The Impact of the Opioid Antagonist Naloxone on Experimentally Induced Craving in Nicotine-Dependent Individuals

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Keywords

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Abstract

Objective: Preclinical and clinical findings suggest a substantial association of the endogenous opioid system in nicotine dependence. The present study investigates the possible dose-dependent influence of naloxone, an unspecific opioid-receptor-antagonist, combined with cue exposure on the physiological state, locomotor activity, craving and the hypothalamic-pituitary-adrenal axis in nicotine-dependent humans. **Methods:** Twenty nicotine-dependent, out-patient participants were deprived of nicotine for over 4 h, before receiving challenges with naloxone (1.6 mg or 3.2 mg q70 kg IV) or the placebo. Additionally, following drug administration, either smoking-related cues or neutral images were presented. Nicotine withdrawal was monitored by evaluating the following objective signs – skin conductance, heart rate, temperature, respiration, locomotor activity, cor-

tisol, prolactin and ACTH levels as well as craving. **Results:** With respect to subjective effects, participants administered a higher dosage of naloxone and those who were shown smoking-related cues were significantly less pleased ($p = 0.019$), felt more depressed ($p = 0.033$) and thought smoking would make them feel better ($p = 0.028$) than participants given naloxone and shown neutral cues. Participants given no naloxone but with smoking-related cues felt a higher urge to smoke than participants given naloxone and shown neutral cues ($p = 0.042$). Naloxone – in both dosages – also decreased the desire and intention to smoke in comparison to placebo. Compared to the placebo group, significantly higher cortisol, prolactin and ACTH values were observed after administration of lower and higher dosage of naloxone followed by smoking-related cues. **Conclusion:** Naloxone influenced nicotine withdrawal and strengthened significantly by cue exposure, both on objective measurement and on craving scales. These findings suggest an involvement of the endogenous opioid system in the development and maintenance of nicotine dependence.

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Introduction

Nicotine is a strongly addictive drug and after the initial exposure to occasional cigarette smoke, first symptoms of nicotine dependence are likely to appear within days or weeks [1]. It is well known from experimental data that there are numerous similarities between nicotine dependence and opioid dependence [2]. In humans, opioids like heroine and methadone also lead to an increased nicotine self-administration. The efficacy of morphine is promoted by nicotine, while nicotine as well as opioid withdrawal symptoms can be reversed by morphine. Interestingly, nicotine exhibits a biphasic effect: while having opioidergic properties at higher levels, its potency at lower levels seems to be anti-opioidergic [3]. Activating opioid-receptors with the aid of nicotine at different levels provokes the question of whether it is a primary action of this drug on these receptors or whether it is only tributary to a neuroregulatory cascade. Previous studies have shown that different neurotransmitters, such as Acetylcholine, dopamine, opioid peptides, GABA, serotonin, glutamate and norepinephrine, interact in nicotine's action [4, 5]. Results from previous studies suggest 2 possible modes of action between nicotine and endogenous opioids: First, the synthesis of beta-endorphin and met-enkephalin, especially in the nucleus accumbens, is altered after nicotine intake or withdrawal. Second, the activity of κ -opioid and δ -opioid receptors is altered in the same brain region [6].

In addition, animal studies of nicotine withdrawal have been shown to explain mechanisms associated with nicotine withdrawal behaviours. Somatic signs, affective signs and cognitive changes have all been found to be affected in the rodent model of nicotine withdrawal [7].

In rats, nicotine binds directly to opioidergic, paraventricular corticotropine-releasing hormones releasing neurons, leading to a dopamine increase in the mesolimbic accumbal nucleus [8]. Some resulting effects are changes in locomotor activity [9], physiologic parameters [10] and craving, which is of specific importance in promoting a possible relapse [11].

Since nicotine dependence is related to the opioid system, some authors have suggested that an opioid antagonist, for example naloxone, can be used to block the effects caused by nicotine in the brain. One study showed that heavy smokers treated with an opioid antagonist significantly reduced their cigarette consumption compared to a placebo group. Regarding the investigated pituitary

hormones, only prolactin was increased in the opioid antagonist group [12]. However, other research groups did not find significant changes of ACTH, prolactin or cortisol in acute nicotine's action, withdrawal or opioid receptor blockade [13, 14]. A Cochrane review concluded that opioid antagonists did not show any effect alone or as an adjunct nicotine replacement treatment on long-term smoking abstinence [15].

However, not much is known about the effects of opioid antagonists on craving.

In the present double-blind, placebo-controlled study, we investigated whether experimentally induced craving (physiological and perceived) can be modified by the administration of naloxone. One pilot study suggests dose-dependent effects [16]; therefore, properties of naloxone, a presentation of smoking-related cues and the combination of these two was of special interest to us.

