

Deep TMS on alcoholics: effects on cortisolemia and dopamine pathway modulation. A pilot study

Marco Ceccanti, Maurizio Inghilleri, Maria Luisa Attilia, Ruggero Raccah, Marco Fiore, Abraham Zangen, and Mauro Ceccanti

Abstract: The hypothalamic pituitary adrenal axis and dopamine have a key role in transition from alcohol social use to addiction. The medial prefrontal cortex was shown to modulate dopaminergic activity and cortisol releasing factor (CRF) release in hypothalamic and extra-hypothalamic systems. The recent advancements in non-invasive neurostimulation technologies has enabled stimulation of deeper brain regions using H-coil transcranial magnetic stimulation (TMS) in humans. This randomized double-blind placebo-controlled pilot study aims to evaluate H-coil efficacy in stimulating the medial prefrontal cortex. Cortisol levels and prolactinemia were evaluated as effectiveness markers. Alcohol intake and craving were considered as secondary outcomes. Eighteen alcoholics were recruited and randomized into 2 homogeneous groups: 9 in the real stimulation group and 9 in the sham stimulation group. Repetitive TMS (rTMS) was administered through a magnetic stimulator over 10 sessions at 20 Hz, directed to the medial prefrontal cortex. rTMS significantly reduced blood cortisol levels and decreased prolactinemia, thus suggesting dopamine increase. Craving visual analogic scale (VAS) in treated patients decreased, as well as mean number of alcoholic drinks/day and drinks on days of maximum alcohol intake (DMAI). In the sham group there was no significant effect observed on cortisolemia, prolactinemia, mean number of alcoholic drinks/day, or drinks/DMAI. Thus, deep rTMS could be considered a potential new treatment for alcoholism.

Key words: alcohol, prefrontal cortex, transcranial magnetic stimulation, cortisol, prolactin.

Résumé : L'axe hypothalamo-hypophysaire-surrénalien et la dopamine jouent un rôle clé dans la transition d'une consommation sociale d'alcool vers une dépendance à l'alcool. Le cortex préfrontal interne module l'activité dopaminergique et la libération de corticolibérine (CRF) dans les systèmes hypothalamique et extra-hypothalamique. Un progrès récent sur le plan des technologies de neurostimulation non-invasive permet de stimuler ces régions profondes du cerveau à l'aide de la stimulation magnétique trans-crânienne (SMT) chez l'humain. Cet essai pilote aléatoire à double aveugle comparatif avec placebo visait à évaluer l'efficacité de stimulation du cortex préfrontal interne de la bobine solénoïde. La cortisolémie et la prolactinémie ont été évaluées en tant que marqueurs d'efficacité. La consommation d'alcool et l'état de besoin ont été considérés comme résultats secondaires. Dix-huit personnes alcooliques ont été recrutées et réparties de manière aléatoire en deux groupes homogènes, 9 personnes avec stimulation réelle et 9 personnes avec stimulation simulée. La STM répétitive (STMr) a été appliquée au moyen d'un stimulateur magnétique en 10 sessions de 20 Hz, et dirigée sur le cortex préfrontal interne. La STMr pouvait réduire significativement le niveau de cortisol sanguin et la prolactinémie, suggérant ainsi un accroissement de la dopamine. L'échelle visuelle de l'état de besoin chez les patients traités diminuait, de même que le nombre moyen de consommations par jour et le nombre de consommations lors des jours de consommation d'alcool maximale. Chez le groupe simulé, aucun effet significatif n'était observé sur la cortisolémie, la prolactinémie, le nombre moyen de consommations par jour et le nombre de consommations lors des jours de consommation maximale. Le STMr profonde pourrait être considérée comme un outil supplémentaire pour traiter l'alcoolisme. [Traduit par la Rédaction]

Mots-clés : alcool, cortex préfrontal, stimulation magnétique trans-crânienne, cortisol, prolactine.

Introduction

Background

Impulse-control disorders and antisocial personality disorders are very common in patients affected by substance dependence, particularly alcoholics (Hasin et al. 2011; Lewis 2011). In the context of alcohol consumption there are different categories of consumers. In the United States, 65% of the adult population are alcohol consumers (National Institute on Alcohol Abuse and Alcoholism 1997), and 12% of them can be classified as abusers or addicts (Merikangas and McClair 2012).

According to Koob (Koob and Le Moal 1997), addiction is characterised by (i) compulsive substance seeking and intake, (ii) loss of control over the amount of substance that is consumed, and (iii) the emergence of a negative emotional state when access to the substance is prevented.

In terms of clinical interventions, it is also beneficial to identify addiction as a cycle consisting of 3 stages (Koob et al. 2009): intoxication, withdrawal, and preoccupation-anticipation (craving). The first stages are dominated by impulsivity, characterised by gratification or relief after committing an act. In the later stages,

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compulsiveness is added to impulsivity, and is characterised by anxiety and stress, which disappear with the completion of the compulsive act.

The 3 stages of addiction are linked to impairment of different brain areas and circuits, and to the action of hypothalamic-releasing factors (Sanchis-Segura and Spanagel 2006; Koob et al. 2009). During withdrawal there is a decrease in the function of the dopamine (DA) circuits that are involved in the phase of acute intoxication: this leads to a decreased motivation toward stimuli that are unrelated to the abuse, and a greater sensitivity toward those that are associated with it (Melis et al. 2005). Indeed, decreased dopaminergic activity in the mesolimbic areas and depleted serotonergic neurotransmission in the nucleus accumbens have been demonstrated during drug withdrawal (Rossetti et al. 1992). Another aspect of withdrawal is stress circuit activation, mediated by corticotropin-releasing factor (CRF), which increases adrenocorticotrophic hormone (ACTH) and corticosterone levels. CRF is also released in the central nucleus of the amygdala, thus activating the noradrenergic system that induces an anxiety-like state, which is reversible by the administration of CRF antagonists (Funk et al. 2006; Koob 2008). The medial prefrontal cortex (PFC) of rats contains many glucocorticoid receptors and controls the hypothalamic–pituitary–adrenal (HPA) axis (Diorio et al. 1993; Figueiredo et al. 2003). The dorsal portion (anterior cingulate and prelimbic cortex) of the medial PFC inhibits the HPA axis (Garrido et al. 2012), thereby reducing stress, whereas the ventral portion (infralimbic cortex) activates the HPA axis, thus enhancing stress (Radley et al. 2006). The shift in balance between stress-circuit activation and inhibition may mirror the shift between the psychological state of “withdrawal” and that of “gratification” after a compulsive act, respectively.

