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ORIGINAL INVESTIGATION

Effects of the 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine on appetite, food intake and emotional processing in healthy volunteers

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Abstract
Rationale The treatment of obesity is an increasing global health priority, yet few effective drug treatments are currently available. The discovery of novel anti-obesity therapies could be assisted by the validation of experimental (translational) medicine models in healthy volunteers that assess efficacy and safety at an early stage of drug development.
Objectives The aim of this study was to examine the effects of the 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine (mCPP) in an experimental medicine model assessing both appetite and mood.
Methods Using a between-subjects, double-blind, placebo-controlled design, 24 male and 24 female participants were randomly assigned to either placebo, 15- or 30-mg mCPP treatment groups. Lunch was eaten from a Universal Eating Monitor (UEM) that measured eating rate, and the participants completed the P1vital® Oxford Emotional Test Battery (ETB) and a series of appetite and mood ratings.
Results mCPP reduced appetite and, in women, enhanced measures of satiation. The drug also enhanced memory for emotional material in the word recall and recognition memory tasks of the ETB.
Conclusions The results provide new insight into the effects of mCPP on appetite, satiety and memory in humans. In addition, our data provide an illustration of the value of measuring changes in appetite and mood in healthy volunteers to determine the potential efficacy and safety of novel anti-obesity drugs.

Keywords Experimental medicine · Anti-obesity drugs · mCPP · 5-HT_{2C} · ETB · UEM · Appetite · Food intake · Satiation quotient · Emotional processing · Memory

Obesity is an increasing major global health concern, and the American Medical Association, at its annual meeting on 18 June 2013, recognised obesity as a disease with multiple pathophysiological aspects, requiring a range of interventions to advance treatment and prevention (AMA 2013). It is a condition of excessive fat accumulation that is associated with an increased risk of developing type II diabetes, coronary heart disease, hypertension, and some cancers (Finucane et al. 2011). Data from the World Health Organization indicate that more than 1 in 10 of the world's adult population was obese in 2008 (WHO 2012), and rates of obesity are predicted to rise over the next decade. It is estimated that by 2020, seven of 10 British people will be overweight or obese (Sassi 2010). The prevention and treatment of obesity is therefore a worldwide healthcare priority.

An effective means to help weight loss, as part of a comprehensive programme, is to prescribe drug treatments designed to reduce appetite. At present, however, pharmacotherapy options for obesity are limited, and there have been concerns over the long-term efficacy and safety of anti-obesity drugs (Li and Cheung 2009). In Europe, there are no centrally acting drugs on the market, and treatment is limited to the lipase inhibitor, Orlistat (Xenical®; Alli®), which works by preventing the breakdown and absorption of fat in the intestinal system (Davidson et al. 1999). The Food and Drug Administration (FDA) in the USA has recently approved the

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introduction of two new anti-obesity drug treatments, the 5-HT_{2C} receptor agonist Lorcaserin (Belviq®) and a combination therapy of phentermine and topiramate (Qsymia®). However, the weight loss induced by Lorcaserin is relatively modest, and while Qsymia is more efficacious than Lorcaserin as a weight loss agent, it is associated with unpleasant side effects (Heal et al. 2012) and has recently been rejected by the European Medicines Agency (EMA) due to the concerns about cardiovascular and CNS side effects. Therefore, there is a continued need for the development of new drugs that have the potential to deliver greater weight loss than existing compounds and have an acceptable safety profile.

A barrier to successful development of new drug therapies for obesity is that the costs are high, and the chance of a novel compound in phase 1 trials reaching the market is very low largely due to the failure in late-scale clinical trials or during the regulatory approval process (Kola and Landis 2004). The cannabinoid CB_1 receptor antagonist rimonabant (Acomplia®) was approved by the EMA and marketed for a period in Europe but was refused marketing authorisation by the FDA due to concerns regarding adverse psychiatric side effects. Consequently, marketing authorization for rimonabant in Europe was withdrawn (Butler and Korbonits 2009). It is increasingly recognised by the pharmaceutical industry that the introduction of experimental (translational) medicine models at the interface between phase 1 and phase 2 clinical trials provides a more effective approach to assess drug efficacy and safety before large-scale trials are undertaken (Dawson et al. 2011). Initial efficacy testing of novel compounds can also be conducted in healthy volunteers to assess safety issues that might pose risks for vulnerable patient populations (Dourish et al. 2008). For example, a number of previously marketed drugs for obesity were withdrawn due to cardiovascular safety issues, which are a particular concern in obese patients who may have hypertension (see below).

Drugs that induce weight loss by enhancing satiety exert effects on energy intake after acute dosing in volunteers (Dourish et al. 2008; Halford et al. 2010). For example, the noradrenaline and serotonin re-uptake inhibitor sibutramine, which was previously marketed as an anti-obesity agent but withdrawn due to increased prevalence of cardiovascular problems (Sayburn 2010), has been reported to decrease food intake in short-term studies (Halford et al. 2010). Thus, it is possible to evaluate the potential efficacy of anti-obesity drugs at an early stage of clinical development by investigating the acute effects of drug administration on food intake. Furthermore, the acute effects of sibutramine on food intake have been reported to be mediated by decreases in eating rate, suggesting that the analysis of the microstructure of eating (Kissileff et al. 1980; Yeomans 1996; Halford et al. 2010) provides an experimental marker of anti-obesity drug efficacy.

