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Dependence on Smoking and the Acoustic Startle Response in Healthy Smokers

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MUELLER, V., R. F. MUCHA AND P. PAULI. *Dependence on smoking and the acoustic startle response in healthy smokers*. PHARMACOL BIOCHEM BEHAV **59**(4) 1031–1038, 1998.—Recent data from nicotine-dependent rats (14) and healthy smokers (18) would suggest that nicotine withdrawal modulates the acoustic startle reflex in a way similar to that of fear (5,20). We examined this directly using nonsmokers and healthy smokers who had no deprivation, brief deprivation (2–3 h), or prolonged deprivation (15 h). Groups differences in heart rate (HR), alveolar carbon monoxide (CO) levels, and desire and craving for cigarettes confirmed the presence of smoking withdrawal. However, there were no significant differences in the magnitude of the baseline startle response among the differently deprived smokers or between the smokers and the nonsmokers. Subsequent startle tests were carried out in the smokers during repeated sequences of preparing a cigarette for smoking (smoking cues) and then smoking. Whereas we did find statistically significant interactions of smoking deprivation with smoking cues and with renewed smoking, there was only weak confirmation of a priori predictions of motivational effects of smoking cues or of smoking. We conclude that smoking dependence may not affect the acoustic startle response itself; modulation seems to occur, but only after experience with the test situation. Discussed were possible mechanisms of this modulation in both humans and animals and further application of the startle response for providing interdisciplinary assessments of the motivational effects of nicotine withdrawal. © 1998 Elsevier Science Inc.

Startle Smoking Nicotine Cue Motivation Dependence Startle reflex Heart rate Craving

A full understanding of the motivating effects of drug withdrawal requires crossspecies and crossdrug studies (23). The startle reflex is a predictable reaction to stimuli with abrupt onset (6) that lends itself to a comparative analysis of drug exposure and drug withdrawal (11,21). The magnitude of the acoustic startle response also reflects in a systematic way the motivational state of the subject. In both humans and animals the startle response is proportional to the acute and conditioned aversiveness of a test condition (2,12,13,20,26). However, there are no clear data on the startle response indexing the motivating effects of drug withdrawal in either animals or humans.

As a first step towards this, we report changes in startle responses produced by nicotine withdrawal in humans. General interest in the issue of comparative studies of withdrawal motivation arose from data collected in animals, suggesting that nicotine withdrawal may not be similar to that of other abused drugs. In rats, opiate abstinence produced a conditioned aversion, but nicotine withdrawal produced a conditioned preference or no effect (23,31). This observation cannot be easily reconciled with the well-known assumption that smoking deprivation is aversive. However, data from humans are based almost entirely on verbal reports, and any reported aversive effect of nicotine withdrawal in smokers could reflect the following problems: 1) Self-reports on withdrawal in a drug-dependent individual are influenced by cognitive information, such as on secondary gain of further consumption (17). 2) There are also documented interactions of biological, environmental, and cognitive processes of withdrawal-produced behavior that are still not understood (22). 3) Withdrawal may be motivating for a variety of reasons, including the absence of reinforcement [e.g., (29)]. It is also known that the notion of withdrawal being aversive is influenced by learning in children even before smoking has occurred (7). It is clearly necessary to examine smoking withdrawal with objective indicators of motivation.

It is therefore important that rats infused with 3 and 6 mg/kg/day nicotine showed significant withdrawal-produced in-

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creases in startle magnitude and with renewed nicotine exposure a reversal of this effect was seen (14). In a recent report in humans, Kumari et al. (18) also found a decrease in startle amplitude after smoking in deprived smokers. This was in line with the data from rats and with smoke withdrawal being aversive (20). Unfortunately, Kumari et al. (18) had no nonwithdrawn controls, and startle had been repeatedly tested before smoking so one could not rule out an effect of habituation (10). In addition, in the first systematic study of the acoustic startle response and nicotine withdrawal in rats, Acri and her colleagues (1) infused rats with 6 and 12 mg/kg/day nicotine and saw no evidence of nicotine dependence. In the present study we extend the observations from rats by examining systematically nicotine deprivation-related changes in startle amplitude in the smoker. To avoid the interpretation problem of Kumari et al. (18), we examined the effects of smoking deprivation on baseline levels of startle.

Although the measurement of the acoustic startle response is relatively simple, there are different ways that the startle response may be influenced (16). Due to the lack of any systematic work on smoking and the startle response, we designed our study to address several possible effects. Based on the assumed aversive effects of nicotine withdrawal, we predicted that the baseline startle would be proportional to the degree of smoking deprivation. In addition, imagery-produced smoking scenarios increase the magnitude of the startle response (8), thereby suggesting that smoking cues are aversive. Hence, we also predicted that actual smoking cues (lighting up a cigarette) would increase startle magnitude in smoke-deprived smokers. To the extent that the data of Kumari et al. (18) suggest that smoking affected habituation to the startle stimuli (3), we also looked at the effect of smoking after repeated startle tests.

