Title: Association of Stimulants With Dopaminergic Alterations in Users of Cocaine, Amphetamine, and Methamphetamine: A Systematic Review and Meta-analysis

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Abstract:

Importance: Stimulant use disorder is common, affecting between 0.3 to 1.1% of the population, and costs over $85 billion per year globally. There are currently no licensed treatments. Several lines of evidence implicate the dopamine system in the pathophysiology of substance use disorder. Thus understanding the nature of dopamine dysfunction seen in stimulant users has the potential to aid the development of new therapeutics.

Objective: To comprehensively review the in-vivo imaging evidence for dopaminergic alterations in stimulant (cocaine or amphetamine/methamphetamine) drug abuse or dependence.

Data sources: The entire PubMed, EMBASE and PsycINFO databases were searched for studies from inception date to May 14, 2016.

Study selection: A total of 31 studies were identified that compared dopaminergic measures between 519 stimulant users and 512 controls using positron emission tomography or single-photon emission computed tomography to measure striatal dopamine synthesis or release, or dopamine transporter or receptor availability.

Data extraction and synthesis: Demographic, clinical and imaging measures were extracted from each study and meta-analyses and sensitivity analyses were conducted for stimulants combined and cocaine and amphetamines separately where there were sufficient studies.

Main Outcomes and Measures: We determined the difference in dopamine release (assessed using change in the D2/3 receptor availability following administration of amphetamine or methylphenidate), transporter and receptor availability in cocaine, amphetamine and methamphetamine users and healthy controls.

Results: In majority of the studies the duration of abstinence varied from 5 days to 3 weeks.

There was a significant decrease in striatal dopamine release (stimulants combined: Hedge's g= −0.84; cocaine: −0.87, both p<0.001), dopamine transporter availability (stimulants combined: Hedge's g= −0.91, p<0.01; amphetamine and methamphetamine: Hedge's g: −1.47, p<0.001) and D2/3 availability (stimulants combined: Hedge's g= −0.76; cocaine: −0.73; amphetamine and methamphetamine: −0.81, all p<0.001). We did not find consistent alterations in vesicular monoamine transporter, dopamine synthesis or D1 receptor studies.

Conclusion and relevance: Our data suggest that both pre and post-synaptic aspects of the dopamine system in the striatum are down-regulated in stimulant users. We discuss the commonality and difference between these findings and the discrepancies with the preclinical literature as well as their implications for future drug development.

Introduction:

According to World Health Organization estimates amphetamine-like stimulants (predominantly methamphetamine and amphetamine) and cocaine are the second and fourth most common forms of illicit substance abuse respectively (world drug report 2015; https://www.unodc.org). The world-wide prevalence of amphetamine-like stimulant use was estimated at between 0.3-1.1 percent in 2015 (between 13.8 million and 53.8 million users), and for cocaine it was 0.3-0.4 percent of the population aged 15-64 (between 13 million and 20 million users) (world drug report 2015; https://www.unodc.org). Stimulant use is thus a significant burden to society [1]. Dopamine dysregulation is hypothesized to underlie addictive behaviour [2-6] and stimulants such as amphetamine and cocaine act on dopamine transporters and increase extracellular dopamine [7-10]. Furthermore, preclinical models show that the acute rewarding effects of stimulant drugs are linked to the release of dopamine in the nucleus accumbens measured using micro-dialysis or fast scan cyclic voltammetry [6,11]. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) enable us to measure dopaminergic function in-vivo in humans [12]. Using these imaging tools, human studies have found that stimulant drugs increase synaptic dopamine levels in the whole striatum (including ventral striatum which includes the nucleus accumbens) and that increases are associated with the subjective perception of drug reward in non-drug abusing controls [13,14]. However, determining the dopaminergic effects of stimulants in human stimulant users is essential as the neurobiological mechanisms may be different. A number of studies have investigated dopamine release, dopamine transporter and dopamine receptor levels in stimulant addiction. However, to our knowledge, there has not been a previous meta-analysis of these findings. Thus we aimed to synthesize the PET and SPECT imaging findings on dopaminergic

function in cocaine and amphetamine-like (amphetamine and methamphetamine) stimulant addiction and to consider their implications for its treatment. Since both drugs are known to increase extracellular dopamine levels, either by blocking (cocaine) or reversing (amphetamine/methamphetamine) the dopamine transporter, we pooled the data [10]. We group findings into studies of dopamine release, dopamine transporter availability, and dopamine receptor availability. We focus on the whole striatum as it is richly innervated with dopaminergic neurons and reliably imaged with PET and SPECT in humans [15].