Transition from social use to addiction is promoted by DA and glutamate-mediated neuroplasticity (Kalivas and O’Brien 2008). Zhou et al. (2007) demonstrated, in an animal model (rats), that chronic alcohol use modifies glutamatergic transmission within the synapses between axons afferent from the PFC and neurons in the nucleus accumbens. They further demonstrated that, unlike other drugs of abuse such as cocaine, ethanol decreases dendritic spine density within the nucleus accumbens; moreover, the remaining spines seemed to be disoriented, with a narrow “multi-headed” neck and enlarged heads. It has been hypothesized that a single narrow neck alters the transmission of electrical signals coming from the heads. These dendritic spine alterations are attributed to the inhibiting-action of alcohol on the *N*-methyl-D-aspartate (NMDA) channels, which is said to cause synapse restructuring. Hence, pre- and post-synaptic mechanism impairment could be hypothesized.

Cortisol and alcoholism

The role of the CRF–ACTH–cortisol axis is proving increasingly important, particularly in determining the state of abstinence and the amount of alcohol consumed on heavy drinking days (as in, days with a high alcohol consumption) (Roberto et al. 2010).

Koob (2008) suggested the existence of centres, located in the dorsal portion of the medial PFC, that modulate the HPA axis by inhibiting the release of CRF. Injuries in this region can increase the response of the HPA axis to stressors (Radley et al. 2006).

Cortisol has a key role, both in the early stages of abstinence and in long-term abstinence (Mantsch et al. 2003, Zhou et al. 2003a, 2003b). De Timary et al. (2012) demonstrated high cortisolemia in alcohol-dependent subjects during alcohol withdrawal, and this remained higher than the controls until at least day 16 of abstinence. The high levels of cortisol in alcoholics creates a pseudo-Cushing state (Besemer et al. 2011). Importantly, these changes are behaviourally relevant, as alterations to the HPA axis during alcohol withdrawal have been shown to positively correlate with an aggressive tendency in alcoholics (Ozsoy and Esel 2008).

CRF-induced increase in the ventral tegmental area (VTA) dopaminergic neuron activity may in turn enhance DA release in the projection areas, including the PFC, nucleus accumbens (NAc), and some amygdaloid nuclei, potentiating drug-seeking behaviour and the response to reward-predicting stimuli (Wanat et al. 2008). Reduction of the sensitivity of the HPA axis could thus be beneficial in improving alcohol relapse outcomes and the negative symptoms of withdrawal, which are associated with stress (Sinha et al. 2011).

Dopamine and alcoholism

As demonstrated by Damasio et al. (1996), the so-called biological impulses (search for food, sex, etc.) are generally controlled by the PFC in normally developed adults. The PFC, through glutamatergic fibres, activates the dopaminergic VTA (Koob 2008; Stahl 2008) and the NAc, which in turn exerts a GABAergic inhibitory control over the ventral pallidum (Jentsch and Taylor 1999). Reduced blood flow and metabolism in the PFC have been demonstrated in alcoholics (Goldstein and Volkow 2002); this condition can be called hypofrontality and could explain the decreased dopaminergic activity in the mesolimbic areas and in the NAc during drug withdrawal (Rossetti et al. 1992). Serum levels of DA are difficult to evaluate. Prolactin (PRL) release by the adenohypophysis is inhibited by the tubular infundibular DA pathway, acting on D2-receptors. Thus, serum levels of PRL are typically used as a marker of DA activity. Serum levels of PRL have been specifically evaluated and found to be elevated during alcohol withdrawal, probably reflecting reduction in dopaminergic pathway (Wilhelm et al. 2011).

Deep repetitive transcranial magnetic stimulation (deep rTMS)

Transcranial magnetic stimulation (TMS) is a non-invasive technique used for brain stimulation. The strength of a magnetic field generated by the previously used 8-coils decreases rapidly with increased distance from the source (Tofts 1990; Tofts and Branston 1991). Therefore, to stimulate deep brain regions, a very high intensity would be required. This intensity cannot be achieved using the currently available magnetic stimulators. Moreover, the intensity required to effectively stimulate deeper regions would also act on the cortical regions to an extent that would lead to unwanted side effects.

The H-coil has therefore been developed to achieve deep brain stimulation (Zangen et al. 2005). Its design allows us to stimulate neural pathways linked to motivation control, reward, and pleasure. Previous protocols have demonstrated its efficacy by inactivating fibres connecting the PFC and the cingulate cortex to the NAc and the VTA. Several clinical studies have documented the effectiveness of deep TMS in drug-resistant depression (Levkovitz et al. 2009; Rosenberg et al. 2010).

When administered in accordance with current international guidelines, transcranial magnetic stimulation has been shown to be safe (Levkovitz et al. 2007; Rossi et al. 2009), with few, mild, adverse effects.

Absolute contraindications for the use of the equipment are the presence of organic brain pathology, unstable medical conditions, pacemakers, implanted metallic pumps, metallic implants, and epilepsy (or a positive family history). Children and pregnant women were also excluded.

Aims of the study

Primary aims: to assess the effectiveness of deep rTMS for reducing cortisolemia and activating dopaminergic pathway in alcoholics during withdrawal.

Secondary aims: to reduce cravings and average alcohol intake, particularly on days of maximum alcohol intake (DMAI), defined as days in which alcohol assumption exceeded the 95th percentile of mean number of alcoholic drinks consumed by each patient.