Although animal studies demonstrated the impact of nicotine addiction in patterns of motor activity, this is – to our knowledge – the first study to examine this phenomenon in humans.

This study aims to investigate the possible dose-dependent influence of naloxone combined with cue exposure on the physiologic state, locomotor activity, craving and the hypothalamic-pituitary-adrenal (HPA) axis in nicotine-dependent humans.

Materials and Methods

Participants

Thirteen female and seven male, Caucasian, nicotine-dependent, adult outpatient volunteers with a median age of 25 (interquartile range [IQR] 23–29) years participated in the study. All participants were recruited via advertisements and were invited to come to the outpatient facilities of the psychiatric hospital of the Ludwig-Maximilians University in Munich. The study participants reported using nicotine on a daily basis from 17 (IQR 16 to 19) years on, and the first time of consumption happened at the age of 15 (IQR 14–16) years. In our study, group smokers reported a consumption of nicotine on 30 (IQR 29–30) days in the last few months and a continuous use since 5.5 (IQR 4–10) years. All smokers who were enrolled were required to fulfil the 6 ICD-10 criteria for substance (nicotine) dependence: an increased tolerance, sometimes physical withdrawal, a strong desire to take the drug, difficulties controlling their use, a higher priority given to drug use than to other activities and obligations, and a persisting use despite harmful consequences. These 6 criteria were evaluated in all participants. The Fagerstrom test to evaluate the degree of nicotine dependence [17, 18] showed a moderate grade of 2.5 (IQR 1.0–4.3) points. The number of cigarettes smoked per day was 11–20 cigarettes (1.0, IQR 1.0–1.0 points according to the Fagerstrom test). No volunteer stated regular use of alcohol or

Fig. 1. Study procedure. **a** Flow diagram. **b** Time line of one session. QSU, questionnaires of smoking urges; VAS, visual analog scales.

other psychotropic substances. After a first personal interview, eligible candidates completed detailed questionnaires about nicotine and other drug intake and referred to possible comorbid disorders (based on DSM-IV Axis I and ICD-10 chapter V) as well as their general physical health. Since both sexes were included in the study, female participants were explicitly asked about their status related to pregnancy and excluded if they were pregnant. Further exclusion criteria included any psychiatric disorder other than nicotine dependence, any present or past serious somatic disorder requiring long-term medication, and in particular, cardiovascular diseases. In order to exclude other psychiatric disorders, the structured clinical interview for DSM-IV axis I disorders was performed. The use of other substances was excluded only by asking the participants. Urine analyses to control for other substances or cotinine levels were not performed. Volunteers who participated were paid €50 at the end of the study. Participants signed informed consent forms describing the rationale, aims and process of the study as well as potential risks and possible benefits. This study was approved by the Institutional Board of the Department of Psychiatry and the Ethics Committee of the University of Munich and conducted in accordance with the Declaration of Helsinki.

Experimental Sessions: Drugs and Induction of Craving

Individuals were investigated by using the Physio Recorder S, the Mini Motionlogger Actigraph, different immunoassays for cortisol, ACTH and prolactin evaluation as well as visual analog scales (VAS) and questionnaires on smoking urges for subjective effects. In 4 afternoon sessions within a fortnight, participants were taken to a testing room after 4 h of nicotine deprivation. Physiological parameters (skin conductance level [SCL], skin conductance reflex [SCR], heart rate, skin temperature, respiration ampli-

tude around the thorax) and locomotor activity were monitored continuously, while subjective effects and hormone levels were investigated 5 min prior and 5, 15, and 30 min after IV naloxone administration. Smoking-related cues were presented 10 min after drug application. Three steps were performed in smoking-related cues exposure. First, individuals were asked to imagine a personal positive situation of smoking. Second, pictures from tobacco ads (<http://www.gauloises.com/>) were presented and third, a burning cigarette of choice was held by the participants, without being allowed to smoke it.

Naloxone (0; 1.6 mg q70 kg; 3.2 mg q70 kg – naloxon 0.4 mg Curamed, CuraMED Pharma GmbH, 76227 Karlsruhe, Germany) followed by cue exposure was administered randomly in a double blind manner, while all individuals were given naloxone (3.2 mg q70 kg) when combined with neutral not smoking-related cues, that is photos of landscapes (www.naturbilder.de) on the last day of 4 study days. Figure 1 shows details on the study procedure.

