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ORIGINAL RESEARCH

Deep TMS of the insula using the H-coil modulates dopamine release: a crossover [^{11}C] PHNO-PET pilot trial in healthy humans

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Abstract

Modulating the function of the insular cortex could be a novel therapeutic strategy to treat addiction to a variety of drugs of abuse as this region has been implicated in mediating drug reward and addictive processes. The recent advent of the H-coil has permitted the targeting of deeper brain structures which was not previously feasible. The goal of this study was to bilaterally target the insular region using the H-coil with repetitive Transcranial Magnetic Stimulation (rTMS) and subsequently measure changes in dopamine levels using Positron Emission Tomography (PET) with [11C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO). This was a within-subject, crossover, blinded and sham-controlled pilot study. Eight healthy, right-handed subjects, aged 19–45, participated in the investigation. All subjects underwent 3 PHNO-PET scans preceded by rTMS (sham, 1 Hz or 10 Hz), on 3 separate days. Low frequency rTMS (1 Hz), targeting the insular cortex, significantly decreased dopamine levels in the substantia nigra, sensorimotor striatum and associative striatum. Replicating this study in tobacco smokers or alcoholics would be a logical follow-up to assess whether H-coil stimulation of the bilateral insula can be employed as a treatment option for addiction. Trial registration: NCT02212405

Keywords Insula · PET · Dopamine · H-coil · Deep rTMS · PHNO

Abbreviations

RTMS	Repeated Transcranial Magnetic Stimulation
PET	Positron Emission Tomography
PHNO	[11C]-(+)-propyl-hexahydro-naphtho-oxazin
BPND	[11C]-(+)-PHNO specific binding

Introduction

The insular cortex is a neural substrate submerged within the lateral sulcus of the brain. It has reciprocal connections with numerous cerebral regions including the orbitofrontal cortex, thalamus, amygdala, anterior cingulate cortex, and

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globus pallidus, and has functionally been implicated in gustation, interoception, language and speech, emotion, attention and decision making, feeding behavior, verbal memory and as the viscero and somatosensory cortex (reviewed in (Augustine 1996; Gasquoine 2014)). The past decade has extensively favored the insula as a mediator of drug reward and addictive processes (Droutman et al. 2015; Naqvi and Bechara 2009, 2010; Naqvi et al. 2014; Volkow and Baler 2015).

Addiction, or more specifically, substance use disorder, is described as compulsive intake of a drug of abuse despite recognition of the harmful consequences. In humans, the insula has been associated with the facilitation of drug reward as activity within this region correlates with subjective cue-induced drug urges (craving) (Naqvi and Bechara 2009) and damage to this structure disrupts addiction to cigarette smoking (Naqvi et al. 2007). In animals, interference with insular activity, both electrically and chemically, has been shown to alter drug-seeking behavior (Contreras et al. 2007, 2012; Forget et al. 2010; Pushparaj et al. 2013; Scott and Hiroi 2011). Collectively, these data suggest that sensitization of the insular cortex may perpetuate addiction, whereby insular injury restores balanced function. Modulating activity in this structure could therefore potentially be a novel therapeutic strategy to treat addiction.

The neurochemical basis of addiction involves dopamine. The mesolimbic dopamine system, originating in the ventral tegmental area and projecting to the nucleus accumbens, is fundamental for instrumental behavior and drug motivation (Berridge and Robinson 1998; Di Chiara 2000, 2002; Everitt et al. 1999, 2001; Robbins et al. 1989; Salamone 1992; Salamone and Correa 2002; Salamone et al. 2003; Schultz 2002, 2006; Schultz et al. 1997; Shalev et al. 2002). Radiotracer imaging in humans has shown that various drugs of abuse, including tobacco, amphetamine, cannabis and alcohol (Barrett et al. 2004; Boileau et al. 2003; Bossong et al. 2009) result in dopamine release in vivo. Interestingly, evidence suggests that repetitive transcranial magnetic stimulation (rTMS) can also influence the dopaminergic circuitry (Strafella et al. 2001).

rTMS is a non-invasive modality that appears to be promising for the treatment of neuropsychiatric disorders. Technically, rTMS modulates neuronal activity via the induction of electrical currents through time shifting magnetic field pulses. Changes are reported to occur through alterations in cortical excitability, blood flow and/or the release of neurotransmitters or growth factors (Bestmann et al. 2005; Cho and Strafella 2009; Gersner et al. 2011; Noda et al. 2015; Strafella et al. 2003). In humans, rTMS-induced transient inactivation of specific neural substrates may also occur via mechanisms that are associated with potentiation of $GABA_B$ receptor-mediated inhibitory neurotransmission (Daskalakis et al. 2006). Local changes in the dynamic release patterns of various neurotransmitters, including dopamine (Keck et al. 2000, 2002; Strafella et al. 2001, 2003) can also play a role. In vivo evidence that rTMS of frontal brain regions has a modulatory effect on the dopaminergic system, suggests that rTMS may be useful in psychiatric illnesses linked to dopamine dysfunction, such as addiction (Strafella et al. 2001; Volkow et al. 2009). The recent advent of deep rTMS coils, in particular a unique version of the H-coil, has permitted rTMS targeting deeper brain structures such as the insula (Roth et al. 2002, 2007; Zangen et al. 2005). It is thought that adaptation of rTMS parameters enables either stimulation (increased excitability) or inhibition (decreased excitability) of a brain structure (Daskalakis et al. 2006).

