ORIGINAL ARTICLE

Addiction Biology   SSA   **WILEY**

Aversive drug cues reduce cigarette craving and increase prefrontal cortex activation during processing of cigarette cues in quitting motivated smokers

Stefanie L. Kunas[1] | Felix Bermpohl[1] | Irene S. Plank[1,2,3] | Andreas Ströhle[1] | Heiner Stuke[1]

[1]Department of Psychiatry and Neuroscience, Campus Charité Mitte, Charité-Universitätsmedizin Berlin, Berlin, Germany

[2]Einstein Center for Neurosciences, Charité Campus Mitte, Universitätsmedizin Berlin, Berlin, Germany

[3]Berlin School of Mind and Brain, Institute of Psychology, Humboldt-Universität zu Berlin, Berlin, Germany

Correspondence
Stefanie L. Kunas, Department of Psychiatry and Neuroscience, Campus Charité Mitte, Charité-Universitätsmedizin Berlin, Berlin D-10117, Germany.
Email: stefanie.kunas@charite.de

Funding information
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Grant/Award Numbers: 402170461, TRR265; Charité-Universitätsmedizin Berlin

Abstract

Aversive drug cues can be used to support smoking cessation and create awareness of negative health consequences of smoking. Better understanding of the effects of aversive drug cues on craving and the processing of appetitive drug cues in abstinence motivated smokers is important to further improve their use in cessation therapy and smoking-related public health measures. In this study, 38 quitting motivated smokers underwent functional magnetic resonance imaging (fMRI) scanning while performing a novel extended cue-reactivity paradigm. Pictures of cigarettes served as appetitive drug cues, which were preceded by either aversive drug cues (e.g., smokers' leg) or other cues (neutral or alternative reward cues). Participants were instructed to rate their craving for cigarettes after presentation of drug cues. When aversive drug cues preceded the presentation of appetitive drug cues, behavioural craving was reduced and activations in prefrontal (dorsolateral prefrontal cortex) and paralimbic (dorsal anterior cingulate cortex [dACC] and anterior insulae) areas were enhanced. A positive association between behavioural craving reduction and neurofunctional activation changes was shown for the right dACC. Our results suggest that aversive drug cues have an impact on the processing of appetitive drug cues, both on a neurofunctional and a behavioural level. A proposed model states that aversive drug-related cues activate control-associated brain areas (e.g., dACC), leading to increased inhibitory control on reward-associated brain areas (e.g., putamen) and a reduction in subjective cravings.

KEYWORDS
control network, craving, cue reactivity

1 | INTRODUCTION

Smoking is a leading cause for cancer, respiratory and cardiovascular diseases and related to an estimated 12% of deaths in the adult population worldwide.[1,2] These approximately 4.8 million cases of premature death each year are preventable, highlighting the importance to identify new and enhance already existing prevention and treatment strategies. One promising approach to target smoking in

public health measures and cessation therapy is the use of aversive drug cues that show negative consequences of smoking in the form of an image or text (e.g., on cigarette packets).

Aversive drug cues have been proven to reduce craving in smokers,[3] to curtail the number of smoking initiators,[4] to augment quitting rates and to raise awareness for health issues related to tobacco consumption.[5] To investigate the neurofunctional mechanisms involved in these beneficial effects, previous functional magnetic resonance imaging (fMRI) studies[6–8] investigated aversive drug cue reactivity. They showed activation of a brain network including regions involved in executive control (e.g., dorsolateral prefrontal cortex [DLPFC]), motor planning regions (e.g., supplementary motor area), limbic regions involved in memory and affect (e.g., hippocampus and thalamus) and visual processing regions (e.g., cuneus and precuneus). These results are complemented by an investigation that found that prefrontal cortex (DLPFC) activation in smokers was associated with increased reward anticipation, poorer learning from errors and decreased attention control.[9] However, while the elucidation of aversive drug cue and punishment processing in smokers is still in its early stages, reactivity towards appetitive drug cues (cigarettes) has already been well investigated.[10,11]

Previous studies, examining cue reactivity in smokers, suggest that the mesolimbic brain reward system (e.g., midbrain, putamen, pallidum, nucleus accumbens [NAc] and ventral striatum) gradually becomes sensitized to drug-related stimuli and desensitized to nondrug-related alternative rewards.[12,13] Increased activation of these reward-associated areas has been directly linked to subjective craving and relapse risk.[14–16]

Neural correlates of resisting craving for tobacco have been linked to prefrontal cortex areas, associated with higher executive functioning and cognitive reward control.[17,18] Importantly, brain regions involved in executive and cognitive reward control processes (e.g., PFC, anterior cingulate cortex [ACC], and anterior insula) possess a rich set of connections to cortical and subcortical areas that are key to emotional and reward processing as well as to craving, and this connectivity is assumed to underlay craving regulation processes.[17,19–21] In line with results of aversive drug cue reactivity and the aforementioned processes, Do and Galván[22] showed negative functional connectivity patterns between prefrontal (DLPFC) and limbic (bilateral amygdala) brain regions in smokers while viewing graphic health warning labels, which was interpreted as improved regulatory control over emotionally responsive brain regions.

However, it is still unclear how aversive drug cues influence the subsequent processing of appetitive drug cues and the related craving. Based on the described prior findings on neurofunctional underpinnings of appetitive and aversive drug cue reactivity as well as craving and its control, an aversive cue model of tobacco use disorder (TUD) can be proposed: aversive drug-related cues change the processing of rewarding drug stimuli by (1) decreasing activation of reward areas (e.g., ventral striatum and putamen), (2) increasing activation of control and self-regulation areas (PFC, ACC and anterior insula) and (3) increasing the down-regulation of reward areas by control areas (Figure 1). In the current study, we aimed to test specific
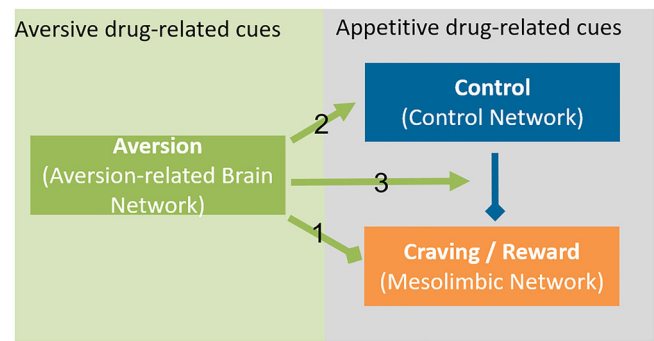

FIGURE 1 Aversive cue model of tobacco use disorder. The model highlights the effects of aversive drug-related cues on the processing of appetitive drug cues in tobacco use disorder subjects (pointed arrows indicate activation; blunt arrows indicate inhibition). It proposes three pathways through which aversive drug-related cues impact on appetitive drug cue reactivity: drug-related aversive cues (1) directly reduce craving and decrease reactivity of the mesolimbic reward network towards appetitive drug cues, (2) increase activation in control areas during the processing of appetitive drug cues and (3) increase down-regulation of reward areas by control areas

hypotheses derived from the aversive cue model of TUD presented in Figure 1, in quitting-motivated TUD subjects.

