Research Paper

Enhanced corticobulbar excitability in chronic smokers during visual exposure to cigarette smoking cues

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Background: Neuroimaging studies of chronic smokers report altered activity of several neural regions involved in the processing of rewarding outcomes. Neuroanatomical evidence suggests that these regions are directly connected to the tongue muscle through the corticobulbar pathways. Accordingly, we examined whether corticobulbar excitability might be considered a somatic marker for nicotine craving. **Methods**: We compared motor-evoked potential (MEP) amplitudes recorded from the tongue and the extensor carpi radialis (control muscle) of chronic smokers under drug withdrawal and intake conditions as well as a nonsmoker group. All participants were tested during passive exposure to pictures showing a smoking cue or a meaningless stimulus. In the intake condition, chronic smokers were asked to smoke a real cigarette (CSn: group 1) or a placebo (CSp: group 2). **Results**: Results show that MEP amplitudes recorded from the tongues of participants in the CSn and CSp groups under the withdrawal condition were selectively enhanced during exposure to a smoking cue. However, this effect on tongue MEP amplitudes disappeared in the intake condition for both the CSn and CSp groups. **Limitations:** Limitations include the fact that the study was conducted in 2 different laboratories, the small sample size, the absence of data on chronic smoker craving strength and the different tastes of the real and placebo cigarettes. **Conclusion**: These results suggest that, in chronic smokers, tongue muscle MEP amplitudes are sensitive to neural processes active under the physiological status of nicotine craving. This finding implicates a possible functional link between neural excitability of the corticobulbar pathway and the reward system in chronic smokers.

Introduction

In chronic tobacco smokers, craving can be elicited readily in the laboratory using cigarette-related cues.[1] Several research groups have examined regional brain activation associated with the presentation of these stimuli. The most commonly activated areas during presentation of pictures of cigarettes include the frontal[1,2] and the anterior cingulate cortex (ACC).[1,3] The dorsal portion of the ACC together with the orbitofrontal cortex (OFC) have frequently been linked to the subjective experience of craving, and a number of previous studies have demonstrated a correlation between cue reactivity in the dorsal ACC and tobacco craving.[1,4] Moreover, increased smoking cue–related neural activity was reported in the insula.[5,6] These findings suggest that abnormal activity corresponding to the reward system may interact synergistically in the establishment and consolidation of craving for nicotine.

Beyond these areas implicated in craving, the dorsolateral prefrontal cortex (DLPFC) is thought to exert cognitive control over craving and reward related to smoking.[7] For instance, Amiaz and colleagues[8] have shown that repeated

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high-frequency transcranial magnetic stimulation (rTMS) over the DLPFC reduces cigarette craving and consumption. The therapeutic efficiency of rTMS in modulating craving might be at least partially related to its impact on the mesostriatal and mesolimbic dopaminergic system, regions of the reward system that are known to be disturbed in chronic smokers.[2,9]

This suggestion is supported by the study of Strafella and colleagues[10] showing that rTMS of the prefrontal cortex (PFC) induces the release of endogenous dopamine (DA) in the ipsilateral caudate nucleus. Moreover, a recent functional magnetic resonance imaging (fMRI) study[11] has shown that the DLPFC is the exclusive entry point of information about reward in the mesolimbic network and that anticipated reward availability causes ventral tegmental area activation only via its effect on the DLPFC.

Nicotine-related craving or withdrawal effects might also involve wider cortical areas that are not directly related to craving, such as the motor cortex, and thus might have a more general impact on brain functions. For example, Lang and colleagues[12] reported corticospinal hypoexcitability in chronic smokers. On the other hand, increased corticospinal excitability in chronic smokers during nicotine withdrawal compared to nonsmokers was recently reported in another study.[13]

Here we applied single pulse TMS to compare motor cortex excitability in chronic smokers under withdrawal and nicotine intake conditions. Based on research showing anatomo-functional links between the corticomotor representation of the tongue muscle and several neural structures of the reward system we sought to demonstrate abnormal and specific corticobulbar excitability in chronic smokers that could relate with craving for nicotine.