Given the involvement of the dopamine system in addiction and the putative role of the insula in regulating addiction-like behaviors, the goal of the present study was to investigate whether low (1 Hz) and high (10 Hz) frequency H-coil-based rTMS targeting the insular region bilaterally alters synaptic concentration of dopamine in reward-processing brain regions in humans as measured with Positron Emission Tomography (PET), using [11C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO) as the receptor ligand. PHNO is a potent $DRD_{2/3}$ receptor agonist (Wilson et al. 2005) which has been shown to provide an accurate and reliable estimate of the $DRD_{2/3}$ specific binding signal. There is significant binding in $DRD_{2/3}$ -rich areas, while there is no specific binding in the cerebellum, a neural substrate virtually devoid of these receptors (Graff-Guerrero et al. 2008). Known properties of ligand–receptor interactions suggest that agonist radiotracers, such as PHNO, are more sensitive than antagonist radiotracers (e.g. raclopride) for measuring fluctuations in dopamine, and this is supported by empirical evidence (Cardenas et al. 2004; Carson et al. 1997; Ginovart et al. 2004, 2006, 2007; Narendran et al. 2006; Tsukada et al. 2002; Willeit et al. 2008). Accordingly, data suggest that PHNO has advantageous properties for exploration of DRD_3-preferred sites (Rabiner et al. 2009). Based on the current literature and using the PHNO-PET methodology, we expected low frequency deep rTMS to decrease striatal dopamine levels and high frequency deep rTMS to increase them (i.e. increase and decrease PHNO binding potential, respectively).

Materials and methods

Subjects

All study procedures were approved by the Centre for Addiction and Mental Health's (CAMH) Research Ethics Board. Eight healthy adult subjects (4 female, 4 male) were recruited from the community and completed all study related procedures at CAMH. After providing written

informed consent, participants underwent a comprehensive clinical assessment involving both physical and psychiatric examinations, routine blood tests, urine toxicology screen and a 12-lead electrocardiogram. Exclusion criteria included the following: (1) Abnormal blood work, ECG or toxicology screen, (2) contraindications to MR scanning or TMS, such as presence of ferromagnetic objects in the body (3) claustrophobia, (4) cardiovascular or cerebrovascular diseases, (5) major psychiatric disorder(s) (i.e. Axis I Disorders as per DSM-IV) (First et al. 2002), (6) history of, or current neurological illness(es), including seizure disorders, (7) gross structural brain abnormalities as revealed by T1 weighted images, (8) current use or use during the previous month of medication that may affect the CNS, (9) learning disability, amnesia or other conditions that impede memory and attention, (10) exposure to radiation in the last 12 months exceeding limits for subjects participating in research with PET and (11) pregnancy (for females). Cognitive questionnaires (namely, the Barratt Impulsivity Scale, and Kirby's Task) were administered to measure decision and impulsive behavior. Alcohol and caffeinated beverage consumption during the 7 days leading up to their initial assessment visit was recorded using the Timeline Followback (TLFB) tool. Subject recruitment started July 2014 and all data collection was complete by March 2015.

Paradigm

This was a within-subject, cross-over, double-blinded and sham-controlled pilot study. Subjects underwent three PET scans paired with rTMS on separate days. The three rTMS conditions were sham, 1 Hz and 10 Hz. The sham condition was administered on PET day 1 while the 1 Hz and 10 Hz conditions were counterbalanced over PET days 2 and 3. The randomization code was established by a researcher who was not otherwise associated with the investigation, and participants and trial personnel alike were blinded to the sessional treatment. To this effect, a sealed envelope specifying the sequence of the rTMS treatments (sham, 1 Hz or 10 Hz) was provided directly to the technician administering the stimulation. Sessions were a minimum of 4 days apart. In order to minimize the delay between the end of stimulation and administration of the radiotracer, participants were fitted with an intravenous line for the injection prior to the rTMS procedure. On average, the time lag was approximately 19 min. Subjective assessments of rTMS