There is also evidence that drug-induced effects on emotional processing that are predictive of changes in mood associated with depression can be detected after acute or 7-day dosing in healthy volunteers (Harmer et al. 2004; Miskowiak et al. 2007; Browning et al. 2007). Furthermore, recent studies have demonstrated increased negative emotional bias following the administration of the CB_1 receptor antagonist rimonabant that is consistent with the increased incidence of depression observed in obese patients treated with the drug (Horder et al. 2009, 2010).

Our aim in the present study was to validate an experimental medicine model for assessing the efficacy and safety of potential anti-obesity therapies in healthy volunteers. The model combines the assessment of eating microstructural variables using the Sussex Ingestion Pattern Monitor (Yeomans 1996) alongside measures of emotional processing using the P1vital® Oxford Emotional Test Battery (ETB) (Harmer et al. 2004, 2009; Murphy et al. 2008). We examined the effect of a compound that has been reported to alter food intake and mood-related behaviour in rodents, the preferential 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine (mCPP) (Kitchener and Dourish 1994; Kennett et al. 1994; Hewitt et al. 2002). The anorectic effects of mCPP in humans have been reported previously (Walsh et al. 1994; Sargent et al. 1997), but detailed effects of this compound on appetite have yet to be investigated. Similarly, it has been known for some time that mCPP can increase anxiety in healthy volunteers and in patients with panic disorder (Charney et al. 1987; Kahn et al. 1990), but this is the first detailed assessment of the effects of mCPP in a battery of psychological tasks. We predicted that mCPP would decrease food intake and enhance microstructural measures of satiety as well as increase anxiety and negative mood measured by the ETB.

Materials and methods

Participants

Forty-eight healthy student volunteers (24 men and 24 women, mean age 20.92 years (range 18–27), and mean BMI 22.38 (range 18.93–26.30)) were recruited from the University of Birmingham. The study was advertised via posters, as an 'Appetite and Mood Study examining the effects of mCPP'. Participants received £80 compensation upon completion. Informed consent was obtained, ethical approval was provided by the South Birmingham Research Ethics Committee (National Research Ethics Service), and the study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were screened to exclude the following: under 18 or over 65 years old, body

mass index (BMI) under 18.5 or over 27.5, English not first language as determined by the National Adult Reading Test (NART; Nelson 1982), taking psychotropic medication, past or current Axis 1 disorder as determined by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P; Spitzer et al. 2004), pregnant or breast feeding, smoker, dyslexic, food allergies, vegan or vegetarian, diabetic, not using contraception (women), cognitive restraint score higher than 10 as measured by the Three-Factor Eating Questionnaire (TFEQ; Stunkard and Messick 1985), and low rated pleasantness (<65) or low consumption of the test meal (<150 g) on the food screening day. Women were not tested during their premenstrual week.

Design

The study used a parallel group, double-blind, placebo-controlled design. A 30-mg oral dose of mCPP was selected, as similar doses have been shown to reduce appetite, and, in some cases, induce nausea and anxiety (Sargent et al. 1997; Walsh et al. 1994; Cowen et al. 1995; Kahn et al. 1990). A 15-mg dose was also used, as this dose is not associated with nausea but may still affect appetite (Smith et al. 1994). The study had three conditions: placebo, 15-mg mCPP, and 30-mg mCPP (mCPP was supplied by the Guy's and St Thomas' NHS Foundation Trust's (GSTFT) Pharmacy Manufacturing Unit). Each group comprised 16 participants (8 men and 8 women), and both medication and placebo were prepared in identical capsules to maintain blinding.

Sussex Ingestion Pattern Monitor (SIPM)

Test meals were served on a SIPM. This consisted of a balance (Sartorius Model CP4201, Sartorius Ltd., Epsom, UK; 0.1 g accuracy) placed underneath, but protruding through, the surface of a table. A placemat on the table was used to hide the balance from the participants' view. The balance was connected to a laptop computer. Using the procedure described by Yeomans (1996, 2000), plates containing 200 g of pasta were placed on the mat, and the amount of food eaten every 2 s was recorded covertly. The SIPM software (version 2.0.8) was configured to interrupt the participants each time they had eaten 50 g of the meal and ask them to complete visual analogue scale (VAS) ratings of hunger, fullness and pleasantness of the pasta. After 150 g had been consumed, the participants were interrupted, and the plate was replaced with a fresh 200 g plate of pasta. The participants were asked to eat in this manner until they felt 'comfortably full'. The test meal consisted of pasta shells in a tomato and herb sauce (both Sainsbury's brand), served at 55–60 °C (233 kcal per 200 g serving).

P1vital® Oxford Emotional Test Battery (ETB)

The ETB is a computerised battery that comprises five validated cognitive tests (see http://www.p1vital.com/Oxford%20Emotional%20Test%20Battery/index.html) which have been used in previous acute drug studies of emotional behaviour (e.g. Harmer et al. 2004; Murphy et al. 2008).

Facial expression recognition task (FERT) Faces with one of six emotional expressions (happiness, fear, anger, disgust, sadness and surprise) or a neutral expression were displayed. The pictures (from Ekman and Friesen 1976) were morphed from neutral to 100 % expressions (Young et al. 1997) in 10 % stages, producing 10 intensities for each emotion. Each intensity was presented four times for each emotion, along with 10 presentations of neutral expressions, yielding 250 stimuli. These were presented for 500 ms, followed by a blank screen. The participants classified each expression as quickly and accurately as possible, using the button box provided. Accuracy, response bias (bias towards one emotion over another) and target sensitivity (ease in detecting the target stimulus from other stimuli) were calculated.