On the basis of the above framework, we hypothesized a reduction of cigarette-cue-induced craving in TUD subjects by prior presentation of aversive drug cues reflected in subjective craving ratings. On a neurofunctional level, we expected reduced activation of the mesolimbic brain circuit (e.g., ventral striatum and putamen) during the processing of appetitive drug cues after presentation of aversive drug cues. On the other hand, we hypothesized greater activations in craving-regulating control areas (e.g., PFC, ACC and anterior insula). Finally, examining functional connectivity patterns, using significantly activated brain regions identified in the group-level analysis as seed regions, we hypothesized negative functional connectivity between control and reward areas after presentation of aversive drug cues.

2 | MATERIALS AND METHODS

2.1 | Participants

The study was part of the German Collaborative Research Center (TRR 265: losing and regaining control over drug intake), a consortium comprising three German universities funded by the German research foundation (DFG).[23] Here, we present data from one of the main projects of the consortium from Berlin, focusing on understanding specific neural underpinnings underlying human TUD. Whereas previous analyses of the project focused on drug versus alternative reward cue reactivity, the present study investigated the impact of aversive drug stimuli on drug cue reactivity in TUD subjects. Thirty-nine TUD subjects (21 female) were included in the study. One participant had to be excluded due to technical issues with the

autoalignment process during fMRI acquisition, resulting in 38 analysed datasets. Participants were recruited in Berlin through (online and subway) advertising and flyers. Inclusion criteria were current DSM-5 diagnosis of TUD using a structured clinical interview for DSM-5[24] and an age range between 18 and 65 years. Exclusion criteria were comorbid DSM-5 mental disorders within the last 12 months, a lifetime history of any substance use disorder other than TUD, bipolar disorder or psychotic disorder according to DSM-5, current suicidal intent, concurrent psychopharmacological treatment, or psychotherapeutic/psychiatric treatment, a history of brain injury and pregnancy. Additionally, MRI-related exclusion criteria (e.g., ferromagnetic mental implants) were applied. Participants received financial compensation (50 euros) and a 6-week smoking cessation intervention, as all of them were motivated to quit. Additionally, half of the participants were randomized to a sport intervention, of which they were informed before the assessment. The study was approved by the local ethics committee, and all subjects gave written informed consent before participating in the study.

2.2 | Clinical assessments

The Fagerstroem Test for Nicotine Dependence (FTND; range 0–10)[25] was used to measure severity of nicotine dependence. To assess participants' global level of intelligence, subjects completed a 35-item multiple choice vocabulary test (MWT; range 0–37).[26] Furthermore, the Alcohol Use Disorder Identification Test (AUDID; range 0–40)[27] was applied to measure everyday alcohol intake and drinking behaviours. Additionally, participants answered five questions assessing their therapy expectancies, evaluating their motivation to take part in the programme and their assessment of its success (range 0–50), and formulated three individualized goal attainments, using the goal attainment scaling.[28]

2.3 | fMRI paradigm

A novel fMRI paradigm was established to compare self-reported craving ratings and brain responses to cigarette cues preceded by aversive drug cues with those preceded by other cues (neutral cues or alternative rewarding cues). TUD subjects were instructed to refrain from smoking and eating for 3 h prior to the session. Conventional photographs displaying smoking-related items were used as appetitive drug cues, pictures of attractive food were used as alternative reward cues, pictures showing long-term consequences of smoking (e.g., smokers' leg and lung cancer) were used as aversive drug cues and pictures displaying neutrally valenced items were presented during the neutral control condition. Before the assessment, 140 pictures of each category (appetitive drug cues, alternative reward and aversive drug cues) were rated, with the questions 'how strong is your desire to consume this now?' (appetitive drug cues and alternative reward) and 'how deterrent do you experience this picture?' (aversive drug cues), by each participant

using an 8-point Likert scale. The 50% most rewarding/threatening stimuli were automatically selected for the experiment, so that each of the four categories was composed of 70 pictures. In this investigation, we are focusing on the appetitive drug-related and aversive drug-related condition only. Stimuli were presented in the scanner using back-projection. Four pictures of one category were presented per block. Each block lasted 16 s and ended with the presentation of a fixation cross (intertrial interval [ITI]), jittered around 2.5 s. In one run, two blocks of each of the four categories were presented. Within each run, the two blocks with appetitive drug-related cues were preceded once by the aversive drug-related condition and once by one of the other two conditions (either alternative reward or neutral condition). Subjects were instructed to attend to all stimuli and were once per run asked to rate their current desire to consume a cigarette after presentation of the appetitive drug condition and to rate their desire to consume the food after presentation of the alternative reward condition by pressing one of eight buttons covering an 8-point scale ranging from *not at all* to *very strongly*. At the end of each run, participants were additionally asked to rate how strongly they desire to smoke a cigarette, using the same rating scale. In total, the task consisted of nine runs, which altogether lasted maximal 38 min (for an example run, see Figure 2).

2.4 | Statistical analysis of behavioural data

Statistical analysis of behavioural data was performed using IBM SPSS statistics 27.0. To quantify the impact of aversive drug cues on subjective desire for cigarettes, we calculated the difference between craving ratings when appetitive drug cues were preceded by aversive drug cues in comparison with other categories (alternative rewards or neutral cues) using a paired-samples *t*-test. In the following, we will refer to this difference as craving reduction induced by aversive drug cues.