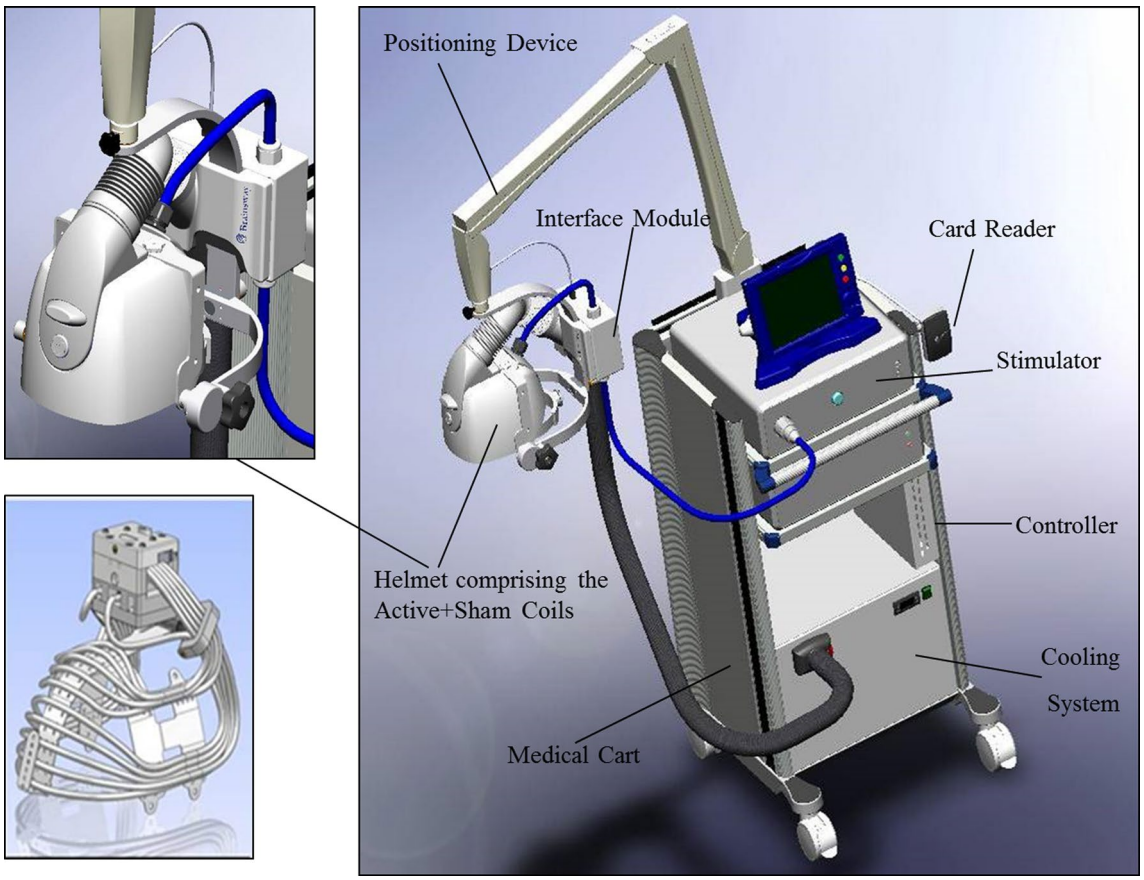

Fig. 1 H-coil deep TMS system (Brainsway, Jerusalem, Israel; figure courtesy of Professor Abraham Zangen)

effects were collected at each experimental condition, and ten point visual analog scales rating mood and appetite were administered at three time points: before rTMS, after rTMS and after PET. This data was collected to determine if rTMS and/or PET modulated these measures. Heart rate and blood pressure were monitored at regular intervals during the scan (0, 30, 60 and 90 min). A MRI scan was performed on a separate day.

rTMS procedure

rTMS was administered over the insula bilaterally using an H-coil (Model 102B, Brainsway, Jerusalem, Israel; Fig. 1). This product is composed of the HLRIADD electromagnetic coil and is designed to target the insular cortex bilaterally, with hemispheric symmetry. rTMS was given for a duration up to 30 min, prior to the PET scan. The resting motor threshold (RMT) was determined by stimulation of the left motor strip and finding the minimum intensity to cause activation of the right abductor pollicis brevis muscle in a minimum of 5 of 10 trials. Low-frequency stimulation was performed using 20 trains of 50 s each, with an inter-train interval of 15 s. Each stimulation train consisted of 50 pulses at 1 Hz. High-frequency stimulation parameters comprised 34 trains of 3 s each at 10 Hz and 30 pulses per train. Stimulation intensity was delivered at 120% RMT. An advanced sham rTMS condition using a pseudo coil (HLRIADD sham coil) built into the same helmet housing the active coil was employed. The sham coil has a circular shape and is placed perpendicular to the scalp within the helmet. This coil mimics the active coil with regards to acoustics and scalp sensation, and it is designed to generate comparable activation of facial muscles, without actually stimulating the brain. A magnetic card reader selectively activated either the sham or the real coil. Participants were given ear plugs to wear to counter the "clicking" noises produced by the coil during stimulation discharge.

PET image acquisition

Neuroimaging data was acquired on the CPS-HRRT PET camera system (Siemens Medical Imaging, USA), which has an in-plane resolution of approximately 2.8 mm full-width at half-maximum (FWHM). In order to minimize motion, all participants received a tailor-made thermoplastic mask (Tru-Scan Imaging, USA) prior to scanning. A ^{137}Cs ($T_{½}$=30.2 year, E=662 keV) single photon point source was utilized to obtain the transmission data, while the emission data was reconstructed by filtered-back projection. Total scanning time was 90 min and 30 frames were specified. The first 15 frames were 1 min each and the subsequent 15 were 5 min each. The radiosynthesis of [^{11}C]-(+)-PHNO has been previously reported (Wilson et al. 2005).