Methods:

Study selection

To be included in the meta-analysis, an article needed to investigate the striatal dopaminergic system in cocaine or amphetamine-like stimulant users (this included amphetamine and methamphetamine) and a control group, including the mean and standard deviations for both groups (see supplementary figures 1 and 2 for the study selection and supplementary methods for further details on the search and inclusion criteria). We focused on amphetamine and methamphetamine as these are the most widely used amphetamine-like drugs.

Data extraction

The main outcome measure was the difference in the dopaminergic imaging index between stimulant users and controls. The following variables were extracted from all the studies: authors, year of publication, subject characteristics of the control and stimulant users group (group size, age, sex, substance use characteristics, comorbid substance abuse, method of abstinence confirmation, duration of abstinence, diagnosis), imaging characteristics

(method, radiotracer, scanner type and resolution), route of administration of drug challenge and modelling method.

Data analysis

The main outcome measure was the effect size for the dopaminergic index for the whole striatum in the stimulant users (cocaine and amphetamine-like stimulants studies combined) using a random effects model. Separate secondary meta-analyses were conducted for the studies of dopamine transporter (DAT), dopamine release and dopamine receptor availability in cocaine and amphetamine-like substance users to determine if the effects were consistent across categories of stimulants. Publication bias was assessed using funnel plots as well as regression tests. Heterogeneity was estimated using the I^2 value (I^2 values <50% indicate low to moderate heterogeneity, whereas I^2 >50% indicate moderate to high heterogeneity). Leave-one-out sensitivity analyses were conducted. A significance level of $p<0.05$ (2-tailed) was taken as significant (see supplementary materials for further methodological details).

Results

Dopamine release

There were 7 studies (5 in cocaine users and 2 in amphetamine-like stimulant users) assessing dopamine release in 164 stimulant users with 139 healthy controls [16-22]. The meta-analysis showed a significant reduction in dopamine release in the stimulant users relative to controls with an effect size of −0.84 ([95% confidence interval (CI), −1.08 - −0.60], p <0.001). This was also seen when the meta-analysis was restricted to cocaine users, effect size −0.87 ([95% CI, −1.15 - −0.60], p<0.001). There were too few studies of amphetamine-

like stimulant users for a meta-analysis, but the effect sizes in the two studies were in the same direction (standardized mean difference (SMD): −1.05 [95% CI, −1.76 - −0.34] and −0.40 [95% CI, −1.11 - 0.32]) [20,21]. The results of heterogeneity and sensitivity analysis are provided in supplementary material.

Dopamine transporter:

There were 12 studies (3 in cocaine users[24-26] and 9 in amphetamine-like stimulant users[27-35]) assessing dopamine transporter availability in 177 stimulant users and 191 healthy controls. The meta-analysis showed a significant reduction in dopamine transporter availability in the stimulant users relative to controls with an effect size of −0.91 ([95% CI, −1.5 - −0.32], p<0.01). For sub-analysis, there were 9 studies in amphetamine-like stimulant users [27-35] assessing dopamine transporter availability in 108 stimulant users and 126 healthy controls. The meta-analysis showed significantly reduced dopamine transporter availability in amphetamine-like stimulant users (effect size: −1.47 ([95% CI, −1.83 - −1.1], p<0.001). There were 5 studies in cocaine users [24-26,36,37]. However, one study was excluded as it included cocaine users with potential CNS co-morbidity (HIV) [36]. This left too few studies for a separate meta-analysis in cocaine users. The results of these studies were inconsistent, with the two PET studies (where there was an overlap of samples) showing no significant difference in the DAT availability in cocaine users [26,37], whilst the two SPECT studies reported elevated DAT in cocaine users who were acutely abstinent [24,25]. The two SPECT studies in cocaine users had durations of abstinence of a maximum of 4 days[25] and mean of 7 days. [24] As such, residual cocaine could block radiotracer binding to DAT, resulting in a slight underestimation of DAT levels in these studies. The results of heterogeneity and sensitivity analysis are provided in supplementary material.

