Cortical inhibition within motor and frontal regions in alcohol dependence post-detoxification: A pilot TMS-EEG study

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Abstract

Objectives. Preclinical studies suggest that cortical alterations within the prefrontal cortex (PFC) are critical to the pathophysiology of alcohol dependence. Combined transcranial magnetic stimulation (TMS) and electroencephalography (EEG) allows direct assessment of cortical excitability and inhibition within the PFC of human subjects. We report the first application of TMS-EEG to measure these indices within the PFC of alcohol-dependent (ALD) patients post-detoxification.

Methods. Cortical inhibition was assessed in 12 ALD patients and 14 healthy controls through single and paired-pulse TMS paradigms. Long-interval cortical inhibition indexed cortical inhibition in the PFC. In the motor cortex (MC), short-interval intracortical inhibition and cortical silent period determined inhibition, while intracortical facilitation measured facilitation, resting and active motor threshold indexed cortical excitability. Results. ALD patients demonstrated altered cortical inhibition across the bilateral frontal cortices relative to controls. There was evidence of altered cortical excitability in ALD patients; however, no significant differences in MC inhibition. Conclusions. Our study provides first direct evidence of reduced cortical inhibition in the PFC of ALD patients post-detoxification. Altered cortical excitability in the MC may reflect hyper-excitability within the cortex associated with chronic alcohol consumption. These findings provide initial neurophysiological evidence of disrupted cortical excitability within the PFC of ALD patients.

Key words: transcranial magnetic stimulation, electroencephalography, alcohol dependence, dorsolateral prefrontal cortex, cortical inhibition

Introduction

Alcohol has widespread effects across the brain, including alterations in multiple neurotransmitter systems within the mesocorticolimbic circuitry (Addolorato et al. 2012). The mesocorticolimbic dopaminergic circuitry plays a critical role in the development of addiction (Koob 1988). The mesocorticolimbic “addiction” circuitry contains dopaminergic (DA) neurons (Diana et al. 1993) originating in the ventral tegmental area (VTA), projecting to limbic structures involved in the incentive-sensitization of drugs (Robinson and Berridge 2003) via the nucleus accumbens (NAcc), amygdala and hippocampus, and to cortical areas, such as the prefrontal cortex, proposed to regulate inhibitory control over drug seeking (Goldstein and Volkow 2002). Neurotransmitter inputs, such as γ-aminobutyric acid (GABA) and glutamate (GLU) act by differentially regulating the activity of the DA neurons within the addiction circuitry (for a review, refer to Morikawa 2010).

Acute alcohol exposure indirectly increases extracellular dopamine levels in the mesocorticolimbic cir-
circuitry via the intervening principal neurotransmitters, which include GABA and GLU. A prominent effect of acute alcohol administration is the overall dampening of brain cortical excitability through: (1) facilitation of GABAAergic inhibitory neurotransmission, and (2) suppression of glutamate release and glutamate receptor activity (in dopaminergic cells) (Morikawa 2010). Repeated alcohol exposure induces compensatory neuroadaptations which oppose the inhibitory effect of acute alcohol administration (Pulvirenti and Diana 2001; Kalivas 2009), resulting in the suppression of (GABA) inhibitory neurotransmission (Enoch 2008; Filip and Frankowska 2008) and potentiation of (GLU) excitatory neurotransmission (Gass and Olive 2008). Therefore, altered cortical inhibition (CI), the neurophysiological mechanism by which cortical GABA inhibitory interneurons selectively suppress the activity of other neurons in the cortex, plays a critical role in the development of alcohol dependence (Diana et al. 2003). However, despite extensive preclinical and neuromolecular evidence of these neuroadaptative changes, supporting human evidence for altered CI within the mesocorticolimbic “addiction” circuitry in the human brain is lacking.

Advances in brain stimulation techniques have demonstrated that CI of the cortex can be measured through the application of transcranial magnetic stimulation (TMS; Barker et al. 1985; Hallett 2000). Recently, these TMS techniques have been employed to index the effects of acute ethanol exposure, as well as chronic ethanol consumption on localized changes in CI. Acute alcohol exposure was found to potentiate GABAAergic inhibitory mechanisms in the motor cortex (Ziemann et al. 1995; Conte et al. 2008a), reduce cortical excitability in the prefrontal cortex (Kahkonen et al. 2003), and influence functional connectivity between the motor and prefrontal cortices (Kahkonen et al. 2001; Kähkönen and Wilenius 2007). Further studies have identified altered motor cortical activity in clinical populations with alcohol dependence (Conte et al. 2008a), including those experiencing alcohol withdrawal (Nardone et al. 2010). However, these previous studies were largely confined to measuring CI of the motor cortex, as recording CI from other brain regions, which relate directly to the pathophysiology of dependence, was technically difficult.