MRI image acquisition

To enable the localization of ROIs for the PET analysis, subjects underwent a standard proton density-weighted brain magnetic resonance imaging (MRI) scan on a General Electric Discovery MR750 3.0T MRI scanner (slice thickness: 2 mm; interleaved; slice number: 86; repetition time: 6004 ms; echo time: 8 ms; number of excitations, 2; acquisition matrix: 256×192; FOV: 22×16.5).

PET image analysis

Region of interest (ROI)-based analysis

ROI mapping and time activity curve analyses were performed using the Regions of Mental Interest (ROMI) software (Rusjan et al. 2006). Demarcated regions comprise the globus pallidus (GP; whole), substantia nigra (SN) and the striatum (Boileau et al. 2012; Martinez et al. 2003). Functional striatal subdivisions that were selected include the limbic striatum (LST), the sensorimotor striatum (SMST) and the associative striatum (AST) (Martinez et al. 2003). [^{11}C]-(+)-PHNO specific binding (BP_{ND}) was quantified in each ROI using the simplified reference tissue method (Lammertsma and Hume 1996) (SRTM), employing the cerebellar cortex (minus the vermis, lobules IX and lobule X) as the reference region. Parameter estimation was realized using PMOD (Version 2.8.5; PMOD Technologies Ltd, Zurich, Switzerland).

Receptor occupancy, defined as the percentage change in [^{11}C]-(+)-PHNO BP_{ND} from sham scan to the active rTMS scan (1 and 10 Hz, separately) was calculated for each subject as per the following equation:

$$\%Occupancy = \frac{BP_{ND}sham - BP_{ND}(1or10Hz)}{BP_{ND}sham} \times 100$$

Comparisons between [^{11}C]-(+)-PHNO $D_{2/3}$ BP_{ND} in the selected regions of interest, were conducted using a repeated-measures ANOVA with two within-subject factors (*ROI* X rTMS conditions*)*. Sphericity was assessed using the Mauchly test and, corrections were made with Greenhouse-Geisser adjustments, if required. When appropriate, Bonferroni post-hoc tests were applied to determine the significance of regional differences in BP_{ND} between conditions and groups. Subsequent to a two-way ANOVA, one way ANOVAs (followed by Bonferroni correction) were performed on the above-specified ROIs. All statistical analyses were performed using Graphpad Prism Version 6.0.

Results

Of the eight subjects that completed the study, one female individual was excluded from the final analysis as she was an outlier in the ROI analysis. Analyzed participants (n = 7) were all healthy non-smokers with a mean age of 30 ± 9.9 (21–44 years old) and a body mass index of 25.3 ± 3.4 kg/m^2 (mean ± SD). Plasma cotinine, hydroxycotinine and serum nicotine were all < 1 ng/ml for all individuals. Average hematocrit (L/L) was 0.42 (SD: 0.03). Timeline followback scores (number of drinks ± SD) were 3.29 ± 5.6 for alcohol, and 5.57 ± 5.7 for caffeine. Subjects tolerated the rTMS and PET sessions without any serious adverse effects. Specifically, rTMS was not associated with any side effects such as headaches, tinnitus or general discomfort. Anecdotally, some patients experienced jaw twitching due to the activation of the temporalis muscle during stimulation, but reported feeling no pain and the stimulation was well endured. Likewise, the PET sessions were not associated with any medical issues other than PHNO-induced nausea in a single subject (at the 1st PET scan). The nausea, a recognized effect of PHNO administration, was of mild severity and dissipated within 15 min of the PHNO injection. Scan parameters were consistent across the three rTMS treatment conditions (mean mass Injected (μg) across all scans 2.31 ± 0.24; mean amount Injected (mCi) 8.8 ± 1.3; mean specific activity (mCi/μmol) 966.2 ± 221.6). Vital signs information (temperature, blood pressure, respiration and heart rate) collected at each visit was within the normal range.

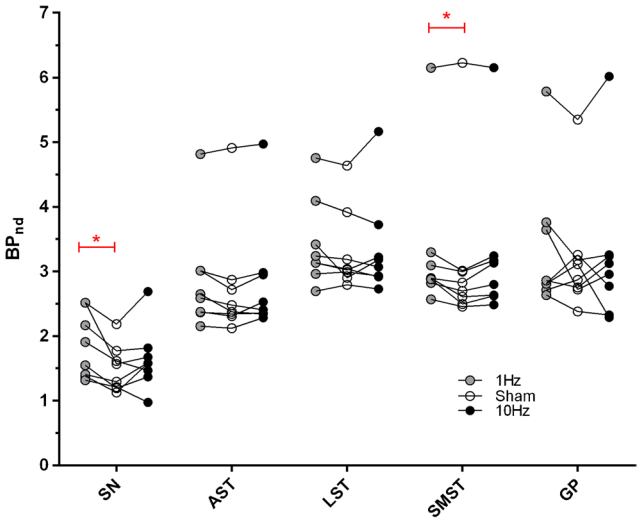

Fig. 2 Regional [11C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO) binding (BP_{ND}) following H-coil-based repeated Transcranial Magnetic Stimulation (rTMS) of the bilateral insula in healthy subjects. Eight subjects (4 male, 4 female) underwent three rTMS sessions (Sham, 1 and 10 Hz) on separate days followed by Positron Emission Tomography using PHNO. Excluding the outlier (n = 7), two-way ANOVA showed a main effect of condition (sham versus 1 Hz). Subsequent 1-way ANOVAs revealed significant differences between the sham and 1 Hz conditions in the substantia nigra and the sensorimotor striatum. SN: substantia nigra; AST: associative striatum; LST: limbic striatum; SMST: sensorimotor striatum; GP: globus pallidus (*p < 0.05)