2.5 | fMRI data acquisition and analysis pathway

Scanning was carried out on a 3T MRI scanner (Siemens Magnetom Prisma) using a 64-channel head coil. Functional images were acquired using a Siemens simultaneous multislice T2*-weighted gradient-echoplanar imaging (EPI) sequence (TR = 869 ms, TE = 38 ms, 60 slices, slice thickness = 2.4 mm, voxel size 2.4 × 2.4 × 2.4 mm, no interslice gap, field of view [FoV] = 210 mm, matrix size 88 × 88, acquisition orientation T > C, interleaved slice order, acceleration factor slice = 6, flip angle = 58°, bandwidth = 1832 Hz/Px, prescan normalize, weak raw data filter, fat sat). Field map images were obtained using a Siemens dual gradient-echo sequence (TR = 698 ms, TE1 = 5.19 ms, TE2 = 7.65 ms, 64 slices, slice thickness = 2.4 mm, no slice gap, voxel size 2.4 × 2.4 × 2.4 mm, FoV = 210 mm, matrix size 88 × 88, acquisition orientation T > C, interleaved slice order, flip angle = 54°, bandwidth = 279 Hz/Px). High-resolution anatomical images were acquired using a T1-weighted MPRAGE sequence
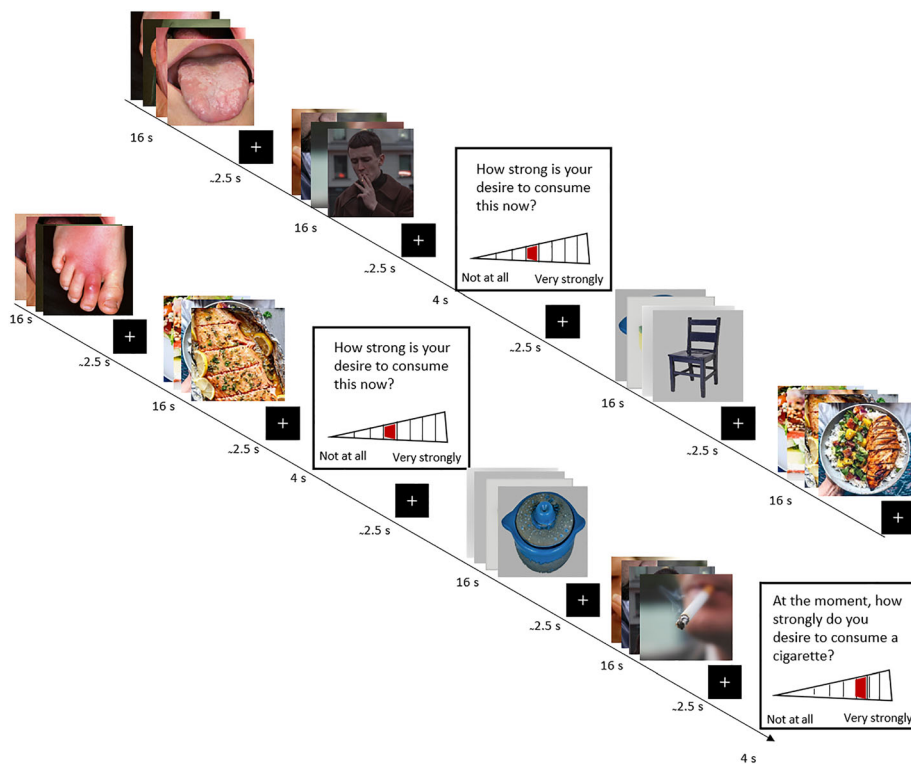
FIGURE 2 Illustration of an example run of the extended cue-reactivity task. Each condition was presented twice per run (aversive drug related, appetitive drug related, neutral and alternative reward). The appetitive drug-related category was preceded once per run by the aversive drug-related condition (see above) and once by either the neutral or alternative reward condition. Once per run, participants were asked how strong they desire to consume this now (referring to the appetitive drug-related condition). At the end of each run, they were asked how strongly they desire to consume a cigarette now. The task consisted of nine runs with a maximum duration of 38 min

(TR = 2000 ms, TE = 2.01 ms, TI = 880 ms, FoV = 256 mm, 208 sagittal slices, voxel size 1 × 1 × 1 mm, flip angle = 8°, GRAPPA factor 2 [PE], 24 ref. lines, prescan normalize, 23.1% slice oversampling, bandwidth = 240 Hz/Px). To minimize movement artefacts, participants' heads were positioned on a pillow and fixated using foam pads surrounding the head. Image preprocessing was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12), implemented in MATLAB R2020a (MathWorks, Sherborn, Massachusetts) and comprised slice timing with reference to the middle slice, SPM12 standard realignment and unwarping including correction for field deformations based on a previously acquired field map, co-registration, normalization to MNI stereotactic space using unified segmentation based on the SPM tissue probability map for six tissue classes, and spatial smoothing with 8-mm full-width at half-maximum isotropic Gaussian kernel (similar to previous studies in our field[29,30]). Following preprocessing, all nine runs were visually inspected, for each subject separately, for a visual quality control.

On the subject level, brain activation differences related to presentation of the different stimuli were analysed using the general linear model (GLM) in SPM12. The blood oxygen level-dependent response was modelled by a canonical haemodynamic response function (HRF) for each of seven conditions: neutral cues, alternative reward cues, aversive drug related cues, nicotine cues preceded by aversive drug-related cues (Nico[+]), nicotine cues preceded by other cues (Nico[−]), button presses and ratings, resulting in three regressors of interest (Nico[+], Nico[−] and neutral) for the current analysis. Model parameter estimates and the resulting t-statistic images (condition against baseline) were submitted to the group-level analysis.

Within-group differences were assessed using paired-samples t-tests on the second level. To test the effects of presentation of aversive drug-related cues on appetitive cue reactivity, we analysed the contrast Nico[+] > Nico[−] and vice versa. Whole-brain analyses as well as an anatomical region of interest (ROI) analysis of a priori defined brain areas were conducted. To investigate the mesolimbic brain reward system, same ROIs as described in previous investigations were included (e.g., Lin et al.[11]): the ventral striatum (NAc), thalamus, pallidum, caudate and midbrain (including ventral tegmental area [VTA]). Furthermore, brain areas responsible for executive and cognitive reward control processes were selected for a second ROI of control areas, including the middle frontal gyrus (DLPFC), orbitofrontal gyrus (ventromedial prefrontal cortex [VMPFC]), superior medial frontal gyrus (dorsomedial prefrontal cortex [DMPFC]), ACC and insula (e.g., Brandl et al.[17] and Morawetz et al.[31]). Combining the definitions from the Automated Anatomical Labeling Atlas,[32] which is implemented in the toolbox 'Wake Forest University PickAtlas',[33] in SPM12, the bilateral ROIs were investigated using one mask. To further specify the regions, we report the corresponding Brodmann areas. Small volume correction was applied using this ROI mask and a family-wise error (FWE) corrected threshold of $p_{fwe} < 0.05$ with a minimum cluster size of $k = 10$ continuous voxels. ROI analyses were followed by whole-brain analyses, thresholded at $p < 0.001$ uncorrected. Furthermore, as sensitivity analysis and to ensure that the experimental manipulation worked, we investigated an activation of the selected ROIs of the reward system in response to appetitive drug cues not preceded by aversive drug cues in comparison with the neutral control condition (Nico[−] > neutral).