Recently, a novel technique, which combines TMS with electroencephalography (TMS-EEG), was found to successfully measure CI in the prefrontal cortex (Daskalakis et al. 2008) through a paired pulse TMS paradigm known as long interval CI (LICI; Valls-Solé et al. 1992). LICI involves stimulation of the cortex with a suprathreshold conditioning stimulus 50–150 ms prior to the suprathreshold test stimulus, resulting in the inhibition of cortical evoked activity by approximately 30% (Daskalakis et al. 2008). A number of studies suggest that LICI relates to activity at the GABAReceptor (Sanger et al. 2001; McDonnell et al. 2006), allowing researchers, for the first time, to probe altered GABAAergic neurotransmission within the prefrontal cortex (PFC), a key structure of the addiction circuitry.

The current study utilised these novel TMS inhibitory paradigms to investigate the presence of altered CI within the frontal (the dorsolateral prefrontal cortex) and motor cortices of patients diagnosed with alcohol dependence post-detoxification. A secondary objective was to expand previous TMS studies in alcohol-dependent populations and investigate whether alcohol dependence was associated with concomitant changes in excitability within the motor cortex.

Materials and methods

This collaborative study between Monash Alfred Psychiatry Research Centre and Turning Point Alcohol and Drug Centre was approved by the Alfred Human Subjects Research and Ethics Committee. Active enrolment ran from October 2010 through September 2011. Consenting participants signed a detailed informed consent form prior to study enrolment; they were informed that participation was voluntary and they could withdraw at any time without prejudice.

Subjects

Alcohol-dependent sample. Twelve patients (mean age = 40.1 years, SD = 13.4 years; eight males and four females) meeting criteria for DSM-IV-TR alcohol dependence (DSM-IV-TR, American Psychiatric Association 2000) were recruited within 2 years of successful completion of a detoxification program (Range = 8–668 days, Mean = 145 days, SD = 202 days, Median = 50). Screening procedure included a psychiatric history and medical phone interview conducted by a trained research assistant. Participants were recruited through treatment agencies by self or clinician referral. Posters and cards advertising the study were presented at participating centres and offered to clients who met the study entry criteria. Alcohol-dependent subjects were required to: (1) have a current Wechsler Test of Adult Reading standardized score higher than 100, as an indication of no significant intellectual disability; (2) no self-reported drug or alcohol use since completing the detoxification program; and (3) have no current comorbid mental health disorder (including co-morbid depression and polysubstance use). As a secondary screening procedure to confirm detoxification, participants were required to complete the
Timelines for Back, a 4-week calendar which asks the participant to retrospectively estimate their alcohol consumption over the previous month. Exclusion criteria included individuals with head injury, acute medical or physical illness, major depression, epilepsy or history of seizures, metal implants, other drug dependence, engaging in pharmacotherapy programs (including anti-craving or anti-depressant treatment), or reporting psychotic symptoms or suicidal ideation.

Healthy control sample. Fourteen healthy control subjects (mean age = 31.1 years, SD = 5.3 years; seven males and seven females), without any previous or current history of psychiatric illness, or alcohol/drug dependence or abuse, head injury, epilepsy or seizures, were recruited through local advertisements and posters.

All participants were aged between 18 and 60 years and received $40 reimbursement for their participation. At screening, participants completed a general demographics questionnaire, the obsessive-drinking scale (OCDS; Anton et al. 1996) and the severity of alcohol dependence questionnaire (SADQ; Stockwell et al. 1979) to assess overall craving and degree of dependence on alcohol, the Beck Depression Inventory (BDI; Beck and Steer 1987) to measure severity of depressive symptoms and the Wechsler Test of Adult Reading (WTAR; Wechsler 2001) to assess pre-morbid level of intellectual functioning; relevant clinical demographic and participant characteristics for both alcohol-dependent and control groups are summarized in Table I.