Objective and Subjective Measures

Physiological Response

The Physiorecorder S, as a part of the Wiener Testsystem (Dr. G. Schuhfried GmbH, 2340 Mödling, Austria, www.schuhfried.co.at) was used to record specific physiologic parameters. Electrodermal activity with 2 parameters – SCL and SCR – was assessed on the non-dominant palmar. While SCL quantified the tonic level, SCR represented changes of skin conductance. The heart rate was measured by Photoplethysmography on the earlap, the skin temperature with a sensor on the non-dominant lower arm, and changes in respiration patterns by assessing an ultrasonic distance measurement around the thorax. Data was recorded continuously and their mean and SD were calculated for each session.

Actigraph

The Mini Motionlogger Actigraph® -Basic- (Ambulatory Monitoring, Inc., 731 Saw Mill River Road, Ardsley, NY 10502-0609, USA) was applied in 6 participants by using Zero Crossing Mode at a data collection time of 1 min each [19]. Actigraphs were placed on the dominant wrist, comparable to Physiorecorder S, at the beginning of each session. Again, the mean and SD were calculated.

Subjective Effects: Craving Measures: VAS and Questionnaires of Smoking Urges

The first instrument in order to investigate subjective effects was VAS [20] by using 12 10 mm horizontal lines, labelled with the following items: “anxious,” “pleased,” “tranquil,” “depressed,” “hungry,” “tired,” “I’d like to smoke,” “smoking would reduce unpleasant feelings now,” “smoking would make me feel better,” “I intend to smoke soon,” “being able to control smoking,” “feeling the urge to smoke”.

Second, the questionnaires related to smoking urges (QSU)-G (German translation), representing 2 factors “desire to smoke” and “intention to smoke,” was used to study craving at baseline, after naloxone administration, after cue exposure and after further 15 min. This 32-item questionnaire contains answers with 7 degrees from total agreement to total disagreement.

Sensitivity to nicotine deprivation and reactivity to smoking-related cues has been demonstrated before [21].

HPA Axis

Three hormones of the HPA were investigated at the same time as filling out the questionnaires. Blood samples were taken in order to evaluate cortisol levels by using cortisol double antibody DPC®, cortisol antiserum (COD1), ¹²⁵I cortisol (COD2), cortisol calibrators (COD3-8), precipitating solution (N6; DPC® Diagnostic Products Corporation, 5700 West 96th street, Los Angeles, CA 90045-5597, USA), ACTH levels with ACTH 100T Kit – No. 60–4175, Chemiluminescence Immunometric Assay (Nichols Institute Diagnostics, San Juan Capistrano, CA 92675 USA) and prolactin levels with the aid of the A018-301 DELFIA® Prolactin kit and time-resolved fluoroimmunoassay (PerkinElmer Wallac GmbH, Jechtinger Strasse 9, 79111 Freiburg, Germany).

Statistical Analyses

SPSS and SAS (version 9.4) were used for data analyses. Physiorecorder S and Actigraph data was analyzed using 2-sided *t* tests. For the analysis of cortisol, prolactin and ACTH levels as also for both the domain forms – the QSU questionnaire and the VAS results – we used linear mixed models with subject as random effects. Mixed models account for the correlation between repeated measurements on the same person through their covariance matrix structure. For this analysis, a covariance matrix with compound symmetry was used. This assumes constant correlation within an individual over time and zero correlation between individuals. A further advantage of mixed models is that for the analysis of a single time point, the information from all time points is used. Specifying random effects at individual level allows for different baseline values for single individuals. This also reduces the “gender effect”: if one gender generally has higher levels of, for example, cortisol, the model takes this into account through the random effects assumption. We also tested for a

presence of a gender effect formally by including gender of the participant as a covariate in a linear mixed model. If the covariate’s effect was non-significant, we concluded that we could not find a gender effect. When conducting several tests on the same data, we applied Bonferroni *p* value adjustment to account for multiple testing. Results were considered statistically significant at *p* < 0.05.

Results

Effects of naloxone on physiological parameters: skin conductance, heart rate, temperature, respiration and locomotor activity.

Two-sided *t* tests revealed statistically significant differences in heart rate (*p* = 0.045) and temperature (*p* = 0.043) when both dosages of naloxone were compared, and when high dosages of naloxone with cue exposure compared with dosages without cue exposure.

Locomotor activity was significantly higher after placebo administration in comparison to both 3.2 mg (*p* < 0.001) and 1.6 mg naloxone q70 kg (*p* = 0.004) administration, whereas the dosage comparison did not reach a significant level (*p* = 0.072; Table 1).

Subjective Effects: Induced by Drug and Cue Exposure

With respect to subjective effects, as measured by VAS, participants administered a higher dosage of naloxone and those who were shown smoking-related cues were significantly less pleased (*p* = 0.019), felt more depressed (*p* = 0.033) and thought smoking would make them feel better (*p* = 0.028) than participants who were given naloxone and shown neutral cues.