2.6 | Correlation analysis

To test associations between behavioural and neurofunctional effects of aversive drug cues, we computed the Pearson correlations between beta values at significant peaks of the ROIs activated in the group-level analysis and craving reduction induced by aversive drug cues (craving rating after Nico$^-$ minus craving rating after Nico$^+$), implicating that higher values reflect an increased influence of aversive drug cues on subjective cravings. Beta values of the significant ROIs were extracted using the toolbox marsbar[34] with a 5-mm sphere around the peak voxel.

2.7 | Generalized psychophysiological interaction analysis (gPPI analysis)

The Functional Connectivity Toolbox (CONN toolbox v18.4)[35] for Matlab and SPM12 was used to perform functional connectivity analyses using the implemented gPPI procedure. This analysis was conducted post hoc to explore the connectivity profile of seed regions identified in the former group-level analysis (bilateral anterior insulae and bilateral dorsal anterior cingulate cortex [dACC] and left DLPFC). A gPPI analysis allows the description of connectivity alterations between brain regions due to an experimental context. The selected seed regions were created as 5-mm spheres

TABLE 1 Sociodemographic and psychometric characteristics of the TUD sample

Sample characteristic	TUD subjects N = 38
Age (M [SD])	35.18 (10.57)
Female gender (n [%])	21 (55.26)
Right-handedness (n [%])	38 (100)
Level of education	
A level[a] (n [%])	30 (78.95)
Monthly income in € (n [%])	
<1000	7 (18.42)
1000–2000	12 (31.58)
2000–3500	16 (42.11)
3500–4500	2 (5.26)
>4500	1 (2.63)
MWT (M [SD])	28.32 (4.67)
TE (M [SD])	40.92 (6.91)
FTND (M [SD])	4.03 (2.27)
Pack years (M [SD])	10.75 (9.55)
Cigarettes/day (M [SD])	14.40 (6.05)
AUDID	5.92 (4.69)

Note: Missing values = monthly income: 1.
Abbreviations: AUDID, Alcohol Use Disorder Identification Test; FTND, Fagerstroem Test of Nicotine Dependence; MWT, Mehrfachwahl-Wortschatz Test; TE, therapy expectancies; TUD, tobacco use disorder.
[a]Abitur.

around the peak voxel identified in our group-level analysis in the contrast Nico$^+$ > Nico$^-$ (for MNI coordinates, see Table 2). We used a seed-to-voxel approach to conduct gPPI analyses on the Nico$^+$ > Nico$^-$ condition. In the first-level analysis, the BOLD time course of all seeds was extracted from each participant and condition, and then, a seed-to-voxel beta map was calculated including the interaction between the seed regions BOLD time series and the Nico$^+$ > Nico$^-$ contrast condition. Afterwards, the seed regions and beta images were entered into a regression model at the second level. Our goal was to investigate possible stronger negative relationships between control and reward areas. Therefore, we used a one-sided FWE corrected $p < 0.05$ at the cluster level and an uncorrected $p < 0.001$ at the voxel level (as implemented in the CONN toolbox software) to guard against false-positive findings. Cerebrospinal fluid, white matter and six rigid-body parameters were regressed out of the whole-brain grey matter activity.

3 | RESULTS

3.1 | Sample characteristics and subjective craving ratings

Demographic and smoking characteristics of the TUD sample are shown in Table 1. Craving ratings within the task were significantly lower in the Nico$^+$ condition compared with the Nico$^-$ condition, $t(37) = -4.03$, $p < 0.001$, $d = 0.922$ (see also Table 1 and Figure 3). Participants showed a high motivation to quit smoking and expected the therapy to be helpful to reach this goal (see also Table 1 and Text S1).

3.2 | fMRI results

Contrasting the Nico$^+$ > Nico$^-$ condition, we found greater activations in the bilateral anterior insulae and dACC and in the left DLPFC in the ROI analysis. No significant activations in the VMPFC and DMPFC were observed. On the whole-brain level, significant activations were found in the left middle frontal gyrus (DLPFC), insula, superior frontal gyrus (pre-supplementary motor area, SMA), precentral gyrus, SMA, angular gyrus and caudate as well as in the right calcarine sulcus, cerebellum, SMA, angular gyrus, middle occipital gyrus, middle fontal gyrus (DLPFC), ACC and thalamus (Table 2 and Figure 3). The contrast Nico$^-$ > Nico$^+$ revealed no significant results, neither in the ROI analysis nor in the whole-brain approach.

3.3 | Correlation results

We found a positive correlation between craving reduction induced by aversive drug cues (Figure 3B) and right dACC activation
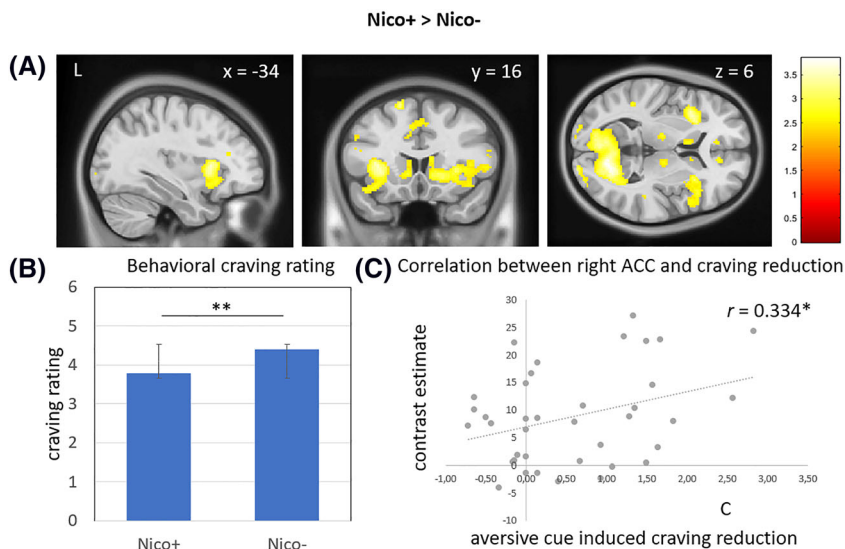
Nico+ > Nico-

FIGURE 3 (A) Significantly activated brain regions during the processing of appetitive drug cues, preceded by aversive drug cues in the whole-brain analysis ($p < 0.001$ unc.). (B) Behavioural craving ratings after Nico+ and Nico−. (C) Positive correlation between significantly activated right anterior cingulate cortex (ACC) in the contrast Nico+ > Nico− and behavioural craving reduction induced by aversive cues. $*p < 0.05$, $**p < 0.001$

($r = 0.386$, $p = 0.040$) and a trend for the left DLPFC ($r = 0.334$, $p = 0.056$) for the contrast Nico+ > Nico− (Figure 3C). No other correlations reached significance (left dACC and bilateral insulae).