Among the neural targets of the corticobulbar tract, the hypoglossal motor neurons and the nucleus of the solitary tract (NTS) are of particular relevance. Interestingly, both neural structures are sensitive to nicotine. For example, a recent study[14] has shown that chronic exposure to nicotine, which affects the activity of the reward system[15] during both embryonic development and the first week of life, alters neurotransmission and excitability of hypoglossal motor neurons of rats. Moreover, Simons and colleagues[16] have reported nicotine suppression of gustatory responses of neurons in the NTS of rats. Further evidence in support of this link between the motor tongue area and reward system is provided in a study by Alipour and colleagues,[17] which documented the presence of projections between the corticomotor tongue area and several cortical and subcortical regions involved in the processing of rewarding outcomes, such as the ventrolateral prefrontal cortex (VLPFC), OFC, ACC, agranular and granular insula, ventral putamen, caudate nucleus and amygdala in the Saguinus fuscicollis monkey. By contrast, limb regions of the motor cortex do not project to the OFC or insula in this species. Finally, Chung and colleagues[18] have recently shown that deep brain stimulation of the globus pallidus internus, a neural region firing in response to reward contingencies during task[19] and reinforcement learning,[20] normalized tongue movements in 2 patients affected by tongue protrusion dystonia.

Based on these observations, one might expect corticobulbar excitability to be sensitive to, and modulated by, altered reward representation of several compounds[21] (e.g., food craving in individuals with anorexia nervosa[22]) as well as being sensitive to rewarding/punishing outcomes.[23]

In the present study, we explored corticobulbar and corticospinal excitability of 2 groups of chronic smokers under nicotine withdrawal and intake conditions, with intake involving either nicotine (CSn) or placebo (CSp). An age-matched nonsmoker group underwent a single experimental session, and data were compared with that of both chronic smoker groups.

We predicted selective enhancement of corticobulbar excitability the CSn and CSp groups in the withdrawal condition during visual exposure to smoking cues compared to control stimuli, enhanced corticobulbar excitability in the CSn and CSp groups in the withdrawal compared to the intake condition, and enhanced corticobulbar excitability in the CSn and CSp groups in the withdrawal condition compared to the control group during visual exposure to smoking cues.

Methods

Participants

We recruited chronic smokers from the Universities of Trieste and Verona (Italy); those recruited from the University of Trieste composed the CSn group and those from the University of Verona composed the CSp group. We recruited an age- and sex-matched group of healthy, nonsmoking controls from the University of Trieste, whom we tested in a single session with the same paradigm. All experiments were run by the same researcher; a second researcher and laboratory was involved when testing the CSp group.

General exclusion criteria were cardiac pacemaker; metal implants in the head; age younger than 18 or older than 40 years; current medication; current or previous neurological, psychiatric, or medical conditions; pregnancy or breastfeeding; and current or previous drug (other than nicotine) or alcohol abuse. Chronic smokers had to be continuous and uninterrupted consumers of at least 10 cigarettes per day in the 4 years preceding the study. To induce a mild but clinically important physiological sensation of withdrawal, we asked chronic smokers to smoke their last cigarette 4 hours before the beginning of the individual experimental session. Nonsmokers had to be without a history of continuous smoking and without occasional nicotine consumption in the 4 years preceding the study. The study was conducted according to the Declaration of Helsinki. All participants gave their written informed consent. The study was approved by the ethical committee of the University of Verona, and the process of ethical evaluation performed by the University of Verona was examined and accepted by the International School for Advanced Studies (ISAS) in Trieste.

Electromyographic and TMS recording

We obtained electromyographic (EMG) recordings using a Biopac system MP 150 device (ISAS laboratory) or a Digitimer D360 8-channel and CED Power 1401 device (University of Verona laboratory). The EMG signals were band

filtered (20 Hz–2.5 kHz, sampling rate 10 kHz), digitized and stored for offline analysis. Two different types of electrodes montages were proposed for obtaining motor-evoked potentials (MEPs) from the extensor carpi radialis (ECR) muscle (corticospinal stimulation) and the tongue (corticobulbar stimulation). For the ECR, pairs of Ag-AgCl surface electrodes (1 cm diameter) were placed over the muscle belly (active electrode) and the associated joint or tendon (reference electrode) in a classic belly–tendon montage. The ground electrode was placed over the dorsal part of the elbow. For the tongue, a pair of Ag-AgCl surface electrodes (1 cm diameter) was pasted on plastic buttons and fixed on a spring of iron zinc (see the Appendix, Fig. S1, available at jpn.ca).

