Startle cue-reactivity differentiates between light and heavy smokers

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Appetitive drug cue effects in former and light smokers, but not in heavy smokers

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ABSTRACT

Aims It is assumed that the startle amplitude in smokers is reduced while viewing pictures of smoking, suggesting that smoking cues are appetitive. The goal of the present study was to investigate (1) whether smoking scenes induce appetitive cue effects in smokers, and (2) whether smoking intensity is related to cue reactivity.

Design Smokers and non-smokers participated in a single session.

Participants A total of 62 individuals participated: 36 smokers and 26 non-smokers.

Measurements Participants took part in an acoustic startle experiment using standardised pleasant, neutral and unpleasant scenes from the International Affective Picture System (IAPS), as well as pictures of smoking. The effect of smoking cues was assessed by comparing neutral and smoking scenes (termed cue-related startle suppression, CSS).

Findings While there was no overall difference between smokers and non-smokers regarding the CSS, the light smokers showed statistically increased cue reactivity towards smoking-related cues, as compared with heavy smokers and non-smokers. In addition, light smokers also displayed stronger appetitive responses towards positive stimuli.

Conclusions This novel finding supports recent theories which discriminate between habit-based and incentive-based drug abuse. This distinction may have consequences for the assessment and treatment of drug-addicted subjects. Furthermore, incentive-based light smoking seems to have general effects on the reward system.

Keywords: Smoking, cue reactivity, startle reflex, smoking intensity.
Conditioned responses towards drug-associated stimuli and consumption habits play an important role in explaining the motivation for drug intake and the occurrence of relapses [1,2]. Classical conditioning theories of cue reactivity differ with regard to the nature of those drug-associated stimuli. On the one hand, cues are supposed to elicit aversive withdrawal-like responses [3], which may resemble homeostatic compensatory responses to drug effects [4-6]. On the other hand, cues may serve as appetitive cues or even incentives leading to similar behavioural and neurological responses like the drug itself [7-9]. The incentive sensitisation theory [1,10], which outreaches the scope of classical conditioning models, proposes that repeated drug consumption increases the motivational salience of drug-associated cues via a sensitisation of mesolimbic dopamine pathways, especially its projections into the nucleus accumbens. This sensitisation turns drug-associated stimuli into powerful incentives, which produce compulsive drug-taking behaviour on a psychological level (i.e. wanting or craving). The authors further assume that as addiction proceeds, hedonic effects of drug consumption (i.e. liking) decrease, whereas wanting of the drug increases.

There are several well-established indirect measures of cue reactivity that do not rely on self-report [1,11]. For example, the attentional bias towards smoking-related relative to neutral cues has been studied as an indirect index of cue reactivity by means of the visual dot probe task or the Stroop Test [e.g., 12-15]. Recently, some neuroimaging experiments also found stronger activations in the ventral striatum or in other parts of the mesolimbic network during the processing of smoking in contrast to neutral pictures [e.g., 16,17]. Another well-known method to uncover the indirect quality of drug-associated cues is the startle reflex [18]. The acoustic startle reflex consists of an automatic response to a loud burst of sound and has the sudden eye blink as its most reliable measure. This reflex is modulated by the affective value of a foreground stimulus: Compared to neutral pictures, the reflex is inhibited when an
individual views pleasant pictures and is potentiated when an individual views unpleasant pictures [19,20]. By including pictures depicting drug paraphernalia or drug intake rituals, the affect-modulated startle methodology has been repeatedly applied in drug research [21-28]. In smokers, two studies confirm incentive theories of drug addiction showing that smoking cues suppress the startle amplitude relative to neutral stimuli [27,28]. Others did not find such a smoking cue-related startle suppression (CSS) [21,26], although smoking-related stimuli produced lower startle amplitudes than negative stimuli. However, a direct comparison of smoking cue-related startle responses of active smokers and non-smokers is lacking so far.

