Endogenous Opioid Release in the Human Brain Reward System Induced by Acute Amphetamine Administration

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Background: We aimed to demonstrate a pharmacologically stimulated endogenous opioid release in the living human brain by evaluating the effects of amphetamine administration on [^{11}C]carfentanil binding with positron emission tomography (PET).

Methods: Twelve healthy male volunteers underwent [^{11}C]carfentanil PET before and 3 hours after a single oral dose of d-amphetamine (either a "high" dose, .5 mg/kg, or a sub-pharmacological "ultra-low" dose, 1.25 mg total dose or approximately .017 mg/kg). Reductions in [^{11}C]carfentanil binding from baseline to post-amphetamine scans (ΔBP_{ND}) after the "high" and "ultra-low" amphetamine doses were assessed in 10 regions of interest.

Results: [^{11}C]carfentanil binding was reduced after the "high" but not the "ultra-low" amphetamine dose in the frontal cortex, putamen, caudate, thalamus, anterior cingulate, and insula.

Conclusions: Our findings indicate that oral amphetamine administration induces endogenous opioid release in different areas of human brain, including basal ganglia, frontal cortex areas, and thalamus. The combination of an amphetamine challenge and [^{11}C]carfentanil PET is a practical and robust method to probe the opioid system in the living human brain.

Key Words: Amphetamine, carfentanil, dopamine, neuroimaging, opioids, PET, psychostimulants

The endogenous opioid system consists of three distinct opioid receptors (ORs; μ-, Δ-, and κ-OR) and four families of endogenous peptides (β-endorphin, enkephalins, dynorphins, endomorphins). It is thought to modulate many aspects of human behavior, including the reward system (1), pain responses (2), eating (3), negative emotions (4,5), and social behaviors (6). Endogenous opioids have been implicated in the pathophysiology of disorders such as chronic pain (7), depression (8), anxiety (4), and borderline personality disorder (9). Furthermore, the endogenous opioid system has been proposed to mediate impulsivity (10) as well as the effects of multiple drugs of abuse (11).

Opioid receptors and peptides are highly expressed in brain areas involved in reward and motivation, such as the ventral striatum, putamen, caudate, frontal and cingulate cortex, hypothalamus, amygdala, and ventral tegmental area. Other brain areas with high OR expression are those involved in pain regulation such as thalamus, insula, and periaqueductal grey (PAG) (12). Endogenous opioid signaling in the brain is hypothesized to possess intrinsic rewarding properties and to mediate some of the effects of psychostimulant drugs. Animal studies demonstrating a facilitation of striatal dopamine (DA) release by OR stimulation (13–16) have led to the proposal that the mediation of rewarding behaviors by endogenous opioids is secondary to dopamine release; however, human studies of interactions of opioids with dopamine have been few, and no consistent findings have emerged (17–20).

Although opioid modulation of dopaminergic systems has been recognized, the dopaminergic modulation of opioid systems might also be important in mediating reward. Acute administration of amphetamine, a monoamine-releasing agent, induces the release of β-endorphin in the rodent ventral striatum (21) and increases striatal enkephalin and dynorphin precursor messenger RNA levels (22). Clinical data support the role of endogenous opioids in mediating the pharmacological effects of amphetamine, because the opioid antagonist naltrexone attenuates the subjective effects of amphetamine administered orally (23), and positron emission tomography (PET) studies have demonstrated a role for the opioid system in the mechanisms underlying psychostimulant addiction (24,25). However, no direct evidence for induction of endogenous opioid release by psychostimulants in the living human brain has been reported.

Positron emission tomography can detect endogenous neurotransmitter release on the basis of the competition between synaptic neurotransmitters and PET radiotracers at receptor level (26,27). [^{11}C]carfentanil is a PET agonist radioligand with high and selective affinity for μ-opioid receptors (MOR) (28). Changes in the brain binding of [^{11}C]carfentanil after physiological and psycholog-

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ical interventions designed to induce the acute release of opioid peptides have led to the suggestion that it is sensitive to endogenous opioid fluctuations (2,5).