Visual stimuli

The experimental visual stimuli consisted of 2 target figures depicting lit cigarettes and a control picture obtained by creating a scramble of the 2 target figures (see the Appendix, Fig. S1). The stimuli subtended a visual angle of about 9.3° and were perceived effortlessly by the participants at a distance of 60 cm from the computer screen. During the experimental blocks, participants were seated comfortably in a dimly lit room at a distance of 80 cm from a computer screen (ISAS laboratory: Samsung, 17 inches, 60 Hz refresh rate; University of Verona laboratory: Asus, 17 inches, 60 Hz refresh rate).

Procedure

Chronic smokers were tested in 2 consecutive sessions lasting approximately 120 min in total. Nonsmoking controls were tested in a single session lasting approximately 80 min. The MEP amplitudes from either the ECR or the tongue muscle were recorded in separate blocks. The muscle stimulation order was counterbalanced across participants. Each session consisted of 1 experimental block of 32 trials (16 smoking cues and 16 scramble configurations) and lasted for 5 minutes. To be sure that participants correctly recognized the displayed visual stimuli, first the stimuli were presented, and the participants were asked to report what they had seen. Therefore, they were instructed to pay attention to the visual stimuli presented on the screen. The inter-trial interval was 7000 ms. During each experimental block, participants were presented with 16 pictures of lit cigarettes (2 figures repeated 8 times) and 16 times with the scrambled pictures. Thus, 32 MEPs per block were obtained (16 MEPs obtained for each muscle–stimulus combination). Smoking cues and control items were presented randomly within each block, which diminishes habituation effects. Each stimulus appeared at the centre of the screen for 1500 ms. During stimulus presentation, a single pulse of TMS was delivered over the participants' muscle in the optimal scalp position at an intensity of 120% of resting motor thresholds (rMT). The magnetic stimulation was delivered randomly between 1000 and 1300 ms from stimulus onset to avoid priming effects that might influence MEP amplitude.[24,25] The TMS frequency during the experimental blocks was less than 0.1 Hz to avoid the possible influence of TMS on motor cortex excitability.[26] For inducing nicotine withdrawal, chronic smokers were required to smoke their last cigarette no later than 4 hours before the beginning of the testing session. During the intake phase, participants were led out of the laboratory to smoke a cigarette (nicotine v. placebo) until they felt satisfaction. The CSn participants were required to smoke a cigarette of their usual brand. The CSp participants were required to smoke a nicotine- and tobacco-free cigarette (NTB herbal & peppermint cigarettes, 0% tobacco, 0% nicotine, 3 mg tar) bought in a pharmacy.

Data analysis

The MEP amplitudes that fell 2.5 standard deviations (SDs) above or below the mean for each experimental condition (1.2% of trials) or single trials contaminated by muscular preactivation (3.6% of trials) were excluded as outliers and precontracted trials. One chronic smoker participant recruited at the University of Trieste, whose mean MEP amplitudes were outliers (MEP amplitudes > 3 SDs from the mean) with respect to the group average was excluded. Raw MEP amplitudes were converted into z scores and analyzed. We decided to standardize raw data to control for the between-group difference in the baseline condition (see the Appendix for details), probably due to some difference in the experimental setting of the 2 laboratories.

We performed a repeated-measures analysis of variance (ANOVA) to compare MEP amplitudes of chronic smokers under the withdrawal condition with those of controls. We entered MEP amplitudes in a $3 \times 2 \times 2$ factorial design ANOVA with group (CSn, CSp, control), muscle (tongue, ECR) and visual stimulus (smoking cue, scramble) as main factors. We performed a second $3 \times 2 \times 2$ repeated-measures ANOVA to compare chronic smokers under the intake condition and controls. Finally, we compared both groups of chronic smoker participants in a $2 \times 2 \times 2 \times 2$ ANOVA with group (CSn, CSp), condition (withdrawal, intake), muscle (tongue, ECR) and visual stimulus (smoking cue, scramble) as main factors. Post hoc comparisons were performed using the Duncan test, and we considered results to be significant at $p < 0.05$.