Recently, the contribution of individual factors, such as smoking deprivation and the intention to quit, received some attention in startle research [21,28]. However, the relationship between the severity of addiction and the magnitude of CSS is still unclear. Recent studies suggest that the indirect index of reactivity to smoking cues might be lower in individuals who smoke more cigarettes per day (CPD) [12-14]. These studies tested the attentional bias towards smoking-related pictures compared to neutral pictures using a visual dot probe task, and the behavioural approach towards those cues using a stimulus-response compatibility task. The inverse correlation is consistent with theories of habit responding [e.g. 29,30], which assume that the effect of incentive processes on behaviour declines during the progress of addiction.

First, we hypothesised that smokers have a greater CSS index, as compared to non-smokers because smoking cues produce appetitive effects in smokers, but not in non-smokers. Second, because other bias measures have been shown to be negatively correlated with the number of CPD, we hypothesised a negative relationship between CSS and this measure of smoking intensity within active smokers. This means that light smokers are supposed to show stronger cue reactivity as compared to heavy smokers. Beside these two hypotheses, we tested whether smokers show stronger startle suppression during pleasant relative to neutral scenes because smoking may lead to general changes in the processing of natural rewards [31,32].
METHODS

Subjects
The investigation was carried out with a group of 36 active smokers (18 women) and a group of 26 non-smokers (16 women) who were matched with respect to age, education and gender proportion. Active smokers were either current participants in a clinical smoking cessation programme and were tested before their final quit day \((\text{quitters}, n = 27, 6 – 55 \text{ CPD})\) or recruits who had responded to an advertisement \((\text{sustainers}, n = 9, \text{ at least } 15 \text{ CPD})\) and did not explicitly wish to achieve abstinence. Non-smokers were defined as persons who did not smoke more than 100 cigarettes life-time. The present study was approved by the local ethics committee of the Medical Faculty of the University of Bonn and is in accordance with the Declaration of Helsinki. After providing informed written consent, all subjects were screened for psychiatric disorders. They received €20 for their participation.

Stimulus material and presentation
The testing material consisted of 56 colour slides, presented with a 12-in. monitor Notebook and a screen resolution of 600 x 800 pixels at 1 m in front of the participant. Sixteen pictures, which were taken from previous studies \([33,21]\), depicted either the beginning or the end of smoking a cigarette and control pictures, which were taken from the International Affective Picture System (IAPS) \([34]\), depicted 8 unpleasant \((9410, 3000, 3010, 3102, 3170, 3150, 3530, 6230)\), 8 neutral \((5534, 6150, 7002, 7030, 7050, 7190, 7002, 78203)\) and 8 pleasant \((8030, 8080, 8370, 4180/4490, 4290/4510, 4660, 2840)\) objects or scenes. Pleasant pictures mainly displayed sportive events and erotic scenes. Unpleasant stimuli consisted of pictures depicting violence and injuries. Furthermore, the set contained 16 pictures showing alcohol-related scenes, which were not analysed here. A 2 (group) x 4 (stimulus) repeated measures analysis of variance (ANOVA) for preliminary ratings of the stimulus material by
16 smokers and 15 non-smokers showed that all IAPS pictures were rated according to their assumed valence \((F_{(3,87)} = 144.077, P < .001)\) and arousal \((F_{(3,87)} = 67.147, P < .001)\) [34]. Both groups rated smoking scenes as more arousing than neutral scenes, and as less arousing than positive or negative scenes, but smokers judged smoking scenes as more positive than the non-smokers \((F_{(3,87)} = 5.585, P = .001)\).

After a 4-min habituation period of 70-dB background white noise, pictures were presented for 7 to 8 s in a fix-randomised order. Interstimulus intervals (black screen) lasted from 16.5 to 25.5 s. An acoustic startle response was evoked during 75% of the pictures from one category by 40 ms burst of 116-dB white noise presented binaurally through headphones (Maico, TDH-39-P) 2.5, 4.0 or 5.5 s after picture onset. The order of picture type and startle onset (no startle, 2.5 s, 4.0 s, or 5.5 s after picture onset) were counterbalanced for the first and the second half of the experiment. Consecutive stimuli were not from the same picture type.