A mixed design was used. All subjects had a baseline test for startle followed by repeated tests for the effect of smoking preparation and smoking. Smokers were tested under prolonged (minimum of 15 h), brief (at least 2 h), or no smoke deprivation. One group of nonsmokers sham smoked a nonlight cigarette to test for any effects of smoking-related behavior itself. Because it is almost impossible to find subjects who have no information about smoking (7), a second control was run without any reference to smoking. Finally, alveolar carbon monoxide levels (CO) and HR were used to confirm recent smoke and nicotine intake. Measures of desire for cigarettes and scales of a cigarette craving questionnaire [QSU, Questionnaire of Smoking Urges, (30)] were applied as subjective indicators of cigarette motivation; the third scale (intention to smoke for anticipation of immediate relief from nicotine withdrawal or relief from negative affect) was expected to be correlated with startle magnitude.

METHOD

Subjects

Sixty-five men and women located through advertisements distributed in the city and in local newspapers were used to generate five groups of subjects of 11 or 12 each. Five subjects did not complete the study because of problems with the physiological recording; one subject was dropped for not inhaling the cigarette smoke during the experiment. The deprivation conditions of the experimental groups were manipulated in part by specifically recruiting different groups of smokers. Thus, three types of individuals were recruited: smokers of more than 20 cigarettes per day, smokers of 6–15 cigarettes per day, and nonsmokers; the nonsmokers and the heavy smokers were, in turn, used to generate two groups of randomly assigned subjects (see Table 1). It should be noted, however, that the heavy smokers were not recruited at the same time as the other smokers. We had originally recruited the groups of light smokers and had expected that a brief period of abstinence would allow testing of our hypothesis. As this was not the case, the two groups of heavy smokers were added and we implemented what we thought would be the most appreciable manipulation of smoke deprivation, a prolonged and no deprivation. Because the hypotheses remained identical, as did the protocol, the three groups of smokers were subjected to a combined analysis.

Experimental Setting and Physiological Recording

The subjects were tested under conditions of dim lighting and sound attenuation while sitting on a normal chair at a table. The acoustic startle reflex was elicited by a 50-ms burst of 95 dB white noise with an instantaneous rise–fall, presented through Stinetron (STH-7 EX) ear phones. The eye blink component of the startle response was measured from the lower portion of the left orbicularis oculi muscle using miniature Ag/AgCl electrodes (4 mm) filled with Hellige electrode gel. A digital, multichannel recorder (Vitaport, Becker Engineering, Karlsruhe, Germany) was used. The settings of our recorder for the EMG signal had high- and low-pass filter settings of 0.015 s and 2200 Hz, respectively. The eye-blink responses were sampled at 512 Hz, rectified, integrated, and then stored at 64 Hz.

Heart rate (HR) as beats per minute was measured using Arbo (H93) one-way electrodes. Carbon monoxide levels (CO) in alveolar air were measured after 20 s breath holding using a Belfont Microsmokerlyzer. The subjects were also required to smoke through a cigarette holder; the recorded data were only used to ensure that the subject drew properly on their cigarette. Instructions were presented to the subjects

TABLE 1

MEAN (±SEM) AGE AND DEPENDENCE SCORE (FTQ) AND SEX OF THREE GROUPS OF SMOKERS TESTED UNDER PROLONGED DEPRIVATION (pd), BRIEF DEPRIVATION (bd), OR NO DEPRIVATION (nd) AND TWO GROUPS OF NONSMOKERS WHO SHAM SMOKED (ns) OR SERVED AS A NO SMOKING CONTROL (nc)

Parameter	pd (n = 12)	bd (n = 11)	nd (n = 12)	ns (n = 12)	nc (n = 12)
Age (years)	36.6 ± 2.7	30.6 ± 2.0	30.3 ± 2.3	30.2 ± 2.1	32.2 ± 2.8
Female/male	4/8	6/5	5/7	6/6	7/5
FTQ score	7.2 ± 0.3	4.7 ± 0.5	6.2 ± 0.4	—	—

FTQ: Fagerström Tolerance Questionnaire.

over the earphones using prerecorded messages. Subjects also had a keypad during the experiment.

Subjective Tests

Momentary desire for cigarettes, eating, drinking, sexual arousal, alcohol, sleeping, salt, relaxation, excitement, movement, and fresh air was assessed using Visual Analog Scale (VAS, ranged from absolutely not to strong on a 75-mm scale). Emotional state was tested using the Self-Assessment Mannequin (SAM) of Bradley and Lang (4), using a nine-point scale to score momentary levels of pleasure, arousal, and dominance.

Urges and craving for smoking were assessed using translated items of the 32-item Questionnaire of Smoking Urges (QSU). Answers on a seven-point scale provided scores for Tiffany and Drobes' (30) theoretically derived scales: QSU1: desire to smoke; QSU2: anticipation of immediate positive outcome from smoking; QSU3: anticipation of immediate relief from nicotine withdrawal or relief from negative affect and QSU4: intention to smoke. The Fagerström-Tolerance-Questionnaire (FTQ) was used to score smoking dependence (9).

Procedure

Before beginning the study, subjects gave informed consent in accordance with the guidelines of the University ethics review board. They were told that they were to serve as experimental subjects or controls in an investigation of smoking and noises on body and mental activities. A reaction test was given to the subjects during the startle tests to confirm this, which we also believed would reduce variability by focusing all the subjects on the same activity; attention to a startle stimulus is known to modulate a normal startle reaction (24). One group of nonsmokers were asked to sham smoke during the experiment; they were labeled the ns group. The subjects of the second group of nonsmokers served as a control for smoking-related information, and they were offered light reading material during the smoking phase of the experiment; they were labeled the nc group. All heavy smokers were asked to present themselves with at least 15 h of smoking abstinence. One group of heavy smokers were nondeprived (the nd group) and were allowed upon arrival in the lab to smoke at their leisure. The second group of heavy smokers received no special treatment and were tested in the full 15-h deprivation; this was termed prolonged deprivation, and this was the pd group. The final group of smokers were tested with only brief deprivation and were simply asked to abstain from smoking for at least 2–3 h before the experiment; they were the bd group.