Results

Twenty university students participated in the study; 11 (5 men and 6 women, mean age 23.3 ± 2.54 yr) in the CSn group and 9 (5 men and 4 women, mean age 22.5 ± 2.18 yr) in the CSp group. There were 10 participants (6 men and 4 women, mean age 23.2 ± 5.18 yr) in the control group. Two participants (1 in the CSn group and 1 in the control group), were left-handed according to the Standard Handedness Inventory,[27] and all participants had normal or corrected-to-normal visual acuity. With the exception of 5 participants (2 in the CSn group, 1 in the control group and 2 in the CSp group), all participants were new to TMS methodology.

We compared rMT of both tongue and ECR muscles between chronic smokers and controls. We detected a main effect for the group factor ($F_{1,27} = 5.85$, $\eta p^2 = 0.178$, $p = 0.022$) with a lower rMT in chronic smokers (mean 43.4 ± 1.347) than controls (mean 49 ± 1.857). The group × muscle interaction factor was also significant ($F_{1,27} = 4.75$, $\eta p^2 = 0.149$, $p = 0.038$). Post

hoc comparisons showed that rMT of the tongue was significantly lower in chronic smokers (mean 41.5 ± 2.172) than controls (mean 52.5 ± 2.994, $p = 0.003$). No difference was observed for the ECR ($p = 0.97$). Finally, the main effect of muscle was not significant ($F_{1,27} = 0.403$, $\eta p^2 = 0.014$, $p = 0.53$).

We also compared raw MEP amplitudes for the scramble configuration stimulus (i.e., baseline), to evaluate between-group differences not related to the experimental manipulation (i.e., smoking cue). Results showed a significant group × condition interaction. In particular, we found wider MEP amplitudes in the CSn group than the CSp group under the intake condition (refer to the Appendix for details).

The MEP amplitude analysis for the CSn and CSp groups under the withdrawal condition and for the control group showed no significant main effect of group ($F_{2,26} = 2.309$, $\eta p^2 = 0.150$, $p = 0.12$) or muscle ($F_{1,26} = 0.556$, $\eta p^2 = 0.020$, $p = 0.46$). No significant differences occurred for the group × muscle ($F_{2,26} = 1.55$, $\eta p^2 = 0.106$, $p = 0.23$) and stimulus × group ($F_{2,26} = 0.984$, $\eta p^2 = 0.070$, $p = 0.39$) interactions. The main effect of stimulus ($F_{1,26} = 4.86$, $\eta p^2 = 0.157$, $p = 0.036$), muscle × stimulus ($F_{1,26} = 9.98$, $\eta p^2 = 0.277$, $p = 0.004$) and muscle × stimulus × group ($F_{2,26} = 3.37$, $\eta p^2 = 0.206$, $p = 0.049$) interactions were significant. Post hoc comparisons showed increased tongue muscle MEP amplitudes in both the CSn and CSp groups under the withdrawal condition during presentation of smoking cues compared to the scramble stimuli (CSn: $p = 0.006$; CSp: $p = 0.005$). This difference was not present in the control group ($p = 0.94$). The between-group analysis showed that tongue MEP amplitudes were significantly enhanced in both the CSn and CSP groups compared to controls during visual exposure to smoking cues (CSn: $p = 0.022$; CSp: $p = 0.013$). No significant differences were detected for the scramble stimulus ($p = 0.08$ and $p = 0.09$; Fig. 1A).