Materials and methods

Participants

Eighteen male patients (mean age 45.0 ± 11.07 years; median 44.0 years), fulfilling the DSM-IV criteria for alcohol dependence were recruited between September 2012 and March 2013 (Table 1). Patients with psychotic disorders, and abusers of substances other than alcohol, nicotine, or cannabis were excluded, as well as patients with organic brain disorder, metal prostheses, clinical history of complicated withdrawal symptoms (convulsions, delirium tremens, etc.), and treatment with anti-craving drugs or mood stabilisers. Patients with epilepsy or a family history of this pathology were also excluded. Patients were not administered any drugs and no psychological and (or) psychiatric therapy was provided during the trial period. TMS was the only intervention allowed.

Participants were recruited by the Alcohol Unit of the Department of Internal Medicine, Sapienza – University of Rome, and were stimulated with deep rTMS in the Neuromuscular Disease Centre (Sapienza – University of Rome). The subjects were randomly distributed among 2 homogeneous groups: 9 in the real stimulation group, and 9 in the sham stimulation group. Randomisation was performed by blocks, with a block size of 4; the random allocation sequence was generated by personnel outside the trial using a random-numbers table. Ethical approval was provided by the institutional review board of Sapienza – University of Rome, and the research complies with the World Medical Association – Declaration of Helsinki.

Psychiatric evaluation

Patients underwent psychiatric and psychological analysis, whereby tests were administered for the diagnosis of Axis I and DSM-IV Axis II personality disorders (SCID II).

Stimulation procedure

rTMS was delivered through a high-frequency biphasic magnetic stimulator (Magstim Rapid2; The Magstim Company, Whitland, South West Wales, UK). Eighteen magnetic cards encoding for real or sham stimulation were used to activate the deep rTMS device. A blind protocol was applied to both patients and experimenters so they did not know the stimulation type, and the sham and real stimulation produced identical sounds during the session (Isserles et al. 2013).

Pulses in the real stimulation condition were administered over the medial PFC. They were applied over 10 sessions (5 per week) of 30 consecutive trains of 50 stimuli delivered at an excitatory frequency of 20 Hz (Modugno et al. 2001) and an intensity of 120% of the resting motor threshold, at 30 s inter-train intervals, to the medial PFC at 5 cm anterior to the hot-spot for the first dorsal interosseus (FDI). The hot-spot for a muscle indicates the scalp area with the lowest motor threshold, corresponding to the cortical representation for that muscle. By positioning the coil 5 cm anterior to the hot-spot for FDI, we simultaneously stimulated the 2 medial prefrontal areas, thereby reducing the chance of possible lateralisation of the CRF control areas. The resting motor threshold (RMT) was calculated as the lowest stimulus intensity to evoke a motor-evoked potential (MEP) of at least 50 μ V in 5 out of 10 consecutive trials.

An olfactory–visual provoking stimulus was dispensed to patients just before each stimulation. Participants were asked to raise a glass filled with their favourite alcoholic drink (wine, beer, spirits), take a good look at it and sniff it for 5 s, and to repeat this action for a total of 15 times in 3 min (Van Den Wildenberg et al. 2007). The glass and the bottle containing the patient's favourite drink remained in front of the patient during the treatment. It has been demonstrated (Amiaz et al. 2009) in nicotine addiction that deep TMS alters the relevant neurocircuit when patients are exposed to alcohol cues prior to the stimulation.

Table 1. Demographic characteristics and behavioural data.

	Real (<i>n</i> = 9)	Sham (<i>n</i> = 9)
Mean age (SD)	43.22 (11.10)	47.29 (11.46)
Ethnicity (<i>n</i>)		
European	8	8
Non-European	1	1
Employment status (<i>n</i>)		
Employed	6	7
Unemployed	3	2
Marital Status (<i>n</i>)		
Married	3	4
Single	4	3
Divorced	2	2
Mean years of education (SD)	11.32 (3.48)	10.41 (3.52)
Preferred alcoholic beverage		
Wine	6	6
Beer	2	1
Spirits	1	2
Mean years of at risk consumption (SD)	26.0 (8.7)	25.28 (11.16)
Mean number of drinks/drinking day in at risk consumption years (SD)	19.22 (9.64)	17.61 (11.16)

Note: There were 9 males in each group. Real, group that were administered deep repetitive H-coil transcranial magnetic stimulation (rTMS); Sham, group exposed to the machine but not receiving rTMS.

All patients abstained from alcohol the day before being admitted to hospital. During the first week, benzodiazepines were dispensed for the treatment of acute alcohol withdrawal (Lejoyeux et al. 1998). The first rTMS session was performed 10 days after admission to hospital, to allow the benzodiazepines to be flushed out.