Following preparation, the subjects were seated in the test area. Extensive instructions on the experiment were then given over a 5-min period to acquaint the subjects with the procedures. The subjects also received two startle stimuli during this period to help familiarize them with the startle test. The smokers and the sham nonsmokers (ns) were then told to complete the QSU questionnaire. They were then instructed to place on the table before them three of their cigarettes; the ns subjects were given three commercially available cigarettes to prepare for sham smoking. The read-only, nonsmoking control (nc) group were given the entertainment section and want ads of a regional newspaper. The actual experiment started with a CO measure. Then following a period with no specific instructions, the protocol started after the instruction to relax for 1 min (see Fig. 1). The subjects were presented at 6-min intervals with six tests for their startle response; each test consisted of four presentations of the startle stimulus at intervals of 15 to 30 s. During the startle test the subjects were also given six tests of reaction time (data were not collected). The subjects were required to press a reaction button as soon as a red light was turned on. There was also a green warning light, which proceeded the red signal by 3–5 s. A startle stimulus was presented approximately 9 s after the first, the second, the fourth and the sixth reaction test.

The first startle test served as a baseline test. Just prior to the second startle test, the subject was asked to prepare a cigarette (prep. cig. 1 in Fig. 1); the person was required to light up their cigarette but was told to smoke only if instructed to do so; this served as a smoking-cue condition. Following this, the subjects smoked the lighted cigarette. In the case of the nonsmokers, the ns subjects were instructed to pretend lighting up and smoking; the nc subjects got instructions to prepare for and then engage in reading. At 4 min and 15 s after being instructed to smoke, the subjects were told to extinguish their cigarette (put away reading material). This general sequence was initiated two more times, once after the fourth and once again after the sixth startle test. The experiment ended after the third cue test. Exactly 3.5 min before lighting up each cigarette the subject was instructed to complete the SAM test and the needs scales. Following the last startle test, CO was measured again; the smokers and the ns subjects filled out the QSU once again.

Data Reduction and Analysis

The magnitude of an individual startle response was measured following visual identification of the peak signal found in the period 21 to 188 ms after onset of the startle stimulus. As baseline, we determined the average over the 47 ms prior to the tone. The actual data for a test condition comprised an average signal of the four startle reactions making up each test, scored as A–D units (one A–D unit corresponds to approximately 1 microvolt). When an extraneous blink started during a baseline period of a startle probe or within 20 ms after onset of the startle stimulus, the data for that startle were not included and only the remaining startles were averaged.

The various predictions regarding startle amplitude and nicotine withdrawal were tested as follows: the baseline startle data were first evaluated using a one-way between-group ANOVA with five levels (groups). The data from the entire session was then evaluated using a mixed ANOVA with five groups and six within-subjects startle tests (ST1–ST6). These were then examined for linear trends to look for habituation of the startle over the tests. Work by Friedman et al. (10) suggested that there should be a decrease in the habituation in

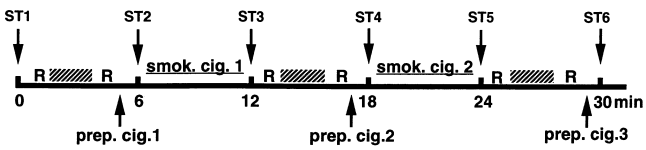

FIG. 1. Schema of the events in a typical session for a smoking subject presented as a function of minutes in which the subjects were required to prepare for smoking on three occasions and smoke a cigarette on two. ST(1– 6) refers to startle tests; each R is a period of relaxation when the HR was measured; the crosshatching refers to periods for completing questionnaires on momentary emotional state and desire for goal objects. Control subjects were requested to read or sham smoke during the smoking periods.

TABLE 2

MEAN (±SEM) ALVEOLAR CO CONCENTRATION AND SCORES FOR THE DIFFERENT QSU SCALES TAKEN BEFORE AND AFTER THE EXPERIMENTAL SESSION IN SMOKERS TESTED UNDER PROLONGED DEPRIVATION (pd), BRIEF DEPRIVATION (bd), AND NO DEPRIVATION (nd)

	pd (n = 12)		bd (n = 11)		nd (n = 12)		
Parameter	pre	post	pre	post	pre	post	
CO (ppm)	8.2 ± 0.7	18.9 ± 1.5	9.2 ± 0.9	16.2 ± 1.3	19.7 ± 3.5	28.0 ± 4.0	pre*: nd > bd¶, pd# pre-post†: pd > bd§
QSU1‡	4.8 ± 0.3	2.4 ± 0.4	3.8 ± 0.5	1.8 ± 0.3	3.3 ± 0.4	1.9 ± 0.3	pre: pd > nd¶
QSU2	5.0 ± 0.3	3.5 ± 0.5	4.3 ± 0.2	2.6 ± 0.3	3.8 ± 0.4	2.4 ± 0.4	pre: pd > nd§
QSU3	4.1 ± 0.3	2.7 ± 0.5	2.8 ± 0.3	2.2 ± 0.3	2.6 ± 0.2	2.1 ± 0.2	pre: pd > bd¶, nd¶
QSU4	5.7 ± 0.3	3.9 ± 0.4	5.3 ± 0.3	3.4 ± 0.4	4.9 ± 0.4	3.3 ± 0.4	

CO: carbon monoxide; QSU: Questionnaire of Smoking Urges

*pre: baseline differences.

