

Title:

Single cocaine exposure does not alter striatal presynaptic dopamine function in mice: an [¹⁸F]-FDOPA PET study

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Abstract

Cocaine is a recreational drug of abuse that binds to the dopamine transporter (DAT), preventing reuptake of dopamine into presynaptic terminals. The increased presence of synaptic dopamine results in stimulation of both pre- and postsynaptic dopamine receptors, considered an important mechanism by which cocaine elicits its reinforcing properties. However, the effects of acute cocaine administration on presynaptic dopamine function remain unclear. Non-invasive imaging techniques such as positron emission tomography (PET) have revealed impaired presynaptic dopamine function in chronic cocaine users. Similar impairments have been seen in animal studies, with microdialysis experiments indicating decreased basal dopamine release. Here we use μ -PET imaging techniques in mice to measure dopamine synthesis capacity and determine the effect of acute cocaine administration on presynaptic dopamine function. We show that a dose of 20mg/kg cocaine is sufficient to elicit hyperlocomotor activity, peaking 15-20 min post treatment ($p < 0.001$). However, dopamine synthesis capacity in the striatum was not significantly altered by acute cocaine treatment (K_i^{Cer} : 0.0097 min⁻¹ vs. 0.0112 min⁻¹ in vehicle controls, $p > 0.05$). Furthermore, expression levels of two key enzymes related to dopamine synthesis, tyrosine hydroxylase and aromatic l-amino acid decarboxylase, within the striatum of scanned mice were not significantly affected by acute cocaine pre-treatment ($p > 0.05$). Our findings suggest that while the regulation of dopamine synthesis and release in the striatum have been shown to change with chronic cocaine use, leading to a reduced basal tone, these adaptations to presynaptic dopaminergic neurons are not initiated following a single exposure to the drug.

Key words: Behaviour, Cocaine, Dopamine, Mouse, PET

Abbreviations:

AADC - aromatic l-amino acid decarboxylase

COMT - catechol-O-methyltransferase

DAT – dopamine transporter

[¹⁸F]-FDOPA - [¹⁸F]-fluoro-3,4-dihydroxyphenyl-L-alanine

K_i^{Cer} - influx rate constant, index of dopamine synthesis capacity

K_i^{mod} - modified influx rate constant, incorporating k_{loss}

k_{loss} – rate constant of radio-metabolite loss

ROI – region of interest

SUV – standardised uptake value

TAC – time-activity curve

TH – tyrosine hydroxylase

μPET – micro Positron Emission Tomography

V_d – distribution volume, index of dopamine storage capacity

VTA – ventral tegmental area

Introduction

Cocaine is a drug commonly abused for recreational purposes, with approximately 18.3 million users worldwide in 2014 (United Nations Office on Drugs and Crime, 2016). Cocaine binds to dopamine transporters (DAT), preventing the reuptake of dopamine from the synaptic cleft back into presynaptic terminals (Kuhar et al., 1991). As a result, cocaine rapidly increases the levels of extracellular dopamine, leading to stimulation of dopamine D1 and D2 receptors. This D1-binding is considered a crucial process in the reward pathways activated by drugs of abuse (Caine et al., 2007; Gether et al., 2006). In animal studies, the blocking of DAT by various psychostimulants is associated with increased locomotor activity (Tilley et al., 2007). The initial rewarding effects of drugs such as cocaine are considered the first of three spiralling steps on the pathway towards addiction; followed by withdrawal and anticipation/drug seeking behaviours (Koob and Le Moal, 2001; Koob and Volkow, 2016).

Presynaptic dopamine function is regulated by a number of factors including the activities of tyrosine hydroxylase (TH) and aromatic l-amino acid decarboxylase (AADC), enzymes responsible for the *in vivo* synthesis of L-Dopa and conversion to dopamine respectively, and by autoreceptors (Cumming et al., 1995, 1997). Stimulation of presynaptic dopamine D2 autoreceptors by agonists leads to a reduction in dopamine neuron firing and down-regulation of dopamine synthesis (Ford, 2014). Thus, by increasing synaptic dopamine, cocaine may initiate auto-regulatory feedback that reduces dopamine synthesis. By reducing the availability of dopamine for release, this could contribute to escalating patterns

of cocaine use and the development of addiction. Supporting this, chronic cocaine use in humans has been associated with a reduction in striatal dopamine release following a stimulant-challenge in a number of studies with large effect sizes (Ashok et al., 2017; Martinez et al., 2007, 2011, Volkow et al., 1997, 2001, 2014). Moreover, chronic human cocaine use is associated with reduced dopamine synthesis capacity, as indexed by the rate constant for [^{18}F]-fluoro-3,4-dihydroxyphenyl-L-alanine (FDOPA) uptake and its conversion to dopamine (Wu et al., 1997).