Participants given no naloxone but with smoking-related cues felt higher urge to smoke than participants given naloxone and shown neutral cues (*p* = 0.042). Details are shown in (Table 2a, b).

By using QSU, both factors (“desire to smoke,” “intention to smoke”) were increased after a higher dosage administration followed by cue exposure, in comparison to naloxone alone (Fig. 2, 3). Naloxone – in both dosages – also decreased the desire and intention to smoke in comparison to placebo. However, the results were not significant (Table 3).

In the placebo group, the intention to smoke was significantly higher after further 20 min after cue presentation compared to baseline values (*p* = 0.023). The intention to smoke among the participants administered naloxone and shown neutral cues was significantly lower after naloxone administration when compared to baseline (*p* = 0.022). Regarding the desire to smoke, in the

Table 1. Effects of naloxone on SKL, heart rate, temperature, respiration and locomotor activity

	Placebo + cue, mean $\pm$ SD	3.2 mg naloxone + cue, mean $\pm$ SD	1.6 mg naloxone + cue, mean $\pm$ SD	3.2 mg naloxone – cue, mean $\pm$ SD
SCL, $\mu\text{S}/\text{cm}^2$	11.39 $\pm$ 6.15	10.52 $\pm$ 7.87	11.88 $\pm$ 5.93	10.45 $\pm$ 5.81
SCL, $\mu\text{S}/\text{cm}^2$	0.065 $\pm$ 0.13	0.079 $\pm$ 0.11	0.089 $\pm$ 0.074	0.095 $\pm$ 0.13
Heart rate, per min	15.63 $\pm$ 6.67	13.00 $\pm$ 1.45	13.05 $\pm$ 1.47	12.40 $\pm$ 0.82
Temperature, $^{\circ}\text{C}$	30.27 $\pm$ 1.77	30.29 $\pm$ 1.85	30.72 $\pm$ 1.71	30.89 $\pm$ 1.90
RESP-1, cm	4.24 $\pm$ 1.77	3.98 $\pm$ 2.26	4.68 $\pm$ 2.12	4.80 $\pm$ 2.37
RSPA-1, cm	0.59 $\pm$ 0.13	0.59 $\pm$ 0.17	0.59 $\pm$ 0.15	0.61 $\pm$ 0.18
Locomotor activity (total count)	89.97 $\pm$ 43.95	68.58 $\pm$ 35.71	73.65 $\pm$ 45.06	72.03 $\pm$ 43.91

Values for physiological effects and locomotor activity collected in 3 h sessions with placebo or naloxone administration combined with smoking related cues or neutral cue exposure (+ cue = smoking related cues, – cue = neutral cues, all naloxone doses q70 kg).

SCL, skin conductance level.

placebo group, the values recorded after 20 min of cue presentation were significantly higher compared to baseline values ($p = 0.021$; data not shown).

Changes in HPA Axis

Compared to the placebo group, significantly higher cortisol values were observed after the administration of lower and higher dosage of naloxone followed by smoking-related cues ($p = 0.006$, $p < 0.001$, respectively), and after administration higher naloxone dosage followed by neutral cues ($p = 0.001$). Greater difference in the cortisol values for higher naloxone dosage was observed suggesting dose dependence. Differences between lower and higher dosage of naloxone did not gain significance. Cortisol values within a group tend to decrease over time, except for the participants shown neutral cues, where cortisol values increase after cue exposure and remain higher (Fig. 4 and 6). Regarding prolactin, compared to the placebo group, significantly higher prolactin values were observed after the administration of 3.2 mg naloxone followed by smoking-related cues ($p < 0.001$). There was a significant increase in prolactin values in the higher naloxone dosage group compared to the lower dosage group ($p = 0.022$). A dose dependence pattern can be suggested for prolactin. Prolactin values increase over time for participants given naloxone, but for the placebo group, the values decrease (Fig. 5 and 7).

Compared to the placebo group, significantly higher ACTH values were observed after the administration of lower and higher dosage of naloxone followed by smoking-related cues and after administration of 3.2 mg naloxone followed by neutral cues ($p < 0.0001$ for all 3 comparisons). Also for ACTH, dose dependency seems possible. In the course of time, for the naloxone-

treated groups, ACTH level remains the same or increase after naloxone; in the placebo group, ACTH values decrease.

ACTH values seemed to be higher in males than in females ($p = 0.048$). Males had lower levels of cortisol and prolactin than females; however, the effects were not significant ($p = 0.0760$ and $p = 0.218$, respectively). The used mixed models account for the gender effect to a great amount.

Discussion

In this study, we investigated the interaction of nicotine and the endogenous opioid system. We challenged smokers with naloxone and identified that naloxone increased the dose-dependent nicotine withdrawal, strengthened significantly by cue exposure on objective measurement and craving.