We investigated the interactions between monoamines and endogenous opioids in the human brain in vivo by examining the effects of single oral dose of amphetamine on the binding of [^{11}C]carfentanil. To control for the effects of expectation, we compared the effect of a pharmacologically relevant dose of amphetamine with that of a pharmacologically inactive dose. We hypothesized that that only the administration of the "active" dose of amphetamine would result in an increase in the synaptic concentration of endogenous opioids and hence a decrease of [^{11}C]carfentanil binding. We also evaluated the correlation between the subjective effects of amphetamine administration and the changes in [^{11}C]carfentanil binding.

Ten anatomically defined regions of interest (ROIs) were chosen a priori, on the basis of the known distribution of MOR (Figure S1 in Supplement 1). The ROIs examined were: the ventral striatum, caudate, putamen, amygdala, thalamus, hypothalamus, frontal cortex (including up to the primary motor cortex), insula, anterior cingulate cortex, and PAG. The brain areas where changes were predicted include those involved in reward and motivation processes, such as ventral striatum, putamen, caudate, frontal and cingulate cortex, hypothalamus, and amygdala.

Methods and Materials

Study Design

This was a single-blind, nonrandomized, between-groups design study. Twelve healthy male volunteers (details in Supplementary Methods and Table S1 in Supplement 1) were examined with [^{11}C]carfentanil PET before and 3 hours after an oral administration of either a high (.5 mg/kg, $n = 6$) or an ultra-low dose (1.25 mg total dose or approximately .017 mg/kg; $n = 6$) of d-amphetamine. The ultra-low dose was included to control for any effects of expectation of receiving amphetamine and was not expected to produce pharmacological effects (29) (Figure S2 in Supplement 1).

Procedure

All subjects received identical instructions and were told they were receiving an oral dose of amphetamine. Subjects underwent magnetic resonance imaging (MRI) at approximately 9:00 AM. Ten of the subjects underwent baseline and post-amphetamine PET scans in the same day, 5 hours apart, at approximately 10:30 AM and 3:30 PM, respectively. In two cases, one within each dose group, the post-amphetamine scan was done on the day after the baseline scan, at the same time of day as the baseline scans. The oral dose of d-amphetamine sulfate (Dexedrine, UCB) was administered on an empty stomach 3 hours before the start of the post-amphetamine scan. The choice of the time for the post-amphetamine scan was based on the hypothesis that the expected effects on the opioid system are secondary to monoamines released by amphetamine. Amphetamine plasma levels after oral amphetamine administration reach a peak at 3 hours, and a previous PET study conducted in our center demonstrated dopamine release at the same time point (30).

Systolic and diastolic blood pressure and heart rate were measured 2 hours before the baseline PET scan, 30 min before amphetamine administration and immediately before and after the post-amphetamine PET scans.

The subjective response to amphetamine administration was rated by the subjects with a simplified version of the amphetamine interview rating scale (SAIRS) (31). The SAIRS consisted in self-ratings for euphoria ("feel good"), restlessness ("feel like moving"), alertness ("feel energetic"), and anxiety ("feel like moving") on an analogue scale ranging between 1 ("least ever felt") and 10 ("most ever felt"). The SAIRS was administered at the following time-points relative to amphetamine administration: 0 (before amphetamine administration), 1 hour, 2 hours, 3 hours (before PET scan), and 4.5 hours (after PET scan).

PET Protocol

Subjects were positioned in the PET scanner, after insertion of a venous cannula in an antecubital vein, and a head-fixation device was used to minimize head movements during data acquisition. All dynamic [^{11}C]carfentanil PET scans were acquired on a Siemens HiRez 6 PET/computed tomography scanner (Siemens Healthcare, Erlangen, Germany). A low-dose computed tomography scan was performed immediately before each PET study for subsequent attenuation and scatter correction. Dynamic emission data were collected continuously for 100 min (1×30 sec, 6×15 sec, 3×60 sec, 5×120 sec, 5×300 sec, and 6×600 sec), after an intravenous injection of a bolus over 20 sec of up to 350 MBq of [^{11}C]carfentanil. Maximum injected carfentanil mass was .03 μg/kg. The synthesis of [^{11}C]carfentanil has been described in details in Supplement 1.