As these studies were all done in chronic users it is not known if acute use results in reduced dopamine synthesis capacity in humans but there is some evidence from two animal studies that acute use results in dose-dependent decreases in presynaptic dopamine activity with increasing cocaine concentrations (Fang et al., 2013; Yeh et al., 2014). However, these studies used quantitative autoradiography techniques to measure specific binding ratios at a fixed time (2hrs) after [^{18}F]-FDOPA administration to determine tracer uptake. Whilst this *ex vivo* technique gives an index of the total accumulation of radiolabeled F-DOPA, it is not a direct equivalent to the *in vivo* rate constant derived from the human imaging study, determined through dynamic acquisitions over the complete scan length. A separate PET study in monkeys using [β - ^{11}C]-DOPA has previously demonstrated time-dependent changes in dopamine synthesis rates following cocaine, from an initial decrease 30 min post treatment, to a transiently increased synthesis rate 3-5 hours after treatment (Tsukada et al., 2000). However, it is not clear if acute cocaine administration would result in altered dopamine synthesis capacity over the same period used

in human studies and thus, it remains unknown if acute cocaine results in the same reduction in dopamine synthesis capacity seen in chronic human cocaine users.

In view of this, we conducted a PET study of [^{18}F]-FDOPA accumulation in the striatum of mice acutely treated with cocaine to estimate presynaptic dopamine synthesis capacity derived from *in vivo* data acquired over the duration of the scan. Based on the evidence above, we hypothesised that mice treated acutely with cocaine sufficient to elicit a behavioural response would exhibit reduced striatal dopamine synthesis capacity compared to saline treated controls.

Experimental Procedures

Subjects:

Young adult male C57Bl/6J mice (9-11 weeks old, mean weight 25.4 ± 0.6 g, RRID: IMSR_JAX:000664) were obtained from Charles River (Kent, UK) and group housed under a 12:12hr light:dark cycle with food and water provided *ad libitum*. All experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and approved by the Animal Welfare and Ethical Review Board of Imperial College London. We selected a dose of 20mg/kg i.p. cocaine based on previous evidence this leads to robust increases in dopamine levels and a marked locomotor response (Tilley et al., 2007).

Open field:

We first sought to confirm that the 20mg/kg cocaine dose resulted in a locomotor response using an open field test. The open field arena consisted of a plain wooden box (45x45x30cm) with sawdust covering the base. Mice were individually placed in the centre of the open field and habituated for 20 min. At the start of the test phase, mice were given 20mg/kg cocaine (Sigma-Aldrich, UK) i.p. (n=10) or 0.9% saline vehicle (n=10) in a similar volume. Locomotor activity was tracked from above using EthoVision XT tracking software (Noldus, Nottingham, UK) and total distance travelled over the 60 min test phase was analysed in 5 min bins. All open field tests were carried out in dim white light during the light cycle.

Radiotracer Production:

[¹⁸F]-FDOPA was produced as previously described by Füchtner et al., (2008).

The specific activity of [¹⁸F]-FDOPA injected was 0.03 ± 0.01 GBq/ μ mol. The radioactive concentration received was 108 ± 12 Mbq/ml. The injected dose of [¹⁸F]-FDOPA was 4.5 ± 0.6 MBq (mean $\pm$ SEM) given intravenously in a volume of 5ml/kg.

PET Scanning:

A second cohort of drug naïve mice was used for PET scanning and western blot analysis. All scanning was performed at Imanova Ltd (London, UK). Mice were anaesthetised and maintained at 1.5-2% isoflurane in 1L/min oxygen throughout surgery and the scan with temperature and respiration continually monitored (BioVet, m2m Imaging Corp, OH, USA). One hour prior to scanning, mice underwent surgery to cannulate the external jugular vein for intravenous delivery of [¹⁸F]-FDOPA. The catechol-O-methyltransferase (COMT) inhibitor Entacapone (40mg/kg in 10% DMSO, i.p., Sigma-Aldrich), and the aromatic l-amino acid decarboxylase (AADC) inhibitor Benserazide hydrochloride (10mg/kg in sterile water, i.p., Sigma-Aldrich) were given 45 min and 30 min prior to [¹⁸F]-FDOPA respectively to prevent peripheral metabolism of the radiotracer (Walker et al., 2013a, 2013b). A bolus injection of 20mg/kg cocaine in 2ml/kg (n=10) or saline in equal volume (n=10) was administered i.p. 30 min prior to [¹⁸F]-FDOPA dosing.

Following surgery, animals were transferred to the Inveon μ -PET/CT scanner (Siemens, Surrey, UK). Mice were given a CT scan (20 min) for attenuation

correction and to provide structural references. Intravenous delivery of [^{18}F]-FDOPA coincided with the start of a 120 min dynamic PET scan. At the end of the PET scan, the animals were sacrificed and, in a subset, the striata were isolated and stored at -80°C for downstream analysis of tyrosine hydroxylase (TH) and aromatic l-amino acid decarboxylase (AADC) enzyme expression levels by western blot.