### Table I. Description of demographic and clinical data of participants (mean and standard deviation).

| Characteristic                              | Participants (n = 12) | Healthy control participants (n = 14) | P value |
|--------------------------------------------|-----------------------|--------------------------------------|---------|
| Age, years                                 | 40 ± 13               | 31 ± 5                               | 0.031*  |
| Gender ratio (M:F)                         | 8:4                   | 7:7                                  | 0.45    |
| Daily standard drinks                      | 15.5 ± 5.20           | 0.08 ± 0.28                          | 0.00**  |
| Years of alcohol dependence               | 16 ± 13               | NA                                   | NA      |
| SADQ                                       | 28.7 ± 11.4           | 0.4 ± 0.8                            | 0.00**  |
| OCD total                                  | 26.4 ± 9.0            | 1.6 ± 2.2                            | 0.00**  |
| W TAR (standardized)                       | 112.1 ± 6.42          | 117.47 ± 3.79                        | 0.010** |
| BDI                                        | 12.9 ± 12.3           | 1.9 ± 1.7                            | 0.003** |

NA, not applicable; SADQ, Severity of Alcohol Dependence Questionnaire; OCDS, Obsessive Compulsive Drinking Scale; W TAR, Wechsler Test of Adult Reading; BDI, Beck Depression Inventory. *P < 0.05, **P < 0.01.

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### Experimental design/procedure

Active TMS was administered over the left motor cortex (Experiment 1) (always first), the right dorsolateral prefrontal cortex (DLPFC) and the left DLPFC (Experiment 2) in a single session. The order of left and right DLPFC stimulation was counterbalanced across participants. All subjects participated in both Experiments 1 and 2; however, data from two controls, relating to stimulations to the right DLPFC, were not processed due to excessive movement.

### Transcranial magnetic stimulation

Biphasic TMS pulses were administered to the cortex using a 7-cm figure-of-eight cooled coil connected to a MagPro R30 stimulator with a MagOption unit (Magventure, Denmark). The coil was held over the scalp, the handle of the TMS coil pointed backward, perpendicularly to the presumed direction of the central sulcus, and angled approximately 45° away from the midsagittal line. In Experiment 1, participants were administered a number of motor cortical TMS paradigms as measured by electromyography (EMG), including: resting motor threshold (RMT), active motor threshold (AMT), 1 mV measure (1 mV), cortical silent period (CSP), short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). In Experiment 2, participants were administered single and paired-pulse TMS; long-interval cortical inhibition (LICI) of DLPFC electroencephalography (EEG) activity, both in the left DLPFC and right DLPFC, was examined.

### Motor cortical TMS paradigms

#### Electromyography (EMG)

For all motor cortical TMS parameters and measures, EMG recorded motor-evoked potentials (MEPs) during stimulation of the left motor cortex via disposable disc electrodes placed over the contralateral first dorsal interosseous (FDI) muscle. All EMG activity was acquired through Signal software (Cambridge Electronics design, CED Micro 1401 mk II analogue-to-digital converting unit, Cambridge, UK), amplified and filtered (low pass 2 kHz, high pass 10 Hz) by a PowerLab/4SP system (AD instruments, Colorado Springs, CO), and stored in a laboratory computer for offline processing.

#### Measurement of motor cortical inhibition/facilitation

**Single-pulse stimulations.** At the beginning of each experiment, single pulse stimulation was applied to the motor cortex to determine the RMT. RMT refers to the minimum stimulus intensity required
to induce peak-to-peak MEPs > 50 μV of the contralateral FDI muscles in at least five out of 10 trials (Rossini et al. 1994). Following this, we measured AMT, the minimum stimulation intensity, during FDI muscle contraction, required to produce peak-to-peak MEPs of > 100 μV in three out five consecutive trials. Voluntary muscle contraction was elicited by participants pressing down their index finger against a spring; a press of approximately 20% of maximum voluntary contraction was maintained throughout delivery of the AMT stimulations. Next, the stimulator output intensity was increased to induce peak-to-peak MEPs of approximately 1 mV over 10 consecutive trials (i.e., 1 mV measure). The 1 mV measure was also determined by stimulating the motor cortex over the EEG cap and electrodes (i.e., 1 mV measure over cap) to provide appropriate stimulation parameters for Experiment 2. Refer to Table II for stimulator output values.