†pre-post: differences between measures taken before and after the test session.

‡Scales of QSU: QSU1, desire to smoke; QSU2, anticipation of positive outcomes; QSU3, relief of negative affect; QSU4, intention to smoke.

§$p < 0.05$, ¶$p < 0.01$, #$p < 0.001$.

the smokers as a function of deprivation; we expected this to be reflected in significantly different slopes of the regression lines over the test session.

We then tested for two interactions: 1) the interaction of deprivation with the effect of the smoking cues, and 2) the interaction of deprivation with the effect of actual smoking. Regarding the former, data from the startle tests, ST1, ST3, and ST5, were relevant. The work of Kumari et al. (18) suggested that there would be a progressive fall in the magnitude of the startle from the baseline over the two postcigarette tests, and that this would be proportional to the level of the deprivation. Regarding the interaction of the deprivation with smoking, data from startle tests, ST2, ST4, and ST6, were relevant. It was predicted from the work of Elash et al. (8) that the startle reaction measured in the presence of smoking cues would be potentiated; we had further expected that this would be greater at the beginning of the session when the subjects were deprived and than at the end after they had smoked. For the interaction tests, we used 3 × 3 mixed ANOVAs with the three groups of smokers and three repeated tests.

The HR data were averaged for the 1-min periods during the six relaxation phases (each designated as "R" in Fig. 1). Baseline changes due to deprivation are well known and were analyzed with a priori tests. To look for effects of other manipulations in this study, ANOVAs similar to those of the startle tests were also carried out. The data from the questions about desire for different goal objects were analyzed with mixed ANOVAs. The QSU data were analyzed using a MANOVA procedure. The relation of startle responses to the magnitude of craving was tested with Pearson-product moment correlations. The accepted level of significant of this study was $p < 0.05$, two tailed and Greenhouse-Geisser corrections were applied in the case of repeated measures. Unless otherwise noted, the data were given as mean ± the standard error of the mean (SEM).

RESULTS

Baseline Measures of Smoke Intake and Withdrawal-Related Symptoms

In accordance with our subject recruitment, the five groups differed on various parameters. The groups of smokers showed different degrees of dependence (see Table 1); the FTQ-scores of the pd and nd groups (heavy smokers) differed significantly from that of the bd group, $t(21) = 4.24$ and 2.23, respectively, both $p < 0.05$. As seen in Table 2, the baseline CO values of the nd smokers were significantly higher than those of the other groups of smokers, which in turn, were higher then those of the nonsmokers (CO value in the ns group was 1.8 ± 0.2 ppm). The bd and pd subjects did not show CO values that were different from one another, $t(21) = 0.25$. It was also found that the pd smokers differed from the bd groups on the QSU-3 (relief of withdrawal or negative affect) and from the nondeprived on all scales except the QSU-4 (see Table 2).

Startle Responses Before Smoking, After Preparing for Smoking, and Following Smoking. On the baseline measure of startle the mean values ranged from 33.8 ± 7.3 A–D units in the bd group to 41.0 ± 4.3 A–D units in the nd group (see Fig. 2). Statistical examination indicated no significant differences, $F(4, 54) = 0.35$. For all the data from the test session, we noted a significant effect of condition, $F(5, 270) = 17.36$, $p < 0.0001$, and a significant interaction of group by condition, $F(20, 270) = 2.33$, $p < 0.004$. Trend analysis of the data from the individual groups over the test session revealed further significant changes. There was a negatively accelerating linear function, suggestive of habituation, but this was only seen for the two groups of nonsmokers, $F(1, 71) = 5.18$ and 8.36, both $p < 0.02$. No linear trend even approached significance in the smokers; the nd group of smokers gave the impression of a fall, but this was not significant, $F(1, 71) = 1.49$, $p > 0.2$.

We also evaluated the magnitude of the startle response in the smokers in the presence of smoking cues by looking at the three tests taken just after cigarette preparation (see Fig. 2 and ST2, ST4, and ST6). The ANOVA indicated a group by condition interaction, $F(4, 64) = 3.16$, $p < 0.02$. As seen from the appropriate startle tests in Fig. 2, this was probably not due to any differential decrease in the startle magnitude in the deprived groups (open circles and triangles); rather, we attributed the interaction to a fall in startle magnitude in the nd group (closed circles) and an increase in the bd smokers (open triangles).

