of the CONT group (Wald chi-square contrast p value $=0.0057$) and it remained significantly below CONT levels for up to 1 year of continued abstinence (Wald chi-square contrast p value $=0.0165$; Fig. 1). There was no evidence of any interaction between time and decibel level.

In order to further explore the potential effects of smoking status on the results, ASR magnitude in non-smoking CONT and COC subjects was compared. ASR magnitude was equivalent in the two subject groups in early abstinence, but COC subjects exhibited a statistically significant decline in ASR magnitude over time among non-smokers. By approximately day 90 of abstinence, ASR magnitude in non-smoking COC subjects ($n=23$) was significantly lower than that of the non-smoking CONT group ($n=25$; Wald chi-square contrast p value $=0.0174$) and it remained significantly below non-smoking CONT levels for up to 1 year of continued abstinence (Wald chi-square contrast p value $=0.0274$). Because there were only five smoking CONT subjects, a meaningful comparison of smoking COC vs. CONT subjects could not be done.

Startle latency

In addition to exhibiting lower ASR magnitudes, COC subjects demonstrated significantly longer startle onset latency at all decibel levels tested, even after controlling for smoking status, as compared with CONT subjects (Wald chi-square contrast p value $=0.0293$; Fig. 3). For the COC subjects, there were statistically significant differences across days clean (Wald chi-square p value $=0.0065$; Table 2) as well as for decibel level (Wald chi-square p value $=0.02$; Table 2). In CONT subjects, onset latency remained stable across the three test

sessions (Wald chi-square p value=0.474; Table 2) and no significant difference in latency was observed across decibel level (Wald chi-square p value=0.130; Table 2, Fig. 3).

In order to determine whether the increased onset latency observed in the COC group was independent from the decreased ASR magnitude, onset latency was reanalyzed in a GEE model that included ASR magnitude in addition to the variables described earlier and the difference between COC and CONT groups persisted (Wald chi-square contrast p value=0.015). Based on this model, there was a statistically significant association between ASR magnitude and onset latency (Wald chi-square p value<0.0001), such that for every one unit increase ASR magnitude there was a corresponding 0.09 ms decrease in onset latency (95% CI -0.12, -0.06).

Startle latency as a predictor of time to relapse

Based on the analysis described above, the median time to relapse for COC subjects was determined to be 44 days (95% CI 11.8, 76.3). Log-rank tests were used to assess the bivariate effect of possible predictors of time to relapse. ASR magnitudes on the first test day or throughout the follow-up period were not found to be predictive of subjects' ability to maintain longer abstinence. This negative finding is likely due to a floor effect of ASR; the COC subjects had such low ASR magnitude that there was not sufficient variance to allow detection of predictive effects on time to relapse. However, we found that startle onset latency was predictive of time to relapse (hazard ratio=0.97, p =0.02 for onset latency for the 112-dB trial types) indicating that subjects with faster latency had lower risk of relapse, such that, for every 1 ms of faster latency, a given subject had a 3% reduction in risk of relapse. Therefore, for onset latency at 112 dB, where the differences in means for COC subjects across time intervals range from 4 to 11 ms, this would translate to a 12–33% difference in risk of relapse.

Discussion

The major findings of this study are that, as compared with CONT subjects, COC subjects exhibited reduced ASR magnitudes and increased onset latency that persist for up to a year of continued abstinence. Additionally, these differences in startle latency were found to be predictive of relapse risk.

More specifically, while ASR magnitude in CONT and COC subjects were equivalent in early abstinence, COC subjects exhibited a statistically significant decline in ASR magnitude with increased duration of abstinence. By approximately 40 days of abstinence, ASR magnitude in COC subjects fell below that of CONT, and remained significantly below CONT levels for up to 1 year of continued abstinence. ASR magnitude in CONT subjects remained stable when tested repeatedly over a period of 3 months, indicating that the reduction in startle magnitude observed in COC subjects cannot be explained by the effect of exposure to repeated ASR testing over time. The reduction of ASR magnitude in abstinent COC subjects seen here is consistent with previous findings (Efferen et al. 2000).

In addition to exhibiting lower ASR magnitudes, COC subjects also demonstrated significantly longer onset latency at all decibel levels tested as compared with CONT subjects. Interestingly, the longer onset latency observed in COC subjects did not follow the same time course as the startle magnitude, where reductions emerged after approximately 40 days of abstinence. Latency was generally longer in COC subjects regardless of time clean. The mechanisms underlying this temporal disconnect between startle latency and magnitude are unclear and warrant further investigation. There were no significant changes in onset latency across the three stimulus intensity levels or over the three test sessions in the CONT group. Latency is generally slower for lower ASR magnitudes (Hoffman and Ison 1980).