ROI analysis

Two-way repeated measures ANOVA examining ROI-specific [^{11}C]-(+)-PHNO binding and condition (sham, 1 Hz, 10 Hz) was performed. ROIs included the substantia nigra (SN), sensory motor striatum (SMST), associative striatum (AST), globus pallidus (GP) and limbic striatum (LST). Treatment order (sham-1 Hz-10 Hz versus sham-10 Hz-1 Hz) was initially incorporated into the statistical

Table 2 Mean receptor occupancy (percentage change in [^{11}C]-(+)-PHNO BP_{ND} from sham scan to the active rTMS scan (1 and 10 Hz, separately))

Region of interest	% Occupancy 1	% Occupancy 2
SN	24.4	8.3
AST	5.2	3.7
LST	3.7	−0.09
SMST	7.2	5.0
GP	5.4	−0.9

*Occupancy 1 = (1 Hz/Sham-1)*100; Occupancy 2 = (10 Hz/Sham-1)*100

SN substantia nigra, *AST* associative striatum, *LST* limbic striatum, *SMST* sensorimotor striatum, *GP* globus pallidus

Table 1 Mean regional BP_{ND} values across 3 conditions (n = 7)

	Sham		1 Hz		10 Hz	
	Mean	SD	Mean	SD	Mean	SD
SN	1.4	0.25	1.8	0.46	1.5	0.27
AST	2.5	0.26	2.6	0.33	2.5	0.29
LST	3.1	0.37	3.2	0.44	3.1	0.32
SMST	2.7	0.23	2.9	0.23	2.9	0.31
GP	2.9	0.31	3.0	0.46	2.9	0.41

SN substantia nigra, *AST* associative striatum, *LST* limbic striatum, *SMST* sensorimotor striatum, *GP* globus pallidus

Table 3 Visual analogue scale data (mean and SD). T1: pre-rTMS; T2: post-rTMS; T3: post-PET

	Sham			1 Hz			10 Hz		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Hunger	4.07 (2.2)	3.6 (2.3)	4.04 (1.9)	1.7 (1.1)	1.13 (0.6)	2.94 (2.4)	1.56 (1.3)	0.99 (1.0)	2.19 (1.6)
Bored	3.5 (1.7)	3.7 (1.6)	2.13 (0.97)	1.81 (0.8)	2.59 (1.5)	2.19 (1.7)	1.5 (1.0)	1.86 (1.4)	2.24 (2.3)
Irritable	0.59 (0.7)	0.94 (1.0)	0.99 (0.6)	0.76 (0.3)	1.73 (1.4)	2 (1.8)	1.09 (0.8)	1.77 (1.0)	1.89 (1.7)
Discomfort	1.77 (1.5)	2.34 (1.7)	1.43 (1.0)	1.3 (1)	1.83 (1.3)	2.57 (2.5)	1.64 (1.5)	1.96 (1.5)	2.31 (2.5)
Craving salt	1.61 (1.2)	1.27 (0.8)	1.97 (1.7)	1.07 (0.7)	1.27 (1.2)	2.04 (2.3)	0.9 (0.7)	0.99 (0.9)	1.7 (1.7)
Craving sweet	1.49 (0.6)	1.3 (0.3)	1.3 (0.5)	1.37 (1.2)	1.07 (0.5)	1.76 (2.1)	0.86 (0.7)	0.86 (0.6)	1.67 (1.9)

model yet since it was not significant ($F(8,40) = 0.62$, $p = 0.75$), it was discarded from the analysis. There was a significant effect of condition ($F(2,12) = 5.6$; $p = 0.02$) and ROI ($F(4,24) = 80.3$, $p < 0.0001$) but no interaction effect ($F(8,48) = 0.83$, $p = 0.58$). Bonferroni's multiple comparisons test revealed a significant difference between sham and 1 Hz conditions. Subsequent repeated measures one way-ANOVAs comparing the above selected ROIs across