Image Analysis:

PET data was histogrammed (43 frames: 10x3sec, 6x5sec, 8x30sec, 5x60sec, 6x300sec, 8x600sec) and images were reconstructed using filtered back projection, correcting for CT attenuation, randoms, scatter and radiotracer decay. The percentage injected dose was corrected for body weight and activity to normalise to standardised uptake values (SUV). Using the Inveon Research Workspace software (Siemens), PET and CT images were checked for alignment and 3D regions of interest (ROIs) were drawn around the left and right striatum (0.06cm^3) as well as the cerebellum (0.1cm^3), which was used as a reference region in place of an arterial input function. Time-activity curves (TACs) were generated from the dynamic PET data and modelled by extended Patlak graphical analysis with the cerebellum as a reference region (Patlak and Blasberg, 1985) using an in-house pipeline developed in Matlab (Mathworks, MA, USA). This pipeline derived two previously published outcome measures of dopamine synthesis capacity from the Patlak analysis. The first value, K_i^{Cer} is the influx rate constant, which provides an estimate of fluorodopamine accumulation within the striatum from a linear regression of data between 10-60 min relative to a reference region, the cerebellum (Kyono et al., 2011). We have

used the notation K_i^{Cer} to be consistent with our previous clinical imaging publications, although it should be noted that other notations have been used in the field (Bloomfield et al., 2014; Egerton et al., 2010; Howes et al., 2011). The second value, K_i^{mod} , is a modified version of the uptake constant corrected for k_{loss} (Holden et al., 1997; Patlak and Blasberg, 1985; Walker et al., 2013a). K_i^{mod} was derived from an extended period of data (15-120 min) in order to account for the loss of activity within dopaminergic neurons (k_{loss}), as the irreversible trapping becomes reversible after the first hour. This extended form of Patlak analysis estimated a value for k_{loss} and adjusted the uncorrected K_i^{Cer} value accordingly. Prior to data analysis, a blind independent researcher determined that the optimal window of analysis, in terms of lowest between-subject variability, was 15-120 min for the extended Patlak analysis (data available upon request). Parametric images of K_i^{Cer} were generated by applying the Patlak graphical analysis to individual voxel time activity curves within matlab for visual purposes. Using data from the extended Patlak analysis, dopamine storage capacity, indexed as the distribution volume, was calculated as $V_d = K_i^{mod} / k_{loss}$.

Western Blotting:

Striatal brain tissue from vehicle (n=5) and cocaine (n=6) treated mice collected following the scan (120 min post [^{18} F]-FDOPA administration) were homogenised in RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, and 50 mM Tris-HCl, pH 7.2, Sigma-Aldrich). In addition, a separate cohort of mice treated identically to scanned animals were collected 15 min after [^{18} F]-FDOPA administration (vehicle and cocaine, n=4 per group) or 60 min after the radiotracer (vehicle n=3, cocaine n=4 per group). A further cohort,

given vehicle in place of the inhibitors entacapone and benserazide was collected 120 min after [^{18}F]-FDOPA to rule out possible interactions of the inhibitors with cocaine (vehicle and cocaine, $n=4$ per group). 10 μg of protein from each sample was separated on a 4-10% Bis-Tris gel and subsequently transferred to a methanol-activated PVDF membrane (Millipore, Hertford, UK). The membrane was blocked with 5% bovine serum albumin (BSA, ThermoFisher, UK) in tris-buffered saline with 0.1% tween 80 (TBS-T) for 1 hour prior to immunoblotting with antibodies selective for tyrosine hydroxylase (1:5000 dilution, AB9983, Millipore, UK, RRID: AB_1587573) or AADC (1:5000 dilution, ab131282, Abcam, UK, RRID: AB_11157524) overnight. Membranes were incubated in an HRP conjugated goat anti rabbit secondary antibody (1:20,000, AP307P, Millipore, RRID: AB_11212848) prepared in 1% BSA, TBST, for 1 hour. Pierce ECL western blotting substrate (Sigma-Aldrich) was applied to the membranes for chemiluminescence detection of TH and AADC on Carestream Kodak Biomax film (Sigma-Aldrich). After development, the membrane was stained with Amido Black solution (Sigma-Aldrich) to confirm equal loading of lanes. Relative quantification of TH and AADC were normalised using the membrane on ImageJ software (NIH, USA).