Measurement of motor inhibition/facilitation. To determine CSP, 20 single TMS pulses at 125% of AMT were administered to the motor cortex; during voluntary muscle contraction, the suppression of the FDI muscle was measured via EMG (Cantello et al. 1992). Following this, inter-neuronal activity in the motor cortex was assessed using SICI and ICF paradigms with paired pulse TMS. To obtain a measure of SIC, two pulses were delivered in rapid succession; the subthreshold conditioning pulse (90% of AMT) was first applied, then occurred a brief inter-stimulus interval (ISI) of 2 ms, followed by the suprathreshold test pulse (1 mV measure) (Kujirai et al. 1993). For ICF, the same two pulse intensities were administered, but with an increased ISI of 15 ms between the pulses. Twenty trials of each condition were applied in a random counterbalanced order.

**EMG measurement of motor inhibition/facilitation processing.** The peak-to-peak amplitudes of MEPs evoked by paired-pulse stimulations were analysed by Signal 3.8 (Cambridge Electronic Design, Cambridge, UK). The CSP duration was measured by calculating the time (ms) from the beginning of the MEP until the end of CSP (i.e., the resumption of tonic activity). For SICI and ICF, to assess their inhibitory and facilitatory effects on a test pulse, the mean MEP amplitudes were quantified as a percentage relative to the single pulse measure (i.e., 1 mV measure). Therefore, all EMG measures are relative to the single pulse, whereby, a response of 100% reflects no effect of TMS, a response less than 100% refers to an inhibitory effect and a response greater than 100% is suggestive of a facilitatory effect.

**Frontal cortical TMS paradigms**

**Electroencephalography (EEG).** To evaluate TMS induced cortical evoked activity in the frontal regions, EEG recordings were acquired through a Synamps EEG system (Compumedics Neuroscan, TX, USA). A custom-made 24-channel EEG cap was used to record the cortical signal, and individual electrodes were placed on the outer side of each eye, and above and below the left eye, so as to closely monitor eye movement artefact. All electrodes (sintered Ag/AgCl) were fastened in plastic electrode clips according to the standard 10–20 positions (EASYCAP GmbH, Germany) were referenced to an electrode placed posterior to the Cz electrode. These electrodes are not adversely affected by heating and present similar TMS artifacts to other TMS-compatible electrodes such as the C ring electrodes. EEG signals were recorded DC at a sampling rate of 20 kHz and filtered through a low pass filter of 3500 Hz to minimize TMS-related artefacts. Following every TMS discharge, the recharging capacitors were delayed by 1 s to avoid additional artefacts in the EEG data. The dynamic range of amplifiers was sufficient to avoid saturation during the TMS pulse. To reduce the effect of TMS click-induced auditory activation on the cortical evoked potentials, white noise (95 dB) was played through inserted earphones (Fitzgerald et al. 2008).

**Measurement of frontal cortical inhibition.** The LI CI paradigm involved delivering two suprathreshold pulses:

### Table II. Mean (unadjusted) and standard deviation of stimulus intensities and motor-evoked MEPs following single and paired-pulse stimulation.

| Condition | Participants detoxified from alcohol dependence (n = 12) | Healthy control participants (n = 14) | P value |
|-----------|------------------------------------------------------|-----------------------------|-------|
| RMT       | 50.67 ± 6.47                                         | 52.79 ± 8.20               | 0.006** |
| AMT       | 44.08 ± 6.56                                         | 44.64 ± 8.02               | 0.011* |
| 1 mV measure | 59.92 ± 7.73                                         | 62.43 ± 10.07             | 0.04*  |
| CSP       | 117.05 ± 30.00                                       | 113.39 ± 32.72            | 0.97   |
| SICI % of SP | 50.65 ± 43.44                                     | 33.95 ± 22.03             | 0.55   |
| SICI variability | 0.95 ± 0.33                                    | 0.73 ± 0.21               | 0.048* |
| ICF % of SP | 97.73 ± 74.39                                      | 142.78 ± 103.14           | 0.75   |
| ICF variability | 0.64 ± 0.41                                    | 0.55 ± 0.23               | 0.46   |
| 1 mV measure over cap | 70.58 ± 5.35                                   | 73.29 ± 7.82              | 0.026* |