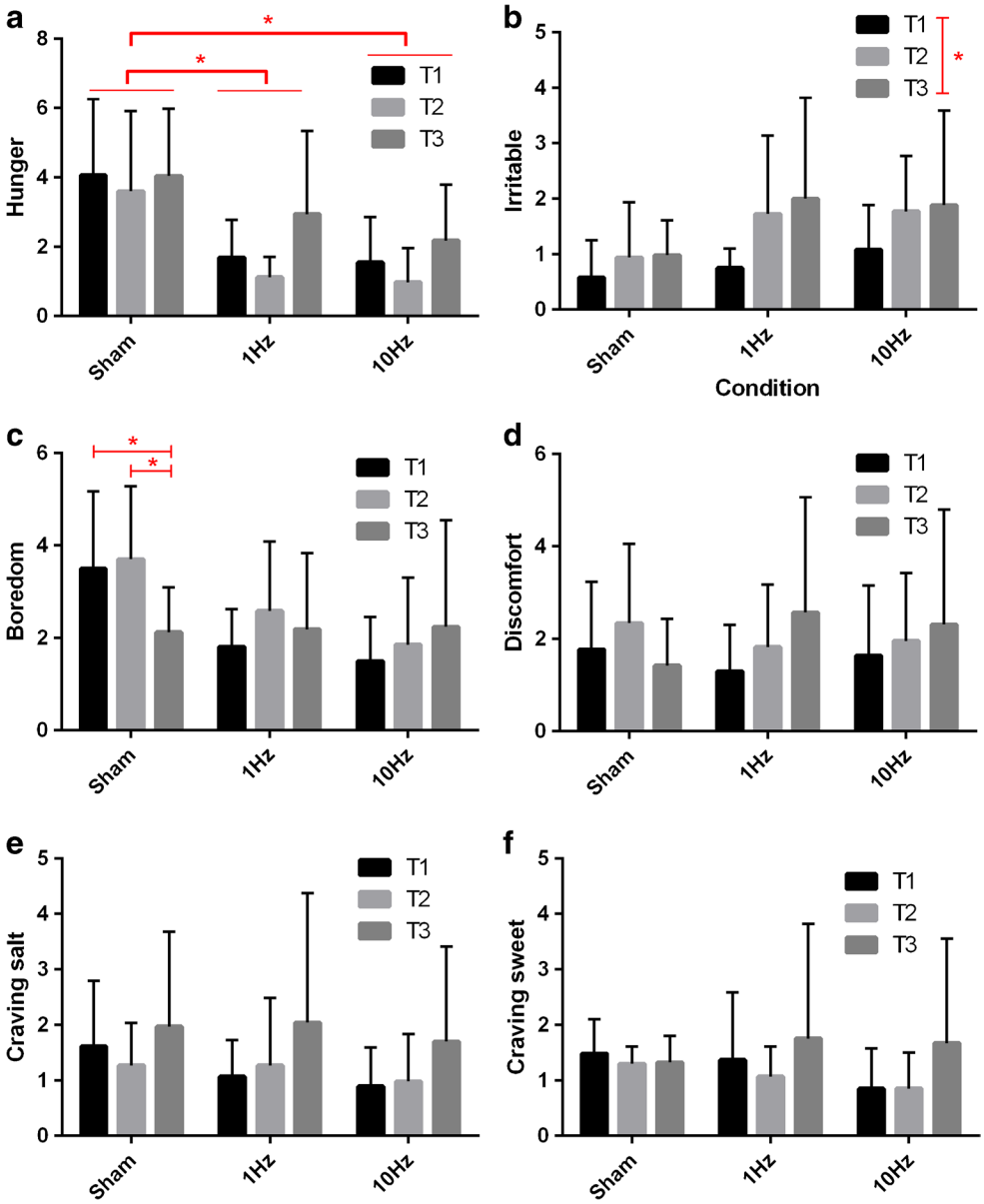

Fig. 3 Visual Analog Scale data across time and condition (n = 7). All values represent the mean score ± standard deviation: **a** hunger, **b** irritability, **c** boredom, **d** discomfort, **e** craving salt and **f** craving sweet. Two-way ANOVAs showed no significance for measures of discomfort, craving sweet and craving salt. A main effect of condition (sham > active) was observed for the hunger measure. Time and condition were main effects for irritability scores, with pairwise comparisons showing increased irritability at T3 versus T1. Boredom showed an interaction effect (sham: T1 > T3 and T2 > T3) (*$p < 0.05$)

the three treatment conditions showed a significant effect across condition in the SN ($F(2,20) = 5.06$, $p = 0.026$) and SMST ($F(2,20) = 7.47$, $p = 0.0078$). The AST approached significance ($F(2,20) = 3.63$, $p = 0.058$). Bonferroni's pairwise multiple comparison test showed a significant difference between sham and 1 Hz, with an increase in [^{11}C]-(+)-PHNO BP_{ND} in the 1 Hz condition, in both the SN and the SMST (Fig. 2 and Table 1). Receptor occupancy (percentage change in [^{11}C]-(+)-PHNO BP_{ND} from sham scan to the active TMS scan) was calculated for each subject. Mean regional values are summarized in Table 2.

VAS data

Mean VAS data is summarized in Table 3 and Fig. 3. Two-way repeated measures ANOVA was used to examine visual analog scale (VAS) scores at 3 different time points (pre-rTMS (T1), post-rTMS (T2) and post-PET (T3)) during the 3 testing conditions (sham, 1 Hz, 10 Hz). A separate ANOVA was conducted for each measure (hunger, irritable, bored, discomfort, craving sweet, craving salt). No significance was observed for the measures of discomfort, craving sweet, or craving salt. There was a main effect of condition ($F(2,12) = 7.34$, $p = 0.008$) for the hunger measure, such that scores were higher in the sham condition relative to the active conditions, yet no effect of time ($F(2,12) = 2.55$, $p = 0.12$) nor an interaction ($F(4,24) = 2.049$, $p = 0.12$). The irritability scores showed a main effect of both condition ($F(2,12) = 3.90$, $p = 0.05$) and time ($F(2,12) = 6.42$, $p = 0.013$) but no interaction effect ($F(4,24) = 0.47$, $p = 0.76$). Pairwise comparisons only showed increased irritability in T3 versus T1. The boredom scores exclusively showed an interaction effect ($F(4,24) = 3.18$, $p = 0.031$; condition: ($F(2,12) = 2.77$, $p = 0.10$; time: $F(2,12 = 0.75$, $p = 0.49$)). Bonferroni's multiple comparison test revealed a difference in T1 versus T3, and T2 versus T3 in the sham condition.