With regard to the ANOVA in which we compared MEP amplitudes between the chronic smokers under the intake condition and controls, no significant main effects of muscle ($F_{1,16} = 0.358$, $\eta p^2 = 0.013$, $p = 0.56$), group ($F_{2,26} = 2{,}21$; $\eta p^2 = 0.145$, $p = 0.13$) and stimulus ($F_{1,26} = 0.210$, $\eta p^2 = 0.008$, $p = 0.65$) were detected. Likewise, the group × muscle ($F_{2,26} = 0.196$, $\eta p^2 = 0.014$, $p = 0.82$), muscle × stimulus ($F_{1,26} = 0.003$, $\eta p^2 < 0.001$, $p = 0.95$), group × stimulus ($F_{2,26} = 0.654$, $\eta p^2 = 0.047$, $p = 0.53$) and group × muscle × stimulus ($F_{2,26} = 0.094$, $\eta p^2 = 0.007$, $p = 0.91$) interactions were not significant (Fig. 1B).

Finally, we compared MEP amplitudes of the CSn and CSp groups under withdrawal and intake conditions. No main effects of group ($F_{1,17} = 0.797$, $\eta p^2 = 0.044$, $p = 0.39$), muscle ($F_{1,17} = 0.001$, $\eta p^2 < 0.001$, $p > 0.99$) and condition ($F_{1,17} = 2.31$, $\eta p^2 = 0.119$, $p = 0.15$) were detected. The main effects of stimulus ($F_{1,17} = 5.151$, $\eta p^2 = 0.232$, $p = 0.037$), muscle × stimulus ($F_{1,17} = 7.291$, $\eta p^2 = 0.300$, $p = 0.015$) and condition × muscle × stimulus ($F_{1,17} = 9.288$, $\eta p^2 = 0.353$, $p = 0.007$) interactions were significant. Post hoc analyses showed that smoking cues enhanced tongue MEP amplitudes compared to scramble stimuli in the withdrawal condition ($p < 0.001$). Moreover, tongue MEP amplitudes recorded during exposure to smoking cues under the withdrawal condition were significantly larger than those recorded under the intake condition ($p = 0.020$). On the other hand, tongue MEP amplitudes recorded during exposure to the scramble stimuli under the withdrawal condition were significantly lower than those recorded under the intake condition ($p = 0.034$; Fig. 1C). No other significant differences were detected.

Discussion

The results of our study show stimulus-related cortical excitability alterations in chronic smokers, specifically in motor neurons projecting to a muscle that has strong connections with the reward system. Corticomotor excitability of chronic smokers has been explored recently in two TMS studies.[12,13] Lang and colleagues[12] investigated chronic smoker excitability with single and paired pulse TMS to the left primary motor cortex as well as short latency afferent inhibition by combining median nerve stimulation and motor cortex TMS. They found that, compared with nonsmoking controls, chronic smokers showed a significantly larger amount of short latency afferent inhibition, which is thought to depend on the activity of cholinergic inhibitory circuits produced by somatosensory inputs. Moreover, the TMS-evoked inhibitory cortical silent period was prolonged, whereas paired pulse intracortical facilitation and MEPs during moderate contraction were reduced. The results of the study by Grundey and colleagues[13] document enhanced short interval afferent inhibition and diminished intracortical facilitation in smokers under a nicotine withdrawal condition.

Our data are consistent with the pattern of increased inhibition reported in these studies with regard to the baseline stimulus (i.e., scramble stimulus). However, several important methodological differences between the present study and these investigations (i.e. the study of corticobulbar system, the use of smoking cues, the use of nicotine or placebo cigarette in contrast to the nicotine patch used in the study of Grundey and colleagues[13]) limit interstudy comparability.

We chose the present experimental design to test the hypothesis that corticobulbar excitability could be a somatic marker sensitive to abnormal activity of the reward system in our clinical population (e.g., chronic smokers), which relates to their addiction to nicotine.

The main result of the present study is that, compared with meaningless scrambled stimuli, visual exposure to smoking cues selectively increased corticobulbar motor excitability in both the CSn and CSp groups under the withdrawal condition. In contrast, no changes of corticobulbar motor excitability were reported in nonsmoking controls. On the other hand, corticobulbar excitability of both the CSn and CSp groups did not differ with regard to the nicotine content of the cigarettes (nicotine or placebo). In these conditions, cortical excitability of chronic smokers was similar to that of the nonsmokers. Critically, no changes of excitability were detected for any group of participants. Finally, rMT of chronic smokers was lower than in controls, and this difference was selective for the tongue muscle.