Faces dot probe task (FDOT) Pairs of photographs (from Matsumoto and Ekman 1988) were presented comprising either one emotional (either happy or fearful) and one neutral facial expression or two neutral expressions. The faces appeared above or below a central fixation point. For unmasked trials, the pair was displayed for 100 ms, whereas for masked trials, the pair was displayed for 16 ms then replaced with a mask for 84 ms; for both trials, the images were then replaced with a probe, located in the position of one of the faces (or masks). The probe consisted of a pair of dots either in a vertical (:) or in a horizontal (··) orientation. The participants indicated the orientation as quickly and accurately as possible using the corresponding buttons on the button box. There were 192 trials: 32 happy+neutral, 32 fear+neutral, and 32 neutral+neutral for both masked and unmasked trials. Vigilance (sustained attention) scores were calculated.

Emotional categorisation task (ECAT) Sixty positive and negative self-referent personality descriptors (e.g. "cheerful" vs "hostile") (Anderson 1968) were displayed for 500 ms. Words were matched for meaningfulness, length and frequency of occurrence. The participants indicated using the button box whether they would like or dislike to be described as such, as quickly and accurately as possible. Accuracy and reaction times were measured.

Emotional recall task (EREC) The participants were asked to recall as many words as they could remember from the ECAT within a 4-min period. This task was partly computerised;

instructions were given via computer, but words were written down using pen and paper. The number of correct words recalled and their valence was measured.

Emotional recognition memory task (EMEM) The participants were presented with a series of words on a computer screen, containing the 60 personality descriptors from the ECAT, along with 60 matching novel distracter words. The participants were instructed to use the button box, pressing 'yes' or 'no' to indicate whether the word had been presented during the ECAT. Accuracy, response bias and target sensitivity were calculated.

Biological samples

Salivary cortisol Saliva was collected using salivettes (SARS TEDT). The participants abstained from drinking water for 30 min prior to giving the sample and then chewed on a synthetic cotton wool swab for 60 s, before the sample was taken to be centrifuged for 2 min at 1,000 g and then frozen at −80 °C until analysed. Salivary cortisol was measured by liquid chromatography–mass spectrometry (LC–MS/MS) by the Clinical Laboratory Services, University Hospitals Birmingham NHS Foundation Trust. The Waters AQUITY UPLC system was used for chromatography connected to a Waters Premier XE tandem mass spectrometer with an electrospray ion source. Intra-assay coefficients of variation (CVs) were <5.9 % and inter-assay CVs <9.8 % between 3 and 47 nmol/L. The lower limit of quantification (defined as the lowest concentration at which the CV is <20 %) was 1.6 nmol/L.

Procedure

The experimental procedure is summarised in Fig. 1.

Screening days The participants underwent an initial screening at the School of Psychology (University of Birmingham) for exclusions and completed the Eysenck Personality Questionnaire (EPQ; Eysenck and Eysenck 1975) to determine baseline personality traits. They returned approximately 1 week later for a trial run with the test meal served; those who liked and ate a sufficient amount of the pasta proceeded to the test day.

Test day The participants arrived at the Welcome Trust Clinical Research Facility (University Hospitals Birmingham NHS Foundation Trust–Queen Elizabeth Hospital) at 8.00, 9.00, or 10.00 a.m. It was ensured that the participants had eaten their normal breakfast 2 h earlier by asking them to complete a breakfast questionnaire detailing their food intake that morning. The participants then completed a physical health check with a physician (including blood pressure and an electrocardiogram (ECG)), were breathalysed, and completed a pregnancy test if female. Only individuals who passed the health and alcohol check, and women who were not pregnant, were allowed to continue. The participants also completed baseline VAS for mood and appetite that comprised the following: 'alertness', 'disgust', 'drowsiness', 'light-headed', 'anxiety', 'happiness', 'nausea', 'sadness', 'withdrawn', 'faint', 'hungry', 'full', 'desire to eat' and 'thirst'. One and a half hour after the start of the session, the participants completed a pre-dose Beck Depression Inventory (BDI; Beck et al. 1961), Befindlichskeit Scale of mood and energy (BFS; von Zerssen et al. 1974), and Positive and Negative Affective Schedule (PANAS; Watson et al. 1988) as measures of subjective mood and energy. They also completed a State-Trait Anxiety Inventory (STAI; Spielberger 1983) as an index of their anxiety, and another set of VAS (as described above). After 2.0 h, further VAS were completed, and a saliva sample was taken, after which participants were administered the test dose (either placebo, 15-mg mCPP or 30-mg mCPP). VAS were completed every 30 min. Four hours after the start of the session, the participants were given ad libitum access to the test meal on the SIPM and completed the computerised VAS during the meal. Following the meal, the participants completed post-dosing: BDI, BFS, PANAS, STAI and VAS. A saliva sample was taken 1 h after lunch, and the participants then completed the ETB (which took approximately 90 min). A final set of VAS and a saliva