STAI and Kirby's task

Mean score on State Trait Anxiety Inventory (STAI) was 28.43 (SD: 8.2), and on the Kirby's task 0.025 (SD: 0.02). No correlations were observed between BP_{ND} in the 5 pre-selected ROIs and these measures.

Discussion

To the best of our knowledge, this is the first study to pair deep rTMS using the H-coil targeting the insula bilaterally, with dopamine-related PET imaging. The main finding of this investigation was that dopamine levels were significantly decreased following low frequency rTMS in the substantia nigra (SN) and the sensorimotor striatum (SMST), in healthy subjects. The associative striatum (AST) approached significance. In these regions (SN, SMST, AST), the mean receptor occupancy, expressed as percentage change in BP_{ND} from sham scan to the 1 Hz scan, was 24.4, 7.2 and 5.2, respectively. No rTMS-specific changes in self-reported behavioral measures were observed.

Deep rTMS with the H-coil is a novel approach that is now being explored as a potential treatment for addiction. In a 2014 study by Dinur-Klein and colleagues, high frequency (10 Hz) deep rTMS of the insular and prefrontal cortices with the H-coil was shown to significantly decrease cigarette consumption and nicotine dependence in heavy smokers (Dinur-Klein et al. 2014). While this effect was not observed with low frequency (1 Hz) stimulation, the elevated frequency (10 Hz) yielded a 33% abstinence rate 6 months post termination of the treatment. A reduction in cigarette consumption was also observed in this group's earlier study however the effect was temporary and involved 10 Hz stimulation of the dorsolateral prefrontal cortex (DLPFC) using the standard figure 8 coil (Amiaz et al. 2009). Notably, post-treatment abstinence was also attained in nicotine smokers when 1 Hz stimulation of the DLPFC (with the figure 8 coil) was paired with nicotine replacement therapy in smokers motivated to quit (Trojak et al. 2015). The authors suggest that rTMS may be effective in reducing "compulsivity". The present work employing the H-coil showed the opposite effect to low and high frequency stimulation relative to the Dinur-Klein investigation; however, the main point of divergence between the two studies is that our population consisted of healthy individuals. It is not surprising then that the response between the two groups would vary. Multiple studies have shown that changes in affect or behavior induced by rTMS differ in healthy versus clinical populations (e.g. depressed or addicted) and likely reflect differences in neurochemistry and/or structural organization/connectivity/architecture (Ko et al. 2013). Also, while it is widely accepted that low frequency rTMS inhibits neural excitation, the mechanism of action of high frequency stimulation remains controversial. A number of investigations state that it increases neural excitability (Feil and Zangen 2010; Hoogendam et al. 2010; Pascual-Leone et al. 1994; Pell et al. 2011) while others argue that it may also enhance inhibition (de Jesus et al. 2014; Fitzgerald et al. 2006).

Craving is a common and persistent feature of addiction that appears to emerge from activation of the reward circuitry. Neuroimaging studies have shown that numerous brain regions are implicated in this phenomenon including the anterior cingulate cortex, insula, amygdala and DLPFC (Naqvi et al. 2014; Parvaz et al. 2011). Studies involving repeated TMS of the DLPFC generally favor a decrease in spontaneous or cue-induced nicotine craving subsequent to high frequency stimulation (reviewed in (Gorelick et al. 2014; Jansen et al. 2013)). Notably, one study revealed

that inactivation of the DLPFC with low frequency rTMS reduced both cue-induced craving, and functional MRI signal in the OFC, ventral striatum and anterior cingulate (Hayashi et al. 2013). The DLPFC, especially the left side, has been a frequent target of rTMS paradigms primarily because of its role in treating depression and addiction, but perhaps also because it is relatively accessible as a target site (Daskalakis et al. 2008; Grall-Bronnec and Sauvaget 2014). Importantly, evidence suggests that the frontal cortex can alter dopamine release in the striatum, OFC and anterior cingulate cortex (Cho and Strafella 2009; Karreman and Moghaddam 1996; Keck et al. 2002; Pogarell et al. 2006, 2007; Strafella et al. 2001, 2003) and this may partially explain its role in 'craving'. Here, we attempted to examine the role of the insula in craving (Naqvi et al. 2014) following deep rTMS at various frequencies using the H-coil which can penetrate brain structures and/or circuits 5–7 cm deep. We did not see any relevant change in self-reported craving for food in general, nor for salty or sweet foods, specifically. This is not surprising given that metabolic state (i.e. fasted or satiated) was not controlled in this investigation, nor were these subjects afflicted with any malady that would affect their craving for nutrients.