3.4 | Sensitivity results

To ensure that the experimental manipulation worked, we investigated the contrast Nico− > neutral as sensitivity analysis. We could show an activation of the brain reward system to appetitive drug cues not preceded by aversive drug cues in the ROI analysis (left ventral striatum [NAc] and caudate as well as bilateral pallidum, midbrain and thalamus) and that these activations could be positively associated with behavioural craving ratings (see Tables S1 and S2).

3.5 | gPPI results

To exploratively examine functional connectivity patterns of significantly activated brain regions identified in the group-level analysis (left DLPFC as well as bilateral dACC and bilateral anterior insulae), a gPPI analysis was conducted for the contrast Nico+ > Nico−. We found a stronger negative functional connectivity between the left DLPFC and the right supramarginal gyrus, fusiform gyrus, superior occipital gyrus and the left cerebellum. The right anterior insula showed a significant negative functional connectivity to the right nucleus caudatus and the left anterior insula to the right superior occipital gyrus. The right dACC showed a significant negative functional connectivity to the left putamen and the left dACC to the right brain stem (see Table 3). These stronger inverse couplings point towards an aversive cue-induced down-regulation process on mesolimbic brain reward areas (putamen and caudate) by prefrontal and paralimbic control areas (dACC and anterior insula) in TUD subjects.

4 | DISCUSSION

The present study proposed an aversive cue model of TUD (Figure 1), describing different pathways through which drug-related aversive cues impact on the processing of appetitive drug cues, based on the literature. According to this aversive cue model of TUD, aversive drug-related cues modulate subsequent drug cue reactivity by (1) reducing subjective craving and neural responsivity in mesolimbic reward areas, (2) enhancing activation in prefrontal control areas and (3) increasing prefrontal top-down-regulation of mesolimbic reward areas. To test our hypotheses, derived from this model in quitting motivated TUD subjects, we employed a novel extended cue-reactivity paradigm, where aversive drug cues (displaying negative consequences of tobacco consumption) preceded the presentation of appetitive drug cues. When appetitive drug cues were preceded by aversive drug cues, we found (1) reduced cigarette craving, but not reduced reactivity in mesolimbic reward areas towards appetitive drug cues; (2) enhanced activation of prefrontal and paralimbic control areas (DLPFC, dACC and anterior insulae), with a positive association between aversion-related reduction of craving and prefrontal activation; and (3) down-regulation of mesolimbic reward areas (putamen and caudate) by prefrontal and paralimbic control areas (dACC and anterior insula). Overall, these findings support our hypotheses referring to all three pathways proposed by the model.

4.1 | Impact on craving and mesolimbic reward areas

The craving reduction induced by aversive drug cues is in accordance with our hypothesis (Pathway 1 in Figure 1), consistent with findings from a previous investigation that used graphic health warning labels with different emotional contents[5] and was expected a priori. However, contrary to our hypothesis, we found no corresponding activation reduction of reward-associated brain areas (e.g., NAc and

TABLE 2 Significant activated brain regions during the processing of appetitive drug cues preceded by aversive drug cues (Nico$^+$) or other cues (Nico$^-$)

Contrast/region	Side	Voxels	x	y	z	t	BA	p < 0.001
Nico$^+$ > Nico$^-$								
Region of interest analysis								
Insula	L	460	−34	16	6	4.92	13	0.013*
Insula	R	241	32	16	4	4.02	13	0.049*
ACC	L	59	−10	42	10	4.14	32	0.026*
ACC	R	92	8	34	22	4.32	32	0.017*
DLPFC	L	59	−40	36	18	4.77	46	0.030*
Whole-brain analysis								
Calcarine sulcus	R	3503	14	−70	8	5.44	17	<0.001
Insula	R	938	31	18	4	4.65	13	<0.001
Cerebellum	R	678	6	−44	−16	4.80	-	<0.001
Insula	L	650	−34	16	6	4.92	13	<0.001
Cerebellum	R	311	2	−46	−16	4.59	-	<0.001
Supplementary motor area	R	286	16	4	62	4.56	6	<0.001
Angular gyrus	L	230	−58	−42	26	4.30	39	<0.001
Angular gyrus	R	211	54	−44	22	4.40	39	<0.001
Supplementary motor area	L	191	−18	10	66	4.83	6	<0.001
ACC	R	192	10	34	20	4.32	32	<0.001
Middle occipital gyrus	R	177	20	−100	0	4.68	18	<0.001
Caudate	L	174	−10	16	0	4.03	-	<0.001
Precentral gyrus	L	143	−16	−32	66	4.70	4	<0.001
Middle frontal gyrus (DLPFC)	L	134	−40	36	18	5.36	46	<0.001
Thalamus	R	129	12	−20	14	4.07	-	<0.001
Superior frontal gyrus (pre-SMA)	L	127	−14	14	70	4.74	6	<0.001
Middle frontal gyrus (DLPFC)	R	105	30	42	38	4.45	9	<0.001
Nico$^-$ > Nico$^+$								
Region of interest analysis				No differential activation				
Whole-brain analysis				No differential activation				

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; L, left; Nico$^+$, appetitive drug-related cues preceded by aversive drug-related cues; Nico$^-$, appetitive drug-related cues preceded by other cues; R, right; SMA, supplementary motor area; voxels, number of voxels per cluster; x, y, z, MNI coordinates.

*$p < 0.05$ family-wise error (FWE) corrected: For ROI analyses, an FWE corrected threshold of $p_{fwe} < 0.05$ with $k > 10$ voxels on the peak level was applied. For whole-brain analyses, an uncorrected threshold of $p < 0.001$ was applied.