Image data were reconstructed with filtered backprojection (direct inversion Fourier transform) with a 128 matrix, a zoom of 2.6, a transaxial Gaussian filter of 5 mm, scatter correction, and attenuation correction. All volunteers had structural MRI, performed on a 3-T MR scanner (Magnetom Trio Syngo MR B13 Siemens 3T; Siemens AG, Medical Solutions), including volumetric T_1-weighted magnetization-prepared rapid acquisition gradient-echo sequences. All structural images were inspected by an experienced clinical neuroradiologist (A.D.W.) 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 volunteers recruited into our study.

Image Analysis

Dynamic PET images were registered to the volumetric MRI dataset of the subject and corrected for motion with a frame-to-frame registration process with a mutual information cost function (SPM5b; Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm).

Most of our prechosen ROIs (see introductory section of this article) and the occipital lobe (used as reference region) were defined with the ICBM152 template (http://www2.bic.mni.mcgill.ca), deriving from an average of standard MRI brain scans from the Montreal Neurological Institute, which was nonlinearly warped to the high-resolution T1-MRI of each individual, with Statistical Parametric Mapping 5 (SPM5; Wellcome Trust Centre for Neuroimaging). The deformation parameters derived were then applied to a corresponding anatomical atlas (32) to bring this into the space of the individual subject. Finally, the MRI image, ROIs, and warped anatomical atlas were resampled to match the PET image resolution. From the atlas, the ROIs were automatically defined with SPM5 (32). Each ROI was then applied to the dynamic PET data to derive regional time-activity curves. The exceptions were the ventral striatum, hypothalamus amygdala, and the PAG—small but important regions that were manually defined on the MRI of each subject with anatomical guidelines. The definitions of "ventral striatum" and "hypothalamus" are as in previously published guidelines (32), whereas "amygdala" and "PAG" are defined as in Supplementary Methods and Figure S3 in Supplement 1.

Regional MOR availability was quantified as the [^{11}C]carfentanil binding potential (BP_{ND}) (33) with the simplified reference tissue

model (34) with occipital cortex as reference region (Figure S1 in Supplement 1). The occipital cortex has been used as a reference region in previous human studies with [^{11}C]carfentanil (35), because it has a very low concentration of MOR (36). In a study conducted in our laboratory, blockade with MOR antagonists induced a generalized, dose-dependent reductions in [^{11}C]carfentanil BP_{ND}, which was observed in all ROIs except the occipital cortex, justifying its selection as a reference region (3). Furthermore, an excellent test-retest reliability of [^{11}C]carfentanil uptake has been previously observed in the occipital cortex (37).

The binding potential relative to the nondisplaceable compartment (BP_{ND}, Equation 1) is equal to the product of concentration of available receptor sites (B_A), affinity of ligand for the target ($1/K_D$), and the free fraction in the brain (f_{ND}):

$$BP_{ND} = f_{ND}\frac{B_A}{K_D} \quad (1)$$

The magnitude of endogenous opioid release induced by the administration of d-amphetamine in each ROI was inferred from the fractional reduction in BP_{ND} (ΔBP_{ND}).

$$\Delta BP_{ND} = \frac{BP_{ND^{Baseline}} - BP_{ND^{Post\ Amphetamine}}}{BP_{ND^{Baseline}}} \quad (2)$$

Differences between baseline BP_{ND} and post-amphetamine BP_{ND} were analyzed by means of two-tailed *t* test for paired samples, separately for each amphetamine dose group. Differences in ΔBP_{ND} between high- and ultra-low-dose groups were tested with two-tailed *t* test for independent samples. An analysis of variance for repeated measures to study the effect of the interaction of time × group on BP_{ND}, with baseline BP_{ND} and post-amphetamine BP_{ND} as dependent variables and amphetamine dose group as between-subject factor was also conducted. The level of significance for all the analyses was set at .05. Data were normally distributed as determined by visual inspection and the Kolmogorov-Smirnov and Shapiro-Wilk tests for normality.

In addition to the predefined regional level analysis, exploratory voxel-level analysis was performed to examine the between-group differences in ΔBP_{ND}, with permutation-based nonparametric inference (Methods in Supplement 1).