Dopamine receptor availability

There were 19 studies assessing dopamine receptor availability in 342 stimulant users and 321 healthy controls (7 studies in amphetamine-like stimulant users [20,21,38-42] and 12 studies in cocaine users [16-19,22,37,43-48]).

The meta-analysis revealed an overall reduction in D2/3 receptor availability in stimulant users relative to controls with an effect size of −0.76 ([95% CI, −0.92 - −0.6], p<0.001). In the separate analyses a reduction in D2/3 receptor availability was noted in both cocaine users (effect size=−0.73 [95% CI, −0.94 - −0.53], p<0.001) and amphetamine-like stimulant users (effect size=−0.81 [95% CI, −1.12 - −0.49], p<0.001) relative to controls. The results of heterogeneity and sensitivity analysis are provided in supplementary material.

Other dopaminergic measures:

There was only one study in stimulant users using 6-[18F]fluoro-dihydroxy-phenylalanine ([18F]-DOPA) assessing dopamine synthesis capacity [49]. This study showed reduced dopamine synthesis capacity in cocaine users and the estimated effect size was found to be 0.46 [95% CI, −0.46 - 1.39]. We could not find any studies on dopamine synthesis capacity in amphetamine-like stimulant users. Four studies assessed vesicular monoamine transporter - 2 (VMAT2) availability, with inconsistent findings. Two studies showed significantly reduced VMAT2 availability, one in cocaine users with two weeks of abstinence [Hedge's g: 1.6, 95% CI: 0.68 - 2.52] [50], and the other in methamphetamine abusers after three months of abstinence [Hedge's g: 1.68, 95% CI: 0.86 - 2.5] [28]. However, two studies in recently abstinent methamphetamine users (mean duration of abstinence: 2.6 days and 19 days) showed elevated VMAT2 levels [Hedge's g: 1.16, 95% CI: 0.56 - 1.76] [51,52]. Moreover, given

that metamphetamine interacts with VMAT2 at the same site as the PET tracers and the relatively short duration of abstinence, it is possible that VMAT2 levels were underestimated in some subjects.

There was only one study on stimulant users and D1 receptors, which used [11C] NNC112 to compare cocaine abusers with controls [53]. Though there were no differences in D1 receptors between groups, the availability of D1 receptors in cocaine abusers was negatively associated with the choice to self-administer cocaine by the cocaine abusers

Discussion:

To our knowledge this is first meta-analysis investigating the nature of dopaminergic dysfunction in stimulant users. Our main findings are that dopamine release, transporter levels and D2/3 receptor availability are all lower in vivo in stimulant users compared to healthy controls with large to very large effect sizes (Hedge's g: −0.84, −0.91 and −0.76 respectively). This indicates that there is a generalised down-regulation of the dopaminergic system in stimulant users, as summarised in Figure 4. Our sensitivity analyses of the dopamine D2/3 receptor availability and dopamine release findings showed consistent results and we noted low heterogeneity across studies of cocaine and amphetamine-like drugs, and across differing radiotracers and techniques. However, there was a difference between results in amphetamine/methamphetamine and cocaine drug users in dopamine transporter availability. In amphetamine/methamphetamine users there were large and consistent reductions in dopamine transporter availability. In contrast for cocaine users, despite the limited number of studies preventing sub-analysis, there were two studies that showed no difference [26,54] and two other studies that reported elevated dopamine transporter availability, both in acutely abstinent cocaine users [24,25]. This may point to a mechanistic difference between the

effects of amphetamine-like drugs and cocaine on dopamine transporters, consistent with preclinical findings[55], and highlights the need for more studies in cocaine users. Cocaine is known to primarily act by blocking DAT, while amphetamine competitively inhibits dopamine re-uptake at DAT, and increases DAT mediated reverse-transport of dopamine from the cytoplasm into the synaptic cleft independent of action potential evoked vesicular release.[7-9] It has also been suggested that amphetamine's actions depend on its concentration, with it acting primarily as a DAT blocker at low concentrations and reversing dopamine transport at high concentrations.[7] In addition, amphetamine-like stimulants are known to trigger internalization of plasmalemmal DAT.[56] Finally, cocaine, amphetamine and methamphetamine are also known to act on serotonin and norepinephrine transporters, although their affinities for these are different.[57] Given these pharmacological differences between stimulants, there could be differences in dopaminergic effects between stimulants that are masked by pooling studies.