So far, animal models have indicated a mutual dependence of opioid antagonists like naloxone and altered behaviour of nicotine-dependent mammals [22, 23]. This led to naloxone challenges in human beings [16], resulting in contradictive findings. Hence, the contribution of opioid antagonists on smoking withdrawal remained unclear. A Cochrane analysis had shown that numerous nicotine replacement treatments using medication like antidepressants, or other forms of nicotine replacement therapies were effective in increasing nicotine abstinence rates [24]. Furthermore, opioid antagonists like naltrexone and naloxone were used for smoking cessation. Another Cochrane review concluded that opioid antagonists alone did not show an effect, nor did they

Table 2.**a.** Subjective effects – induced by drug and cue exposure

VAS item	Placebo + cue				3.2 mg naloxone + cue			
	baseline	after drug	after cue	30 min after drug	baseline	after drug	after cue	30 min after drug
Anxious	0.8 (0.0–3.6)	0.6 (0.0–2.9)	0.3 (0.0–2.4)	0.4 (0.0–3.0)	0.6 (0.0–2.8)	0.5 (0.0–2.0)	0.5 (0.0–2.4)	0.6 (0.0–1.0)
Pleased	8.9 (7.2–10)	9.5 (7.3–10.0)	10.0 (7.3–10.0)	9.4 (7.3–10.0)	9.2 (7.4–10.0)	8.5 (7.3–10.0)	7.4 (5.9–10.0)	8.5 (5.8–10.0)
Tranquil	8.4 (5.8–10)	10.0 (7.3–10.0)	10.0 (7.3–10.0)	10.0 (7.3–10.0)	10.0 (8.1–10.0)	10.0 (7.3–10.0)	8.1 (7.2–10.0)	9.3 (6.0–10.0)
Depressed	0.6 (0.0–2.8)	1.8 (0.1–4.2)	0.6 (0.1–3.2)	1.1 (0.2–3.2)	1.6 (0.1–3.2)	0.6 (0.1–4.4)	0.6 (0.1–6.1)	1.2 (0.0–7.3)
Hungry	3.2 (1.1–9.6)	6.1 (1.4–9.7)	5.9 (1.3–10.0)	3.7 (0.4–10.0)	3.0 (0.3–5.4)	3.6 (0.7–7.8)	3.7 (0.4–7.5)	3.4 (0.3–7.9)
Tired	5.8 (0.0–8.9)	7.3 (1.9–10)	6.7 (1.9–8.8)	3.3 (1.5–10.0)	3.4 (0.0–7.3)	4.4 (1.6–10.0)	6.8 (0.8–9.4)	4.5 (1.7–7.4)
I'd like to smoke	7.5 (4.6–10.0)	7.8 (4.6–10.0)	8.2 (7.3–10.0)	8.8 (5.3–10.0)	7.5 (5.3–9.8)	8.0 (5.7–10.0)	8.9 (7.3–10.0)	9.2 (2.1–10.0)
Smoking would reduce unpleasant feelings now	3.8 (0.1–7.4)	3.5 (0.3–7.6)	5.9 (0.3–10.0)	3.6 (0.4–7.4)	2.7 (0.1–7.3)	2.2 (0.2–5.6)	3.6 (0.2–8.9)	3.6 (0.0–9.2)
Smoking would make me feel better	7.5 (2.6–9.8)	7.0 (0.9–9.8)	6.2 (1.9–10.0)	9.3 (0.8–10.0)	7.4 (2.0–9.2)	6.8 (2.4–10.0)	7.3 (3.6–10.0)	7.5 (1.3–10.0)
I intend to smoke soon	10.0 (7.4–10.0)	10.0 (7.3–10.0)	10.0 (7.3–10.0)	10.0 (7.3–10.0)	8.3 (7.3–10.0)	9.5 (5.5–10.0)	10.0 (5.7–10.0)	10.0 (7.1–10.0)
Being able to control smoking	4.1 (1.2–7.3)	3.5 (1.1–6.3)	3.6 (1.7–7.3)	4.1 (1.3–9.2)	4.8 (1.8–9.0)	4.7 (3.0–8.7)	4.0 (2.9–7.3)	5.4 (1.2–7.7)