RMT, resting motor threshold; AMT, active motor threshold; 1 mV, 1 millivolt; CSP, cortical silent period; SICI, short intracortical inhibition; ICF, intracortical facilitation; SP, single pulse. *P < 0.05, **P < 0.01.
the suprathreshold *conditioning* pulse (1 mV measured over cap), then, a long ISI of 100 ms, followed by a suprathreshold *test* pulse (1 mV measured over cap). Thus, the conditioning pulse inhibits the MEP produced by the test stimulus (Valls-Solé et al. 1992). The suprathreshold output intensity (i.e., 1 mV over the EEG cap) corresponded to 70.09 ± 5.32% in the AD patient group, and 73.29 ± 9.29% in the control group. LICI was delivered across both stimulation sites (left and right DLPFC), with 150 stimuli delivered to each site. The stimuli consisted of 75 single pulses (unconditioned *test* stimuli) and 75 paired-pulses (with *conditioned* stimuli followed by *test* stimuli) with an ISI of 3 seconds and were randomly counterbalanced between subjects to prevent order effects.

To assess LICI in the DLPFC stimulation was directed between AF3 and F3 (left DLPFC), and between AF4 and F4 (right DLPFC). The recording electrode of interest for the left DLPFC was F3, and the right DLPFC was F4, selected to optimally represent Brodmann areas (BA) 8/9 and 46 within the DLPFC on the medial frontal gyrus (Fitzgerald et al. 2009).

**EEG data processing.** The EEG recordings were processed offline with the commercially available software SCAN 4.3 (Compumedics Neuroscan) (refer to Additional Material available online at http://informahealthcare.com/doi/abs/10.3109/15622975.2015.1066512). The average signals at each recording site were calculated from the movement-free epochs (approximately 75 trials per subject) and average EEG waveforms (area under the curve (AUC)) were saved as rectified data and imported into SPSS version 17.

**EEG data analysis.** To obtain a primary measure of LICI, the AUC from averaged event-related potentials (ERPs) were examined. After importing each averaged rectified recording into SPSS via Excel for each subject, the AUC for the time-frame of 50–150 ms post TMS pulse, for both the single and paired-pulse condition, were averaged separately, and compared across the left DLPFC and right DLPFC stimulation sites. The first interval (i.e., 50 ms post-stimulus) was chosen as the earliest artefact-free data recorded post-stimulus.

For each subject, EEG inhibition was indexed by measuring the ratio of the AUC of the average paired-pulse potentials (conditioned) over the average single pulse potentials (unconditioned). The measure of LICI was represented by the following equation.

\[
1 - \frac{\text{Area under rectified curve (conditioned)}}{\text{Area under rectified curve (unconditioned)}} \times 100 \quad (A)
\]

Therefore, for the EEG measure, a LICI score of 0 reflects no TMS effect, while a LICI score greater than 0 reflects an inhibitory effect (with 100 being maximum inhibition), and a LICI score less than 0 suggests a facilitatory effect.

**Statistical analysis**

Comparability of the alcohol-dependent group and the controls subjects was assessed using χ²-tests for categorical and independent *t*-tests for continuous variables (see Table I). For frontal cortical stimulation, two-tailed paired *t*-tests were performed to assess suppression of the rectified EEG activity in the paired LICI condition compared to the single-pulse condition within groups. Analysis of covariance (ANCOVA), while controlling for age, BDI and standardized WTAR score, was also administered to calculate group differences in LICI-related measures. For motor cortical stimulations, an ANCOVA, which controlled for age, BDI and standardized WTAR, was used to measure group differences. The group values were reported as mean ± SD, and tests were run at an alpha level of 0.05. There were no significant violations of homogeneity of regression or unequal variance. All statistical analyses were performed using SPSS version 17 (SPSS Inc, Chicago, IL, USA).

We found that the sample of 12 ALD subjects and 14 CS was large enough to detect, under standard assumptions (minimum of 80% power, significance level of alpha = 0.05), between group differences exceeding an effect size of at least 0.332 (partial eta squared) across all significant findings. The sample size, which is consistent with previous pilot addiction TMS studies (Kähkönen et al. 2001, 2003; Conte et al. 2008b; Nardone et al. 2010), should therefore be sufficiently high to reveal moderate within-group differences.

There is the possibility that length of abstinence might possibly have influenced the findings; however when it was included as an additional covariate, the only effect was to reduce the still-significant difference between the groups in LICI of the right DLPFC from *P* = 0.006 to *P* = 0.036 while also significantly reducing the effect size and power observed. It also reduced the significant difference between the groups in LICI of the left DLPFC from *P* = 0.003 to *P* = 0.002 while increasing the effect size and power observed.