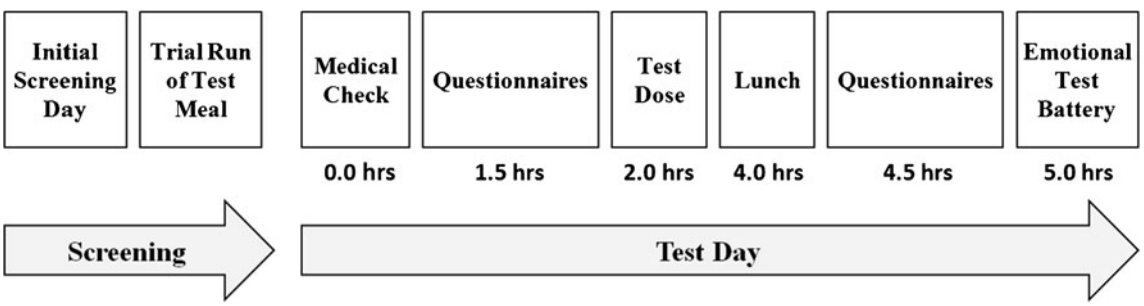

Fig. 1 Flow diagram for *Screening Days* (*Initial Screening Day* and *Trial Run of Test Meal* on a separate day) followed by an overview of *Test Day* timings in hours (*hrs*)

sample were taken at the end of the session, along with a single blood sample. Participants were fully debriefed and questioned to determine if they were aware of the hidden balance or the aims of the study (see Fig. 1 for a summary of the procedure).

Data analysis

General One participant did not complete testing, and their data were removed from all analyses (female, 30-mg mCPP group). For statistical analyses, effects and interactions with condition were determined with analysis of variance (ANOVA) and followed up with planned comparisons; interactions with condition were also analysed if approaching statistical significance. Dunnett's correction was used for all *t* tests, and violations of sphericity were addressed using the Greenhouse–Geisser correction.

VAS To establish a factor structure for the VAS, a principal component analysis (PCA) was run with varimax rotation. Analysis of the 14 items provided five factors with eigenvalues >1, accounting for 70.81 % of the variance. Items that loaded >0.5 onto a factor were included, resulting in four factors of three or more items: appetite (hunger, fullness and desire to eat), physical effects (lightheaded, nausea and faint), negative effects (disgust, anxiety, sadness, withdrawn and thirst) and arousal (alertness, drowsiness and happiness). Scores for each of the factors were calculated by summing the scores for all items in that factor and then dividing by the number of items (items with a negative scale, were inverted to match the other items).

SIPM Microstructural data for 12 participants were lost due to technical issues with the balance, so data for 35 participants were analysed. For total intake and correlational analysis, an estimate of intake was used (e.g. where intake data were missing for a plate, it was assumed that if the plate was finished, the individual had eaten approximately 150 g).

Results

Participant characteristics

The groups did not differ in age, BMI or EPQ scores (all $p>0.05$). There was a main effect of gender for the NART, with men scoring higher than women ($F(1,41)=5.12$, $p<0.05$) and for the TFEQ factor cognitive restraint, with women showing higher restraint than men ($F(1,41)=4.19$, $p<0.05$), but there were no other main effects or interactions at baseline (Table 1).

Salivary cortisol

Salivary cortisol was analysed by gender, condition, and time (baseline measure *t*0; time after dosing *t*60, *t*120, *t*180, and *t*300). There was a main effect of time ($F(4,164)=7.07$, $p<0.001$), condition ($F(2,41)=12.32$, $p<0.001$), an interaction between condition and time ($F(8,164)=2.49$, $p<0.05$), and an interaction between time and gender which was not further analysed ($F(4,164)=3.42$, $p<0.05$). Post hoc analysis showed that there were main effects of condition for *t*60 ($F(2,44)=7.41$, $p<0.01$), *t*120 ($F(2,44)=8.37$, $p<0.01$) and *t*300 ($F(2,44)=6.16$, $p<0.01$). Cortisol was significantly higher in the 15-mg mCPP condition compared with placebo at *t*60 ($t(30)=2.43$, $p<0.05$), while cortisol was higher in the 30-mg mCPP condition compared with placebo at *t*60 ($t(29)=4.18$, $p<0.01$), *t*120 ($t(29)=3.86$, $p<0.01$) and *t*300 ($t(29)=3.36$, $p<0.01$) (Fig. 2). There was no interaction between gender and condition ($F(2,41)=0.32$, $p>0.05$), or gender, condition and time ($F(8,164)=3.29$, $p>0.05$), indicating no differential cortisol response for men versus women to mCPP.

Subjective state questionnaires

Data were analysed by gender, condition, and time (pre- vs post-dosing). There were no main effects or interactions for the BDI, STAI, or PANAS negative scores (all $p>0.05$). There was, however, a main effect of time for PANAS positive scores, with a small decrease in positive affect over the day (from 30.43 to 28.11; $F(1,41)=9.64$, $p<0.01$). For the BFS, there was a significant interaction between time and condition ($F(1,42)=4.363$, $p<0.05$), whereby the placebo group BFS score decreased over time ($t(15)=2.18$, $p=0.05$).

Visual analogue scales

Pre-dosing VAS scores were averaged for a pre-dose baseline. Each factor (negative effects, arousal, appetite, and physical effects) was analysed separately by condition and gender, showing no main effects or interactions (all $p>0.05$). Post-dosing factors were then analysed by gender, condition and time (post-dosing in minutes: *t*30, *t*60, *t*90, *t*120, *t*150, and *t*210).

Negative effects and arousal There were no main effects or interactions for negative effects (all $p>0.05$). For arousal, there were no main effects (all $p>0.05$) but a significant two-way interaction between time and condition ($F(5,107)=2.37$, $p<0.05$); follow-up tests were not statistically significant (all $p>0.05$), although there was a near significant effect of condition for *t*210 ($F(2,44)=2.67$, $p=0.08$), with mean arousal scores of 64.69 for placebo, 75.79 for 15-mg mCPP and 75.22 for 30-mg mCPP.