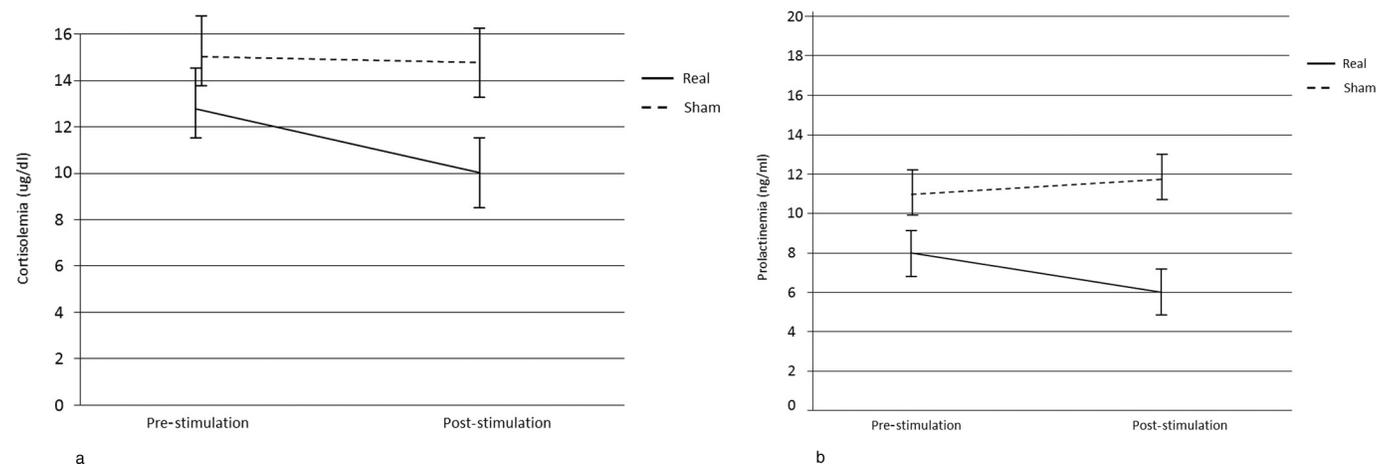
On the day of the first rTMS stimulation, on the day after the last TMS session, and in each follow-up visit (performed once a month, for a total of six months), a blood sample was collected at 0800 h to evaluate cortisolemia and prolactinemia. Prolactinemia was assessed to evaluate activation of the dopamine pathways. In this study, prolactin was evaluated by electrochemiluminescence immunoassay (ECLIA; Roche Diagnostic) (Fahie-Wilson et al. 2000; Gibney et al. 2005), as was cortisol (Chiu et al. 2003).

Moreover, at the first and last rTMS session and at each monthly control follow-up (for 6 months), patients were asked about the average number of drinks consumed daily and the number of alcoholic drinks/day of maximum alcohol intake (DMAI) through time line follow back (TLFB) (Sobel and Sobel 1992); craving was also evaluated using a visual analogue scale (VAS) normalized for the base value. Blood alcohol concentration was also measured, with a breath analyser, at each follow-up.

Data analysis

Preliminary analyses were conducted to compare the baseline values for the enrolled patients assigned to the 2 groups, in order to evaluate the success of our randomization process. Owing to the number of participants and the distribution of the variables, the Mann–Whitney *U* test was used to assess differences for age, years of alcohol dependence, mean number alcoholic drinks consumed daily, and drinks/DMAI consumed before hospitalization. The Mann–Whitney *U* test was used also to compare the baseline values for cortisolemia, prolactinemia, VAS for craving, mean number of alcoholic drinks consumed daily, and drinks/DMAI. A χ^2 test was used to assess differences in the preferred drink consumed (wine, beer, or spirits). We used ANCOVA, covaried for the pre-stimulation values for cortisol and prolactin, to evaluate differences between pre- and post-stimulation for cortisolemia and prolactinemia; post-stimulation values for cortisol and prolactin were considered dependent variables. Cortisol and PRL values from the follow-up visits were evaluated through univariate repeated measures ANOVA in the only real group, because the drop-out rate in the sham group did not allow for further analysis.

Fig. 1. (a) Values for pre- and post-stimulation cortisolemia (\pm SEM) in the real and sham groups. (b) Pre- and post-stimulation levels of prolactinemia (\pm SEM) in the real and sham groups. Real group, group stimulated with repetitive deep H-coil transcranial magnetic stimulation; Sham group, group attached to the machine but not actually treated (noise only).



When cortisol reduction was significant, regression curves were used to verify the existence of a relationship between the levels of pre-stimulation PRL (related to dopamine pathway activity), pre-stimulation levels of cortisol, and absolute reduction of cortisol after treatment (Δ cortisolemia) in the real group.

To evaluate the effects of rTMS stimulation, paired Student *t* tests were applied to assess pre- and post-stimulation differences in craving, mean number of alcoholic drinks consumed daily, and drinks/DMAI.

Results

Baseline differences

All patients completed rTMS sessions with no significant side-effects. The Mann–Whitney *U* test was applied to the data from the 2 groups, and demonstrated no significant differences in terms of age ($p = 0.76$), years of alcohol dependence ($p = 0.84$), VAS for craving ($p = 0.30$), mean number of alcoholic drinks consumed daily ($p = 0.68$), and drinks/DMAI ($p = 0.30$) consumed before hospitalization, and pre-stimulation levels of cortisolemia ($p = 0.55$), or prolactinemia ($p = 0.07$). Samples were also homogenous in terms of the preferred drink (wine, beer, or spirits; $\chi^2 (2, N = 18) = 0.67, p = 0.72$).

Drop-out rate

In the course of the 6 month trial, of the 9 subjects in the real group, 4 subjects dropped out in the first and second months, 5 dropped out in the third month, 6 dropped out in the fourth and fifth months, and 7 in the sixth month. Of the 9 subjects in the sham group, 3 patients dropped out in the first month, 6 in the second and third months, 8 in the fourth and fifth months, 9 in the sixth month.

Cortisol

Baseline cortisolemia was not significantly different between the real and sham groups ($p = 0.55$). One patient belonging to the real group had contracted influenza at the time of the seventh rTMS session, so his cortisolemia value was not included in the statistical analysis. In the real group, the average value for cortisolemia decreased from $13.4 \pm 1.5 \mu\text{g/dL}$ ($\bar{X} \pm \text{SEM}$) to $9.9 \pm 1.0 \mu\text{g/dL}$ after stimulation, with a mean reduction of $3.5 \mu\text{g/dL}$, corresponding to a reduction of 26.1% (Fig. 1a).

The between-subjects effect in the ANCOVA values for cortisolemia was significant ($F_{[1,16]} = 6.180, p = 0.026, \eta^2 = 0.306$, observed power = 64%, $R^2 = 0.39$), with a high effect value. However, pre-test cortisolemia does not significantly correct for the value of the post-test cortisolemia ($F_{[1,16]} = 1.446, p = 0.251, \eta^2 = 0.100$). Thus,

pre-test cortisolemia is not a possible explanation for the variance in post-test cortisolemia. This suggests that the rTMS treatment is effective in reducing cortisol levels in the real group only, regardless of pre-stimulation cortisol levels.

