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Amphetamine induced endogenous opioid release in the human brain detected with [^{11}C]carfentanil PET: Replication in an independent cohort

Short title: Oral amphetamine challenge induces opioid release

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Abstract

This study aimed to replicate a previous study which showed that endogenous opioid release, following an oral dose of amphetamine, can be detected in the living human brain using [^{11}C]carfentanil positron emission tomography (PET) imaging. Nine healthy volunteers underwent two [^{11}C]carfentanil PET scans, one before and one three hours following oral amphetamine administration (0.5mg/kg). Regional changes in [^{11}C]carfentanil BP_{ND} from pre- to post-amphetamine were assessed. The amphetamine challenge led to significant reductions in [^{11}C]carfentanil BP_{ND} in the putamen, thalamus, frontal lobe, nucleus accumbens, anterior cingulate, cerebellum, and insula cortices, replicating our earlier findings. None of the participants experienced significant euphoria/ 'high', supporting the use of oral amphetamine to characterize in vivo endogenous opioid release following a pharmacological challenge. [^{11}C]carfentanil PET is able to detect changes in binding following an oral amphetamine challenge that reflects endogenous opioid release and is suitable to characterize the opioid system in neuropsychiatric disorders.

Key words: Amphetamine, [^{11}C]carfentanil, opioid system, PET

Introduction

The endogenous opioid system is involved in various aspects of human behavior, including pain (Maarrawi et al., 2013), addiction (Williams et al., 2009), reward (Petrovic et al., 2008) and impulsivity (Love et al., 2009) as well as social (Hsu et al., 2013) and emotional behavior (Koepp et al., 2009). Given the widespread use of opioid medication in diverse conditions, including cough suppression, mild and chronic pain, and substance dependence, a fundamental understanding of this neurotransmitter system is essential.

Positron emission tomography (PET) is a sensitive technique that enables the determination of receptor and neurotransmitter levels in the living human brain. The human endogenous opioid system is composed of three subtypes of opioid receptors (mu, delta and kappa), widely distributed throughout the brain. Mu opioid receptors (MOR) are most dense in the caudate and putamen, nucleus accumbens, thalamus, amygdala and the frontal lobe (Mansour et al., 1988). [^{11}C]carfentanil is a highly selective MOR agonist PET radioligand, which can be used to map opioid receptor availability. Some PET radioligands are also able to detect endogenous neurotransmitter release on the basis of competitive binding between the radioligand and the neurotransmitter, or through changes in affinity or expression of the receptor (Paterson et al., 2010).

Recently, we (Colasanti et al., 2012) demonstrated significant reduction in [^{11}C]carfentanil binding in several brain regions following an oral d-amphetamine challenge (0.5 mg/kg) in six healthy volunteers. These results were not replicated by Guterstam et al. (Guterstam et al., 2013) using an intravenous 0.3 mg/kg dose of d-amphetamine in 10 healthy volunteers.

This study aimed to replicate our previous study in an independent cohort of nine male healthy volunteers, using an identical study design.

Method

Participants were recruited by advertisements in daily newspapers or from our database. A telephone eligibility interview was followed by a screening visit to comprehensively assess participants' current and previous medical and mental health as well as history of alcohol,

tobacco, and other substance use. Individuals with current or prior psychiatric disorders (ICD-10 or DSM-IV Axis I diagnostic criteria assessed using the modified international neuropsychiatric interview- MINI) were excluded. No participant scored above the threshold for mild-depression (range 0-7, mean 1± 2.3) on the Beck Depression Inventory (BDI). Current or past history of dependence on substances of abuse, except nicotine, was an exclusion criterion, although previous recreational drug use was allowed. Participants were excluded if they drank more than 21 UK units of alcohol (166 g) per week two weeks before and during study participation. Other drug use (except nicotine) was not allowed two weeks prior and during the inclusion into the study. On both screening and study days, urine drug screen testing for cocaine, amphetamine, methamphetamine, morphine, methadone, benzodiazepines and THC were performed and participants were tested for alcohol using a breathalyzer. Smoking was not allowed at least 1 hour before each scan. All participants had laboratory (haematology, clinical chemistry) and ECG results within normal range. None of the participants were taking regular medication; they had never used antipsychotics or antidepressants. In total, nine male healthy volunteers, including two smokers, mean age 33.1± 6.5 years were included into this study.