Power Analysis:

Previous studies of acute cocaine exposure on striatal [^{18}F]-FDOPA uptake in rodents showed large effect sizes (Cohen's $d = >3.31$) as measured by quantitative autoradiography (Fang et al., 2013; Yeh et al., 2014). In rats, [^{18}F]-FDOPA μ -PET has been used to measure changes in striatal dopamine synthesis capacity in response to chemical lesioning of dopamine neurons, again with large

effect sizes (Cohen's $d = 1.86-2.02$) (Kyono et al., 2011; Walker et al., 2013a). A priori power analysis in G*Power (Faul et al., 2007) indicated that expecting a more conservative effect size of $d = 1.2$ at $>80\%$ power would require $n=10$ per group to detect significant effects on dopamine synthesis capacity at $\alpha = 0.05$. Prior to the experiments, mice were randomly assigned to a treatment group using a random number generator (vehicle = odd, cocaine = even) until groups reached the required size.

Statistical Analysis:

Differences in locomotor activity were determined by two-way ANOVA (treatment x time as main factors), followed by Bonferroni's multiple comparisons post hoc test. For comparisons of dopamine synthesis capacity outcome measures (K_i^{Cer} and K_i^{mod}) the saline treated vehicle group was tested against the cocaine treated group using an unpaired two-tailed t-test. Striatal expression levels of TH and AADC were compared by two-way ANOVA (treatment vs. time). The effect of entacapone and benserazide on TH and AADC expression was tested using a two-tailed t-test. All statistical tests were performed using GraphPad Prism v.6 software (GraphPad, CA, USA). Statistical significance was considered $p<0.05$. No data was excluded from the statistical analysis.

Results

Acute administration of cocaine increases locomotor activity:

Following habituation in the open field arena, administration of 20mg/kg cocaine significantly increased locomotor activity over the 60 min testing period (fig 1) with significant effects of treatment ($p < 0.001$, $F_{(13,234)} = 82.69$), time ($p < 0.001$, $F_{(13,234)} = 28.26$), and an interaction of both ($p < 0.001$, $F_{(13,234)} = 26.41$). Multiple comparisons post hoc analysis confirmed there was no difference between the two groups at the end of the habituation period ($p > 0.05$) and increased locomotor activity was found within the first 5 min bin post cocaine administration.

Dopamine synthesis capacity is unchanged by acute cocaine exposure

After confirming that a dose of 20mg/kg cocaine was sufficient to elicit behavioural responses associated with increased synaptic dopamine, we examined the effect on presynaptic dopamine function using [^{18}F]-FDOPA μ -PET to measure dopamine synthesis capacity, as indexed by the influx rate constant K_i^{Cer} . Previous studies have reported low signal-to-noise and poor distinction of the striatal uptake in mice using [^{18}F]-FDOPA (Honer et al., 2006). Time activity curves of the mean striatal and cerebellar uptake are shown in figure 2a. Specificity of the striatal signal can be seen in representative images in figure 2b, where standardized uptake values of the radiotracer signal were corrected for body weight and time of dosing. The main results are summarised in Table 1. Dopamine synthesis capacity in cocaine treated mice was not significantly different from vehicle treated controls (t-test, $t = 1.952$, $p > 0.05$, fig 3a-b). K_i^{mod} was also unaffected following acute cocaine treatment (fig 3c),

which was not significantly different between the two groups (t-test, $t=1.103$, $p>0.05$). Dopamine storage capacity, indexed by the distribution volume (V_d) of [^{18}F]-FDOPA and its radio-metabolites (Schabram et al., 2014) and similar to the effective distribution ratio (EDVR) (Sossi et al., 2001; Walker et al., 2013a) also did not show significant differences following cocaine exposure (t-test, $t=0.9001$, $p>0.05$).

The regulatory enzymes of presynaptic dopamine function are unaltered by acute cocaine exposure

After PET scanning, striatal expression levels of tyrosine hydroxylase (fig 4a-c) and AADC (fig 4d-f) were determined by western blot analysis. Two-way ANOVA revealed a significant effect of time ($p<0.001$, $F_{(2,19)}=15.89$), but no effect of cocaine pre-treatment ($p>0.05$, $F_{(1,19)}=1.077$) and no interaction ($p>0.05$, $F_{(2,19)}=0.1264$) on TH expression. Similarly, AADC expression showed a significant effect of time ($p<0.05$, $F_{(2,20)}=3.854$), but was not altered by cocaine pre-treatment ($p>0.05$, $F_{(1,20)}=0.08676$), , or an interaction between the two factors ($p>0.05$, $F_{(2,20)}=2.741$).

Discussion

Our main findings are that an acute exposure to cocaine, sufficient to elicit a marked behavioural response, does not significantly affect striatal dopamine synthesis capacity or dopamine synthetic enzyme levels. These findings are not consistent with our hypothesis and suggest that the reduced dopamine synthesis capacity seen in chronic cocaine users (Ashok et al., 2017; Martinez et al., 2007, 2011, Volkow et al., 1997, 2001, 2014) either only becomes apparent after repeated exposure or, alternatively, is an intrinsic vulnerability factor.