Paired Student *t* tests highlighted a significant effect of rTMS stimulation on cortisolemia in the real group ($t = 3.23; p = 0.018$).

By contrast, in the sham group, cortisolemia decreased from $15.0 \pm 1.8 \mu\text{g/dL}$ to $14.7 \pm 1.9 \mu\text{g/dL}$ after stimulation, with a mean reduction of $0.3 \mu\text{g/dL}$, corresponding to a decrease of 2% (Fig. 2). These data, analysed by paired Student *t* tests, are not statistically significant ($t = 0.20; p = 0.84$). No statistically significant decreases in cortisolemia were achieved in the real group in later follow-up visits (1–6 months), as analysed using univariate repeated measures ANOVA ($p > 0.05$).

Prolactin

Baseline prolactinemia was not significantly different between the real and sham groups ($p = 0.07$). In the real group, pre-stimulation levels of prolactin decreased from $8.0 \pm 0.7 \text{ ng/mL}$ ($\bar{X} \pm \text{SEM}$) to $6.4 \pm 1.0 \text{ ng/mL}$ after stimulation, with a mean reduction of 1.6 ng/mL , corresponding to a reduction of 20.0%. In the sham group, prolactin increased from $11.0 \pm 1.2 \text{ ng/mL}$ to $11.7 \pm 0.4 \text{ ng/mL}$, with a mean increase of 0.7 ng/mL , corresponding to an increase of 6.4% (Fig. 1b).

The between-subjects effect in the ANCOVA for prolactinemia was significant ($F_{[1,16]} = 7295, p = 0.019, \eta^2 = 0.378$, observed power = 70%, $R^2 = 0.63$), with a high effect value. Moreover, pre-test prolactinemia does significantly correct the values for post-test prolactinemia ($F_{[1,12]} = 7.295, p = 0.019, \eta^2 = 0.378$), therefore the value of pre-test significantly “corrects” the value of the post-test prolactinemia. No significant data were achieved in real group in later follow up visits (1–6 months), as analysed by univariate repeated measures ANOVA ($p > 0.05$).

The relationship between pre-stimulation PRL, which is related to dopamine pathway activity, and cortisol reduction after treatment in the real group, as evaluated using an inverse regression curve, was highly significant ($F_{[1,6]} = 28.320; p = 0.003; R^2 = 0.850$) (Fig. 2).

An exponential, highly significant ($F_{[1,6]} = 17.020; p = 0.009; R^2 = 0.773$) regression curve was also demonstrated between pre-stimulation cortisol levels and Δ cortisol post-treatment in the real stimulation group (Fig. 3).

A highly significant ($F_{[1,6]} = 51.320; p = 0.001; R^2 = 0.911$) linear regression was demonstrated between pre-stimulation cortisol/prolactin and the decrease in levels of cortisol post-treatment in the real stimulation group (Fig. 4).

Fig. 2. Inverse regression curve between baseline prolactinemia and absolute cortisol reduction (Δ cortisolemia) after repetitive deep H-coil transcranial magnetic stimulation.

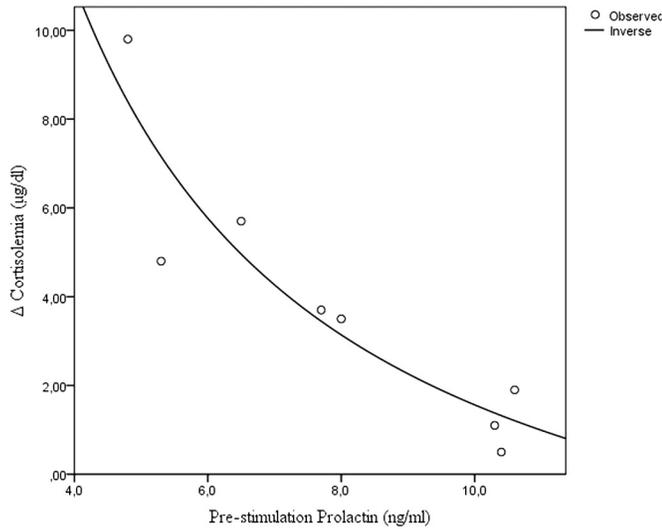
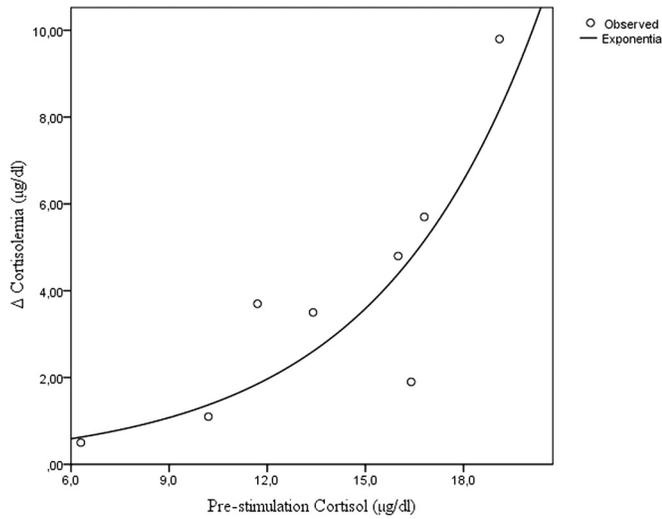


Fig. 3. Exponential regression curve between baseline cortisol and absolute cortisol reduction (Δ cortisolemia) after repetitive deep H-coil transcranial magnetic stimulation.



VAS for craving

Baseline VAS for cravings were not significantly different between the real and sham groups ($p = 0.30$). Analysis of VAS for craving showed a reduction in the real stimulation group, from a mean pre-stimulation score of 26.7 ± 7.3 ($\bar{X} \pm SEM$) to 17.4 ± 7.0 in the post-stimulation follow-up, 13.1 ± 7.3 in the one month follow-up, and 15.5 ± 12.4 in the 2 month follow-up. In the sham group, the pre-stimulation VAS also decreased, from 43.9 ± 12.9 to 33.3 ± 11.0 in the post-stimulation follow-up, to 27.4 ± 9.8 in the one month follow-up, and 49.5 ± 29.5 in the 2 month follow-up.