Accordingly, in this data set, we found a statistically significant association between ASR magnitude and onset latency, such that lower ASR magnitude were associated with longer startle latency, hence confirming the pattern of slower latency associated with lower startle magnitude as described by Hoffman and Ison (1980). However, even after controlling for ASR magnitude, the longer onset latency in COC as compared with CONT subjects persisted.

Onset latency (at 112 dB) was also found to be predictive of time to relapse. COC subjects with faster startle latency had a lower risk of relapse or were able to maintain longer abstinence periods. Specifically, we found that for every 1 ms of faster latency, a given subject had a 3% reduction in time to relapse. Therefore, for onset latency at 112 dB, where the differences in means for COC subjects across time intervals range from 4 to 11 ms, this would translate to a 12–33% difference in risk of relapse.

In this study population, approximately 70% of the COC subjects were smokers, while only 17% of the CONT subjects were. Given this difference in the distribution of smokers across groups, we cannot rule out the effect that smoking status may be playing in the differences in startle magnitude and latency observed between COC and CONT subjects. Duncan et al. (2001) reported that non-withdrawn smokers had reduced startle magnitude and longer onset latency in pulse-alone trials as compared to non-smokers in a study examining the effects of smoking on prepulse inhibition of the ASR. Other studies comparing non-withdrawn smokers to nonsmokers have reported no differences in startle magnitude (Greenstein and Kassel 2010; Della Casa et al. 1998) or latency (Della Casa et al. 1998). Therefore, the effects of smoking on the ASR are not definitive.

In order to assess the role smoking status might have been playing in the group differences observed in startle magnitude and onset latency, additional analyses were performed while controlling for smoking status. When non-smoking COC and CONT subjects were analyzed separately, the decrement in ASR in COC subjects as compared with CONT persisted in the nonsmokers. The significant decrease appeared slightly later in abstinence (by about day 90), but ASR magnitude in COC subjects remained significantly below CONT levels for 1 year of abstinence. In COC subjects, there was an interaction of smoking status with days clean that appeared to be driven by a small number of non-smoking COC subjects in the 0–4 days clean bin. This study did not track the number of cigarettes smoked by the subjects, so it is possible the interaction of days clean with smoking status is indicative of changes in the amount smoked with increased duration of abstinence. Additional studies are needed to explore this possibility further. Smoking status did not appear to impact startle latency. Even after controlling for smoking status, onset latency for COC subjects remained longer than CONT subjects at all decibel levels tested.

Long-term cocaine use and subsequent withdrawal have been shown to result in neuroadaptations of brain DA systems that result in a persistent functional hypodopaminergic state (Kuhar and Pilotte 1996). As mentioned above, DA robustly modulates the ASR. Therefore, a potential explanation for the startle reductions in our COC subjects is the diminished DA function accompanying abstinence following chronic COC use. However, DA's role in modulating ASR is complex (Meloni and Davis 1999) and simple hypodopaminergia does not fully explain the observed deficits. For instance, if this simple explanation held, we should see low startle very soon after COC washout in our subjects rather than the gradual fall in ASR magnitude over the first few weeks of abstinence.

Acute cocaine increases NE levels by blocking reuptake of the membrane-bound NE transporter (Ritz et al. 1990) and startle amplitude is increased by acute NE stimulation via

actions at alpha-1 adrenergic receptors on motor neurons (Davis 1980; Astrachan et al. 1983; Fendt et al. 1994). Hence, if abstinence following prolonged COC exposure results in a functionally low NE tone, this could be the neurochemistry underlying COC-induced lowering of ASR. Acute cocaine administration also increases levels of synaptic 5-HT through inhibition of reuptake at the 5-HT transporter in brain (Cunningham et al. 1996; Essman et al. 1994; Ritz et al. 1990). As described previously, 5-HT modulates ASR magnitude but its effects are complex: forebrain 5-HT inhibits ASR whereas activation of 5-HT₁ receptors in the spinal cord increases startle (Davis et al. 1980a, b). Based on these opposing effects, it is not clear whether alterations in 5-HT resulting from chronic cocaine exposure are involved with the reductions in ASR observed in the COC subjects.