**Experimental procedures**

The active smokers arrived between 4 and 6 h smoking deprived at the laboratory. They were tested for their exhaled alveolar carbon monoxide (ppmCO)-level (Smokerlyzer Micro IV, Bedfont Instruments, Kent, UK). Next, smokers were questioned about their smoking history and daily cigarette consumption. They were asked about their current desire to smoke a cigarette on a percent scale and to complete the Fagerström Test for Nicotine Dependence (FTND) [35, German version 36]. The startle experiment was carried out in a darkened room. Participants were instructed to look at the pictures and to ignore any noises from the headphones. Immediately after the startle experiment, all participants rated the valence of every picture on a 100-mm visual analogue scale (i.e. ‘How pleasant is this picture?’, 0 = very unpleasant, 100 = very pleasant) [21,33]. Active smokers also rated the degree of craving
induced by the pictures (i.e. ‘How much desire does this picture evoke?’, 0 = no desire, 100 = strong desire to smoke). Only ratings for trials where a startle tone was presented were included in the statistical analysis. At the end of the session, smokers were asked again to rate their momentary desire to smoke on a percent scale.

Data recording
The startle response was recorded digitally (EMG-SR-Lab, San Diego Instruments, Inc., San Diego, CA) as electromyographic (EMG) activity from the right orbicularis oculi muscle using two silver/silver chloride electrodes and a ground electrode placed on the glabella. All electrode resistances were less than 10 kΩ. Recorded EMG activity was bandpass filtered (1–1000 Hz). A 50-Hz notch filter was applied to eliminate 50-Hz interference. The EMG activity was recorded from the onset of the acoustic startle stimulus for 250 ms, with a sampling rate of 1 ms.

Data reduction
Spontaneous eye blinks were excluded from further analysis, using the registration parameters described by Braff et al. [37]. The latency to startle response onset was defined by a shift of 2.28 µV (6 digital units) from the baseline value in a time window of 21 to120 ms after the acoustic startle stimulus. Response rejections were made in both in case of onset-to-peak latencies > 95 ms and baseline shifts > 34.2 µV (> 90 digital units). Additionally, startle responses were discarded if the amplitude was more than 3 standard deviations above the individual mean or if the amplitude was less than 25 digital units. The subject was excluded from the study if there were less than two values per picture category after application of these criteria. In summary, six participants had to be excluded from further analyses and are not reported here. Because the amplitude of the startle response underlies a habituation effect over trials [38], and the decline of startle magnitude was stronger for smoking-related scenes
than for the IAPS pictures, we computed separate regression analysis for emotional and smoking-related trials to correct our data against this effect. For both trial types, the habituation was best described by a linear trend. Consequently, the raw data for every subject in the different trials were corrected for the linear trend. Finally, the available responses for the different picture categories were averaged to obtain the actual score.

Data analysis

Because the raw blink magnitude showed strong variations across subjects, individual startle amplitudes were standardised according to the individual mean and SD of the startle amplitudes of pleasant, neutral, and unpleasant scenes. Startle reactions towards pictures depicting the start and the end of smoking did not differ substantially from each other; therefore, they were subsequently analysed together.