Table 1 Participant characteristics, baseline means, and test meal intake (standard error of the mean)

	Men			Women		
	Placebo	15 mg	30 mg	Placebo	15 mg	30 mg
Gender	8	8	8	8	8	7
Age	20.8 (0.4)	21.9 (0.8)	20.4 (0.5)	22.4 (1.0)	20.4 (0.5)	19.9 (0.7)
BMI	23.8 (0.7)	22.1 (0.7)	22.8 (0.8)	21.5 (0.7)	22.0 (0.8)	22.4 (0.9)
NART	114.9 (1.0)	114.6 (1.7)	112.6 (1.6)	112.9 (1.0)	111.9 (1.6)	109.7 (0.9)
TFEQ CR	4.3 (1.4)	3.6 (1.0)	3.1 (1.0)	6.9 (0.9)	5.5 (1.1)	4.1 (1.2)
EPQ N	4.0 (0.8)	4.0 (1.0)	4.0 (1.0)	7.9 (1.1)	3.3 (1.3)	4.1 (0.6)
Test Meal Intake (grams)	649.3 (90.1)	778.2 (111.6)	663.8 (107.7)	510.1 (60.2)	442.8 (31.1)	409.2 (66.9)

BMI body mass index, *NART* National Adult Reading Test, *TFEQ* Three-Factor Eating Questionnaire (*CR* Cognitive Restraint), *EPQ* Eysenck Personality Questionnaire (*N* Neuroticism)

Physical effects There was a main effect of time ($F(3,117)=160.94$, $p<0.01$) and condition ($F(2,41)=5.97$, $p<0.01$; Fig. 3) and a significant two-way interaction ($F(6,117)=3.25$, $p<0.01$). Main effects of condition were observed for $t60$ ($F(2,44)=6.62$, $p<0.01$), $t90$ ($F(2,44)=7.83$, $p<0.01$) and $t120$ ($F(2,44)=4.69$, $p<0.05$), with physical effects rated significantly higher in the 30-mg mCPP group than the placebo (all $p<0.05$; Fig. 3).

Appetite There was a main effect of time ($F(3,115)=237.00$, $p<0.001$) and condition ($F(2,41)=3.78$, $p<0.05$; Fig. 3) but no significant interaction. For the effect of time, all comparisons were significantly different (all $p<0.05$), and for condition, appetite was significantly lower in the 30-mg mCPP group than the placebo ($t(29)=2.30$, $p<0.05$; Fig. 3).

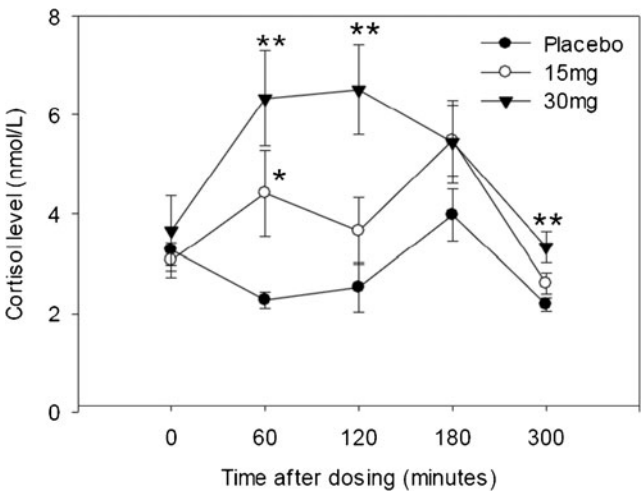

Fig. 2 Salivary cortisol levels over time for placebo, 15-mg mCPP, and 30-mg mCPP. The 15-mg group showed a significant increase in salivary cortisol compared with the placebo group at $t60$, and the 30-mg group showed an increase at $t60$, $t120$ and t300. *Error bars* represent standard error of the mean. Asterisk denotes $p<0.05$; Asterisks denote $p<0.01$

Meal measures

Test meal intake There was a main effect of gender only, with men eating more than women ($F(1,29)=11.32$, $p<0.01$). There was no effect of condition nor any interaction (Table 1).

Meal intake quartiles Amount eaten was analysed by quartile, gender, and condition, showing a main effect of quartile ($F(3,87)=8.18$, $p<0.01$), gender ($F(1,29)=11.32$, $p<0.01$) and a two-way interaction between quartile and condition ($F(6,87)=2.56$, $p<0.05$), although follow up tests were not significant. Men ate more than women (174.09 g vs 106.57 g). Men had a faster eating rate than women (105.72 g/min vs 67.23 g/min; $F(1,29)=13.51$, $p<0.01$) and ate larger mouthfuls than women (12.06 g vs 7.76 g; $F(1,29)=6.56$, $p<0.05$).

Computerised VAS Hunger decreased across quartiles ($F(2,50)=150.46$, $p<0.001$), and men were more hungry than women (47 mm vs 38 mm, respectively; ($F(1,29)=3.81$, $p=0.06$) (all $p<0.001$). Similarly, fullness increased across quartiles ($F(2,47)=176.78$, $p<0.001$), and men were less full than women; (59 mm vs 51 mm, respectively; $F(1,29)=4.46$, $p<0.05$). For pleasantness, there was a main effect of quartile only ($F(2,45)=31.65$, $p<0.001$), with rated pleasantness decreasing across quartiles (all $p<0.05$).