Analysis of Physiological and Subjective Responses

The effect of the time × group interaction on heart rate, systolic and diastolic blood pressure, and subjective measures has been tested with analysis of variance for repeated measures. For the high-dose group only, we studied the correlation (Spearman's nonparametric correlation) between the magnitude of the subjective effects induced by amphetamine and regional ΔBP_{ND}. The magnitude of the subjective effects was computed by calculating the difference between the rating scores at 3 hours after amphetamine administration and the baseline rating scores (Δ scores).

Results

High baseline [^{11}C]carfentanil binding was observed in the ventral striatum, caudate, putamen, thalamus, and cingulate cortex. Other areas with baseline [^{11}C]carfentanil $BP_{ND} > 1$ were the hypothalamus, amygdala, insula, PAG, and medial orbital cortex (Figure S1 in Supplement 1).

Effects of Amphetamine on [^{11}C]carfentanil Binding

Regional ΔBP_{ND} ranged between 10.2% and 2.1% in the high-dose group and between 6.7% and −2.3% in the ultra-low dose group (see Table S2 in Supplement 1 for further details).

The high dose of amphetamine led to reductions in BP_{ND} in the putamen, caudate, frontal cortex ($p < .0001$), thalamus, insula, and anterior cingulate ($p < .005$). No increases in BP_{ND} were observed after the high amphetamine dose. The reductions in BP_{ND} in the putamen ($p < .01$), thalamus, and frontal cortex were greater in the high- than in the ultra-low-dose group ($p < .05$) (Figure 1). Relative differences in ΔBP_{ND} between groups showed similar trends in the ventral striatum, caudate, insula, and anterior cingulate cortex but did not achieve statistical significance ($.05 < p < .09$). No evidence for group differences in ΔBP_{ND} was observed in the hypothalamus, amygdala, and PAG.

The results of an exploratory whole-brain, voxel-wise analysis were consistent with the a priori regional analysis, demonstrating higher ΔBP_{ND} in the putamen; ventral striatum; caudate; thalamus; orbitofrontal cortex; and superior, medial, inferior, and precentral frontal gyri for the high-dose group ($p < .05$, corrected) (Figure 2, Table S3 in Supplement 1).

Effects of Amphetamine on Physiological and Subjective Parameters

We observed significant increases from baseline in heart rate after amphetamine and systolic and diastolic blood pressure in the high-dose group, whereas in the ultra-low-dose group, no effect of amphetamine on physiological parameters was observed (Table S4 in Supplement 1). The changes in heart rate over time were greater in the high-dose group relative to the ultra-low-dose group, with significant group differences in the within-subjects contrasts between post-amphetamine time points (+3 hours and +6 hours) and baseline (0 hours) ($p < .0001$) (Figure S2 in Supplement 1). Similarly, the increase over time in systolic blood pressure was greater in the high-dose group ($p < .05$), whereas the between-groups difference in the change in diastolic blood pressure did not reach statistical significance ($p = .07$).

The changes in subjective ratings after amphetamine administration are presented in Figure S4 in Supplement 1. A mild increase in euphoria and alertness ratings was observed after amphetamine administration, which did not reach statistical significance. The increase in euphoria was more evident in the high-dose group, although the effect of time × group interaction was not significant. A decrease in anxiety ratings after amphetamine administration— greater in the high-dose group ($p < .05$)—was observed, but the restlessness ratings did not change (Figure S4 in Supplement 1).

An exploratory analysis of the relationship between change in subjective ratings and regional ΔBP_{ND} showed significant positive correlations between Δeuphoria and ΔBP_{ND} in the ventral striatum (Figure S4 in Supplement 1), between Δanxiety and ΔBP_{ND} in the putamen, and between Δrestlessness and ΔBP_{ND} in the thalamus.

Discussion

We have demonstrated a reduction in the binding of [^{11}C]carfentanil to the MOR in the human basal ganglia, thalamus, and frontal cortex after an administration of a pharmacologically relevant dose of d-amphetamine. Our results are consistent with preclinical evidence that amphetamine induces a significant increase in the extra-neuronal concentration of opioid peptides with high affinity for MOR (21,22). The availability of the MOR to bind [^{11}C]carfentanil can be reduced by the release of a variety of opioid peptides that have relatively high affinity for MOR, including β-endorphin, endomorphins 1 and 2, met- and leu-enkephalins, and dynorphin B (Table S5 in Supplement 1).