In general, data were evaluated parametrically using Statistical Product and Service Solutions (SPSS) version 12. First, we calculated a one-way analysis of variance (ANOVA) across the whole sample with the three IAPS picture categories as a within-subject variable in order to prove the validity of the startle paradigm as it has been previously shown by Lang et al. [20]. As usual in cue reactivity research [12-17], further analyses were based on the CSS index. Therefore, we subtracted standardised amplitudes during smoking-related trials from neutral trials. In advance, we analysed whether there were any differences in the CSS index of smokers planning to quit smoking soon (quitters) and smokers who did not try to reach abstinence (sustainers) [28]. Then, we tested the overall difference between the smokers and non-smokers in the CSS effect. Afterwards, in line with previous findings [12-14], we examined the nonparametric correlations between the CSS and measures of smoking intensity [i.e., CPD, FTND score, and pack years (based on 20 CPD for one year)]. Based on a significant correlation between CPD and the CSS effect, we made a median split in the group of smokers and subsequently tested differences between light, heavy and non-smokers in a
one-way ANOVA. Accordingly, picture valence ratings where evaluated by a 2 (stimulus; neutral vs. smoking) x 3 (group; non-smoker vs. light vs. heavy smoker) ANOVA. The craving induced by neutral and smoking scenes was compared between heavy and light smokers using a 2 (stimulus) x 2 (group) ANOVA. Analogous to the CSS analysis, we tested whether smoking history is associated with generally altered reward processing by analysing the relative startle suppression during neutral minus positive scenes among the three groups. Throughout the analysis, the level of significance was $P < .05$ (two-tailed). Significant group differences in the one-way ANOVA were followed up by Dunnett T post hoc tests. Effect size calculations between groups refer to Cohens $d$. 


RESULTS

Sample characteristics
The two groups did not differ in terms of gender ($\chi^2(1) = 0.812, P = .368$), level of education ($\chi^2(2) = 5.349, P = .07$) or age ($F_{(1,60)} = 0.865, P = .356$).

Affective startle modulation
As previously reported [20], acoustic cues during negative, neutral and positive pictures from the IAPS evoked a linear startle pattern (Table 1, Figure 1).

Smoking cue-related startle suppression (CSS)
In smokers, there was no difference between the CSS of smokers planning to quit smoking soon (quitters) and that of smokers who did not try to reach abstinence (sustainers) (Table 1). There was no overall difference in the CSS of smokers and non-smokers. Correlation analysis revealed significant correlations between the CSS and CPD ($r_{sp} = -.41, P < .013$) as well as between the CSS and the FTND score ($r_{sp} = -.35, P < .034$). The correlations with pack years failed to reach significance ($r_{sp} = -.23, P < .229$). Subsequently, active smokers were differentiated into light and heavy smokers by means of a median split (median = 25 CPD). The group of light smokers consisted of 17 smokers who smoked less than 25 CPD; the group of heavy smokers comprised 19 smokers who smoked at least 25 CPD. Table 2 summarises the characteristics of smoking behaviour of the two groups. In a three-group comparison, we found significant differences between light, heavy and non-smokers (Figure 2, Table 1). Light smokers showed a stronger CSS effect, as compared to heavy smokers as well as to non-smokers. There was no difference in the CSS of heavy and non-smokers. Effect sizes for differences between the light smokers and the other two groups were large: $d = 1.01$ as compared to heavy smokers, and $d = 0.73$ as compared to non-smokers, respectively. A
comparison of the startle response during the presentation of pleasant relative to neutral stimuli revealed a similar pattern of results (Table 1). Light smokers displayed a higher level of startle suppression, as compared to non-smokers, and a trend towards stronger suppression in comparison to the heavy smokers. There was no difference between heavy smokers and non-smokers.

Explicit picture ratings
A repeated measures 2 (stimulus type) x 3 (group) ANOVA of the valence ratings of the three groups for neutral and smoking scenes showed that all three groups rated smoking pictures as less pleasant than neutral pictures (Table 1). However, heavy smokers rated pictures depicting smoking scenes significantly more pleasant, as compared to non-smokers (Figure 3). Another repeated measures 2 (stimulus type) x 2 (group) ANOVA for the craving ratings of light and heavy smokers showed that both groups report significant more craving while viewing pictures of smoking than neutral scenes (Table 1, Figure 4).
DISCUSSION

Overall, we did not find that the CSS differentiates between smokers and non-smokers. However, a novel finding from our study is that stronger cigarette consumption is related to significantly less CSS (median = 25 CPD): The subgroup of light smokers smoking less than 25 CPD, substantially differed from heavy smokers ($d = 1.01$), and non-smokers ($d = 0.73$). Thus, the lack of an overall effect may be caused by the heterogeneity of the sample of smokers.