Finally, we separately evaluated the startle tests taken at baseline and then just after completion of each of the two cig-

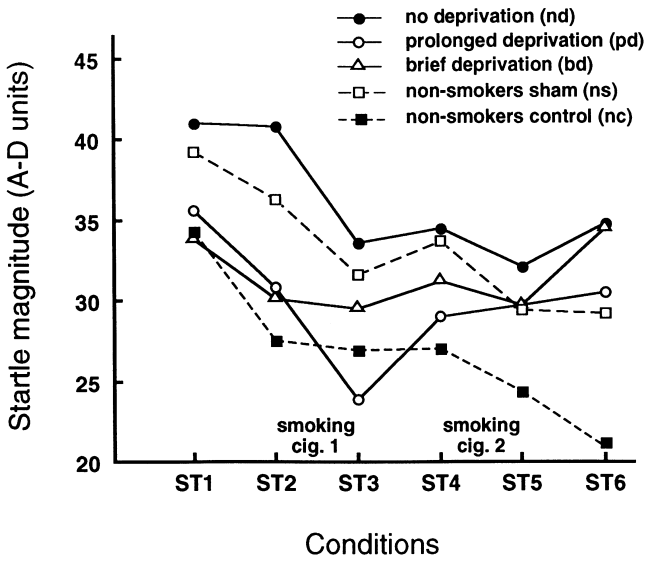

FIG. 2. Mean amplitude of eye EMG (presented as A–D units) in the three groups of smokers and in two groups of controls during the six startle tests presented at 6-min intervals under different test conditions: ST1—baseline, ST2—after the preparation of the first cigarette, ST3—after the smoking of the first cigarette, ST4—after the preparation of the second cigarette, ST5—after the smoking of the second cigarette, ST6—after the preparation of the third cigarette. Data points were from 11 or 12 persons each. See Fig. 1 caption for more details.

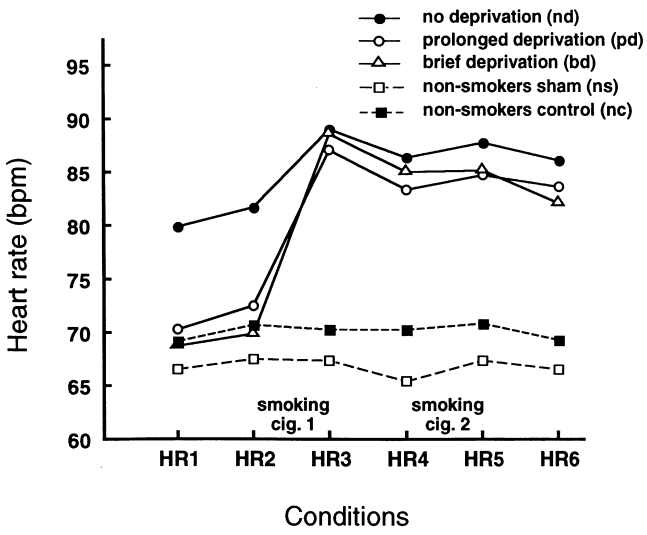

FIG. 3. Mean HR (bpm) in the five experimental groups noted in Fig. 2 during 1-min relaxation periods ("R" points in Fig. 1) at the baseline (HR1), before (HR2, HR4, HR6) and after (HR3, HR5) smoking of the cigarettes. (Further details in Fig. 1 and 2 captions.)

arettes (see Fig. 2; ST1, ST3, and ST5). The ANOVA revealed a significant main effect of condition, $F(2, 64) = 16.69$, $p < 0.0001$. This was most likely due to the significant fall in the startle response after the first cigarette [ST1 vs. ST3, $F(1,32) = 29.75$, $p < 0.0001$)]; however, the absence of a significant group by condition interaction was inconsistent with any simple hypothesis that smoking should reduce the startle. Indeed, the data suggested the opposite effect: for example, the pd group (open circles) when considered separately revealed a fall in the startle after the first and an appreciable increase after the second cigarette, $F(1, 11) = 12.82$ and 13.56, respectively, both $p < 0.004$.

Heart Rate and CO Values as Indices of Smoke Exposure

The data on the startle response contrasts with those on HR (see Fig. 3). On the overall ANOVA of the baseline differences, there was a significant group effect, $F(4, 64) = 3.58$, $p < 0.01$. The nondeprived group of smokers had a baseline (HR1) that was appreciably higher than that of each of the other groups; this was indicated, for example, by the difference between the nd and the pd group, $t(21) = 2.50$, $p < 0.015$. No differences among the baselines of the other four groups were found.

The effect of smoking on HR was clear in all the smokers. This was confirmed by a significant effect of conditions on a separate ANOVA of the data from the three groups of smokers, $F(5, 160) = 80.76$, $p < 0.0001$. A differential increase after the first cigarette was confirmed by a group by condition interaction, $F(10, 160) = 5.03$, $p < 0.003$; this effect was likely due to the smaller increase in the nd subjects. Interestingly, there was no further effect on HR of smoking the second cigarette. The mean pre- and post-HR for the second cigarette (HR4 and HR5) was not significant, $F(1, 32) = 0.89$, $p > 0.3$.

A separate analysis of the data from the two control groups indicated that their HRs remained essentially unchanged throughout the session. There was no significant condition effect, $F(5, 110) = 1.27$, $p > 0.2$.

Analyses of the CO levels after smoking (see Table 2) in the smokers revealed that the change from baseline varied over the groups, as indicated by a significant interaction of group by condition, $F(2, 34) = 3.15$, $p < 0.05$. Thus, the pd group showed a significantly larger increase in CO values than the bd group; surprisingly, the pd group was not appreciably different from the nd group (see Table 2).