This study cannot rule out the possibility that the lower startle magnitude, or the slower startle latency observed in COC subjects may have predated the cocaine dependence. Recent studies have reported high heritability of both ASR magnitude (Anokhin et al. 2003; Hasenkamp et al. 2010) and latency (Hasenkamp et al. 2010). If low ASR and slower startle latency are pre-existing traits in the COC subjects, this may be masked in some way in the early periods of abstinence. Our data indicate that the ASR reduction in COC subjects is not present in very early abstinence. Rather, ASR magnitude is initially similar to that of CONT while COC subjects still have positive urine toxicologies (days 0–4 of abstinence) and does not reach statistical significance until roughly week 6 of abstinence. This time course is consistent with the idea that acute COC withdrawal actually leads to an increase in startle, which then falls to low levels with extended abstinence. While acute COC withdrawal is not associated with obvious physical symptoms seen in opiate and alcohol withdrawal states, chronic COC use and subsequent abstinence leads to alterations in brain stress circuits and is characterized by dysphoria, depression, anxiety and irritability (Gawin and Kleber 1986). Acute withdrawal states are associated with elevations in CRF and HPA axis hormones (Kreek and Koob 1998; Mello and Mendelson 1997). Atypical responses to stress have been well documented in former addicts, suggesting that alterations to the stress systems persist well beyond the acute withdrawal stage (Kreek 1987; Gawin and Ellinwood 1988; Kreek and Koob 1998). Furthermore, substance-dependent subjects have been shown to exhibit elevations in ASR during periods of acute withdrawal from opiates (in rats, Harris and Gewirtz 2004; in humans, Stine et al. 2001) and alcohol (Krystal et al. 1997). Therefore, it is possible that in early abstinence, pre-existing low startle is masked by acute COC withdrawal-induced changes in CRF or other stress circuitry that cause a temporary relative elevation of startle. There may also be abstinence-induced reductions in DA or NE tone further contributing to reductions in startle long into abstinence.

This study is not without limitations. Given the behavioral nature of the study, it is difficult to elucidate the specific mechanistic underpinnings of the ASR differences observed in abstinent COC subjects. Therefore, it is possible that factors not directly related to cocaine dependence are contributing to the ASR changes observed across time in the COC subjects, particularly given the longitudinal nature of the study. For instance, since we did not reassess hearing acuity at each time point, we cannot rule out the possibility that changes in hearing emerging over time could potentially have impacted the findings. Since studies with comparable designs have not yet been published on alcohol or other drugs of abuse, it is not known whether the present findings are specific to cocaine dependence or might be generalized to abstinence from other drugs of abuse.

In summary, this study found that as compared to CONT subjects, COC subjects exhibited a profound ASR reduction and longer startle latency lasting through an entire year of continued abstinence. Additionally, the results indicate that longer startle latency is a predictor of relapse risk. These findings deserve further examination given that, in this time of limited resources, any predictors of clinical course could enable us to target intensive

treatment resources to those at greatest risk for relapse. As a future direction for this work, animal models can be used to uncover the mechanisms involved the ASR reduction accompanying cocaine addiction and can help to clarify whether pre-existing low startle is a vulnerability factor for cocaine dependence.

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Fig. 1.

ASR magnitudes (mean $\pm$ SEM) in CONT and COC subjects in response to noise probes of **a** 104, **b** 112, and **c** 116 dB. CONT data represents the mean of the three test sessions.

* p 0.05, ** p 0.01, and # p 0.001 compared to CONT by GEE (based on the model without interaction with smoking status)

Fig. 2. ASR magnitudes (mean $\pm$ SEM) in CONT subjects in response to noise probes of 104, 112, and 116 dB across three test sessions. There were no significant differences across time when controlling for decibel level (based on the model without interaction with smoking status)

Fig. 3. Onset latencies (mean $\pm$ SEM) of acoustic startle response (time from stimulus onset to onset of startle response) in CONT and COC subjects in response to noise probes of **a** 104, **b** 112, and **c** 116 dB. CONT data represents the mean of the three test sessions. * p 0.05, ** p 0.01, and # p 0.001 compared to CONT by GEE (based on the model without interaction with smoking status)