The high and ultra-low amphetamine doses were chosen to be pharmacologically active and inactive, respectively. The high am-

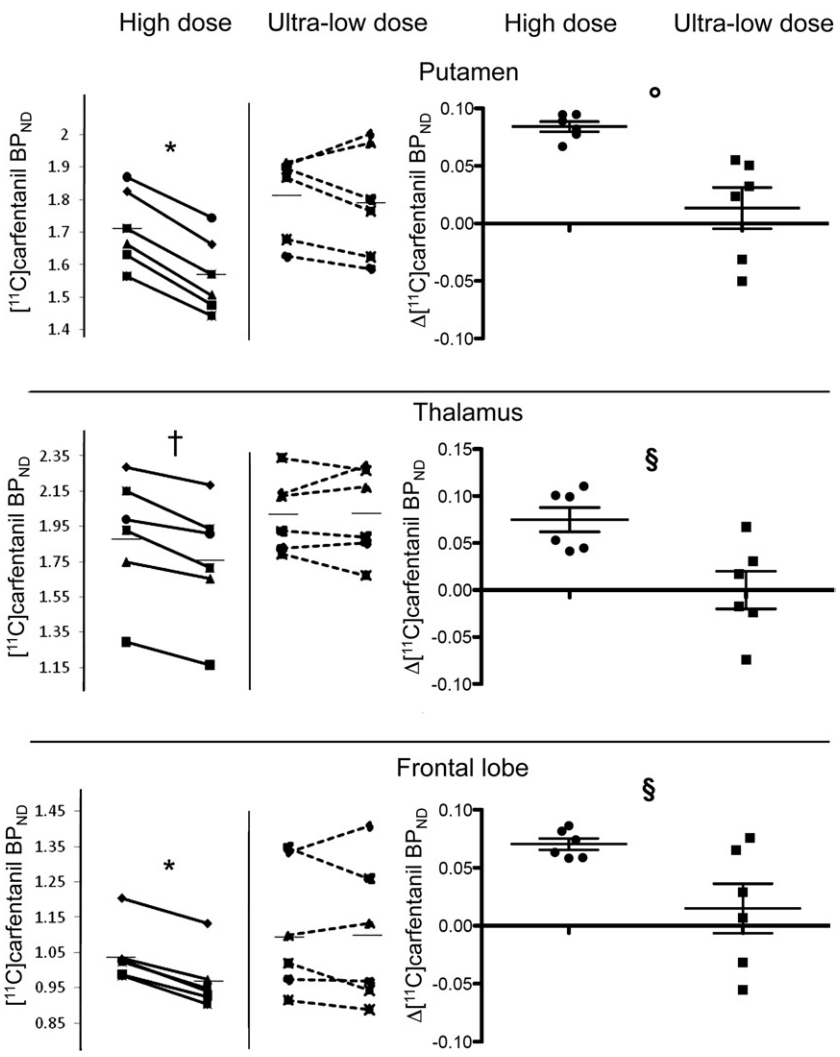

Figure 1. Regional analysis of [^{11}C]carfentanil binding potential (BP_{ND}) across amphetamine dose groups. High dose: .5 mg/kg amphetamine; ultra-low dose: 1.25 mg or .017 mg/kg amphetamine. Left panel displays individual BP_{ND}, before and after amphetamine. The right panel displays ΔBP_{ND}. Bar and markers indicate mean and individual values, respectively. *$p < .0001$; †$p < .005$ (paired samples two-tailed *t* test); °$p < .01$; §$p < .05$ (independent samples two-tailed *t* test).

phetamine dose, .5 mg/kg, has been shown to induce a release of dopamine detectable with PET (38). In contrast, a total oral amphetamine dose of only 1 mg was found to be pharmacologically inactive in humans (39), and a threshold of .07 mg/kg was proposed for pharmacological effects of amphetamine (29). In our subjects, the high dose of amphetamine induced increases in heart rate and systolic and diastolic blood pressure, whereas the ultra-low dose of amphetamine produced no effects on physiologic parameters, providing support for our choice of doses.