Satiation quotient

The satiety quotient (Green et al. 1997; (pre-meal hunger−post-meal hunger)/calories consumed) provides a measure of how satiating a given amount of calories is at time points after an eating episode has finished. Adapting this, we calculated a satiation quotient (SQ) per quartile, a measure reflecting the satiating capacity of a food as it is eaten ((quartile initial hunger−quartile ending hunger rating)/calories consumed during quartile). There was a main effect of quartile ($F(3,87)=11.62$, $p<0.001$) and gender ($F(1,29)=7.11$,

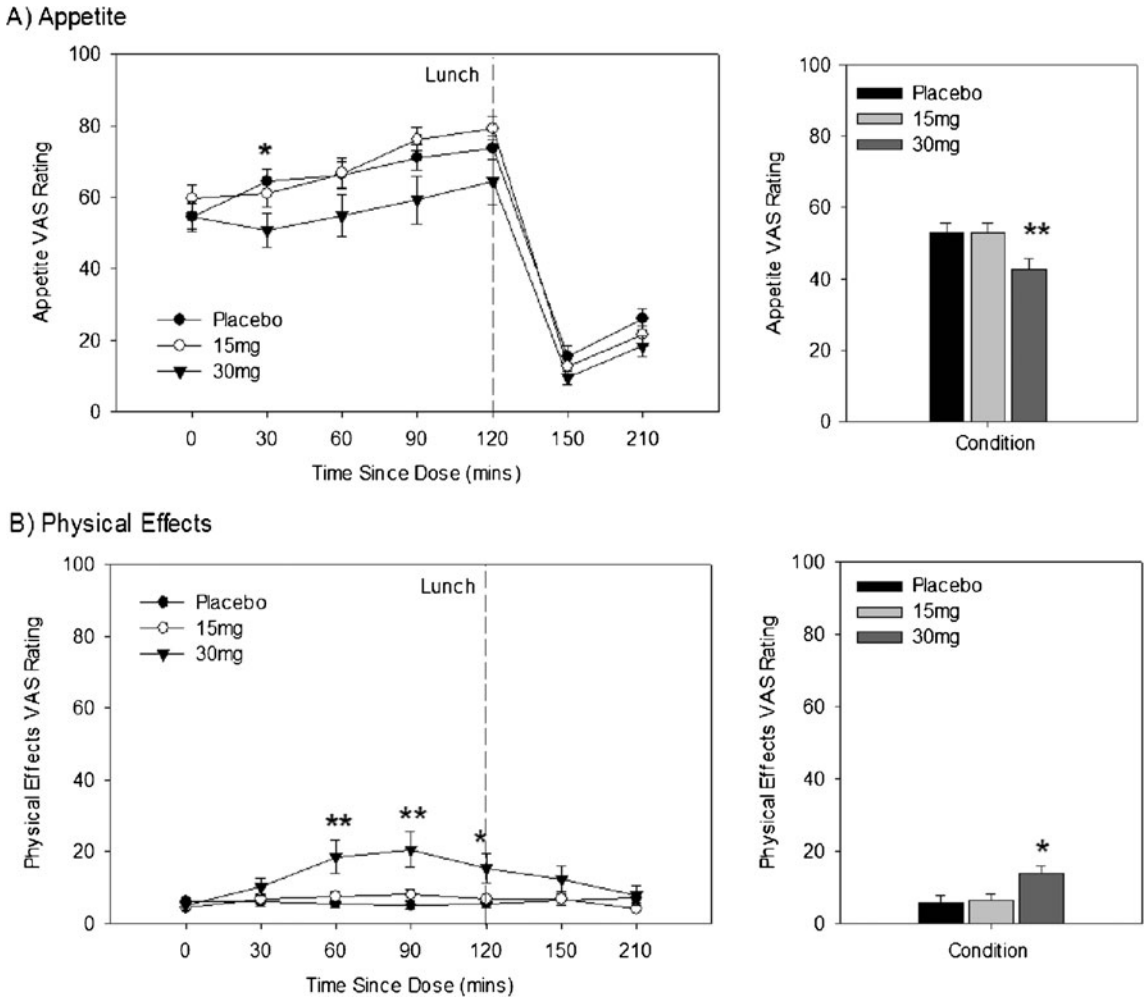

Fig. 3 For appetite (**a**), there was a main effect of condition (*histogram on right panel*) where appetite was significantly lower in the 30-mg group compared with the placebo group, but no significant interaction (*graph on left panel*; post hoc *t* tests showing a difference between placebo and 30 mg at *t*30 only). Physical effects VAS scores (**b**) showed a main effect of condition (*histogram on right panel*) and an interaction between condition and time (*graph on left panel*), where physical effects were significantly higher for the 30-mg group at *t*60, *t*190, and *t*120, compared with the placebo. *Error bars* represent standard error of the mean. Asterisk denotes $p<0.05$; Asterisks denote $p<0.01$

$p<0.05$), a two-way interaction between gender and condition ($F(2,29)=8.61$, $p<0.01$), and a three-way interaction between quartile, gender and condition ($F(6,87)=2.50$, $p<0.05$). For men, there was an effect of quartile ($F(3,36)=6.87$, $p<0.01$) and condition ($F(2,12)=4.30$, $p<0.05$). SQs were significantly lower in the 30-mg mCPP group compared with the placebo ($t(8)=2.72$, $p<0.05$), and there was a significant increase in SQ from quartile 2 to 3 ($t(14)=2.86$, $p<0.05$). For women, there was a main effect of quartile ($F(3,51)=6.53$, $p<0.01$), condition ($F(2,17)=4.84$, $p<0.05$) and an interaction between quartile and condition ($F(6,51)=2.58$, $p<0.05$). Breaking down the interaction by quartile, for quartile 1, SQ was significantly higher for 30-mg mCPP than placebo ($t(11)=2.44$, $p<0.05$), and for quartile 2, SQ was significantly higher for both 15-mg mCPP and 30-mg mCPP, compared with placebo ($t(13)=3.07$, $p<0.01$; $t(11)=2.69$, $p<0.05$) (Table 2).