TABLE 3 Results of the seed-based generalized psychophysiological interaction analysis for the contrast Nico$^+$ > Nico$^-$

Seed	Region	Side	Voxels	x	y	z	t	p < 0.05 FWE*
DLPFC (L)	Supramarginal gyrus	R	227	56	−30	56	−4.52	0.023
	Cerebellum	L	137	−26	−82	−22	−4.58	0.031
	Fusiform gyrus	R	87	22	−82	−12	−4.16	0.049
	Superior occipital gyrus	R	82	16	−66	64	−4.10	0.010
Insula (R)	Caudate	R	76	10	6	16	−5.17	<0.001
Insula (L)	Superior occipital gyrus	R	57	16	−66	64	−4.11	0.045
ACC (R)	Putamen	L	66	−26	0	−8	−4.99	0.027
ACC (L)	Brainstem	R	30	22	−28	−32	−4.80	0.041

Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; FWE, family-wise error; L, left; R, right.

*One-sided, multiple testing correction of $p_{fwe\ (cluster\ level)} < 0.05$.

caudate). This result suggests that activation of the brain reward system through appetitive drug cues is not directly weakened by previous presentation of the negative consequences of smoking, at least not in quitting motivated TUD subjects. Former investigations[36,37] found that a quit interest of smokers modulates smoking cue-reactivity responses in brain reward areas. Thus, it can be assumed that an effect in the mesolimbic reward circuit is more difficult to detect in quitting motivated smokers and may require a lager sample of TUD subjects.

4.2 | Impact on prefrontal control areas

Our finding of increased activation of prefrontal control areas related to aversive drug cues complements previous studies on drug cue reactivity, which found appetitive drug cues to activate prefrontal control areas in addition to mesolimbic reward areas.[18] The present findings also add to studies that have linked cognitive control of craving for hedonic stimuli with activations in lateral fronto-parietal cortices (DLPFC and anterior insulae).[17] In accordance with Pathway 2 of the aversive cue model of TUD (Figure 1), we here demonstrate that aversive drug cues enhance the activity of prefrontal control areas during subsequent processing of appetitive drug cues in quitting motivated TUD subjects. The areas identified here (DLPFC, dACC and anterior insulae) are known to play a role in executive functions and different strategies and goals of emotion regulation and cognitive reward control.[17,31,38–40]

Previous studies found the dACC to be involved in cognitive reappraisal and cognitive modulation of emotion as well as in quitting motivated smokers when instructed to actively suppress their urge for cigarettes.[41,42] These results suggest that dACC activation represents an important substrate of inhibition of cue-induced craving in smokers. On the other hand, the DLPFC was found to be involved in different aspects of (cognitive) emotion regulation[31] such as the down-regulation of different kinds of appetitive desires.[43,44] Furthermore, Kober et al.[21] showed DLPFC activation during cognitive down-regulation of craving for cigarettes in smokers when explicitly applying cognitive strategies to regulate craving. These findings suggest that the DLPFC is involved in deliberate regulation of automatic responses to various kinds of affective cues, including drug cues. In terms of emotion regulation, the anterior part of the insula has been suggested to control activity in other brain regions, to initiate and adjust cognitive control mechanisms.[31,45]

Summarizing the above and integrating our own results, two ways of activating the control network can be distinguished. First, previous studies found that the usage of explicit instructions to exert deliberate control over different kinds of stimuli (positive, negative emotions or drug cues) activates the prefrontal control network. Second, we could demonstrate an indirect, implicit activation of prefrontal control areas through aversive drug-related cues, without the instruction to actively apply any strategies, suggesting a rather subsidiary increase of the control network. This second way, which is consistent with our aversive cue model of TUD, may be relevant for smoking prevention

programmes and cessation therapy. Based on the knowledge that explicitly targeting the control system in smokers through cognitive interventions (e.g., cognitive behavioural therapy) is limited in its success,[40] alternative strategies are clearly needed. Our results suggest an indirect and automatic activation of control processes through the presentation of (unknown) aversive drug cues, preceding appetitive drug cues. Such strategies could complement explicit cognitive approaches through different forms of application. As a novel part of smoking cessation therapy, (unknown) aversive drug cues could be paired with (individualized) appetitive drug cues of quitting motivated smokers in a conditioning paradigm, maybe inducing decreased craving for these favourite drug cues through enhanced cognitive control. Furthermore, it could be beneficial to make aversive drug cues more visible in different places where smokers are used to consume cigarettes (e.g., smoking areas in public places). By applying such strategies, prevention efforts and cessation success may be enhanced, at least in quitting motivated TUD subjects.

4.3 | Impact on top-down control processes

Confirming our hypothesis, we found stronger negative functional connectivity of activated prefrontal control areas to parts of the mesolimbic reward system. Together with the observation of a positive association between right dACC activation and craving reduction induced by aversive drug cues, these findings suggest that aversive drug cues may induce down-regulation processes,[21,22] as proposed by our model (Pathway 3 in Figure 1). A reduction of the overall motivational appeal of smoking may be achieved through balancing the value of cigarettes with the value of the anticipated reward. The value of anticipated reward in mesolimbic brain regions may be down-regulated by prefrontal/paralimbic control areas when aversive drug cues were presented before. However, we only found increased down-regulation of reward areas to appetitive drug cues immediately preceded by aversive drug cues, which might suggest a short-lasting effect of aversive drug cues. This underlines that prevention strategies or cessation interventions, which use aversive drug cues, may benefit from the immediate and contingent presence of aversive drug cues during drug consumption (e.g., on cigarette packets or in novel conditioning paradigms).

4.4 | Impact on extended visual system and (pre-)SMA

In addition to prefrontal control areas, we found activation of the extended visual system (e.g., calcarine sulcus and occipital gyrus) as well as in the SMA and pre-SMA in our whole-brain analysis. While the SMA and pre-SMA have been associated with cognitive reward control across a wide range of rewarding stimuli,[17] the extended visual system has consistently been more responsive to smoking cues than neutral cues in previous investigations (e.g., Engelmann et al.[10]). Former fMRI studies, comparing emotionally arousing stimuli with

neutral stimuli, have found that emotionally arousing stimuli consistently evoke larger responses than neutral stimuli in these brain regions, a finding that has been interpreted as increased allocation of attentional resources to the processing of the arousing stimuli.[46,47] In our study, stronger activation of the (extended) visual system during appetitive cue reactivity, when aversive drug cues preceded the presentation, may suggest that those cues are processed as emotionally arousing, particularly in quitting motivated TUD subjects.

5 | LIMITATIONS AND CONCLUSION

Our findings should be interpreted within the limitations of this study. Our sample consists of quitting motivated TUD subjects who are medium nicotine dependent according to the FTND scores. Including strong, nonquitting motivated smokers may have changed the results and led to other implications. To specify and extend the effects of this investigation, it would be desirable to study a sample of strong smokers who are not intended to quit smoking. Furthermore, we recruited participants through online or subway advertising, which could possibly have led to a selection bias (e.g., recruiting those who are actually working) and therefore probably limit the external validity of the study.