We found a dose of 20mg/kg cocaine was sufficient to cause transient increases in locomotor activity up to an hour after peripheral administration, consistent with previous studies on the locomotor effects and duration of acute cocaine exposure (Gulley et al., 2003; Nelson et al., 2009; Yeh and Haertzen, 1991). This hyperactivity has been previously shown by combined microdialysis/open field studies to correlate with dopamine increases within the dorsal striatum over the same time course (Nelson et al., 2009).

The two previous rodent studies of the effects of cocaine on presynaptic dopamine function both used quantitative [^{18}F]-FDOPA autoradiography, and found dose-dependent decreases in striatal specific binding 30 min and 150 min after cocaine treatment (Fang et al., 2013; Yeh et al., 2014). Previous efforts to use [^{18}F]-FDOPA μPET to measure striatal uptake in mice have reported low signal-to-noise and difficulty in delineation of striatal ROIs (Honer et al., 2006), though see Sharma et al. (2006). The current study shows highly specific striatal uptake in mice using [^{18}F]-FDOPA μPET , and is the first, to our knowledge, to

apply Patlak analyses for determining dopamine synthesis capacity and other translational rate constants. The use of benserazide and entacapone to inhibit peripheral AADC and COMT metabolism respectively, enhances striatal trapping of the tracer, in line with uptake reported previously in several rat studies (Kyono et al., 2011; Walker et al., 2013a).

The differences reported here between our current findings and those of the previous mouse studies are likely attributable to methodological approach. In the previous studies, striatal uptake was measured *ex vivo* at a fixed time 2hr post [^{18}F]-FDOPA delivery. The irreversible nature of [^{18}F]-FDOPA accumulation following i.v. administration is typically present for up to an hour in an *in vivo* system. Beyond this, the subsequent decarboxylation of [^{18}F]-FDOPA through metabolism requires additional modeling for accurate estimation of presynaptic dopamine function (Deep et al., 1997; Kumakura and Cumming, 2009; Sossi et al., 2003). In the present study, we explored two outcome measures derived from graphical analysis that have been used previously to index dopamine synthesis (Bloomfield et al., 2014; Holden et al., 1997; Walker et al., 2013a). Both measures have their strengths and weaknesses, with K_i^{Cer} having lower variance but potentially more bias whilst K_i^{mod} is less biased, but has a higher variance. The extended Patlak analysis used to derive K_i^{mod} was based on data from 15-120 min of the dynamic scan, allowing for correction of k_{loss} . Previously Walker et al. (2013a) have used up to 180 min to derive a value for k_{loss} in rats. Despite the shorter scan length, we show in Table 1 comparable values for k_{loss} and distribution volumes in line with the EDVR reported previously in rats.

Notably, the time of cocaine exposure also differed between studies, with Yeh et al (2014) giving cocaine 90 min post [^{18}F]-FDOPA, and Fang and colleagues (2013) providing cocaine either 30 min prior- or 90 min post-[^{18}F]-FDOPA. In the latter study, inhibition of specific binding by cocaine in the striatum was notably reduced in the pre-treated group relative to the post-treated animals. Since the pre-treated animals more closely resembled the protocol used in the current study, more subtle changes in presynaptic function were to be expected. A power analysis of the two original studies indicated cocaine to have very strong effect sizes (Cohen's $d = >3.3$) on striatal trapping. The few other rodent studies assessing dopamine synthesis capacity also reported large effect sizes of Cohen's $d = >1.8$ following chemical lesioning. To be conservative we powered the study to detect smaller effects, and, consequently, larger group sizes were used in the current study to provide greater than 80% power for these effects. Nevertheless, as the effect size we were powered to detect was still large ($d > 1.2$), we cannot exclude the possibility that we have failed to detect a smaller effect, although its clinical significance may be questionable. Furthermore, Tsukada et al. (2000) demonstrated a time-dependent change in the rate of dopamine synthesis following acute cocaine treatment in awake monkeys. While this study supported our hypothesis for seeing reduced dopamine synthesis over the time range explored, the transient increase in the synthesis rate 3-5 hours after cocaine exposure suggests there may be slower adaptations to the dopamine system occurring.

While our hypothesis, based on previous findings in chronic cocaine users (Wu et al., 1997), was centered around dopamine synthesis capacity as our primary

measure, Schabram et al. (2014), using the alternative psychostimulant methylphenidate, found significant effects of the drug on the k_{loss} parameter, without seeing changes in synthesis capacity. Our own measures of k_{loss} , derived from the extended Patlak analysis, do not indicate any significant change in kinetic parameters following acute cocaine exposure.