Change of corticobulbar excitability during visual exposure to smoking cues: Embodied action or embodied craving?

Prior evidence found increased activity of the action observation network (AON) in chronic smokers looking at smoking cue–related stimuli.[28,29] The AON has been considered recently as

closely related to the mirror neuron system.[30] It is assumed that via this system, visual perception of an action elicits an internal simulation of the same action.[28] Hence, the exposure to smoking cues may automatically trigger action simulation processes of motor representations in chronic smokers that are inherently involved in the execution of the smoking action. In line with this suggestion, one could hypothesize that the exposure to smoking cues increases the excitability of the corticobulbar system owing to a simulation of a tongue action (probably the action of "sucking up"), which is mediated by the motor "requirement" properties of this stimulus. This hypothesis is in accordance with studies documenting enhanced MEP amplitudes while looking at objects that are graspable or pinchable.[31,32]

However, our results require an alternative interpretation. First, enhanced corticobulbar motor excitability in the withdrawal condition disappeared immediately after participants

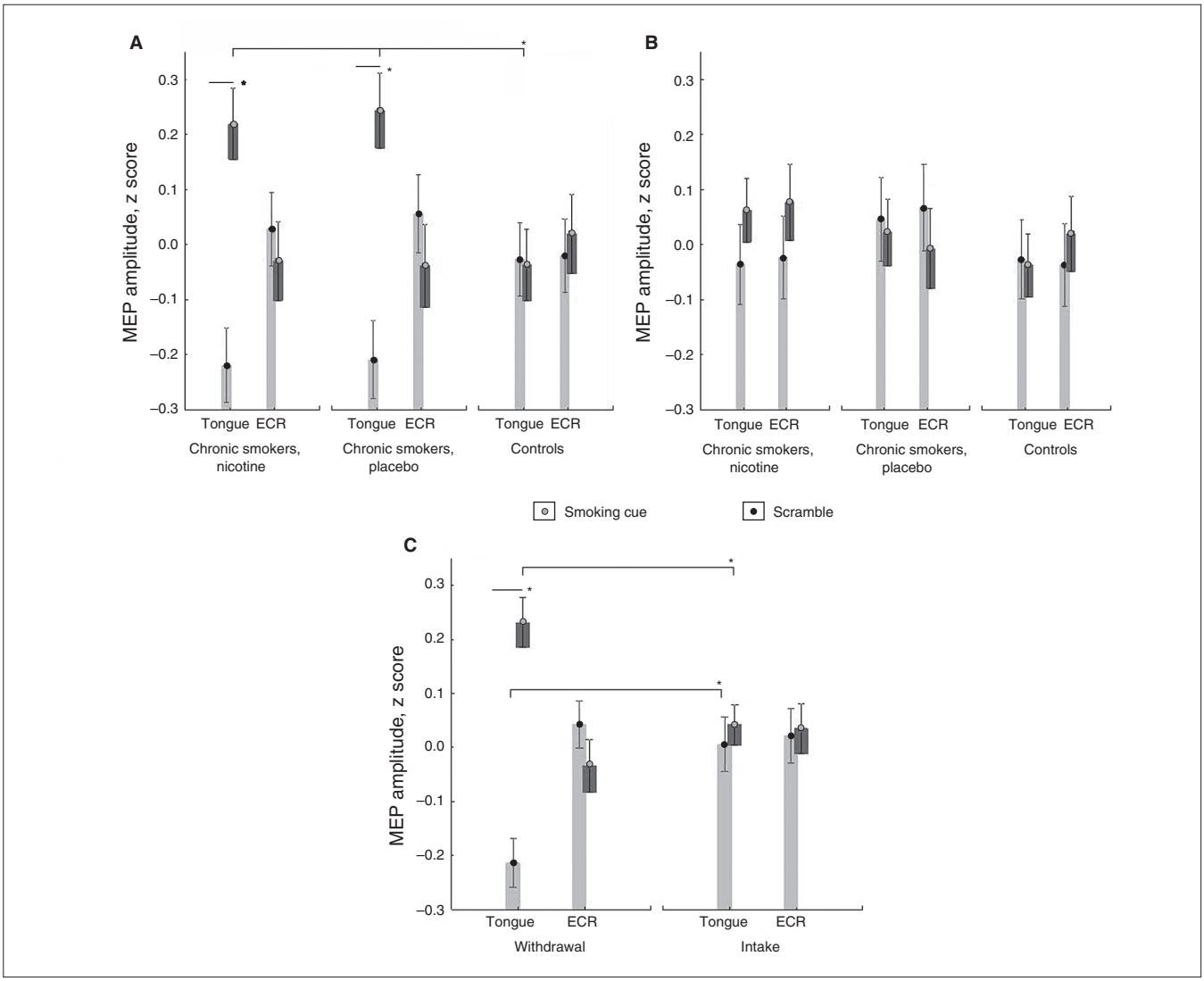

Fig. 1: Motor excitability in chronic smokers and nonsmoking controls. **(A)** Interaction between motor evoked potentials (MEPs) and visual cue factors in the nicotine withdrawal condition. Histograms show that in chronic smokers, but not controls, tongue MEPs were significantly enhanced during the presentation of smoking cues compared to scrambled stimuli. No effect was reported for the extensor carpi radialis (ECR) MEPs in chronic smokers or for both tongue and ECR MEPs in the control group. **(B)** Interaction between muscle and visual cue factors in the nicotine intake condition. Histograms show no difference in the excitability of both tongue and ECR muscles among the groups during the exposure to visual cues (smoking cue v. scrambled stimulus). **(C)** Condition × muscle × stimulus interactions in chronic smokers under withdrawl and intake conditions. Histograms show enhanced tongue muscle excitability under the withdrawal condition during the exposure to smoking cues compared to scrambled stimuli. This difference disappeared after participants smoked a nicotine or placebo cigarette (i.e. intake condition). Moreover, tongue MEPs during exposure to smoking cues were significantly enhanced under the withdrawl condition compared to the intake condition. Conversely, tongue MEPs during the exposure to scrambled stimuli were significantly reduced under the withdrawl condition compared to the intake condition. The ECR MEP amplitudes were identical in both the withdrawal and intake conditions. Raw MEPs were converted to *z* scores. Vertical bars indicate standard error of mean. *$p < 0.05$.