The difference in VAS for cravings before and after rTMS stimulation was only significant in the real group ($t = 2.84$; $p = 0.025$) and was maintained in the first monthly follow-up ($t = 2.65$; $p = 0.038$) (VAS normalized for base value is shown in Fig. 5). No significant difference for VAS values were achieved in the real or the sham groups in later follow-up visits, as analysed with univariate repeated measures ANOVA ($p > 0.05$). In the sham group, univariate repeated measures ANOVA was only performed to the 3 month follow-up, because there was only one patient remaining.

Fig. 4. Linear regression curve between baseline cortisol/prolactin and absolute cortisol reduction (Δ cortisolemia) after repetitive deep H-coil transcranial magnetic stimulation.

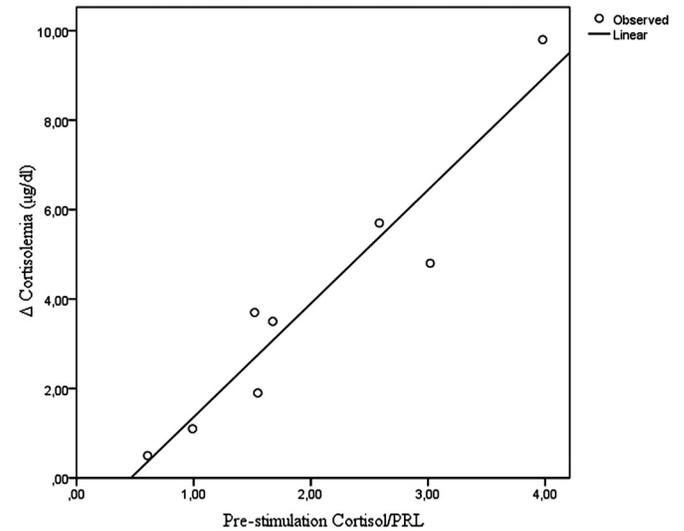
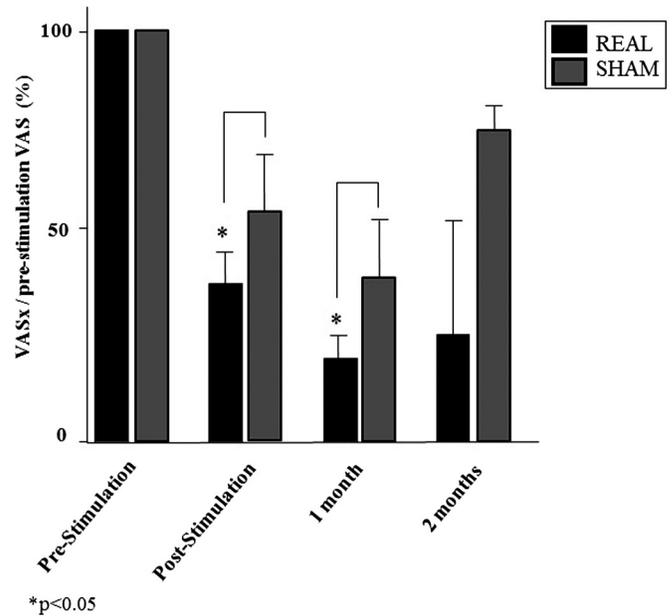


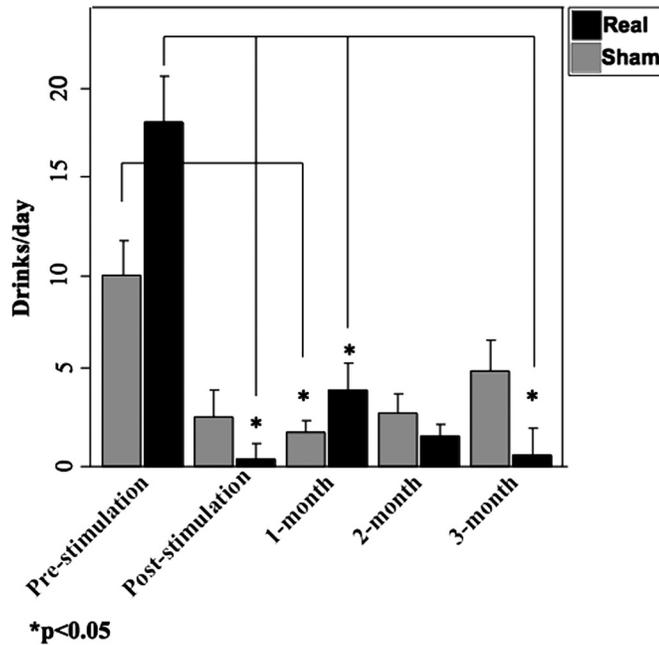
Fig. 5. Normalized craving visual analogic scale (\pm SEM) for the real and sham groups. Real group, group treated with repetitive deep H-coil transcranial magnetic stimulation; Sham group, group attached to the machine but not actually treated (noise only).



Daily alcohol consumption

At baseline, the mean number of alcoholic drinks consumed daily was not significantly different between the real and sham groups ($p = 0.68$). Mean daily alcohol consumption (mean number of drinks/day) showed a reduction in the real stimulation group, from 18.6 ± 4.9 ($\bar{X} \pm SEM$) drinks/day pre-stimulation, to no drinks in any patient post-stimulation, 3.4 ± 2.8 drinks/day in the one month follow-up, 1.0 ± 1.0 drinks/day at the 2 month follow-up, and 0.7 ± 0.7 drinks/day at 3 months. Subjects who continued with the treatment for 6 month ceased consuming alcohol entirely. In the sham group, mean daily alcohol consumption decreased from 10.1 ± 2.8 drinks/day at the pre-stimulation baseline to 2.3 ± 1.5 drinks/day at the post-stimulation follow-up, 1.5 ± 0.8 drinks/day at the one month follow-up, 2.0 ± 1.0 drinks/day at 2 months, and

Fig. 6. Mean number of alcoholic drinks consumed daily (\pm SEM) for the real and sham groups. Real group, group treated with repetitive deep H-coil transcranial magnetic stimulation; Sham group, group attached to the machine but not actually treated (noise only).