Changes in the Desire for Smoking and Other Goal Objects and Emotional State Associated With Smoking

The subjective data were collected just before preparation of each of the three cigarettes (or equivalent periods). There was an effect of emotional state only for the dimension relaxed excited arousal. Thus, on a two-way mixed ANOVA there was a clear group effect, $F(4, 54) = 11.18$, $p < 0.0001$. This effect probably arose because the subjects of the nc group (nonsmoking control subjects; mean = 3.3 ± 0.3) were considerably more relaxed than those of the other groups (means ranged from 5.8 ± 0.3 to 7.0 ± 0.3 in the nd and ns group, respectively).

On the measure of desire for smoking, which was examined in the smokers with a two-way mixed ANOVA, there was a significant effect of group, $F(2, 32) = 3.19$, $p < 0.05$, and condition, $F(2, 64) = 56.36$, $p < 0.0001$. This probably reflected the fact that the craving scores went down over the experimental session (test 1: 45.2 ± 3.5; test 2: 19.2 ± 3.1, and test 3: 10.9 ± 2.4). There were also overall differences between the pd (31.9 ± 4.2) and the bd (17.9 ± 3.5) smokers (post hoc means test, $p < 0.05$). On the measure, desire to drink, there was also an overall effect of condition, $F(2, 64) = 4.69$, $p < 0.01$; this reflected the fact that desire to drink increased in the smokers from 43.0 ± 3.8 to 47.2 ± 3.8 and then 53.8 ± 3.5 on the three tests, respectively.

The QSU data of the smokers taken after the experimental session also revealed changes in the desire for smoking. A two-way MANOVA on all scales together showed a significant main effect of condition [Wilk's Lambda = 0.35, $p < 0.0001$]; on all QSU scales all three groups of smokers showed a decrease in desire to smoke (see Table 2).

Correlation Between Startle Amplitude and Subjective Ratings of Desire for Smoking

To the extent that the negative motivational effect of withdrawal was postulated to be reflected in startle amplitude, we predicted that there would be a correlation between startle magnitude and QSU-3 that would be sensitive to smoking deprivation. In view of the fact that the subjects smoked during the session, this prediction was tested only for the two startle tests taken before smoking and with the data from the initial QSU test. A marginally significant correlation was found between QSU-3 and the second startle measure in the pd smokers ($r = 0.593$, $p < 0.04$) but not in the other tests. There was no significant correlation between the QSU-3 scores and the magnitude of the baseline startles ($r = 0.237$, $p > 0.4$) in the pd smokers, for example.

DISCUSSION

The present work is the first systematic study of the effect of smoking and smoking withdrawal on acoustic startle reflex in humans. We examined baseline startle responses in smokers with different degrees of smoke deprivation. In line with observations of Acri et al. [(1); see Introduction], we found no effects of smoking dependence on the startle magnitude.

A variety of effective manipulations were used. Our observations were not specific to one level of smoking deprivation. In addition to smokers under prolonged deprivation, we used non- and briefly deprived smokers. Smokers were also compared to nonsmokers, and we took special care to ensure that the nonsmokers controlled for a wide range of possible events. We tested one group with sham smoking and the other without any smoking information or activity during the actual test session (see the introductory paragraphs). Also, the test conditions ranged from 15 h smoke deprivation in heavy smokers to ad lib smoking in nondeprived smokers, so it is unlikely that the lack of effects was due to a poor choice of deprivation conditions. Indeed, we showed on a variety of parameters that our deprivation manipulations were effective, including CO levels, HR changes, and data on desire for smoking and the QSU craving test. Our data would indicate, therefore, that smoking withdrawal was effectively manipulated. From these data, together with those from the startle test, we would conclude that smoking deprivation had no appreciable effect on the baseline startle response.

The present results need to be considered in view of the fact that three other bodies of data would suggest different results: 1) The startle response is generally believed to be modulated by the motivational states of the subjects, and smoking withdrawal is said to be aversive (see the introductory paragraphs). 2) Helton et al. (14) showed that in rats nicotine withdrawal increased startle and renewed nicotine exposure reversed this. 3) In a recent study, deprived smokers showed a reduction in startle magnitude just after smoking a cigarette (18).

Our data offer no obvious explanation for the various differences in the effects of nicotine withdrawal on the baseline startle response. However, the literature suggested a number of other effects of deprivation on the startle magnitude, and we did reveal a variety of significant interactions of smoke deprivation and test conditions. Thus, it was previously reported by Elash et al. (8) that smoking imagery evoked an increase in the magnitude of the startle response, which they interpreted to reflect the aversive qualities of the cues as revealed in data of the QSU test. We did find an effect of the smoking cues in our different groups of smokers, but this was not a simple confirmation that smoking cues should evoke more negative feelings. The effect of the cue condition seen was mainly at the end of the test session, and one would expect an aversive effect of the deprivation to be less after smoking two cigarettes than before. In considering the data of the scale of the QSU, which reflected negative affect produced by nicotine withdrawal (QSU3), we also found a correlation with the magnitude of the startle. However, this correlation was only in the heavy smokers and only for the startle measured during smoking cue; this correlation was not present when the same QSU3 data were compared to the baseline startle. It may be helpful to examine this further given that little is known about the effect of drug cues on behavior. One explanation for the results may be that there are two cue processes active in smokers. The two prominent theories of drug cues, that of compensatory response conditioning (28) and incentive motivation (29), actually predict opposite motivational qualities of smoking cues. Through judicious use of the startle test and cue manipulations, one may be able to demonstrate and then characterize a contribution of both theories.