smoked a cigarette (nicotine or placebo). This effect can hardly be explained by the action simulation hypothesis induced by the motor "potential" (i.e. affordance 32) of the smoking cue. In fact, if this were the case, the implied action associated with the cigarette stimulus would not disappear from the motor repertoire of chronic smokers after they smoked a cigarette. Moreover, no alteration of corticobulbar excitability took place in the control group, although all participants recognized the presented stimulus and the related motor action.

Second, ECR excitability was not altered in any of the groups via cue exposure. Arm muscles are directly involved in the execution of a smoking action (i.e., the movement of the hand that leads the cigarette to the mouth); therefore, according to the action simulation hypothesis, we should have found facilitation of the ECR muscle.

Finally, the TMS studies[31,32] that provide evidence for MEP facilitation during exposure to physical properties of objects have shown that this motor enhancement happens in a very early phase (200–300 ms from stimulus onset), whereas longer intervals were reported to have no effect.[32]

Therefore we suggest that the enhanced corticobulbar excitability of chronic smokers might closely reflect the physiological status of craving for nicotine during withdrawal. This hypothesis is supported by studies suggesting a direct link between the tongue muscle representation and the reward system network. As already discussed in the introduction, the hypoglossal nucleus and NTS, 2 key structures of the corticobulbar tract functionally implicated in both motor and sensorial activities related to the mouth region, are sensitive to nicotine.[14,16] Accordingly, one could hypothesize that the enhanced sensitivity to smoking cues under the withdrawal condition, which may reflect physiological craving for nicotine, is mapped onto the corticobulbar system following somatotopic rules that fit with the reward-encoding properties of this anatomical pathway. A study by Wang and colleagues[33] supports this craving embodiment hypothesis of corticobulbar MEP facilitation. In fact, abstinence in smokers increased craving and regional cerebral blood flow in the OFC, a key area of the reward system that directly connects to cortical tongue muscle representations[17] in the Saguinus fuscicollis monkey.