	1.6 mg naloxone + cue				3.2 mg naloxone + cue			
	baseline	after drug	after cue	30 min after drug	baseline	after drug	after cue	30 min after drug
Feeling the urge to smoke	8.5 (3.6–9.9)	4.3 (3.4–8.9)	7.5 (3.1–10.0)	7.3 (3.3–10.0)	2.9 (0.1–6.9)	5.5 (0.8–9.3)	4.7 (0.5–10.0)	6.5 (0.2–10.0)
Anxious	0.7 (0.0–2.3)	0.5 (0.0–1.9)	0.5 (0.0–1.7)	0.3 (0.0–1.3)	0.8 (0.1–1.9)	0.2 (0.0–2.4)	0.2 (0.0–1.2)	0.3 (0.0–2.3)
Pleased	9.6 (7.4–10.0)	9.8 (7.2–10.0)	7.4 (7.3–10.0)	8.5 (7.3–10.0)	10.0 (7.9–10.0)	10.0 (7.3–10.0)	10.0 (8.9–10.0)	9.5 (7.4–10.0)
Tranquil	10.0 (7.4–10.0)	10.0 (7.4–10.0)	7.5 (6.2–10.0)	10.0 (5.2–10.0)	10.0 (7.6–10.0)	9.9 (7.3–10.0)	10.0 (7.3–10.0)	9.7 (7.4–10.0)
Depressed	0.7 (0.0–2.2)	0.9 (0.2–3.0)	0.5 (0.1–2.0)	0.5 (0.0–1.8)	0.3 (0.0–2.3)	0.4 (0.0–3.0)	0.3 (0.0–3.7)	0.3 (0.0–2.2)
Hungry	2.9 (0.5–6.8)	3.2 (0.8–6.8)	3.7 (0.3–8.5)	3.5 (0.6–5.6)	2.7 (0.2–5.6)	2.9 (0.6–6.6)	1.5 (0.0–4.4)	2.7 (0.0–7.0)
Tired	3.8 (0.4–10.0)	5.3 (1.2–10.0)	3.2 (1.4–9.1)	5.9 (0.7–10.0)	4.3 (0.2–8.8)	4.5 (0.0–8.3)	5.8 (0.1–7.4)	6.9 (0.1–9.0)
I'd like to smoke	7.2 (3.6–7.9)	7.5 (2.1–9.9)	7.4 (3.7–10.0)	8.6 (3.6–10.0)	7.3 (3.7–9.5)	7.5 (3.3–9.7)	7.3 (2.3–9.8)	8.6 (7.3–10.0)
Smoking would reduce unpleasant feelings now	3.1 (0.4–4.3)	2.8 (0.8–6.8)	3.3 (0.3–10.0)	4.8 (0.7–10.0)	3.9 (0.3–7.3)	3.7 (0.3–7.3)	2.3 (0.1–6.6)	4.4 (0.1–7.4)
Smoking would make me feel better	6.4 (2.3–8.7)	3.3 (0.7–7.5)	5.0 (0.5–10.0)	7.4 (0.7–10.0)	6.2 (0.8–8.3)	6.9 (0.8–8.8)	3.7 (0.7–8.5)	6.5 (0.4–9.6)
I intend to smoke soon	8.6 (3.9–10.0)	8.2 (3.4–9.5)	8.6 (7.3–10.0)	10.0 (6.9–10.0)	8.1 (5.5–10.0)	9.0 (3.9–10.0)	7.6 (5.7–10.0)	8.9 (7.0–10.0)
Being able to control smoking	3.6 (2.0–7.6)	6.0 (2.2–10.0)	3.1 (0.7–8.9)	3.5 (0.8–8.3)	3.5 (0.7–7.3)	4.5 (0.8–9.4)	5.5 (0.9–7.4)	4.3 (0.7–7.3)
Feeling the urge to smoke	4.3 (0.3–8.0)	5.6 (1.3–7.6)	6.7 (2.0–9.6)	8.1 (2.4–10.0)	5.1 (0.2–7.5)	6.1 (0.3–8.5)	3.7 (0.5–7.6)	6.8 (0.4–8.7)

b. Subjective effects – induced by drug and cue exposure

VAS item	Comparisons between doses and cues					
	3.2 mgnaloxone + cue vs. 3.2 mg naloxone – cue	3.2 mg naloxone + cue vs. 1.6 mg naloxone + cue	placebo + cue vs. 3.2 mg naloxone + cue	placebo + cue vs. 1.6 mg naloxone + cue	placebo + cue vs. 3.2 mg naloxone – cue	1.6 mg naloxone + cue vs. 3.2 mg naloxone – cue
Anxious	1.000	1.000	0.321	0.640	0.139	1.000
Pleased	0.019	0.938	0.898	1.000	0.133	0.196
Tranquil	0.925	1.000	1.000	1.000	0.999	0.415
Depressed	0.033	0.188	1.000	0.925	0.126	0.835
Hungry	0.493	1.000	0.086	0.056	0.006	1.000
Tired	1.000	1.000	1.000	1.000	1.000	1.000
I'd like to smoke	0.441	0.685	1.000	0.193	0.464	1.000
Smoking would reduce unpleasant feelings now	1.000	1.000	1.000	1.000	1.000	1.000
Smoking would make me feel better	0.028	0.278	1.000	0.741	0.152	1.000
I intend to smoke soon	1.000	1.000	0.954	0.050	0.702	1.000
Being able to control smoking	0.209	1.000	0.188	1.000	1.000	1.000
Feeling the urge to smoke	1.000	1.000	0.082	0.426	0.042	1.000