Table 1

Demographic information by group

	Cocaine dependent (COC) <i>n</i>=76		Control (CONT) <i>n</i>=30	
	<i>n</i>	(%)	<i>n</i>	(%)
Gender				
Male	74	(97.4)	26	(86.7)
Female	2	(2.6)	4	(13.3)
Race				
African American	70	(92.1)	26	(86.7)
European American	4	(5.3)	3	(10)
Other ^a	2	(2.6)	1	(3.3)
Smoking status ^b				
Smoker	53	(69.7)	5	(16.7)
Non-smoker	23	(30.3)	25	(83.3)
	Mean	(SD)	Mean	(SD)
Age (years)	47.71	(5.56)	46.46	(8.49)
Education (years) ^c	13.42	(1.51)	15.33	(2.11)
Years cocaine use	17.4	(8.5)	-	-
Money spent on cocaine (per month)	\$854	(\$957)	-	-

^aOther = African American/Cherokee Indian, African American/Hispanic

^bChi-square test of independence $p<0.0001$

^cBetween-group difference by *t* test: $t=5.22$, $p<0.0001$

Table 2

Summary of ASR values by group

Group	n	Magnitude (μV) ^a			Onset latency (ms) ^b			P value ^d	P value ^d
		104 dB	112 dB	116 dB	104 dB	112 dB	116 dB		
Control mean ^c	30	20.46 (8.09)	34.22 (10.86)	47.01 (12.22)	53.65 (1.99)	52.82 (1.84)	52.02 (1.87)		
Baseline	29	15.63 (4.65)	30.36 (7.56)	38.29 (8.84)	53.95 (3.11)	51.94 (1.96)	55.22 (2.61)	Reference	Reference
Day 30	23	17.27 (7.39)	31.73 (10.87)	47.89 (11.72)	52.59 (1.91)	55.63 (2.88)	48.35 (2.27)	0.27	0.348
Day 90	17	33.02 (17.21)	44.17 (20.01)	60.27 (22.48)	54.45 (3.98)	49.92 (2.26)	51.26 (2.57)	0.576	0.277
Overall P value for Decibel level ^e <0.0001					Overall P value for Decibel level ^e =0.1299				
Overall P value for days since baseline ^e =0.5385					Overall P value for days since baseline ^e =0.4738				
Overall P value for smoking status ^e =0.7855					Overall P value for smoking status ^e =0.8502				
Cocaine									
Days abstinent									
0–4	21	14.14 (4.29)	26.20 (7.10)	38.18 (10.27)	60.05 (4.06)	52.93 (2.88)	59.87 (3.71)	Reference ^f	Reference
5–10	25	18.11 (6.92)	27.39 (7.70)	33.02 (8.00)	60.55 (3.85)	63.88 (3.24)	64.47 (2.99)	0.301	0.008
11–18	33	18.98 (3.81)	33.36 (6.88)	44.36 (8.46)	59.36 (3.85)	55.91 (3.42)	57.76 (3.06)	0.5776	0.274
19–39	67	22.48 (9.09)	39.10 (12.95)	57.26 (15.23)	59.88 (3.70)	57.54 (2.03)	58.01 (2.23)	0.0151	0.403
40–93	50	10.73 (3.33)	22.16 (6.36)	32.65 (7.59)	63.30 (4.88)	60.42 (2.59)	62.91 (2.30)	0.0011	0.0868
94–190	30	9.85 (4.72)	23.47 (13.21)	32.14 (15.79)	58.77 (5.42)	56.07 (2.27)	58.77 (5.42)	<0.0001	0.224
191–278	15	4.70 (1.89)	11.73 (6.17)	22.30 (10.11)	52.38 (6.42)	63.94 (4.16)	62.23 (4.24)	0.011	0.8966
>276	20	4.82 (1.53)	12.81 (3.23)	18.22 (5.65)	72.36 (7.66)	64.03 (3.78)	56.83 (3.62)	0.0006	0.1493
Overall P value for Decibel level ^e <0.0001					Overall P value for Decibel level ^e =0.0202				
Overall P value for Days Clean ^e <0.0001					Overall P value for Days Clean ^e =0.0065				
Overall P value for interaction between days clean and smoking status ^e <0.0001									

^aMean (SEM) across all session

^bMean (SEM) across session of block 1

^cRepresent the mean (SEM) of three test sessions. Note: one subject's baseline session data was excluded due to equipment failure

^dWald Chi-square P value from GEE model comparing each time point to the baseline value while controlling for decibel level and smoking status

^eOverall Wald chi-square *P* value from GEE model for each effect in the model

^fWald chi-square *P* value based on model without interaction with smoking status