We observed amphetamine-related changes in [^{11}C]carfentanil

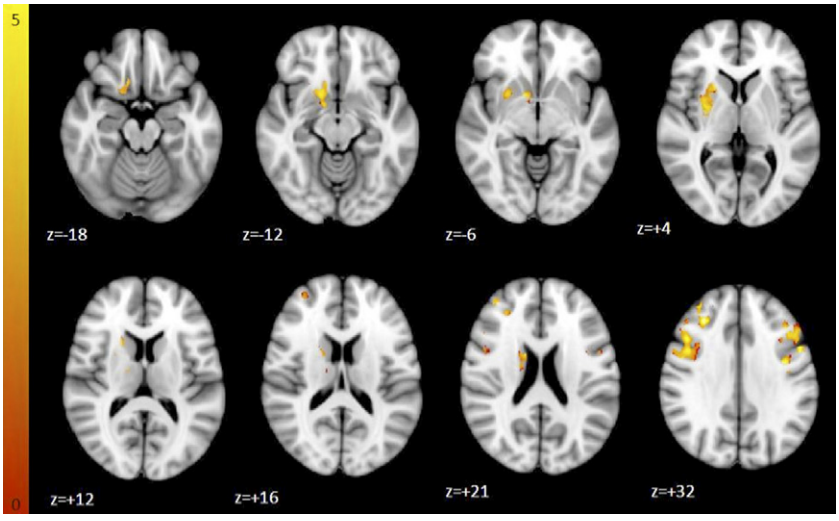

Figure 2. Differences in ΔBP_{ND} between high and ultra-low amphetamine dose groups at the voxel level. ($\Delta BP_{ND\ high} > \Delta BP_{ND\ ultra\text{-}low}$; corrected for multiple comparisons). Parametric statistical testing was carried out at the level of spatially contiguous supra-threshold voxel clusters, while controlling the family-wise probability of type 1 error at $p < .05$, corrected).

BP_{ND} in anatomical regions known to be rich in opioid projections. Opioid neuron fibers positive for β-endorphin, originating in the arcuate nucleus of the hypothalamus, are abundant in all diencephalic structures (40) and have also been described in the whole striatum (41). Small groups of neurons in the frontal cortex, particularly in the cingulate and superior and medial frontal gyri, have a high density of β-endorphin afferents (42). Enkephalin-containing cell bodies and fibers are widely distributed in the striato-pallidal regions and in the diencephalon (41). Endomorphinergic, especially endomorphin-1, fibers are also highly distributed in striatal and thalamic regions (43). However, not all regions with rich opioid innervation displayed the effects of amphetamine. It is not clear why significant changes in [^{11}C]carfentanil BP_{ND} were not seen in regions such as amygdala, hypothalamus, and PAG, where high levels of endogenous opioid terminals have been described (41,43). It is possible that amphetamine-related changes in opioid levels might only occur in areas with significant expression of monoaminergic transporters, which are the primary targets of amphetamine. It is also possible that changes in [^{11}C]carfentanil BP_{ND} in the reference region, induced by amphetamine, might have led to lower measures of ΔBP_{ND} (Discussion in Supplement 1). Because we used simplified reference tissue model as a method for the quantification of the BP_{ND} instead of direct measurement of the arterial plasma input function, we can't rule out this possibility.

In situ hybridization studies have shown that amphetamine administration is associated with increased synthesis of endogenous opioids (22), but there is no evidence of a direct effect of amphetamine on the release of peptides from opioid-synthesizing neurons. It is very unlikely that amphetamine competes directly with [^{11}C]carfentanil. We have examined the affinity of amphetamine at MOR by performing an in vitro receptor binding assay in rat brain tissue (Methods in Supplement 1). Our data suggest that the affinity of amphetamine at MOR is >100 μmol/L, whereas the affinity of amphetamine for the dopamine transporter (DAT) in cultured intestinal cells was found to be 150 times higher at approximately .64 μm (44), suggesting that direct occupancy of the MOR by amphetamine is very unlikely to lead to the changes in [^{11}C]carfentanil BP_{ND} we observed. An amphetamine dose occupying >1% of MOR would produce an occupancy at DAT approaching 100%, which we do not believe would be tolerable in vivo in humans.