5.3 \pm 1.8 drinks/day at 3 months. Just one patient in the sham group was followed-up for more than 3 months (up to the sixth month) and he did not cease alcohol consumption.

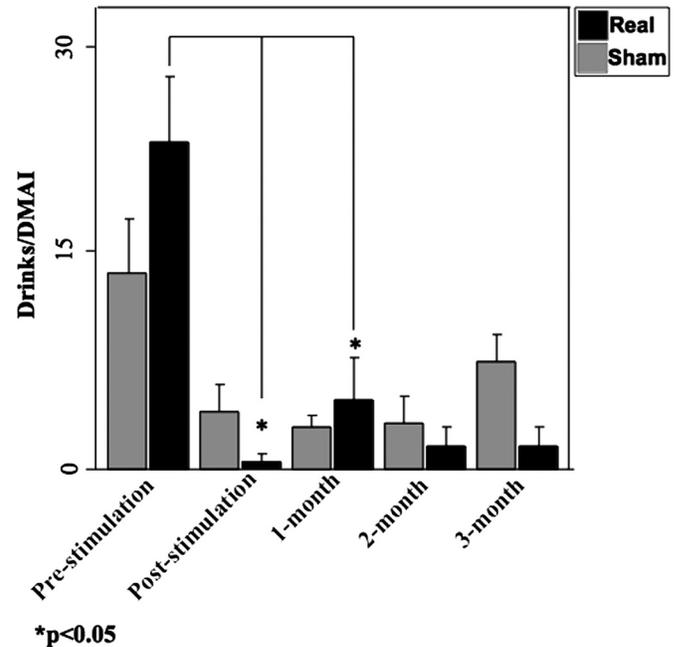
The results showed a significant reduction in mean number of alcoholic drinks/day between pre- and post-stimulation ($t = 3.79$; $p = 0.009$), at one month ($t = 4.25$; $p = 0.008$) and 3 months ($t = 4.50$; $p = 0.046$) in the real stimulation group. Statistical significance was lost in later follow-up tests. In the sham group, the same statistical analysis showed a trend to significance between the pre-stimulation and post-stimulation number of alcoholic drinks/day ($t = 2.34$; $p = 0.058$), and a significant reduction between the number of drinks/day pre-stimulation and one month later ($t = 2.73$; $p = 0.041$) (Fig. 6). Statistical significance was lost in later follow-up evaluations ($p > 0.05$).

Drinks per days of maximum alcohol intake (DMAI)

At baseline, drinks/DMAI were not significantly different between the real and sham groups ($p = 0.30$). In the real stimulation group, drinks/DMAI decreased from 23.4 \pm 7.1 ($\bar{X} \pm$ SEM) pre-stimulation to no alcohol consumed by any patient post-stimulation, 3.4 \pm 2.8 drinks/DMAI in the one month follow-up, 1.0 \pm 1.0 drinks/DMAI at 2 months, and 0.7 \pm 0.7 drinks/DMAI at 3 months. Patients who continued with the treatment for 6 months completely abstained from alcohol. In the sham group, mean drinks/DMAI decreased from 13.7 \pm 5.0 pre-stimulation, to 3.3 \pm 2.2 drinks/DMAI post-stimulation, 2.2 \pm 1.2 drinks/DMAI at the one month follow-up, 2.5 \pm 1.5 drinks/DMAI at 2 months, and 5.3 \pm 1.8 drinks/DMAI at 3 months. As previously mentioned, just one patient in the sham group stayed in the trial for more than 3 months (until the fifth month) and he continued consuming alcohol.

Results showed a significant reduction in drinks/DMAI between pre- and post-stimulation ($t = 3.29$; $p = 0.013$) in the real group. Statistical significance was maintained until one month after rTMS stimulation ($t = 3.22$; $p = 0.018$) and was lost after 2 months ($t = 2.17$; $p = 0.09$). In the sham group, there was no significant reduction in mean drinks/DMAI (Fig. 7) at any post-stimulation follow-up ($p > 0.05$).

Fig. 7. Drinks consumed per day of maximum alcohol intake (DMAI) (\pm SEM) in the real and sham groups. Real group, group treated with repetitive deep H-coil transcranial magnetic stimulation; Sham group, group attached to the machine but not actually treated (noise only).



Discussion

All of the patients enrolled in the study were stimulated (real stimulation or sham stimulation), beginning on the tenth day of abstinence; according to Koob's model we can hypothesize a dopamine reduction at this stage (Koob 2008) with a simultaneous increase in HPA-axis activity and cortisol levels (De Timary et al. 2012). Cortisol is thought to be responsible for the negative withdrawal symptoms (anxiety, aggression, dysphoria, irritability). Indeed, in the sham group, we reported high levels of cortisol in the blood, with no significant differences between the first and last stimulation treatments (Fig. 1). These data confirm the results of previous studies demonstrating high HPA axis activation and high cortisolemia in the first days of withdrawal (De Timary et al. 2012).

The purpose of the study was to find a way to enhance a dopaminergic pathway unlinked to alcohol consumption, and to decrease cortisol associated with negative withdrawal symptoms. It was decided to stimulate the dorsal region of medial PFC. A recent structural MRI study by Rando et al. (2011) demonstrated medial frontal cortex, right PFC, and occipital lobe atrophy in alcoholics, and the extent of atrophy has been proven a good relapse indicator, thus confirming a central role of prefrontal-frontal areas in maintaining abstinence. As demonstrated by Koob (2008) and Stahl (2008), this area has both glutamatergic efferents directed to the NAc and VTA, and stress regulatory centres modulating hypothalamic and extra-hypothalamic CRF secretion, such as the central nucleus of the amygdala.