Correlations

To investigate the relationship between appetite, physical symptoms (both measured prior to lunch) and total food

Table 2 Mean female satiation quotients by quartile and condition (standard error of the mean)

	Condition		
Quartile	Placebo	15 mg	30 mg
1	0.04 (0.04)	0.04 (0.02)	0.20 (0.05)*
2	0.10 (0.02)	0.21 (0.03)**	0.21 (0.04)*
3	0.18 (0.05)	0.21 (0.05)	0.46 (0.16)
4	0.17 (0.04)	0.20 (0.06)	0.09 (0.02)

Compared to placebo: *$p<0.05$; **$p<0.01$

intake, correlations were performed on the three measures. A positive correlation was identified between appetite and intake (r (47)=0.32, p<0.05), but there was no association between physical symptoms and intake or between physical symptoms and appetite (r (47)=−0.05, p>0.05; r (47)=−0.15, p>0.05).

ETB data

FERT and FDOT There were main effects of emotion (F(3,140)=35.46, p<0.001; F(6,246)=130.28, p<0.001) but no other significant effects or interactions for target sensitivity and response bias (all p>0.05). There were no significant main effects or interactions for vigilance scores (all p>0.05).

ECAT Accuracy data showed a main effect of gender only, with women showing greater accuracy than men (96.70 % vs 89.34 %, respectively; F(1,40)=4.36, p<0.05). Reaction time showed a main effect of valence only, with longer reaction times to negative versus positive stimuli (887.36 ms vs 825.36 ms, respectively; F(1,40)=19.47, p<0.001).

EREC More positive than negative words were recalled (5.8 vs 3.8 words; F(1,41)=38.97, p<0.001). There was also a main effect of condition (F(2,41)=4.77, p<0.05), with more words correctly recalled in the 15-mg mCPP and 30-mg mCPP groups than the placebo (5.1 and 5.6 vs 3.6, respectively) (both p<0.05; Fig. 4).

EMEM Target sensitivity showed a main effect of condition only (F(1,41)=5.87, p<0.01), whereby target sensitivity was significantly higher in the 30-mg mCPP group than the placebo (t(23)=4.31, p<0.001; Fig. 4).

Discussion

The 5-HT_{2C} receptor agonist mCPP at doses of 15 mg and 30 mg decreased appetite ratings of both male and female participants and enhanced within-meal satiation quotients (SQs) of female participants in a dose-related manner but had no significant effect on food intake. mCPP also dose dependently increased ratings of negative physical effects (in particular nausea) but had no effects on ratings of negative mood or cognitive measures of anxiety and depression as assessed by the ETB. Interestingly, we provide the first evidence that mCPP may enhance memory for emotional words independent of their valence.

As expected, mCPP dose dependently increased salivary cortisol, confirming activation of 5-HT_{2C} receptors. Peak effects for both doses occurred at 60–180 min post-dosing, which is consistent with previous reports (Kahn et al. 1990). In addition, the 30-mg dose of the drug, but not the 15-mg dose, induced negative physical symptoms in the participants, a finding which also confirms the results of previous studies (Walsh et al. 1994). Taken together, these data suggest that mCPP activated 5-HT_{2C} receptors at concentrations sufficient to exert effects on eating and emotional behaviour.

Appetite ratings were attenuated by 30-mg mCPP, but not by 15-mg mCPP, a finding that is consistent with previous reports (Cowen et al. 1995; Smith et al. 1994). Interestingly, the results for the SQ, showed a dose-dependent effect of mCPP, but only in women. Thus, SQs were enhanced in the first half of the test meal at both 15- and 30-mg mCPP, with an earlier onset of action at the 30-mg mCPP dose. mCPP failed to significantly decrease food intake compared with placebo suggesting that VAS ratings and the SQ measure derived from SIPM microstructural data may be more sensitive to the