On the screening day, participants underwent structural and functional magnetic resonance imaging (MRI); functional MRI results will be reported elsewhere.

Written informed consent was obtained from all participants. The study was approved by the West London Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee, UK.

Procedure

The PET imaging procedures were identical to our previous study (Colasanti et al., 2012). Briefly, participants underwent two [^{11}C]carfentanil PET scans, before and three hours following oral administration of 0.5mg/kg of d-amphetamine. Five of the participants underwent both PET scans on the same day, 5 hours apart (approximately 10.30am and 3.30pm respectively). For four participants, the post-amphetamine scan was acquired on a different day due to failures in the production of the radiotracer. The average time difference

between pre- and post-scans in these cases was 14 days (maximum interval 36 days). The oral dose of 0.5 mg/kg d-amphetamine was administered 3 hours before the post-amphetamine PET scan, after a light meal. The choice of the time for the post-amphetamine scan was based upon the peak of amphetamine plasma levels reached after 3 hours (Shotbolt et al., 2012). Blood samples to assess plasma levels were obtained throughout the study day (pre-dosing and 1; 2; 3 and 4.5 hours post-dosing).

Subjective responses to the amphetamine challenge were rated using the simplified version of the amphetamine interview rating scale (SAIRS) (Van Kammen and Murphy, 1975), consisting of self-ratings for euphoria, restlessness, alertness and anxiety on an analogue scale ranging from 1 (least ever felt) to 10 (most ever felt). The rating scale was administered after the pre-amphetamine PET scan, 15 min pre dosing; 5 min; 1; 2; 3 hours post dosing (before the post-amphetamine PET scan) and 4.5 hours post dosing (after post-amphetamine PET scan). Participants also completed the Spielberger state anxiety inventory (SSAI) before and after each PET scan as well as on the screening day. The BDI was used to rule out significant depressive symptoms and was performed on screening and study days.

PET and MR imaging

We followed our PET previous protocol with a minor change in acquisition periods (Colasanti et al., 2012). The dynamic emission data were collected continuously for 90 minutes (26 frames, 8*15 s, 3*60 s, 5*120 s, 5*300 s, 5*600 s, to a total of 5400 s), following an intravenous injection over 20 s of 217± 51 (mean± SD) MBq of [^{11}C]carfentanil.

All participants underwent a structural MRI, performed on a 3T MR scanner (Magneton Trio Syngo MR B13 Siemens 3T; Siemens AG, Medical Solutions), including a volumetric T1-weighted magnetization-prepared rapid acquisition gradient-echo sequence. All structural images were inspected by an experienced clinical neuroradiologist for unexpected findings of clinical significance or features that might confound PET co-registration or quantitative analysis. No significant findings or features were observed in any of the participants included into the study.

Image analysis

All image processing and modelling was carried out using in-house analysis software developed at Imanova Ltd. (MIAKAT^{TM}). Individual PET frames were corrected for radioactive decay and for head motion using rigid-body co-registration with the 16th frame as the reference image. The T1-weighted MR image was co-registered to the summed PET image, after brain extraction using the Brain Extraction Tool (Smith, 2002). The T1 image was segmented into grey, white matter and cerebrospinal fluid. Non-linear deformation parameters were derived for the mapping of the T1 image into stereotaxic space and this enabled the mapping of a stereotaxic atlas (Tziortzi et al., 2011) into the individual's space. The individualized regions of interest were then applied to the dynamic PET data to derive regional time-activity data for nine regions of interest (ROI). The ROIs examined were: caudate, putamen, thalamus, cerebellum, frontal lobe, nucleus accumbens, anterior cingulate, amygdala and insula cortices. These regions were chosen *a priori* based on high relative density of MOR, including those which showed statistical significance in reduction of [^{11}C]carfentanil binding in our previous study.

Regional [^{11}C]carfentanil specific binding to MOR was quantified as the binding potential relative to non-displaceable binding (BP_{ND}).

BP_{ND} in grey-matter masked ROIs was estimated using the simplified reference tissue model (Lammertsma and Hume, 1996), specifying the occipital lobe as a reference tissue due to the very low regional MOR expression in this region (Hiller and Fan, 1996; Rabiner et al., 2011).