In conclusion, we assume that cues displaying the negative consequences of smoking have an impact on cigarette cue reactivity and craving in TUD subjects who are motivated to quit. On the basis of previous studies, we proposed an aversive cue model of TUD including three different pathways of the impact of aversive drug-related cues on the processing of appetitive drug-related cues through a reduction of subjective craving and neural responsivity in mesolimbic reward areas, enhanced activation in prefrontal control areas and increased prefrontal top-down-regulation of mesolimbic reward areas. Derived from this model, specific hypotheses were tested. We found a reduction of craving for cigarettes in TUD subjects on a behavioural level. The pattern of brain areas activated when aversive drug cues preceded the presentation of appetite drug cues suggests increased cognitive control (of reward), as well as down-regulation of brain reward areas. Thus, from a neurofunctional perspective, TUD subjects automatically and implicitly applied self-regulation and control strategies. Implications for prevention programmes and smoking cessation interventions include the application of aversive drug cues in different ways (e.g., as conditioning paradigm in cessation interventions). Further research is clearly needed to specify the effect and to investigate the applicability of negative drug-associated stimuli in cessation therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The following authors have approved the final article and have participated in the research. Stefanie L. Kunas, Heiner Stuke, Andreas Ströhle and Felix Bermpohl designed the study. Stefanie L. Kunas collected the data. Stefanie L. Kunas and Heiner Stuke analysed the data. Stefanie L. Kunas, Heiner Stuke, Irene S. Plank, Andreas Ströhle and Felix Bermpohl interpreted the results. Stefanie L. Kunas wrote the first draft. All authors revised the article critically.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Stefanie L. Kunas 🔟 https://orcid.org/0000-0003-3788-229X

REFERENCES

1. Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. *Lancet*. 2003;362(9387):847-852. https://doi.org/10.1016/S0140-6736(03)14338-3
2. Ezzati M, Lopez AD. Regional, disease specific patterns of smoking-attributable mortality in 2000. *Tob Control*. 2004;13(4):388-395.
3. Partos T, Borland R, Yong H, Thrasher J, Hammond D. Cigarette packet warning labels can prevent relapse: findings from the international tobacco control 4-country policy evaluation cohort study. *Tob Control*. 2013;22(E1):43-50. https://doi.org/10.1136/tobaccocontrol-2011-050254
4. Villanti AC, Cantrell J, Pearson JL, Vallone DM, Rath JM. Perceptions and perceived impact of graphic cigarette health warning labels on smoking behavior among US young adults nicotine & tobacco research. *Nicotine Tob Res*. 2014;16(4):469-477. https://doi.org/10.1093/ntr/ntt176
5. Wang AL, Romer D, Elman I, Turetsky BI, Gur RC, Langleben DD. Emotional graphic cigarette warning labels reduce the electrophysiological brain response to smoking cues. *Addict Biol*. 2015;20(2):368-376. https://doi.org/10.1111/adb.12117
6. Dinh-Williams L, Mendrek A, Bourque J, Potvin S. Where there's smoke, there's fire: the brain reactivity of chronic smokers when exposed to the negative value of smoking. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014a;50:66-73. https://doi.org/10.1016/j.pnpbp.2013.12.009
7. Dinh-Williams L, Mendrek A, Dumais A, Bourque J, Potvin S. Executive-affective connectivity in smokers viewing anti-smoking images: an fMRI study. *Psychiatry Res Neuroimaging*. 2014b;224(3):262-268. https://doi.org/10.1016/j.pscychresns.2014.10.018
8. Chua HF, Ho SS, Jasinska AJ, et al. Self-related neural response to tailored smoking-cessation messages predicts quitting. *Nat Neurosci*. 2011;14(4):426-427. https://doi.org/10.1038/nn.2761
9. Duehlmeyer L, Hester R. Impaired learning from punishment of errors in smokers: differences in dorsolateral prefrontal cortex and

sensorimotor cortex blood-oxygen-level dependent responses. *NeuroImage Clin*. 2019;23:101819. https://doi.org/10.1016/j.nicl.2019.101819

10. Engelmann JM, Versace F, Robinson JD, et al. Neural substrates of smoking cue reactivity: a meta-analysis of fMRI studies. *Neuroimage*. 2012;60(1):252-262. https://doi.org/10.1016/j.neuroimage.2011.12.024

11. Lin X, Deng J, Shi L, et al. Neural substrates of smoking and reward cue reactivity in smokers: a meta-analysis of fMRI studies. *Transl Psychiatry*. 2020;10(1):1-9. https://doi.org/10.1038/s41398-020-0775-0

12. Nolen-Hoeksema S. *Abnormal Psychology*. Substance Use and Gambling Disorders. seventhed. New York: McGraw-Hill; 2017:404.

13. Robinson T, Berridge K. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev*. 1993;18(3):247-291. https://doi.org/10.1016/01650173(93)90013-P

14. Owens MM, MacKillop J, Gray JC, et al. Neural correlates of tobacco cue reactivity predict duration to lapse and continuous abstinence in smoking cessation treatment. *Addict Biol*. 2018;23(5):1189-1199. https://doi.org/10.1111/adb.12549

15. Due DL, Huettel SA, Hall WG, Rubin DC. Activation in mesolimbic and visuospatial neural circuits elicited by smoking cues: evidence from functional magnetic resonance imaging. *Am J Psychiatry*. 2002; 159(6):954-960. https://doi.org/10.1176/appi.ajp.159.6.954

16. David SP, Munafò MR, Johansen-Berg H, et al. Ventral striatum/nucleus accumbens activation to smoking-related pictorial cues in smokers and nonsmokers: a functional magnetic resonance imaging study. *Biol Psychiatry*. 2005;58(6):488-494. https://doi.org/10.1016/j.biopsych.2005.04.028

17. Brandl F, Corbi ZLH, Bratec SM, Sorg C. Cognitive reward control recruits medial and lateral frontal cortices, which are also involved in cognitive emotion regulation: a coordinate-based meta-analysis of fMRI studies. *Neuroimage*. 2019;200:659-673. https://doi.org/10.1016/j.neuroimage.2019.07.008

18. Hartwell KJ, Johnson KA, Li X, et al. Neural correlates of craving and resisting craving for tobacco in nicotine dependent smokers. *Addict Biol*. 2011;16(4):654-666. https://doi.org/10.1111/j.1369-1600.2011.00340.x

19. Eippert F, Veit R, Weiskopf N, Erb M, Birbaumer N, Anders S. Regulation of emotional responses elicited by threat-related stimuli. *Hum Brain Mapp*. 2007;28(5):409-423. https://doi.org/10.1002/hbm.20291

20. Wager TD, Davidson ML, Hughes BL, Lindquist MA, Ochsner KN. Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron*. 2008;59(6):1037-1050. https://doi.org/10.1016/j.neuron.2008.09.006

21. Kober H, Mende-Siedlecki P, Kross EF, et al. Prefrontal–striatal pathway underlies cognitive regulation of craving. *Proc Natl Acad Sci*. 2010;107(33):14811-14816. https://doi.org/10.1073/pnas.1007779107

22. Do KT, Galván A. FDA cigarette warning labels lower craving and elicit frontoinsular activation in adolescent smokers. *Scan*. 2015;10 (11):1484-1496.