Similarly, on the basis of smoking-produced reductions in startle seen in deprived smokers by Kumari et al. (18), we had expected that with repeated smoking there should be progressively greater reduction in the magnitude of the startle; this was not unrealistic to expect, given that two cigarettes are usually required by a heavy smoker to fully relieve withdrawal (27). Consistent with this, the present study revealed a progressive decline in interest in smoking over the test session. However, if anything, our data appeared to show an increase in the startle magnitude after the second cigarette. Therefore, we could not confirm a view from the Kumari et al. (18) study, that the magnitude of startle in deprived smokers is related in a simple way to the level of withdrawal aversiveness.

Accordingly, this suggested in another way that the changes seen in the startle response were not in line with simple predictions stemming from previous positive findings about nicotine withdrawal and the startle response. It would, however, be premature to argue that the present startle data confirm preference conditioning data in rats, suggesting that nicotine withdrawal is not aversive (see introductory paragraphs). The present data do support a conclusion that the startle response is modulated in a simple way by smoking abstinence, and they also further encourage exploration of the acoustic startle response as a tool for the comparative examination of motivational properties of nicotine and drug withdrawal. Indeed, the need to measure objectively the motivational effects of smoking withdrawal is also seen in clinical data. Shiffman (25) reported that negative affect does not accompany relapse in about 30% of smokers trying to quit. Hughes and Hatsukami (15) also found a smoking withdrawal syndrome that was quite variable.

In reviewing the various studies on nicotine and the startle response, one factor that appears to covary with the disparate findings is the extent of habituation of the subjects to the acoustic stimulation. The study of Kumari et al. (18) and ours differ in that the subjects in the former were well habituated to the startle stimulus at the time that the smoking effect was noted. Our subjects had received two pretest startle stimuli to

prevent any novelty effects, but as seen from the nonsmokers that habituation did occur during the actual test. Habituated subjects also appear to have been used in the positive study of Helton et al. (14); their 25 startle stimuli were applied at 8-s intervals. Acri et al. (1), who did not see a dependence effect on startle, used intervals of 30 s. However, she and her colleagues also presented two different intensities of acoustic stimulation on the test, which would be expected to produce dishabituation (6).

Accordingly, it may be fruitful to consider the modulating effect of nicotine deprivation on startle magnitude as a function of the level of startle habituation. Habituation is itself a complex process, however (6). There are at least two ways that habituation may have contributed to the present results. First, the effect of smoking may be on the habituation process itself. Friedman et al. (10) showed that habituation to acoustic stimuli is inhibited in smoking-deprived subjects. Second, habituation to some component of the startle may be necessary to see a modulation by the deprivation. The startle response is influenced by arousal as well as motivational state (20), and it may be suggested that relatively novel stimuli are arousing at the beginning of a test session; they then interfere with further startle modulation. Nevertheless, any role for habituation in the interaction of the startle response and smoking may be complex. Specific tests carried out here for deprivation-related delays in habituation were not confirmatory, although there was a difference between smokers and nonsmokers. Similarly, on our second startle test, it would be expected that habituation to initial arousing effects of the startle stimulations should have begun to occur; however, there was no evidence of any emerging effect of smoke deprivation.

One further factor to note is the presence of a reaction test during our startle probes. It may be argued that distracting the subjects precluded seeing an effect of deprivation on the startle magnitude. We feel that this is unlikely, however. We used the reaction test to reduce the variability of the startle response, because it is known that expectation of a startle stimulus precludes or reduces the size of the startle reaction (24). Therefore, our tests should have been more sensitive to changes given that during a test all subjects attended to the same activity. Moreover, the reaction test was given throughout the experimental session and several significant findings were seen despite its presence. Finally, our data were in line with those of Acri et al. (1), who also failed to find an effect of nicotine dependence on baseline startle magnitude in nondistracted rats.

In summary, the present study indicated that smoking and smoking withdrawal modulate the magnitude of the acoustic startle response. However, the modulation probably reflects some unknown interaction between smoke deprivation and the nature of the test situation. There are a number of well-defined mechanisms of startle modulation [see (16)], and as suggested here, different smoking-related manipulations may affect the magnitude of the startle. A useful paradigm for investigating specific emotional/motivational influences on a test subject appears to be the fear-potentiated acoustic startle response (16). This test in humans is believed to parallel the well-described model in rats (19) and may supplement the present approach for addressing drug-withdrawal motivation. Accordingly, we are currently investigating smoking-related manipulations on the effect of the startle produced by slides of a known emotional status.