The stimulation of glutamatergic efferents should activate (i) dopaminergic VTA and the NAc with its GABAergic inhibitory efferents on the ventral pallidum, which is crucial for reward-motivated behaviour; (ii) CRF regulatory centres, allowing greater stress control. According to Koob (2008), there are 2 centres regulating CRF release: the dorsal centre, located in the prelimbic cortex, reduces CRF release; whereas the ventral centre, located in the infralimbic cortex, is stress-promoting. Deep rTMS, administered with 120% of FDI resting motor threshold, reaches a depth of 4–5 cm (Roth et al. 2007). It can be assumed that the stimulus will only reach the dorsal medial PFC, including the prelimbic cortex.

PFC stimulation was proved effective in major drug-resistant depression (Nakamura 2012), probably through the induction of neuroplasticity and activation of the dopamine pathways.

Different responses to stimulation can be expected in different patients; an external magnetic field can only activate functionally inactive neurons, acting on neural plasticity with long-term potentiation mechanisms; it cannot act on irreversibly atrophied cells. Moreover, chronic alcohol abuse induces NMDA channel inhibition and density reduction associated with remodelling of dendritic spines (Zhou et al. 2007), thus reducing the efficiency of rTMS at modulating synaptic plasticity. We could thus expect a different response depending on the years of alcohol abuse and average drinks taken during “at risk” consumption years. Estimation of the average number of alcoholic drinks consumed may pose several difficulties such as problems with amnesia or self deception. Reliable and measurable parameters correlating with the average number of alcoholic drinks consumed during the “dependency” years are necessary. Studies conducted by Wilhelm et al. (2011) demonstrate a direct correlation between prolactinemia and severity of alcohol dependence, probably reflecting a modified dopaminergic pattern, also hypothesized in Koob’s model (Koob 2008); therefore, progressive reduction in dopamine pathway activity in chronic abusers promotes prolactin release by the pituitary gland. Prolactinemia decrease after real stimulation suggests dopamine pathway activation, thus rebalancing dopamine–cortisol equilibrium during withdrawal.

We decided to check whether the data from the treated patients could be used to create a regression curve relating cortisolemia decrease and baseline prolactin levels, as measured on the tenth day of withdrawal/abstinence: a highly significant inverse regression has been demonstrated (Fig. 2). Moreover, a highly significant exponential regression between cortisol levels on the tenth day of abstinence and the post-treatment decrease in cortisol levels was demonstrated (Fig. 3). Finally, a linear, highly significant regression was detected between the baseline ratio for cortisol:prolactin and Δ cortisol post-treatment (Fig. 4). This curve could facilitate the selection of patients who would most benefit from rTMS stimulation: patients with a high baseline cortisol:prolactin are the best responders to this treatment, which probably reflects a milder degree of atrophy and dopaminergic impairment, with higher density of dendritic spines with less remodelling, who are still susceptible to the mechanisms of rTMS-induced long-term potentiation.

These data are consistent with Solomon’s “opponent system” theory (Solomon and Corbit 1974) and its adaptation to alcohol dependence (Koob and Le Moal 1997), which would be determined through subsequent steps mediated by plastic changes in neurons. In the first stage, the motivation for drinking is supported by positive reinforcement (reward) mediated by dopamine. At this reinforcement the stress system activity is “opposed”, attempting to maintain a balance between pleasure and social behaviour. Continuous substance use creates an imbalance in the dopaminergic system, which activates only in presence of alcohol; during withdrawal the opponent system prevails, thus inducing negative stress-linked emotions (anxiety, dysphoria) that are considered to favour relapse.

We can speculate that stimulation of the dorsal medial PFC was effective in decreasing the “opponent system” and exciting dopamine-related “reward system” activity in alcoholics.

VAS for craving was only significantly reduced in the real group after stimulation; this effect was maintained at the first monthly follow-up, but not any later than that (Fig. 5), suggesting a limited time-effect for stimulation.

In the real stimulation group, the mean number of alcoholic drinks consumed daily before rTMS treatment significantly declined at post-stimulation, at the one month follow-up, and at the 3 month follow-up. In the sham group, the mean number of alcoholic drinks consumed daily, pre-stimulation, showed a trend to-

ward statistically significant difference ($p = 0.058$) when compared with the mean number of alcoholic drinks consumed daily post-stimulation, which revealed itself at the one month follow-up ($p = 0.041$) (Fig. 6). We interpreted the reduction in the mean number of alcoholic drinks/day for both groups as a bias linked to frequent follow-ups, and greater control over the sample; nevertheless, a significant reduction in mean number of alcoholic drinks/day in the later follow-up visits (3 months) was only found in the real stimulation group.

DMAI analysis showed a significant post-stimulation reduction in the real stimulation group only (Fig. 7), which was maintained until one month after rTMS stimulation. These data are consistent with previous studies (Roberto et al. 2010) demonstrating a key role for cortisol, especially in determining the number of drinks consumed on the heavy drinking days.

The durability of the treatment effect still needs to be determined, but it is apparently important to act on the early abstinence stages, which often turn out to be the most critical. Indeed, the effect of deep rTMS on VAS craving, mean number of drinks consumed daily, and drinks per DMAI seem to last for 1–2 months. Further investigation of the behavioural changes post-treatment that are related to cortisolemia reduction is necessary.

Conclusions

Deep rTMS significantly reduced cortisolemia and prolactinemia, suggesting a rebalancing of the dopamine–cortisol equilibrium during alcohol withdrawal.

The reduction in VAS for craving, mean number of alcoholic drinks/day, and drinks/DMAI suggests a clinical effect lasting 1–2 months, but further clinical trials are necessary to better define this aspect.

The small sample size and the use of an indirect method for measuring dopamine are the major limitations of the study, suggesting further trials are required to confirm our data. Nevertheless, deep rTMS can be considered a useful tool in the treatment of alcohol addiction, alternative to or concomitant with drug therapies with a specific site of action in the brain, and with rare side effects.

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