Bonferroni adjusted *p* values for subjective effects as measured by VAS during 3 h sessions while administrating placebo or naloxone in different doses with or without cue exposure – comparisons between doses and cues (+ cue = smoking related cues, – cue = neutral cues, all naloxone doses q70 kg).

VAS, visual analog scales. Values in bold are significant.

Table 3. Medians (IQR) of QSU for different times and conditions

Time	Placebo + smoking cue			3.2 mg + smoking cue			1.6 mg + smoking cue			3.2 mg + neutral cue		
	baseline (60 min)	after drug (70 min)	after cue (80 min)	30 min after drug (95 min)	baseline (60 min)	after drug (70 min)	after Cue (80 min)	30 min after drug (95 min)	baseline (60 min)	after drug (70 min)	after Cue (80 min)	30 min after drug (95 min)
Intention to Smoke	4.6 (3.6–5.7)	4.7 (4.2–5.8)	5.3 (4.1–5.9)	5.4 (4.9–5.9)	5.1 (3.8–5.9)	4.7 (3.3–5.7)	5.4 (3.5–6.5)	5.7 (4.7–6.5)	4.5 (3.0–5.9)	4.6 (2.8–5.5)	5.5 (3.8–6.3)	5.5 (3.8–6.0)
Desire to smoke	2.5 (1.1–3.8)	2.4 (1.0–3.7)	3.1 (1.2–4.1)	3.1 (1.1–4.3)	2.0 (1.5–3.6)	2.4 (1.4–4.1)	2.2 (1.4–4.2)	2.7 (1.4–4.4)	1.7 (1.1–3.3)	2.3 (1.3–2.8)	3.2 (1.0–4.3)	3.2 (1.5–3.5)
QSU item	Comparisons between doses and cues											
	3.2 mg naloxone + cue vs. 3.2 mg aloxone – cue				3.2 mg naloxone + cue vs. 1.6 mg naloxone + cue				placebo + cue vs. 3.2 mg naloxone + cue			
Intention to smoke, <i>p</i>	1.000				1.000				1.000			
Desire to smoke, <i>p</i>	0.052				1.000				1.000			
VAS item	Placebo + cue vs. 3.2 mg naloxone – cue				1.6 mg naloxone + cue vs. 3.2 mg naloxone – cue							
Intention to smoke, <i>p</i>	0.122				1.000							
Desire to smoke, <i>p</i>	0.171				0.611							
QSU, questionnaires of smoking urges; VAS, visual analog scales.

Fig. 2. Values from QSU, intention to smoke: 3.2 mg naloxone +cue vs. –cue. Subjective effects as measured by questionnaire of smoking urges (QSU; “intention to smoke”) during 3 h sessions while administrating naloxone, followed by smoking-related cues (+cue) or neutral cues (–cue) – all naloxone doses 3.2 mg q70 kg. Bonferoni adjusted $p = 1.000$. **Fig. 3.** Values from QSU, desire to smoke: 3.2 mg naloxone + cue vs. –cue. Subjective effects as measured by questionnaire of smoking urges (QSU; “desire to smoke”) during 3 h sessions while administrating naloxone, followed by smoking-related cues (+ cue) or neutral cues (– cue) – all naloxone doses 3.2 mg q70 kg. Bonferoni adjusted $p = 0.052$.

as an adjunct nicotine replacement treatment on long-term smoking abstinence [15].

However, in our approach, we were interested in the physiological and subjective effects caused by naloxone on nicotine withdrawal in relation to smoking cues. Therefore and for the first time, we combined the presen-

Fig. 4. Cortisol level changes in relation to naloxone with and without smoking-related cue. Changes from baseline cortisol as measured in µg/dL were evaluated during 3 h sessions while administering naloxone followed by smoking-related cues (+ cue) or neutral cues (– cue) – all naloxone doses 3.2 mg q70 kg. $p = 0.077$.

