Appendix 1 to Vicario CM, Komeilipoor N, Cesari P, et al. Enhanced cortico-bulbar excitability in chronic smokers during visual exposure to cigarette smoking cues. *J Psychiatry Neurosci* 2014.

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Supplemental methods

Electromyographic and transcranial magnetic stimulation recording

Transcranial magnetic stimulation (TMS) was performed using a 70 mm figure-eight coil connected to a Magstim Super Rapid Transcranial Magnetic Stimulator (The Magstim Company; ISAS laboratory) or to an STM9000 Magnetic Stimulator (ATES Medical Device, Verona laboratory) placed over the left motor cortex. The coil was held tangentially to the scalp with the handle pointing 45° away from the nasion–inion line in a posterolateral direction[2,3] to find the extensor carpi radialis (ECR) representation area. The tongue area was stimulated with the coil handle oriented at 90° directed straight posteriorly. Pilot work has shown that this orientation is most effective in evoking tongue motor-evoked potentials (MEPs) without spread of activity to hand muscles. To identify individual optimal scalp positions (OSPs; i.e., the stimulation position that induces MEPs of maximal amplitude) for each muscle, the coil was moved in steps of 1 cm over the motor cortex, and the OSP was marked on a bathing cap worn by the participants. For identifying the tongue OSP, first, the vertex was identified according to the international 10–20 electrode system.[4] Starting over the left hemisphere, an area approximately 4 cm anterior and 8–10 cm lateral was then mapped.[5] Once the OSP was found, the resting motor threshold (rMT) of both muscles was defined as the lowest intensity of stimulation that produced 5 MEPs out of 10 consecutive magnetic pulses with an amplitude of at least 50 μV. For measuring motor cortex excitability, single pulse TMS at 120% intensity of the individual's rMT was delivered over the marked OSP. Electromyographic recording started 100 ms before the magnetic pulse in order to control for the absence of muscular preactivation in each trial. The MEPs' peak-to-peak amplitudes (in millivolts) were collected and stored in a computer for offline analysis. The ability to maintain relaxation of the examined muscles during the MEP recording was a crucial aspect for the inclusion of each participant in this study. During the establishment of the OSP and rMT, participants were encouraged to look at their electromyographic trace for visual feedback of the muscle relaxation. This was particularly necessary for the corticobulbar stimulation. To record MEP amplitudes from the tongue, electrodes were immersed in a disinfectant solution (Amuchina, sodium hypochlorite 1.1 g/100 mL of purified water) for 5 minutes and rinsed in drinking water. Participants were asked to introduce their tongue within these 2 electrodes, adjust the spring so that it was perfectly fitting with the tongue and remain as relaxed as possible for the duration of the experiment. The electrodes were placed on the right part of the tongue. The ground electrode was placed on the forehead of the participant.

Supplementary results

Raw MEP amplitudes, while looking at the scramble stimulus (i.e. baseline), were examined to evaluate between-group differences not related to the experimental manipulation (i.e. smoking cue). We first compared all groups with respect to the first TMS session (this because the control group was tested only once) in a 3 (groups) × 2 (muscle) analysis of variance (ANOVA). There was no significant main effect of group ($F_{2,26} = 2.38$, $\eta p^2 = 0.155$, $p = 0.11$) or muscle ($F_{1,26} = 1.90$, $\eta p^2 = 0.068$, $p = 0.18$). The group × muscle interaction was also not significant ($F_{2,26} = 1.37$, $\eta p^2 = 0.095$, $p = 0.27$).

A further analysis was performed by comparing both chronic smoker who received nicotine (CSn) and those who received placebo (CSp) under withdrawal vsersus intake conditions. Thus, we performed a 2 (groups) × 2 (muscles) × 2 (condition) ANOVA. No significant differences were detected for the main effects of group ($F_{1,17} = 2.25$, $\eta p^2 = 0.117$, $p = 0.15$), muscle ($F_{1,17} = 0.17$, $\eta p^2 = 0.010$, $p = 0.68$) and condition ($F_{1,17} = 0.04$, $\eta p^2 < 0.002$, $p = 0.84$). Likewise, no significant differences were reported for the group × muscle ($F_{1,17} = 0.69$, $\eta p^2 = 0.039$, $p = 0.42$), muscle × condition ($F_{1,17} = 0.04$, $\eta p^2 = 0.002$, $p = 0.83$) or group × muscle × condition ($F_{1,17} = 3.28$, $\eta p^2 = 0.161$, $p = 0.09$) interactions; however, the group × condition interaction was significant ($F_{1,17} = 4.76$, $\eta p^2 = 0.219$, $p = 0.043$). Post hoc comparisons showed a significant difference comparing MEP amplitudes of participants in the CSn (mean 1.26 ± 0.20) compared with those in the CSp group (mean 0.58 ± 0.21) under the intake condition ($p = 0.036$).