Recently, the contribution of individual smoking factors received some attention in startle research. Both smoking deprivation and the intention to quit have been discussed in terms of whether or not they affect CSS modulation in active smokers. Whereas Geier et al. [21] found no deprivation effect, Cinciripini et al. [27] demonstrated stronger suppression of the startle response after 12 h of deprivation than immediately after smoking. In the present study, we had required our subjects to refrain from smoking for 4 to 6 hours and found robust CSS effects, suggesting that a relatively mild deprivation is sufficient to elicit the CSS.

Dempsey et al. [28] provided partial support for their new hypothesis that voluntary commitment to quit smoking suppresses CSS effects amongst active smokers. However, we found no CSS difference between smokers who wanted to quit smoking soon and the rest of the group. This finding suggests that startle modulation is probably not under voluntary control.

Whereas the issue of smoking intensity and cue-modulated startle responses has not been considered yet, negative correlations between attentional and behavioural approach biases towards smoking relative to neutral cues and the number of cigarettes smoked daily have been reported in several studies [12-14]. These findings are consistent with integrated incentive-habit theories of addiction e.g. [29,30], which propose a distinction between incentive smokers and habit smokers. Incentive smoking may represent an early stage of
addiction, where external cues trigger pleasant associations and consumption behaviour [2]. By contrast, smoking in habit smokers may be triggered by internal cues, based upon underlying neuronal adaptations. Accordingly, smoking in habit smokers might reflect later stages in addiction, characterised by strong motivational components (i.e. wanting) and compulsive consumption habits [10,30]. Thus, as we measured stronger appetitive effects of cigarette cues in light smokers, startle methodology appears to capture appetitive effects reflecting the hedonic liking of the drug, rather than motivational processes underlying wanting of the drug.

Nevertheless, our data do not conflict with alternative interpretations. For example, heavier smokers have a reduction in their sensitivity for smoking-related cues because they are more affected by the short deprivation period. However, Geier et al. [21] did not find an effect of deprivation on the startle suppression while viewing smoking cues.

Another interesting finding was that incentive-driven light smokers show enhanced startle suppression during the presentation of pleasant scenes relative to the presentation of neutral scenes. There is evidence from conditioning and electrophysiological experiments in animals, that nicotine not only enhances the incentive value of smoking-associated cues, but also the value of natural rewards [31], probably due to an amplification of the difference between tonic and phasic dopamine release in the ventral striatum [32]. Nevertheless, our finding remains preliminary and has to be examined systematically in further studies. For example, the pleasant affective scenes have to be systematically varied with regard to different situations. The pleasant IAPS scenes in our study mainly consisted of sportive scenes and erotic pictures.

**Conclusion**

In summary, the affect-modulated startle paradigm is suited to investigate the implicit valence of smoking-associated cues. Furthermore, the startle suppression by smoking cues
represents a sensitive bias measure that correlates with measures of consumption. Future studies should carefully consider the smoking behaviour in their sample.
References


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### Tables

**Table 1** Summary of tests, statistical values, p-values, and effect sizes for startle analysis.

<table>
<thead>
<tr>
<th></th>
<th>Statistical value</th>
<th>p-value</th>
<th>Effect size</th>
</tr>
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<tbody>
<tr>
<td><strong>Affective Startle modulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(One-way ANOVA with positive, neutral, and negative IAPS scenes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect stimulus</td>
<td>$F_{(2,122)} = 13.379$</td>
<td>$P &lt; .001$</td>
<td></td>
</tr>
</tbody>
</table>

**Dependent variable: CSS effect** (standardised startle amplitudes during neutral minus smoking trials)

(One-way ANOVAs)

- **Within smokers:**
  - **quitters** ($n = 27$) vs. **sustainers** ($n = 9$)
    $F_{(1,34)} = 0.451$, $P < .506$ n.s.

- **Smokers** ($n = 36$) vs. **non-smokers** ($n = 26$)
  $F_{(1,60)} = 1.097$, $P < .299$ n.s.