Fig. 5. Prolactin level changes in relation to naloxone with and without smoking-related cues. Changes from baseline prolactin as measured in mU/L were evaluated during 3-h sessions while administering naloxone, followed by smoking-related cues (+ cue) or neutral cues (– cue) – all naloxone doses 3.2 mg q70 kg $p = 0.022$.

tation of smoking-related cues [25, 26] in a study on human nicotine-dependent subjects. This new approach incorporates results about physiologic changes [27], HPA axis alterations [28] as well as locomotor activity [29] and includes supplementary subjective effects, being the basis

of craving. The current study reveals that there is an overall increased level of activation after low-dose naloxone administration in comparison to higher doses in objective measures like heart rate and locomotor activity, while no significant differences in subjective effects or hormone levels were identified.

Higher dosage of naloxone with smoking-related cues led to feeling significantly less pleased, more depressed and thinking smoking would make one feel better compared to no smoking-related cues. Participants given placebo and smoking-related cues felt higher urge to smoke than participants given naloxone and shown neutral cues.

Desire to smoke and intention to smoke were increased after a higher dosage administration followed by cue exposure, in comparison to naloxone alone. Naloxone – in both dosages – also decreased the desire and intention to smoke in comparison to those in the placebo group.

Overall, the hormone levels of cortisol, ACTH and prolactin were elevated after naloxone administration, being reinforced through cue exposure. In regard to cue exposure, we emphasise the results at higher doses of naloxone with smoking-related cues versus without smoking-related cues: cues led to significantly higher temperature, craving, desire and intention to smoke, as well as a significant increase in prolactin levels. The present study demonstrates changes in craving after high doses of opioid receptor blockades when followed by the presentation of smoking-related cues. Possible implications for behavioural therapy were found in studies investigating the effect of opioid antagonists and cognitive behavioural therapy in alcohol-dependent subjects [30]. Here the authors showed that for those with high cravings compared to placebo, the opioid antagonist cognitive behavioural therapy and the combination increased days of abstinence. Whether a patient is a smoker or not can alter the benefit from an opioid antagonist treatment in alcohol-dependent patients [31]. The authors demonstrated that naltrexone had a stronger effect on reducing alcohol consumption and drinking behaviour among smokers compared to non-smokers. Others found that heavy drinking smokers who were treated with naltrexone showed increased smoking abstinence rates and less smoking urge as well as decreased alcohol use after a 12-week treatment course [32].

The present study also has some limitations: one limitation is the degree of nicotine dependence in our study group. By following the Fagerstrom's classification, the median grade of addiction was found to be moderate. Therefore, our results remain restricted to a moderate addiction level and no conclusions can be drawn for highly

Fig. 6. Boxplots of cortisol for all groups and time points. Shows the measured cortisol values for the placebo followed by the + cue group, 3.2 mg naloxone followed by the + cue group, 1.6 mg naloxone followed by the + cue group and 3.2 mg naloxone followed by the – cue group, and for all time points respectively.

Fig. 7. Boxplots of prolactin for all groups and time points. Shows measured prolactin values for the placebo followed by the +cue group, and 3.2 mg naloxone followed by the + cue group, 1.6 mg naloxone followed by the + cue group and 3.2 mg naloxone followed by the – cue group, and for all time points respectively.

addicted smokers. In addition, the importance of time in interpreting changes in hormone levels needs to be pointed out. One study found peak cortisol levels 60 min and ACTH levels 30 min after naloxone administration [33]. The results from this present study contradict this, lim-

ited by an observation time of 25 min after naloxone administration. Also, one previously published study found a threefold increased activation of HPA axis following an opioid receptor blockade on the basis of an A118G nucleotid exchange in the exon1 of μ -opioid receptor gene. As

a consequence, an Asn40Asp substitution in the receptor's extracellular domain was reported [34]. In our study, participants were not genotyped accordingly, and thus, a possible effect of receptor polymorphisms on hormone levels cannot be ruled out completely. It also appears fundamental to note the feasible influence of the environment on heart rate and skin conductance in smokers. One publication found a higher increase in heart rates but not in skin conductance, in a laboratory compared to a home environment [26]. The present study took place in a lab; therefore, this environment could have influenced physiological changes. However, since the surroundings were equal for all participants receiving naloxone or placebo, these effects might not be significant. Lastly, the small number of study participants might lead to significant effects not being detected due to lack of statistical power. Nevertheless, even after conservative Bonferroni adjustment of the *p* values, some effects were highly significant also in our small study sample. Therefore, this study can

be interpreted as a pilot study and further studies with a higher number of participants can shed more light on the precise effects of naloxone in craving.

Altogether, we confirmed endocrinological and subjective effects after an opioid receptor blockade in smokers. In particular, significant results of stress induction on the level of HPA axis were seen. The described increases – physiological, locomotoric, subjective, endocrinological – in association with naloxone application in combination with cue exposure offer a new perspective on treating craving with opioid antagonists.

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