**Results**

**Frontal-cortical paradigms**

**Site 1. Left DLPFC.** Over the period of 50–150 ms post-stimulus, there was significant suppression of
the rectified EEG activity in the paired LICI condition compared to the single-pulse condition (M = 27.30, SD = 32.89, t = 2.95, df = 13, P = 0.011) in the control group (Table III). There was no significant LICI-related suppression of the rectified EEG activity in the alcohol-dependent group (M = -22.51, SD = 70.89). The difference between the groups in suppression of the rectified EEG activity in the paired LICI condition compared to the single-pulse condition was significant, f(1,21) = 11.68, P = 0.003. While, no significant differences were revealed between the groups in the single-pulse condition, f(1,21) = 0.00, P = 0.98.

Site 2. Right DLPFC. Over the period of 50–150 ms post-stimulus, there was no significant suppression of the rectified EEG activity in the paired LICI condition compared to the single-pulse condition located in either the control or alcohol-dependent group. However, there was a significant difference observed between the alcohol-dependent group (M = -12.98, SD = 73.68) and the control group (M = 16.16, SD = 49.17) in suppression of the rectified EEG activity in the paired LICI condition compared to the single-pulse condition, f(1,19) = 9.45, P = 0.006. While, no significant differences were revealed between the groups in the single-pulse condition, f(1,20) = 0.038, P = 0.85 (Table III).

Motor-cortical paradigms

Single-pulse stimulations. The alcohol-dependent group exhibited a significantly lower RMT, f(1,21) = 9.223, P = 0.006, and a significantly reduced AMT, f(1,21) = 7.669, P = 0.011, relative to controls. The alcohol-dependent group also presented with a significantly lower 1 mV measure, f(1,21) = 4.812, P = 0.04, as well as a reduced 1 mV measure over the EEG cap, f(1,21) = 5.710, P = 0.026, relative to controls (Table II).

Measurement of motor inhibition/facilitation. There were no significant differences between groups on measures of CSP duration, SICI or ICF (Table II). With regards to SICI, there was evidence of significantly increased SICI intra-variability in the alcohol-dependent (M = 0.95, SD = 0.33) relative to control group (M = 0.73, SD = 0.21), f(1,21) = 4.390, P = 0.048.

Discussion

This is the first electrophysiological study to report alterations in CI across both motor and frontal cortices in a clinical sample of patients with alcohol dependence. With regards to frontal excitability, the alcohol-dependent group exhibited reduced LICI in bilateral frontal cortices relative to healthy controls. In terms of motor excitability, the alcohol-dependent group demonstrated increased cortical excitability, as reflected by reductions in RMT, AMT and 1 mV threshold. No significant differences in motor cortex CSP, SICI or ICF were found between groups. However, significantly greater intra-trial-variability in SICI was observed within the alcohol-dependent group.

Frontal cortical inhibition

The present study provides the first direct measure of altered inhibitory neurotransmission in the frontal regions of patients with alcohol dependence. Significant differences in assessment of LICI induced paired-pulse inhibition were observed; the alcohol-dependent group exhibited reduced LICI related to impaired inhibitory neurotransmission in both the left and right DLPFC. This observation is strengthened by the lack of significant difference in cortical excitability between groups with respect to TMS-evoked response to single-pulses applied to the frontal regions. These findings suggest that the reduced LICI reflects altered inhibitory neurotransmission within the frontal regions rather than anatomical differences, such as frontal atrophy, in the alcohol-dependent population.

The current study specifically targeted cortical alterations within the PFC, a key structure within the mesocorticolimbic circuitry; as neural alterations within the PFC have been consistently implicated in

| Condition                          | Participants detoxified from alcohol dependence | Healthy control participants | P value |
|------------------------------------|-------------------------------------------------|------------------------------|---------|
| Left single pulse                  | 12                                              | 14                           | 0.98    |
| % inhibition left DLPFC            | 12                                              | 14                           | 0.003** |
| Right single pulse                 | 12                                              | 14                           | 0.85    |
| % inhibition right DLPFC           | 12                                              | 12                           | 0.006** |

DLPFC, dorsolateral prefrontal cortex. *P<0.05, **P<0.01.
the development and persistence of dependence (Goldstein and Volkow 2002; Feil et al. 2010). These frontal impairments in alcohol dependence (Moselhy et al. 2001) are associated with poorer treatment outcome and increased relapse (Noël et al. 2002). Neuroimaging studies have identified morphological evidence of reduced grey and white matter volume (Chanraud et al. 2007; Makris et al. 2008) reduced cortical thickness (Fortier et al. 2011) and altered regional cerebral blood flow (Noël et al. 2002) within the frontal lobes of alcohol-dependent patients. Our study complements this work by providing direct evidence of altered cortical excitability within the PFC of addicted subjects at a neurotransmitter systems level.