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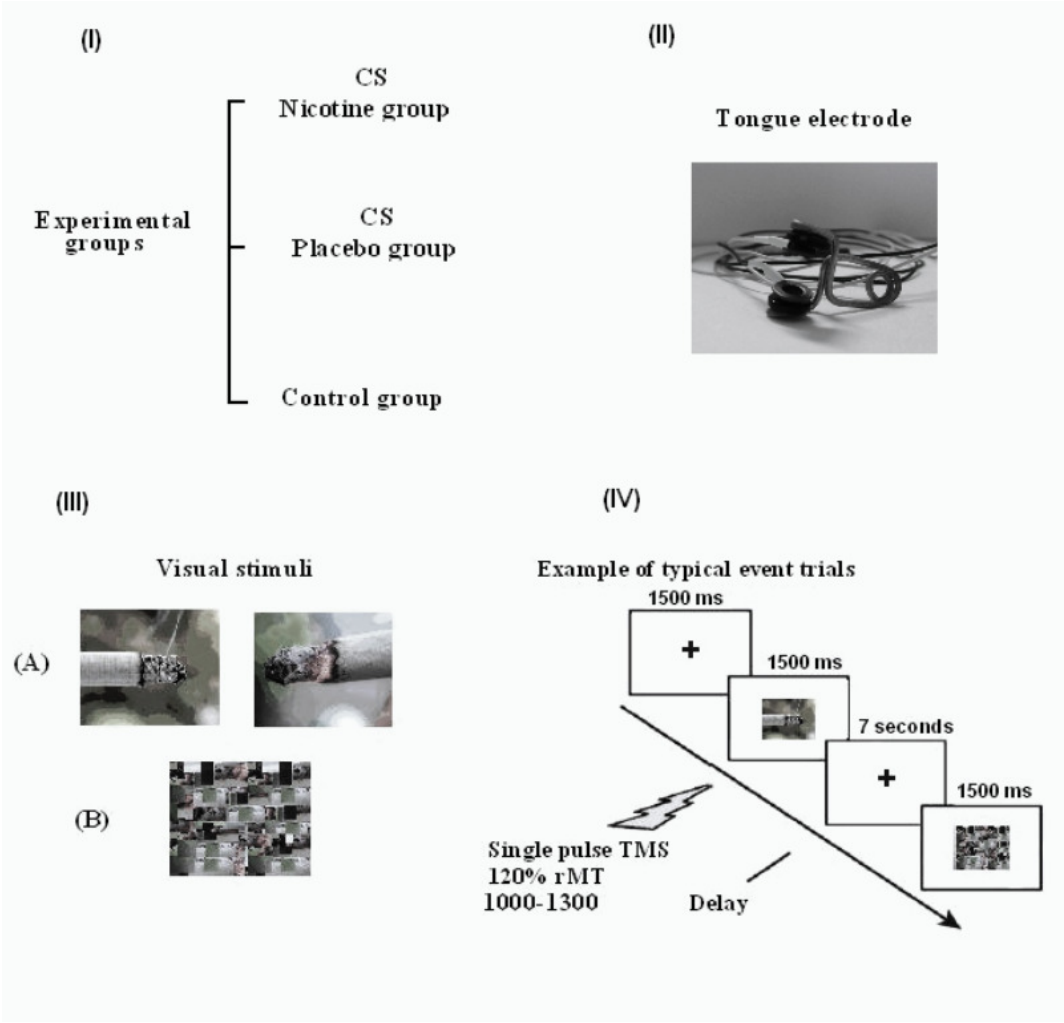

Figure S1: (I) Experimental groups. **(II)** Electrode sample for tongue muscle. **(III)** Visual stimuli: **(A)** Smoking cues and **(B)** Scramble stimulus. **(IV)** Example of typical event trials. CS = chronic smoker; rMT = resting motor threshold; TMS = transcranial magnetic stimulation.

References

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