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

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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. 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, to curtail the number of smoking initiators, to augment quitting rates and to raise awareness for health issues related to tobacco consumption. To investigate the neurofunctional mechanisms involved in these beneficial effects, previous functional magnetic resonance imaging (fMRI) studies 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. 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.

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. Increased activation of these reward-associated areas has been directly linked to subjective craving and relapse risk.

Neural correlates of resisting craving for tobacco have been linked to prefrontal cortex areas, associated with higher executive functioning and cognitive reward control. 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. In line with results of aversive drug cue reactivity and the aforementioned processes, Do and Galván 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 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). 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\(^2\) 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)\(^2\) 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).\(^2\) Furthermore, the Alcohol Use Disorder Identification Test (AUDIT; range 0–40)\(^2\) 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.\(^2\)

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 x 2.4 x 2.4 mm, no interslice gap, field of view [FoV] = 210 mm, matrix size 88 x 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 x 2.4 x 2.4 mm, FoV = 210 mm, matrix size 88 x 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.
(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, coregistration, 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 field29,30). 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, appetitive 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 appetitive 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 $p_{\text{FWE}} < 0.05$ 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 Nico⁰ minus craving rating after Nico⁺), indicating 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 marsbar²⁴ 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 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⁻ contrast 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 calcareus sulcus, cerebellum, SMA, angular gyrus, middle occipital gyrus, insula, middle frontal 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
(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 insula), 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 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)     | R    | 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 \( p_{\text{fwe}} < 0.05 \) 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 (L)  | 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 \( p_{\text{fwe}} (\text{cluster level}) < 0.05 \).
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 found that a quit interest of smokers modulates smoking cue-reactivity 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 larger 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. 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). 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.

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. 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, such as the down-regulation of different kinds of appetitive desires. Furthermore, Kober et al. 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.

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, 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, 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, the extended visual system has consistently been more responsive to smoking cues than neutral cues in previous investigations (e.g., Engelmann et al.). 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.\textsuperscript{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.

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### 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.

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