As a further indication of presynaptic changes, expression levels of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, and aromatic l-amino acid decarboxylase (AADC), the enzyme converting flurodopa to dopamine, were measured in the striatum of mice following PET scanning. A number of studies have described the effects of cocaine treatment on TH regulation, with exposure leading to increased expression within the ventral tegmental area (VTA) upstream of dopaminergic projections to the striatum, and varied results within the nucleus accumbens, indicating either decreases, no change or transient increases in TH expression (Beitner-Johnson and Nestler, 1991; Licata and Pierce, 2004; Rodriguez-Espinosa and Fernandez-Espejo, 2015; Taylor and Ho, 1977; Todtenkopf and Stellar, 2000; Trulson et al., 1987; Vrana et al., 1993). Our results suggest there is no significant change in striatal levels of TH expression following acute cocaine exposure when measured either early in the scan, or at the end. One consideration would be that a number of the studies above have reported changes in the phosphorylation states of TH in response to chronic, but not acute cocaine. This was not tested in the current study. Together, these findings support the observation that an acute cocaine treatment does not alter presynaptic regulation. This is further supported by the lack of change in AADC expression, an enzyme which has been suggested as an

alternative indicator of presynaptic dopamine synthesis capacity (Gjedde et al., 1991, 1993). As with the PET experiments, it is possible that we have failed to detect significance due to the small effect of cocaine on protein expression. A post hoc analysis of our western data indicated large effect sizes on TH (Cohen's $d = 1.3$) and AADC (Cohen's $d = 1.0$) at the earliest 15 minute time point. However, by the two hour time point, the effect of cocaine was minimal for both proteins (TH, Cohen's $d = 0.1$, AADC Cohen's $d = 0.2$). It is important to note that enzyme expression is not necessarily a direct measure of enzyme activity and it is possible that cocaine has effects on activity without affecting expression.

Limitations:

While the current data indicate no significant change in presynaptic dopamine function following acute cocaine treatment, there are a number of limitations to note. Several studies have demonstrated individual differences in the behavioural responses to acute cocaine exposure, often dividing animals into 'high' and 'low' cocaine responders (Hooks et al., 1991; Sabeti et al., 2002). Studies in outbred Sprague Dawley rats have identified predictive traits of high and low responders, including differing locomotor responses to novel environments (Hooks et al., 1991; Marinelli and White, 2000). In the present study, locomotor responses to cocaine were measured in a different cohort to those undergoing PET scanning to restrict cocaine exposure to a single occasion. It is therefore unknown whether inter-individual variability existed in the cohort scanned. However, given the use of an inbred mouse strain and no difference in the activity levels of mice during the habituation phase of the open field cohort

(data not shown), it is unlikely that inter-individual responses play a significant role.

Also, the reversible nature of [^{18}F]-FDOPA striatal accumulation makes accurate estimation of presynaptic dopamine function challenging to model by graphical analysis (Kumakura and Cumming, 2009). The standard estimation of K_i^{Cer} over the first hour of [^{18}F]-FDOPA activity uses a traditional Patlak graphical analysis model assuming no reversibility of accumulation (Kyono et al., 2011; Patlak et al., 1983). By attempting to correct for the increasing reversibility of striatal [^{18}F]-FDOPA over the two hour scan, the extended analysis introduces more assumptions, and therefore more variability, but may reflect a more biologically accurate measure of presynaptic dopamine function (Holden et al., 1997; Patlak and Blasberg, 1985; Walker et al., 2013a).

One final limitation is the need for pre-treatment with inhibitors such as benserazide and entacapone to block peripheral metabolism by AADC and COMT respectively. While providing these or similar inhibitors is common in both animal (Walker et al., 2013b) and human (Bloomfield et al., 2014) [^{18}F]-FDOPA PET studies to enhance striatal uptake and trapping, Jonkers et al. (2001) have demonstrated central effects of benserazide on AADC activity in rats when given at similar concentrations used in the current study. While this dose was shown not to impair extracellular dopamine levels or striatal formation of dopamine following levodopa administration up to 4 hours after, AADC activity was decreased by approximately 40% (Jonkers et al., 2001). We ran additional samples from mice without the inhibitors in separate western blots collected

120 min after [^{18}F]-FDOPA administration, which indicated no change in expression of either TH or AADC levels in response to cocaine pre-treatment. However, as discussed above, changes in activity may not be reflected by total enzyme expression.

Conclusion:

In conclusion, we have demonstrated that a single exposure to cocaine was able to trigger a rapid transient increase in locomotor behaviour, but did not alter presynaptic dopamine function as indexed by dopamine synthesis capacity, dopamine storage capacity, and distribution volume over the time-period assessed in the current study. These data suggest that the long-term down regulation of dopaminergic function found with chronic cocaine use, is not evident after a single cocaine exposure.