The amphetamine challenge is not believed to directly induce opioid release. Amphetamine administration leads to release of a variety of monoamines, including DA, norepinephrine (NE), and serotonin (45). There are strong grounds to implicate DA specifically as the primary mediator of amphetamine for endogenous opioid release. Cocaine-induced release of β-endorphin in the ventral striatum is attenuated by a blockade of the DA D_2 but not D_1 receptors at hypothalamic level (46,47). The DA D_1 and D_2 receptor families on striatopallidal γ-aminobutyric acid-ergic medium spiny neurons also modulate the synthesis of enkephalins and dynorphins, respectively (48). Finally, DA and DA-agonists but not NE enhance the efflux of met-enkephalin from striatal slices (49). Our data therefore support the notion that the brain dopaminergic system modulates opioid neurotransmission.

Because we believe monoamines have a role in mediating the effects of amphetamine on opioid release, the anatomical distribution of monoaminergic terminals is a factor to consider in the interpretation of our findings. The distribution of the observed amphetamine-induced changes in [^{11}C]carfentanil BP_{ND} overlaps partially with the known distribution of the DAT and dopaminergic tracts. The DAT is highly expressed in the putamen, caudate, and ventral striatum and to a lesser extent in the frontal cortex, cingulate cortex, insula, and thalamus. In fact, DAT levels are significantly lower in regions outside the striatum and midbrain (50–52). Although there is a different order of magnitude in dopamine in the frontal areas and striatal regions, similar changes in [^{11}C]carfentanil BP_{ND} across these regions have been observed. This is particularly of interest, because the involvement of other monoamines might be important in brain regions other than striatum, with the insula and the anterior cingulate rich in the serotonin transporter and serotonin receptors (which are also highly expressed in the striatum and thalamus) (53), whereas the norepinephrine transporter is highly expressed in the thalamus (54). However, the relationship between monoamine release and endogenous opioid release might not be straightforward. Opioid release might occur in close proximity to the release of monoamines, such as DA, or can occur distally, via the effects of monoamines on long tracts. Our study cannot inform on the exact mechanisms underlying monoamine-induced opioid release.

We had to exclude the possibility of a direct occupancy of the MOR by nontracer amounts of [^{11}C]carfentanil being responsible for our results. We examined the relationship between the injected mass of carfentanil (range .16–2.33 μg) and BP_{ND} at baseline in 37 subjects examined in our center over the past 2 years. We found no correlation between the injected mass and [^{11}C]carfentanil BP_{ND} in this dose range (Figure S6 in Supplement 1). Thus, despite some minor differences in injected mass between baseline and post-amphetamine scans (Table S1 in Supplement 1) and some statistically significant differences in injected mass at both baseline and post-amphetamine scans between groups (higher injected mass in the ultra-low relative to the high-dose group), we believe that the injected mass of carfentanil is not a significant factor in the interpretation of our data. Thus, we are confident that the effects reported here are mediated through the action of monoamines and, more specifically, DA.

The study of the relationship between subjective effects of amphetamine and changes in [^{11}C]carfentanil binding was limited by our small sample size and has to be considered as preliminary. The presence of a trend indicating a positive correlation of Δeuphoria to ΔBP_{ND} in the ventral striatum (Figure S4 in Supplement 1) is consistent with the notion that the euphoric effects of amphetamine are mediated by release of endogenous opioids (Discussion in Supplement 1).

In conclusion, we have characterized an amphetamine challenge in the context of a [^{11}C]carfentanil PET study as a practical and robust method to probe the opioid system in the living human brain. This represents the first direct demonstration of pharmacologically stimulated endogenous opioid release in the living human brain. The application of this methodology to patient populations has the potential to elucidate the role of opioid peptides in neuropsychiatric disease more broadly.

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Supplementary material cited in this article is available online.

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