The endogenous opioid release induced by the d-amphetamine challenge was derived from the reduction in [^{11}C]carfentanil binding potential (ΔBP_{ND}).

$$\Delta BP_{ND} = (BP_{ND\ pre} - BP_{ND\ post}) / BP_{ND\ pre}$$

Analysis of subjective responses

Differences in subjective responses in regard to the amphetamine-effect using SAIRS and SSAI were calculated:

$$\Delta score = score_{4.5\ hours\ post\ dosing} - score_{baseline}$$

Statistical analysis

Differences between $BP_{ND\ pre}$ and $BP_{ND\ post}$ and between injected $mass_{pre}$ and injected $mass_{post}$ were analyzed using paired t-tests (2-tailed). For correlations between BP_{ND} and subjective effects, we calculated percentage changes in [^{11}C]carfentanil BP_{ND} ($\%\Delta BP_{ND}$) and studied correlations (Spearman non-parametric correlation) between Δscores and regional $\%\Delta BP_{ND}$. All data was normally distributed as determined by visual inspection as well as using the Kolmogorov-Smirnov and Shapiro-Wilk tests for normality. All statistical comparisons were assessed using SPSS version 20.0 and as a nominal level of statistical significance, $p<0.05$ was accepted.

Results

Injected mass and radioactivity

Mean injected [^{11}C]carfentanil $mass_{pre}$ was 1.38± 0.12 µg (mean± SD), mean injected $mass_{post}$ was 1.43± 0.07 µg. There was no significant difference between $mass_{pre}$ and $mass_{post}$ ($p>0.05$). Mean injected $radioactivity_{pre}$ was 234± 57.9 MBq, mean injected $radioactivity_{post}$ was 199± 43.4 MBq. Again, there was no significant difference between $activity_{pre}$ and $activity_{post}$ ($p>0.05$).

Effects of amphetamine on [^{11}C]carfentanil binding

Mean regional percentage reductions in BP_{ND} ranged between -2.2% and -7.2% (see Table 1). The oral d-amphetamine challenge resulted in significant reductions in [^{11}C]carfentanil BP_{ND} in the putamen ($p=0.006$), thalamus ($p=0.002$), frontal lobe ($p=0.005$), nucleus accumbens ($p=0.016$), anterior cingulate ($p=0.002$), cerebellum ($p=0.017$) and insula ($p=0.038$)- see table 1. There were no increases in BP_{ND} observed. A post-hoc analysis showed no impact of delay between 1st and 2nd scan on the results of the intervention ($t(7)=-0.31$; $p=0.93$).

- Insert table 1 here
- Insert figure 1 here

Effects of amphetamine on subjective responses

Changes in subjective amphetamine ratings, including euphoria and anxiety, were only mildly pronounced. The mean change (Δ) in euphoria scores from baseline to 4.5 hours post dosing was +1.11, maximum change was +3. SSAI ratings showed a meanΔ of -6.67, maxΔ of -24. An exploratory analysis of the relationship between changes in subjective ratings and regional $\%\Delta BP_{ND}$ did not show any significant correlations ($p>0.05$).

Pharmacokinetic amphetamine blood sampling

Data from samples for amphetamine plasma concentrations was available for seven participants. At 3 hours post-dosing (just before post-amphetamine PET scan), the calculated plasma amphetamine concentration was 89.7± 19.7 ng/ml (mean± SD) (range from 0 ng/ml at baseline to 80.3± 8.1 ng/ml at 4.5 hours post dosing). The relationship between amphetamine plasma concentrations and regional $\%\Delta BP_{ND}$ did not show any significant correlations ($p>0.05$).

Discussion

We have replicated our previous findings of a reduction in [^{11}C]carfentanil binding following an oral amphetamine challenge in an independent cohort of nine healthy volunteers. Oral amphetamine administration induced a significant reduction in [^{11}C]carfentanil BP_{ND}, consistent with an increase in extracellular endogenous opioids (Colasanti et al., 2012) in the human brain. The regional distribution of significant changes in BP_{ND} was consistent across the two studies, with putamen (-7.24± 5.78) (mean$\%\Delta BP_{ND}$± SD), thalamus (-5.72± 3.60), frontal lobe (-4.93± 3.57), anterior cingulate (-4.42± 2.89) and insula (-3.85± 4.09) cortices showing an effect in both. Additionally, in our larger second cohort, we also found significant reductions in the nucleus accumbens (-5.63± 5.39) and the cerebellum (-4.47± 4.50). Recently, Guterstam et al. (Guterstam et al., 2013) published a study reporting no changes in [^{11}C]carfentanil BP_{ND} after an IV amphetamine challenge of 0.3 mg/kg. Besides the route of administration (0.3 mg/kg IV vs. 0.5 mg/kg oral in our studies), a likely critical difference