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Contributors

The authors confirm that the work carried out here is original and has not been published elsewhere. DB carried out all experiments and wrote the first draft of the manuscript. MK assisted with the PET and behavioural experiments. MV and CC developed the in-house analysis methodology and generated the parametric images. LW and OH contributed to the design of the experiments. All authors contributed to and have approved the final manuscript.

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The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Conflicts of interest

Dr Howes has received investigator-initiated research funding from and/or participated in advisory/ speaker meetings organised by Astra-Zeneca, Autifony,

BMS, Eli Lilly, Heptares, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. Neither Dr Howes nor his family have been employed by or have holdings/ a financial stake in any biomedical company.

The other authors have nothing to declare.

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Figure and Table Legends:

Figure 1: Effects of acute cocaine administration on locomotor behaviour.

20mg/kg cocaine (n=10, closed circles) treatment increased locomotor activity in C57Bl/6 mice with peak activity 15-20 min post administration ($p<0.001$).

Locomotor activity following vehicle (n=10, open circles) treatment remained unchanged. Dotted arrow indicates time of drug treatment.

Figure 2: Uptake of [^{18}F]-FDOPA in vehicle and cocaine-treated mice. A) Time-activity curves show mean striatal (full) and cerebellar (dashed) radiotracer signal over the two hour scan period in vehicle (open circle, grey) and cocaine (closed circle, black) treated mice. Activity presented as standardised uptake values (SUV, mean $\pm$ SEM), corrected for animal body weight and time of injection. B) Representative images of radiotracer uptake in vehicle (left) and cocaine (right) treated mice, demonstrating high signal-to-noise specificity in striatal uptake. SUV activity presented as summed activity from 15min–120min.

Figure 3: Striatal dopamine function in response to cocaine pre-treatment. A) Cocaine (closed circles) 30 min prior to PET scanning did not alter the mean dopamine synthesis capacity, as indexed by the influx rate constant K_i^{Cer} , relative to vehicle (open circles) treated controls. B) Representative images of control (upper) and cocaine (lower) treated mice showing parametric visualisations of K_i^{Cer} in the striatum following [^{18}F]-FDOPA. C) Cocaine (closed circles) 30 min prior to PET scanning did not alter the mean dopamine synthesis capacity, as

indexed by the modified influx rate constant K_i^{mod} relative to vehicle (open circles) treated controls.

Figure 4: Striatal TH and AADC expression in vehicle and cocaine treated mice.

A) TH protein expression was not significantly altered by cocaine pre-treatment at 15 min (n=4), 60 min (n=4), or 120 min (n=6) post [^{18} F]-FDOPA

administration compared to vehicle controls (n=3-5). B) TH expression in inhibitor-free (benserazide and entacapone) striatal samples collected at 120 min were also not significantly changed by cocaine pre-treatment. C)

Representative images showing TH (upper) and total protein transfer (lower). D)

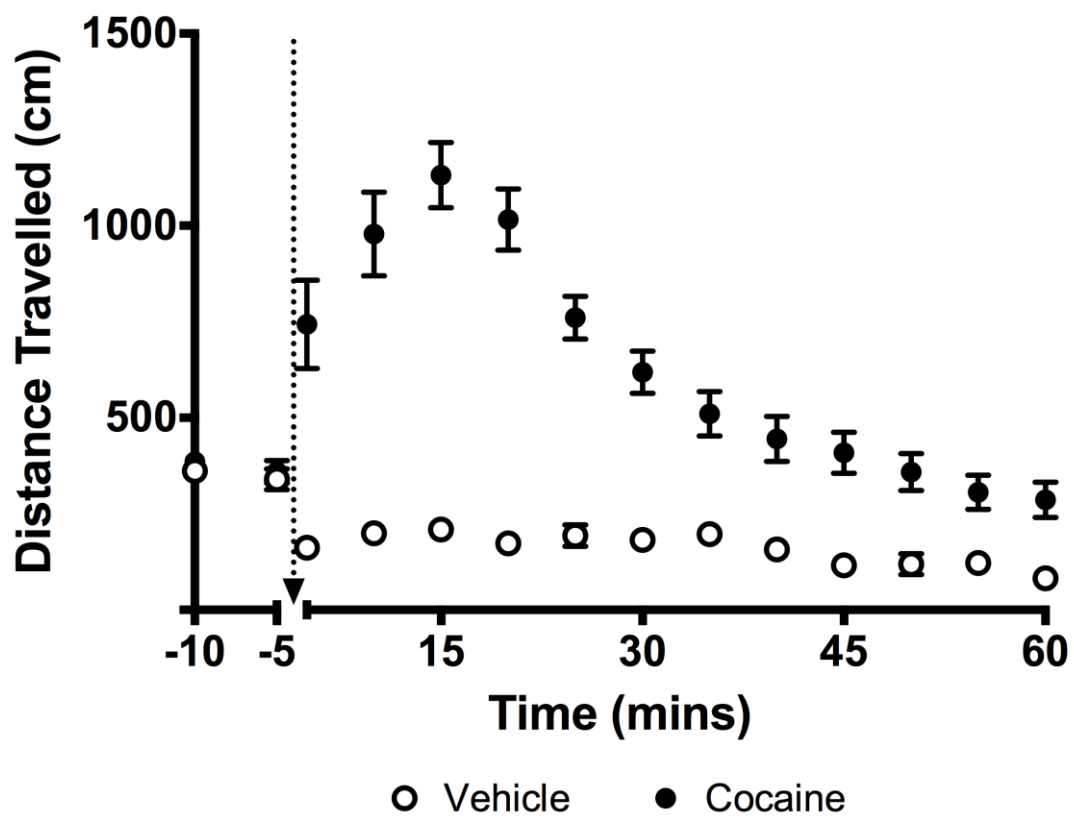
Acute cocaine exposure did not significantly alter striatal AADC expression 15 min (n=4 per group), 60 min (n=3-4), or 120 min (n=5-6) after [^{18} F]-FDOPA dosing. E)