- **Light** ($n = 17$) vs. **heavy smokers** ($n = 19$) vs. **non-smokers** ($n = 26$)
  - **Light smokers vs. heavy smokers**
    post hoc: $T_{(34)} = 3.013$, $P < .025*$, $d = 1.01$
  - **Light smokers vs. non-smokers**
    post hoc: $T_{(41)} = 2.229$, $P < .042*$, $d = 0.73$
  - **Heavy smokers vs. non-smokers**
    post hoc: $T_{(43)} = -0.369$, $P < .899$ n.s.

**Dependent variable: standardised startle amplitudes during neutral minus positive trials**

(One-way ANOVAs)

- **Light** ($n = 17$) vs. **heavy smokers** ($n = 19$) vs. **non-smokers** ($n = 26$)
  - **Light smokers vs. heavy smokers**
    post hoc: $T_{(34)} = 2.013$, $P < .120$ n.s.
  - **Light smokers vs. non-smokers**
    post hoc: $T_{(41)} = 2.682$, $P < .023*$, $d = 0.87$
  - **Heavy smokers vs. non-smokers**
    post hoc: $T_{(43)} = 0.564$, $P < .773$ n.s.

**Dependent variable: Valence ratings**

[2 (stimulus: neutral, smoking) x 3 (group: light smokers, heavy smokers, non-smokers) ANOVA]

- **Main effect stimulus**
  $F_{(1,59)} = 46.512$, $P < .001**$

- **Interaction group x stimulus**
  $F_{(2,59)} = 4.679$, $P < .013*$

**Dependent variable: Craving ratings**

[2 (stimulus: neutral, smoking) x 2 (group: light smokers, heavy smokers) ANOVA]

- **Main effect stimulus**
  $F_{(1,34)} = 31.569$, $P < .001**$

* $P < .05$.
** $P < .001$. 

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Table 2 Characteristics [mean and standard deviation (SD)] of smoking behaviour of light (
\(n = 17\)) and heavy smokers (\(n = 19\)).

<table>
<thead>
<tr>
<th></th>
<th>Light smoker (mean (SD))</th>
<th>Heavy smoker (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTND* (0–10)</td>
<td>4.35 (2.09)</td>
<td>6.53 (1.95)</td>
</tr>
<tr>
<td>Cigarettes smoked daily (CPD)</td>
<td>17.00 (5.12)</td>
<td>31.21 (9.51)</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>22.46 (8.45)</td>
<td>25.47 (8.83)</td>
</tr>
<tr>
<td>Pack years**</td>
<td>17.87 (7.80)</td>
<td>40.18 (25.50)</td>
</tr>
<tr>
<td>ppmCO</td>
<td>11.87 (7.48)</td>
<td>15.47 (10.64)</td>
</tr>
<tr>
<td>Desire to smoke (before startle), 0–100</td>
<td>28.20 (27.07)</td>
<td>47.68 (31.57)</td>
</tr>
<tr>
<td>Desire to smoke (after startle), 0–100</td>
<td>43.53 (29.87)</td>
<td>51.11 (26.87)</td>
</tr>
</tbody>
</table>

*Fagerström Test for Nicotine Dependence.
** Pack years were based on 20 cigarettes per day over one year.
Figure 1 Mean standardised startle amplitudes [and standard error (SE)] during the presentation of different picture categories

Figure 2 Mean smoking cue-related startle suppression (CSS) (and SE) for light smokers, heavy smokers and non-smokers

Figure 3 Mean ratings (and SE) of picture valence for neutral and smoking pictures of light smokers, heavy smokers and non-smokers

Figure 4 Mean ratings (and SE) of induced craving for neutral and smoking pictures of light and heavy smokers
Negative Neutral Positive Smoking

Standardised startle amplitudes

Light smoker (n = 17)

Heavy smoker (n = 19)

Non-smoker (n = 26)

CSS Effect
(smoking cue-related startle suppression)

$d = 0.73$

$d = 1.01$
Neutral Smoking

Valence ratings (0 - 100)

Light smoker

Heavy smoker

Non-smoker

Craving ratings (0 - 100)

Light smoker

Heavy smoker