between the protocols is in the timing of the PET scans. Guterstam et al. started the post-amphetamine scan within minutes of injection, while we waited for three hours post oral dose in order to match the peak plasma concentration. We measured amphetamine levels on several occasions throughout the protocol so we are confident that we captured the plasma peak in the post-amphetamine [^{11}C]carfentanil PET scan. Guterstam et al did not report amphetamine plasma levels though with IV administration it is likely that they also were at peak amphetamine levels close to the start of PET scan.

However capturing the peak of amphetamine levels is not the critical measure, rather it is the increase in endogenous opioids to compete with [^{11}C]carfentanil binding. Comparing the outcome of IV with oral amphetamine administration, it appears that time is required to allow endogenous opioids to increase and accumulate such that IV administration followed closely by injection of [^{11}C]carfentanil is too fast. It is certainly true that if the endogenous opioid system plays an important role in acute rewarding effects, the primary drug dosing effect has to be present within minutes; however, the secondary effect of endogenous opioid release in the brain might not be detectable in such an early stage. These studies suggest that time is needed for endogenous opioids to accumulate to be detectable with [^{11}C]carfentanil PET.

In our current study, there were no significant differences in injected [^{11}C]carfentanil mass or injected radioactivity between pre and post-amphetamine scans. This addresses a concern previously raised by Gusterstam et al. and rejects any differences between the previous studies being down to tracer mass or activity.

Another major difference between the two protocols is the participants' subjective response to the amphetamine challenge. The participants in Guterstam et al. study consistent with fast, IV administration reported strong subjective effects, which were not seen in either of our cohorts receiving oral amphetamine. Nevertheless, we were able to detect changes in [^{11}C]carfentanil BP_{ND} without participants experiencing a potentially adverse 'high', as evidenced by the lack of significant changes in the euphoria scores.

In summary, we have replicated our previous findings that endogenous opioid release following an amphetamine challenge can be detected in multiple regions in the living human

brain using [^{11}C]carfentanil PET imaging. Importantly, we did not find that an oral amphetamine challenge produces euphoria/'high', which reduces the possibility of inducing unwanted behavioral adverse effects in vulnerable patient groups. This supports the use of our PET protocol in further defining the opioid system in neuropsychiatric disorders.

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Statement of Interest

Dr Colasanti was supported by a GSK/Wellcome Trust Fellowship in Translational Medicine and Therapeutics awarded through Imperial College London. Dr Clark provides consultancy work for Cambridge Cognition Ltd. Professor Gunn Roger Gunn is a consultant for GSK, Abbvie and UCB. Professor Lingford- Hughes has received research funding from Lundbeck, NET Device Corp – consultant for imaging protocol, GSK – funding for imaging and medication for MRC funded ICCAM grant; for Wellcome Clinical Training Fellowship, for MRC funded project grant. She also received honoraria paid into University discretionary for talks from Lundbeck Institue UK, Janssen- Cilag, Pfizer and Servier for CINP Certificate in Psychopharmacology. All other authors report no conflict of interest.

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Table 1: [^{11}C]carfentanil BP_{ND} pre- and post-amphetamine in nine regions of interest

Brain region	Mean pre-amph	Mean post-amph	Mean diff	SD	Mean % decrease	SD	Sig (2-tailed)
Caudate	1.495	1.400	.095	.144	-7.236	10.316	.083
Putamen	1.875	1.746	.129	.104	-7.240	5.775	**.006**
Thalamus	2.090	1.976	.115	.075	-5.722	3.605	**.002**
Cerebellum	.798	.769	.029	.029	-4.474	4.501	**.017**
Frontal lobe	1.158	1.102	.056	.044	-4.934	3.574	**.005**
Accumbens	2.821	2.671	.151	.148	-5.630	5.391	**.016**
Ant Cingulate	1.508	1.442	.066	.043	-4.420	2.889	**.002**
Amygdala	1.801	1.756	.045	.146	-2.245	7.956	.381
Insula	1.450	1.397	.053	.065	-3.852	4.094	**.038**