In the absence of inhibitors, AADC expression was not significantly altered by cocaine pre-treatment. F) Representative images of AADC blotting (upper) and amido black stain (lower).

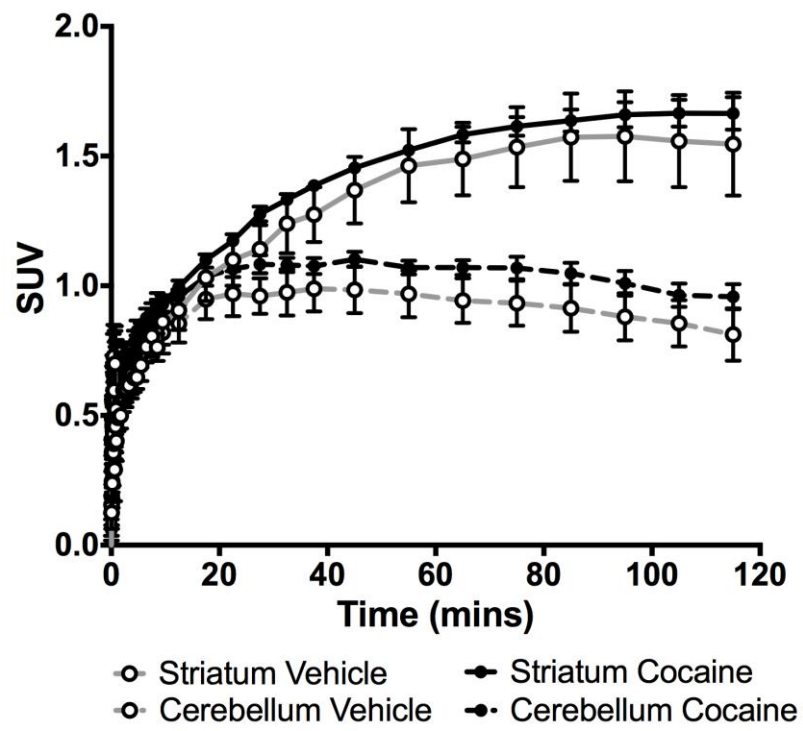
Table 1: Macroparameters showing the effects of acute cocaine treatment on striatal [^{18} F]-FDOPA accumulation. Standardised Uptake Values (SUV) and cerebellum-adjusted SUV (SUVR) were adjusted for injected dose and weight. No significant effects of cocaine pre-treatment were seen in the parameters reported.

Table 1:

Parameter	Control	Cocaine
Striatal SUV (120min)	1.546 ± 0.198	1.665 ± 0.063
Striatal SUVR (120min)	1.915 ± 0.105	1.751 ± 0.041
K_i^{Cer} (min ⁻¹) (10-60min)	0.0112 ± 0.0007	0.0097 ± 0.0003
K_i^{mod} (min ⁻¹) (15-120min)	0.0230 ± 0.0022	0.0194 ± 0.0025
k_{loss} (min ⁻¹) (15-120min)	0.0234 ± 0.0038	0.0289 ± 0.0039
V_d (ml/g)	0.95 ± 0.12	0.79 ± 0.14

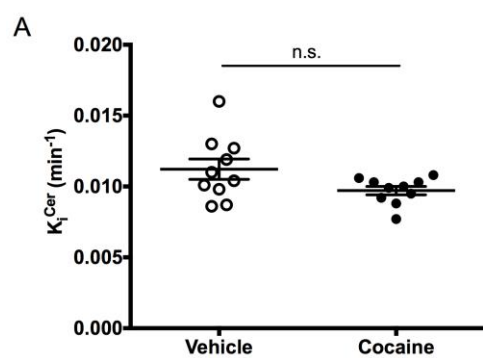


A



B





B