A number of limitations are evident in this study. First, we cannot definitively confirm that we are actually stimulating the insular cortex with the H-coil, nor rule out the fact that surrounding tissue and/or structures are not being modulated. In fact, it is generally accepted that TMS-induced excitation of one region will likely influence other areas. While there is limited data with the insula/ H-coil, several reports have disclosed that the standard TMS method of targeting of the DLPFC is often up to 2 cm off mark (reviewed in (Gorelick et al. 2014)). Neuroanatomical variability across subjects cannot be overlooked either (Hanlon et al. 2012; Mylius et al. 2013; Peleman et al. 2010). Second, the acute rTMS paradigm (single session) may have been sub-optimal for detecting maximal changes in the dopamine system in healthy subjects even though measures were in place to minimize the time lag between the end of the stimulation protocol and the start of the PET scan. Notably however, low-frequency repetitive TMS is believed to disrupt the target cortex for a minimum of 30 min (Lee et al. 2003; O'Shea et al. 2007). Indeed, we did not observe changes following the high frequency stimulation. Third, the study design was flawed in that it was only partially counterbalanced. While the active rTMS conditions were counterbalanced across participants, sham rTMS was always administered first. Nonetheless, a statistical effect of treatment order was not observed. Fourth, being a pilot study, the sample size (n = 7) was small. Though we were able to see significant changes in BP_{ND} in the SN, SMST and AST (borderline), replicating the study in more subjects and controlling for gender would further validate our findings as well as possibly capture changes in other dopaminergic brain regions (Camprodon et al. 2007; Grall-Bronnec and Sauvaget 2014). For instance, strong PET binding changes observed in the globus pallidus (GP) in some subjects, warrants further exploration given the potential role of the GP in drug addiction (Boileau et al. 2016). Fifth, with regards to laterality, our bilateral stimulation of the insular region using the H-coil may have been a limitation for testing addictive and dopaminergic-related behaviors. Finally, the PHNO-PET technique itself has some shortcomings related to the employment of non-tracer doses of the radioligand, and cerebellum-specific binding (Shotbolt et al. 2012).

In conclusion, the present investigation indicates that low frequency rTMS of the bilateral insular cortex decreases dopamine in selective sub-cortical regions. The well-recognized role of the insula in addictive behavior (Garavan 2010; Naqvi and Bechara 2010; Naqvi et al. 2014), warrants validation of this study in a larger sample, followed by replication in specific addicted populations, including tobacco smokers. To optimize achieving maximum efficacy of rTMS, it would be ideal to use MRI-guided localization of the target zone (Fitzgerald et al. 2009; Gorelick et al. 2014). Image-guided stereotactic systems (e.g. Brainsight) use the subjects' MRI to improve target accuracy for superficial coils, where the focal area of stimulation is relatively small. It is possible that image-guiding systems could locate the insula more accurately and thereby guide H-coil placement. This would help account for some individual anatomical variability. Further, since acute stimulation of the bilateral insular cortex yielded a response, it will be interesting to examine the effects of chronic or unilateral (left versus right) stimulation, on changes in dopamine. It will also be useful to map the changes in brain connectivity using magnetic resonance imaging. With the insula as the seed voxel, comparing differences in connectivity between sham and active rTMS conditions will provide significant insights on the effect(s) of insula-specific rTMS on the reward and salience networks. Once a greater understanding of the impact of insular rTMS on the dopaminergic system is obtained, this non-invasive neuromodulation approach may be implemented as a routine therapeutic intervention for addiction in the clinical realm.

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Compliance with ethical standards

Conflict of interest Dr. Malik, Dr. Cho, Dr. Boileau, Dr. Strafella, Dr. Wilson, Dr. Heilig and Mark Jacobs reported no biomedical financial interests or potential conflicts of interest. Dr. Le Foll has received in-kind support from Brainsway that provided the equipment used in this

study. In addition, Dr. Le Foll received in kind donation of drug supplies from Pfizer or GW-Pharma. He received grant and salary support for other unrelated studies from Pfizer Inc. and Bioprojet laboratory. Dr. Le Foll has been a consultant or has received honorariums for lectures from Richter Pharmaceuticals, Lundbeck, Mylan, Ethypharm, Metrum and Pfizer. Dr. Daskalakis has received research and equipment in-kind support for an investigator-initiated study through Brainsway Inc, and has also served on the advisory board for Sunovion, Hoffmann-La Roche Limited and Merck and received speaker support from Eli Lilly. Dr. Blumberger has received research support from the Canadian Institutes of Health Research (CIHR), National Institute of Health (NIH), Brain Canada and the Temerty Family through the Centre for Addiction and Mental Health (CAMH) Foundation and the Campbell Research Institute. He receives research support and in-kind equipment support for an investigator-initiated study from Brainsway Ltd. and he is the site principal investigator for three sponsor-initiated studies for Brainsway Ltd. He also receives in-kind equipment support from Magventure for an investigator-initiated study and receives medication supplies for an investigator-initiated trial from Invidior. Dr. Zangen is a co-inventor of the deep TMS H-coil system, serves as consultant for, and has financial interests in Brainsway.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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