It is likely that the recorded prefrontal cortical effects reflect reduced GABAergic activity within the prefrontal components of mesocorticolimbic circuitry in the alcohol-dependent patients (Diana 2003; Addolorato et al. 2012). In assessing the motor cortex, a number of studies suggest that LICI relates to activity at the GABA<sub>B</sub> receptor. For example, the GABA<sub>B</sub> agonist baclofen potentiates LICI (McDonnell et al. 2006), leading to the proposition that LICI is GABAergic. Additionally, LICI inhibits SICI (Sanger et al. 2001), which is consistent with in vivo findings of suppression of GABA<sub>1</sub> receptor-mediated inhibition by presynaptic GABA<sub>B</sub> receptors (Werhahn et al. 1999). Furthermore, a strong relationship between LICI measured with EMG and EEG has also been identified (Farzan et al. 2010). As such, suppressed LICI in the prefrontal cortex in the alcohol-dependent group quite possibly reflects reduced GABA<sub>B</sub> receptor inhibitory neurotransmission within the frontal regions of alcohol-dependent patients. These findings provide initial clinical support for the neuromolecular models of alcohol dependence that suggest repeated alcohol use disrupts the delicate balance of cortical excitability.

Acute alcohol facilitates the GABAergic system by acting through GABA receptors (Enoch 2008; Filip and Frankowska 2008), while also suppressing glutamate release and glutamate receptor activity (in dopaminergic cells) (Gass and Olive 2008; Kalivas 2009); resulting in an overall inhibitory effect. After chronic alcohol exposure, the brain attempts to restore equilibrium in neuronal cell function (Tambour and Quertemont 2007), which leads to neuroadaptations within the mesocorticolimbic circuitry (Brodie 2002; Diana 2003). This leads to suppression of GABAergic neurotransmission and upregulation of glutamatergic neurotransmission and potentiation of NMDA receptor release (subtype of glutamate receptors). However, despite promising neuromolecular data regarding these compensatory alterations in cortical excitability, our study is the first to provide clinical evidence of compensatory mechanisms via suppressed inhibitory neurotransmission within the mesocorticolimbic circuitry of alcohol-dependent patients. Additionally, the persistence of these cortical alterations within the frontal cortex following detoxification provides initial support for allostatic models of addiction. During abstinence, it appears that altered frontal cortical excitability persists in the alcohol-dependent brain, with deviation from regular homeostatic operating levels (Koob and Le Moal 2001, 2005; Koob 2009). However, despite these promising findings, further large-scale studies are required to confirm our preliminary results.

Various treatment agents for alcohol dependence are being developed to target these alterations in neurotransmission; however, current pharmacotherapies provide only moderate clinical success (Tambour and Quertemont 2007; Addolorato et al. 2012). In terms of direct GABA<sub>B</sub> agonists, baclofen (Bucknam 2007; Leggio et al. 2010) or gabapentin (Bonnet et al. 1999; Bozikas et al. 2002) act by potentiating GABA<sub>B</sub> receptors on the cell body of dopamine neurons; designed to restore the balance of cortical excitability within the brain and alleviate symptoms associated with alcohol dependence. These pharmacotherapies however, have been primarily developed based on preclinical models, rather than from direct studies of altered CI in clinical subjects. In development of improved treatment agents, future pharmacological studies could utilize the frontal TMS-EEG technique to provide a pathophysiological account of how these GABA<sub>B</sub> agonists affect the balance of cortical excitability within the frontal cortex, and whether these changes relate to attenuated symptoms of alcohol dependence.