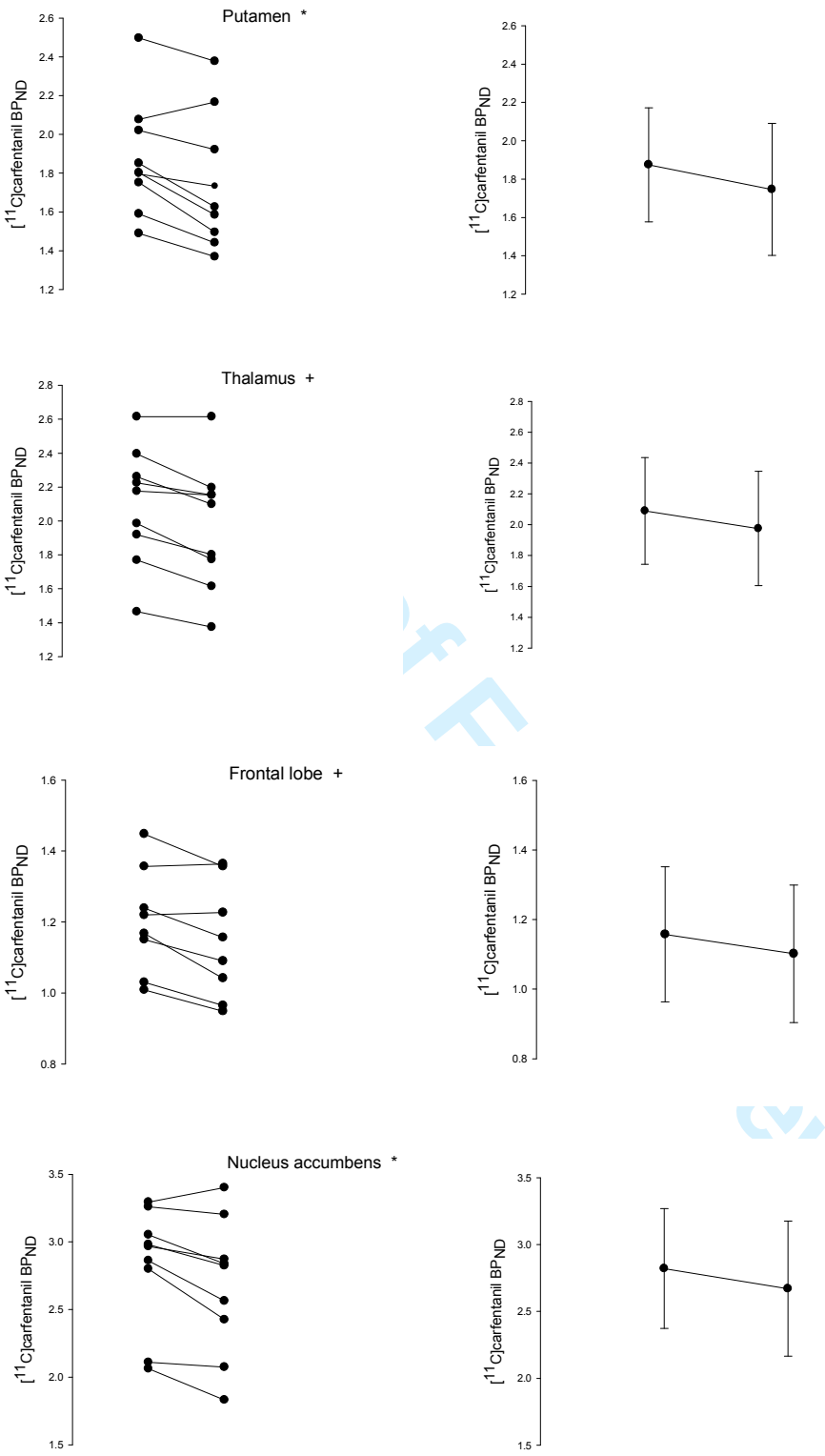
Putamen *
Thalamus +
Frontal lobe +
Nucleus accumbens *
[11C]carfentanil BPND

Fig 1: Regional analysis of [^{11}C]carfentanil binding potential (BP_{ND}). Left panel displays individual BP_{ND}, before and after amphetamine challenge. The right panel displays mean and SD of [^{11}C]carfentanil BP_{ND}. + p≤0.005, * p<0.05