23. Heinz A, Kiefer F, Smolka MN, et al. Addiction Research Consortium: losing and regaining control over drug intake (ReCoDe)—from trajectories to mechanisms and interventions. *Addict Biol*. 2020;25(2):e12866. https://doi.org/10.1111/adb.12866

24. First MB, Williams JB, Karg RS, Spitzer RL. *User's Guide for the SCID-5-CV Structured Clinical Interview for DSM-5® Disorders: Clinical Version*. Arlington, VA: American Psychiatric Association; 2016.

25. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerström test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*. 1991;86(9):1119-1127. https://doi.org/10.1111/j.1360-0443.1991.tb01879.x

26. Lehrl S, Triebig G, Fischer BANS. Multiple choice vocabulary test MWT as a valid and short test to estimate premorbid intelligence. *Acta Neurol Scand*. 1995;91(5):335-345. https://doi.org/10.1111/j.1600-0404.1995.tb07018.x

27. Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. *The Alcohol Use Disorders Identification Test, Guidelines for Use in Primary Care*. 2nd ed. Geneva, Switzerland: Department of Mental Health and Substance Dependence, World Health Organization; 2001.

28. Kiresuk TJ, Sherman RE. Goal attainment scaling: a general method for evaluating comprehensive community mental health programs. *Community Ment Health J*. 1968;4(6):443-453.

29. Karoly HC, Schacht JP, Meredith LR, et al. Investigating a novel fMRI cannabis cue reactivity task in youth. *Addict Behav*. 2019;89:20-28. https://doi.org/10.1016/j.addbeh.2018.09.015

30. Wiers CE, Stelzel C, Gladwin TE, et al. Effects of cognitive bias modification training on neural alcohol cue reactivity in alcohol dependence. *AJP*. 2015;172(4):335-343. https://doi.org/10.1176/appi.ajp.2014.13111495

31. Morawetz C, Bode S, Derntl B, Heekeren HR. The effect of strategies, goals and stimulus material on the neural mechanisms of emotion regulation: a meta-analysis of fMRI studies. *Neurosci Biobehav Rev*. 2017; 72:111-128. https://doi.org/10.1016/j.neubiorev.2016.11.014

32. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002;15(1):273-289. https://doi.org/10.1006/nimg.2001.0978

33. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage*. 2003;19(3):1233-1239. https://doi.org/10.1016/S1053-8119(03)00169-1

34. Brett M, Anton JL, Valabregue R, Poline JB. Region of interest analysis using an SPM toolbox. Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan. Available on CD-ROM in. *Neuroimage*. 16(2).

35. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect*. 2012;2(3):125-141. https://doi.org/10.1089/brain.2012.0073

36. Veilleux JC, Skinner KD, Pollert GA. Quit interest influences smoking cue-reactivity. *Addict Behav*. 2016;63:137-140. https://doi.org/10.1016/j.addbeh.2016.07.017

37. Wilson SJ, Sayette MA, Fiez JA. Quitting-unmotivated and quitting-motivated cigarette smokers exhibit different patterns of cue-elicited brain activation when anticipating an opportunity to smoke. *J Abnorm Psychol*. 2012;121(1):198-211. https://doi.org/10.1037/a0025112

38. Lubman DI, Yücel M, Pantelis C. Addiction, a condition of compulsive behaviour? Neuroimaging and neuropsychological evidence of inhibitory dysregulation. *Addiction*. 2004;99(12):1491-1502. https://doi.org/10.1111/j.1360-0443.2004.00808.x

39. Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci*. 2001;24(1):167-202. https://doi.org/10.1146/annurev.neuro.24.1.167

40. Langner R, Leiberg S, Hoffstaedter F, Eickhoff SB. Towards a human self-regulation system: common and distinct neural signatures of emotional and behavioural control. *Neurosci Biobehav Rev*. 2018;90:400-410. https://doi.org/10.1016/j.neubiorev.2018.04.022

41. Kalisch R, Wiech K, Critchley HD, Dolan RJ. Levels of appraisal: a medial prefrontal role in high-level appraisal of emotional material. *Neuroimage*. 2006;30(4):1458-1466. https://doi.org/10.1016/j.neuroimage.2005.11.011

42. Brody AL, Mandelkern MA, Olmstead RE, et al. Neural substrates of resisting craving during cigarette cue exposure. *Biol Psychiatry*. 2007; 62(6):642-651. https://doi.org/10.1016/j.biopsych.2006.10.026

43. Ochsner KN, Gross JJ. Cognitive emotion regulation: insights from social cognitive and affective neuroscience. *Curr Dir Psychol Sci*. 2008; 17(2):153-158. https://doi.org/10.1111/j.1467-8721.2008.00566.x

44. Kim SH, Hamann S. Neural correlates of positive and negative emotion regulation. *J Cogn Neurosci*. 2007;19(5):776-798. https://doi.org/10.1162/jocn.2007.19.5.776

45. Dosenbach NU, Visscher KM, Palmer ED, et al. A core system for the implementation of task sets. *Neuron*. 2006;50(5):799-812. https://doi.org/10.1016/j.neuron.2006.04.031

46. Bradley MM, Sabatinelli D, Lang PJ, Fitzsimmons JR, King W, Desai P. Activation of the visual cortex in motivated attention. *Behav Neurosci*. 2003;117(2):369-375. https://doi.org/10.1037/0735-7044.117.2.369

47. Sabatinelli D, Bradley MM, Lang PJ, Costa VD, Versace F. Pleasure rather than salience activates human nucleus accumbens and medial prefrontal cortex. *J Neurophysiol*. 2007;98(3):1374-1379. https://doi.org/10.1152/jn.00230.2007

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.