**Motor cortical inhibition**

**Single-pulse stimulation.** The alcohol-dependent group demonstrated significantly reduced RMT, AMT and 1 mV thresholds relative to the control group, suggestive of increased excitability within the motor cortex. Our study is the first to identify these reductions in threshold and are consistent with the chronic effects of alcohol on glutamate receptors (Gass and Olive 2008); i.e., compensatory neuroadaptations counterbalance the acute effects of alcohol exposure, suppress cortical inhibition, and lead to the upregulation of NMDA receptors (Tzschenkte and Schmidt 2003). As such, these reductions in threshold are likely to represent hyper-excitability within the cortex associated with chronic alcohol consumption.

**Paired-pulse stimulation.** Short interval intracortical inhibition (SICI) is a paired-pulse stimulation
measurement which is employed to reflect activity of inter-neurons in the motor cortex (Kujirai et al. 1993). SICI is proposed to represent GABA_A receptor-mediated inhibitory neurotransmission in the motor cortex (Ziemann et al. 1996). Although acute ethanol exposure was found to dose-dependently facilitate SICI (Ziemann et al. 1995), no observable differences in SICI have been identified within alcohol-dependent populations (Conte et al. 2008a; Nardone et al. 2010). In the present study, the alcohol-dependent group showed near significant reduced SICI (i.e., lack of inhibition) and significantly increased SICI intra-trial-variability. However, the ability of SICI to identify alcohol-dependent-related cortical alterations from the motor cortex remains unclear. To our best knowledge, there have been no previous reports describing increased SICI intra-trial-variability in alcohol-dependent groups. The lack of reported variation may have not been observed or statistically addressed. Therefore, we advise that future studies, which assess SICI within addiction samples, should also evaluate intra-trial variability to improve our understanding of the SICI measure and its relevance within addiction populations.

Study limitations

Although our study indicates the presence of altered cortical excitability within an alcohol-dependent sample post-detoxification, there are several limitations that should be noted. Firstly, although we suggest that LICI is mediated through GABA_B receptors, the potential involvement of additional neurotransmitters cannot be ruled out and requires further assessment. In terms of the TMS-EEG technique, although we suggest that inhibition of TMS-induced EEG activity is cortical in nature, other sources, such as the click sound delivered concomitantly with each pulse, may also stimulate the auditory cortex and associated areas. However, previous studies have reported that white noise at 95 dB, as we employed, is sufficient to abolish any such effect (Daskalakis et al. 2008). Additionally, despite being addressed statistically, significant group differences were observed in terms of reported age, depressive symptoms and standardized WTAR scores. It is important to note that elevated depressive symptoms are common in addicted samples and to address this issue we excluded any individuals who met clinical criteria for depression. Similarly, with the WTAR, although there were significant differences between groups, relative to standardized assessment of the WTAR, the alcohol-dependent group was well within the normal range of intellectual functioning. In our preliminary study, abstinence was determined by an over-the-phone medical interview with a trained research assistant and followed by a secondary screening procedure to confirm detoxification through completion of the Timeline Follow Back calendar. However, we recommend that future larger scale studies also include a measure of ethyl glucuronide to provide a physiological measure of abstinence. Finally, given the cross-sectional nature of the study, it is difficult to disentangle whether altered cortical excitability is a direct result of chronic alcohol exposure, relates to pre-existing vulnerabilities, or rather a combination of both (Feil et al. 2010). Moreover, it is plausible that alcoholism-related changes in macroscopic anatomy could contribute to the results; however, given that there was no significant difference between the groups in cortical response to the single pulse, it seems more likely that our results are indicative of differences in cortical inhibition (i.e., response to the paired-pulse). Despite these limitations, our study is the first to demonstrate altered neurotransmission within the frontal regions of alcohol-dependent individuals post-detoxification. While our study sample was small in size, and the results preliminary in nature, the study provides an initial proof of concept, with further studies required to replicate and extend our findings.

Conclusion

Our study demonstrated that TMS-EEG is a promising approach to indexing cortical excitability within the frontal cortex of addicted populations. In particular, we found suppressed CI in the frontal cortex and increased cortical excitability within the motor cortex of alcohol-dependent patients post-detoxification. Our finding of suppressed inhibitory neurotransmission (altered CI) within the frontal cortex is the first report of altered cortical excitability within the frontal cortex of alcohol-dependent patients. Further research is required to confirm and extend our findings, as well as assessing the potential role of TMS-EEG in indexing the impact of pharmacotherapy on frontal function across a range of substance-dependent populations.

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