Alternatively, one could explain this pattern of hyperexcitability as a phenomenon of action simulation related to the reward contingency of the smoking cue stimuli owing to the withdrawal condition. This account would explain why the tongue MEP amplitudes were selectively enhanced during the withdrawal condition. These 2 hypotheses could also explain why we found a modulation of MEP amplitudes at an onset time (1100–1300 ms after visual cue exposure) that may be considered too late for the detection of an action simulation induced by the physical properties of a stimulus.[32]

We propose that the enhanced corticobulbar motor excitability can be interpreted as a physiological derivative of craving for cigarette consumption. We speculate that craving positively correlates with corticobulbar excitability because it is increased under high-craving withdrawal conditions, but normalized after cigarette consumption.

An alternative potential explanation for the reduced tongue MEP amplitudes after smoking a nicotine or placebo cigarette is a simple habituation effect of repeating the experiment. However, we believe this possibility is unlikely since a reduction of MEP amplitudes was selectively detected for the tongue but not the ECR muscle, and habituation should have no selective impact on specific muscle representations.

Corticobulbar excitability as a somatic marker to monitor the effect of placebo on the reward system

Beyond the impact of nicotine-containing cigarettes on corticobulbar excitability, the impact of the placebo cigarette on the same system is intriguing. To the best of our knowledge this study is the first showing that corticomotor excitability is sensitive to placebo treatment. Neuroimaging studies have shown an impact of placebo on the reward system network.[34] For example, placebo is able to affect reward system regions, such as the cingulate cortex, the insula and the ventral striatum,[35] which are also activated by nicotine consumption.[15] The tongue MEP normalization following placebo makes it improbable that this pattern is caused by nicotine. It might, however, reflect the satisfaction derived from the habit of smoking and/or the expectation of receiving a drug compound.

Limitations

The main limitation of the present study is the fact that the experiments were conducted in 2 different laboratories, which might represent an important source of variability owing to the presence of 2 researchers and the use of different devices for testing the placebo group. However, the presence of the same investigator in all experimental sessions ensured the maintenance of similar experimental settings for all participants. This includes electrode montage, the procedure for identifying the optimal scalp position for TMS and determining rMT.

Other limitations include the relatively small number of participants and the absence of data on the intensity of cravings in chronic smokers. Information on craving intensity would have made a more detailed identification of the association between craving and cortical excitability possible. Moreover, the placebo cigarettes had a peppermint flavour, while the nicotine cigarettes had no flavour. However, because nicotine and placebo cigarettes had similar effects on excitability, this factor seems to have had no major impact on our results. Finally, the control sample was tested only once, and a single blinded protocol (only participants were blinded) was adopted.

Conclusion

In chronic smokers, corticobulbar excitability might embody a neural marker linking neural processes related to nicotine craving and intake with motor output. Our results suggest that the reported modulations of corticobulbar excitability under intake conditions are not related to a specific compound (i.e. nicotine), but probably reflect the experience of gratification induced by craving-reducing behaviour.

Our study is relevant to addiction research. It suggests a possible clinical potential of this method for diagnostic purposes. Specifically, excitability of tongue muscle representations might

be suitable somatic markers of the activity of the reward system. Excitability measures derived from TMS have the important advantage of being able to provide information about the excitatory or inhibitory nature of the examined neural processes with high temporal resolution, which is difficult to achieve with neuroimaging methods.[36] Thus, they add important information to addiction-related physiological alterations of central nervous system functions. Therefore, exploring tongue MEP amplitudes in this connection can be considered an interesting new tool to improve our knowledge of the neural mechanisms of addiction.

Competing interests: None declared.

Contributors: C.M. Vicario, P. Cesari, R.D. Rafal and M.A. Nietsche designed the study. C.M. Vicario and N. Komeilipoor acquired the data, which C.M. Vicario analyzed. All authors wrote and reviewed the article and approved the final version for publication.

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