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REFERENCES

1. Acri, J. B.; Grunberg, N. E.; Morse, D. E.: Effects of nicotine on the acoustic startle reflex amplitude in rats. Psychopharmacology (Berlin) 104:244–248; 1991.
2. Bonnet, M.; Bradley, M. M.; Lang, P. J.; Requin, J.: Modulation of spinal reflexes: Arousal, pleasure, action. Psychophysiology 32:367–372; 1995.
3. Bradley, M. M.; Lang, P. J.; Cuthbert, B. N.: Emotion, novelty, and the startle reflex: Habituation in humans. Behav. Neurosci. 107:970–980; 1993.
4. Bradley, M. M.; Lang, P. J.: Measuring emotion: The self-assessment Manikin and the semantic differential. J. Behav. Ther. Exp. Psychiatry 25:49–59; 1994.
5. Davis, M.: The role of the amygdala in conditioned fear. In: Aggleton, J., ed. The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction. New York: Wiley-Liss; 1992:255–306.
6. Davis, M.; File, S. E.: Intrinsic and extrinsic mechanisms of habituation and sensitisation: Implications for the design and analysis of experiments. In: Peeke, H. V. S.; Petrinovich, L., eds. Habituation, sensitisation and behaviour. San Diego, CA: Academic Press; 1984:287–324.
7. Eiser, C.; Walsh, S.; Eiser, J. R.: Young childrens' understanding of smoking. Addict. Behav. 11:119–123; 1986.
8. Elash, C. A.; Tiffany, S. T.; Vrana, S. R.: Manipulation of smoking urges and affect through a brief-imagery procedure: Self-report, psychophysiological and startle probe responses. Exp. Clin. Psychopharmacol. 3:156–162; 1995.
9. Fagerström, K. O.: Measuring degree of physical dependence to tobacco smoking with reference to individualisation of treatment. Addict. Behav . 3:235–241; 1978.
10. Friedman, J.; Horvath, T.; Meares, R.: Tobacco smoking and a "stimulus barrier." Nature 248:455–456; 1974.
11. Gibbins, R. J.; Kalant, H.; LeBlanc, A. E.; Clark, J. W.: The effects of chronic administration of ethanol on startle thresholds in rats. Psychopharmacology (Berlin) 19:95–104; 1971.
12. Grillon, C.; Ameli, R.; Woods, S. W.; Merikangas, K.; Davis, M.: Fear-potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex. Psychophysiology 28:588–595; 1991.
13. Hamm, A. O.; Greenwald, M. K.; Bradley, M. M.; Lang, P. J.: Emotional learning, hedonic change, and the startle probe. J. Abnorm. Psychol. 102:453–465; 1993.
14. Helton, D. R.; Modlin, D. L.; Tizzano, J. P.; Rasmussen, K.: Nicotine withdrawal: A behavioural assessment using schedule controlled responding, locomotor activity, and sensory motor reactivity. Psychopharmacology (Berlin) 113:205–210; 1993.
15. Hughes, J. R.; Hatsukami, D.: Signs and symptoms of tobacco withdrawal. Arch. Gen. Psychiatry 43:289–294; 1986.
16. Koch, M.; Schnitzler, H.-U.: The acoustic startle response in rats—Circuits mediating evocation, inhibition and potentiation. Behav. Brain Res. 89:35–49; 1998.
17. Kolb, L.; Himmelsbach, C. K.: Clinical studies of drug addiction, III. A critical review of the withdrawal treatments with method of evaluating abstinence syndromes. Am. J. Psychiatry 94:759–799; 1938.

18. Kumari, V.; Checkley, S. A.; Gray, J. A.: Effect of cigarette smoking and prepulse inhibition of the acoustic startle reflex in healthy male smokers. Psychopharmacology (Berlin) 128:54–60; 1996.
19. Lang, P. J.: The emotion probe: Studies of motivation and attention. Am. Psychol. 50:372–385; 1995.
20. Lang, P. J.; Bradley, M. M.; Cuthbert, B. N.: Emotion, attention, and the startle reflex. Psychol. Rev. 97:377–395; 1990.
21. Mansbach, R. S.; Gold, L. H.; Harris, L. S.: The acoustic startle response as a measure of behavioural dependence in rats. Psychopharmacology (Berlin) 108:40–46; 1992.
22. Mucha, R. F.: What is learned during opiate withdrawal conditioning? Evidence for a cue avoidance model. Psychopharmacology (Berlin) 104:391–396; 1991.
23. Mucha, R. F.: Preference for tastes paired with a nicotine antagonist in rats chronically treated with nicotine. Pharmacol. Biochem. Behav. 56:175–179, 1997.
24. Patrick, C. J.; Berthot, B. D.: Startle potentiation during anticipation of noxious stimulus: Active vs. passive response sets. Psychophysiology 32:72–80; 1995.
25. Schiffman, S.: Relapse following smoking cessation: A situational analysis. J. Consult. Clin. Psychol. 50:71–86; 1982.
26. Schmid, A.; Koch, M.; Schnitzler, H.-U.: Conditioned pleasure attenuates the startle response in rats. Neurobiol. Learn. Mem. 64:1–3; 1995.
27. Schuh, K. J.; Stitzer, M. L.: Desire to smoke during spaced smoking intervals. Psychopharmacology (Berlin) 120:289–295; 1995.
28. Siegel, S.: Evidence from rats that morphine tolerance is a learned response. J. Comp. Physiol. Psychol. 89:498–506; 1975.
29. Stewart, J.; deWit, H.; Eikelboom, R.: The role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol. Rev. 91:251–268; 1984.
30. Tiffany, S. T.; Drobes, D. J.: The development and initial validation of a questionnaire on smoking urges. Br. J. Addict. 86:1467–1476; 1991.
31. Villanueva, H. F.; Arezo, S.; James, J. R.; Rosecrans, J. A.: Withdrawal from chronic nicotine fails to produce a conditioned taste aversion to saccharin in rats. Pharmacol. Biochem. Behav. 37:59–61; 1990.