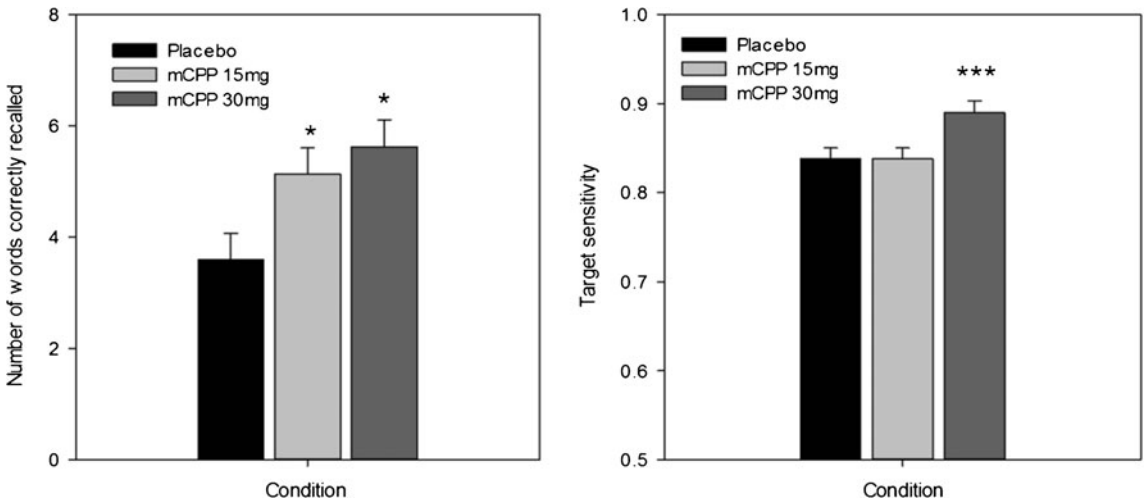

Fig. 4 Mean number of words correctly recalled (*left panel*) and mean target sensitivity (*right panel*) for the placebo, 15-mg mCPP and 30-mg mCPP groups in the emotional recall task. Word recall was enhanced in both the 15- and 30-mg groups compared with the placebo, and target sensitivity was enhanced in the 30-mg group compared with the placebo. *Error bars* represent standard error of the mean. Asterisk denotes p<0.05; Asterisks denote p<0.001

effects of mCPP (at least in women) on eating than measurement of food consumption. The apparent acceleration of satiation by mCPP in lean women in the present study is similar to that observed with the 5-HT reuptake inhibitor sibutramine in obese women (Halford et al. 2010) and may be part of a common mechanism by which 5-HT_{2C} receptor activation reduces food intake.

We observed differences between male and female paricipants in eating patterns and their response to mCPP. Men ate more than women and at a faster rate. While both male and female participants had reduced appetite ratings after mCPP, only women showed enhanced satiety. These differences are unlikely to be explained by male/female differences in body weight, drug distribution, or metabolism because there were no gender effects observed for mCPP-induced increases in cortisol. One possibility is that although the men experienced an effect of mCPP on appetite, they are less sensitive to the drug effects on actual food intake measured in the context of the laboratory. Several male participants ate close to (or more than) 1 kg of pasta, which suggests that they may have been eating larger amounts than usual. It would be informative to assess the response of male participants to the effects of mCPP on intake at usual meals taken in the home.

It appears unlikely that the effects of 15-mg mCPP on appetite and satiety were confounded by nausea, as this dose did not induce significant physical effects. However, it is possible that nausea could have confounded the effects on appetite seen at the 30-mg dose of mCPP. This does not seem plausible, however, as neither appetite nor food intake correlated with physical symptoms. This interpretation is consistent with the results of a previous study in which nausea also failed to correlate with food intake or appetite ratings (Walsh et al. 1994). In addition, the pattern of effects for appetite and nausea are distinct, as 30-mg mCPP attenuated appetite earlier, and for a longer duration, than the induction of nausea.

On the basis of previous research (Charney et al. 1987; Kahn et al. 1990), we predicted that mCPP might induce anxiety and/or depression-like symptoms. The pattern of results from the ETB, however, is not consistent with the induction of anxiety or depression-like responses by mCPP. Thus, there was no significant effect of mCPP administration on responses to fearful or angry facial expressions which are generally indicative of heightened anxiety (Mogg and Bradley 2002; Harmer et al. 2003), nor were there any responses indicative of depression, such as the reduced recall of positive stimuli that has been observed after acute treatment with the CB_1 receptor antagonist rimonabant (Horder et al. 2009). Previous studies that have reported anxiogenic effects of mCPP generally administered the drug as an intravenous bolus, and so it is possible that effects on anxiety are less evident with the oral route of administration used in the present study (Kahn et al. 1990; Silverstone and Cowen 1994; Cowen et al. 1995; Anderson et al. 2002). The lack of an mCPP-induced depressogenic or anxiogenic effect on the ETB tasks is consistent with the general absence of effects observed on the questionnaire-based measures of anxiety and mood.

Most interestingly, however, mCPP dose dependently enhanced memory for emotional words, an effect that was independent of valence. Previous studies in rodents suggest that mCPP may impair memory function (Khaliq et al. 2012). However, studies in human volunteers have linked tryptophan depletion to memory impairment and citalopram enhancement of serotonergic function with facilitation of memory consolidation (Hayward et al. 2005; Harmer et al. 2002). The present results are consistent with these findings and suggest that 5-HT_{2C} receptors may mediate the facilatory effects of citalopram on memory.

Previous research has shown that enhancing memory of an eating episode decreases subsequent food intake (Higgs and Donohoe 2011). Thus, it is possible that long term use of mCPP and other serotonergic drugs could enhance memories of food consumption and reduce subsequent food intake (possibly having an additive effect with the acute drug response observed in the present study). This theory of drug-induced memory enhancement as a mechanism to reduce food intake is supported by findings with the NMDA receptor antagonist memantine. Thus, memantine has been reported to enhance memory in animals and humans and to enhance satiety in non-human primates (Parsons et al. 2007; Foltin et al. 2008). This suggests that memory enhancement might play a role in enhanced satiation and could provide a novel target for future weight loss drugs.

In conclusion, the present results provide an informative profile of the preferential 5-HT_{2C} agonist mCPP in an acute experimental medicine model that can measure drug effects on eating, satiety and mood in humans. For the first time, we show that effects of a low (15 mg) dose of mCPP on appetite and satiety are detectable using a SIPM to measure drug-induced enhancement of within-meal satiation which appears to be a promising marker of an efficacious anti-obesity drug. The model can also detect drug-induced changes in emotional processing. Thus, an enhancement of memory for emotional stimuli by mCPP was observed for the first time supporting a role for 5-HT_{2C} receptors in cognition. Further research is needed with obese participants to ensure translation of results in this model to relevant clinical populations and with other drug classes to identify markers of their potential efficacy and safety.

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