There are some specific issues that affect interpretation of the results. For studies of D2/3 receptors, the tracers generally used do not distinguish between D2 and D3 receptors, or between the high and low affinity forms of the D2 receptor, so the reduction could reflect a change in one, or more of these. However, two studies used [11C]-PHNO, which is selective for the D2 high affinity form and shows a higher affinity for D3 receptors over D2 receptors. These studies did not show significant differences between stimulant users and controls in the striatum [44,46]. This could suggest that the reduction seen in our meta-analysis reflects a reduction in the low affinity form of the D2 receptor availability. Also, given that the radiotracers used to measure D2/3 receptor availability are sensitive to endogenous dopamine levels [58], one possible interpretation of our finding of reduced D2/3 receptor levels is that this reflects elevated synaptic dopamine levels. However, a dopamine depletion study in cocaine users has shown that baseline synaptic levels are also reduced[43]. This indicates that the reduction in D2/3 receptor availability represents a reduction in D2/3 receptor levels. Furthermore, when our findings are taken with the observation of reductions in synaptic dopamine levels [43], and dopamine synthesis capacity [49], they suggest there is a generalised reduction

in presynaptic dopaminergic activity. Although, with the available data we could not specifically rule out the possibility of upregulation D3 receptor.

Limitations

In common with other meta-analyses of psychiatric imaging studies [59-63], there are variations between studies in terms of both the sample characteristics, such as the inclusion of current or abstinent users, co-morbid use of other substances such as nicotine and alcohol and variations in the durations of abstinence, and methods, in particular in the radiotracer used and delineation of the striatum (see supplementary discussion).

Nevertheless, there was low heterogeneity across the analyses, with the exception of the dopamine transporter, and the random effects model we used allows for variations in effects. Furthermore, if anything, these variations between studies would obscure rather than account for the effects we observed. A general limitation of the literature, apparent in the funnel plots, is that there are few studies with large sample sizes. It should also be noted that there have been a relatively small number of studies on dopamine release and we could not investigate potential differences between oral and intravenous routes of drug challenge to elicit dopamine release. Although, in absolute terms the oral challenge studies showed lower release than those using an intravenous route, both indicated blunted release in stimulant users compared to controls.

Implications for understanding stimulant misuse and dependence

Preclinical studies using *in vivo* micro-dialysis and chronoamperometry conclusively demonstrate that acute administration of stimulants increases extra-cellular dopamine concentrations in the striatum, and nucleus accumbens. [11] Furthermore, *in vivo* fast-scan cyclic voltammetry and implantable micro-sensor studies, which are able to quantify the dopamine signalling over a sub-second timescale, have shown that stimulants increase phasic dopamine release [6,11], and human *in vivo* imaging studies also show evidence consistent with acute exposure of stimulants leading to increased synaptic dopamine either through cue induced dopamine release or blockade of DAT, and

that this is linked to subjective 'high' [54] and craving[66,67]. Moreover, change in in vivo dopamine imaging indices, following amphetamine administration, has been shown to be directly related to change in microdialysis measures [68], providing convergence across methods. Thus there is consistency between the preclinical and clinical findings indicating that acute administration of stimulants results in increased extra-cellular dopamine either by stimulating release (amphetamines) or by DAT blockade (cocaine).

Our meta-analysis shows a consistent reduction in dopamine release in people who have been exposed to chronic stimulants. In contrast, preclinical models of chronic use are inconsistent, with some studies showing no change in basal dopamine output after withdrawal of chronic amphetamine [69-72] and cocaine [73-77], whilst others have reported *increases* in dopamine output after cocaine withdrawal [78-81]. The first major implication of our meta-analysis is thus that the findings in many preclinical models of chronic use do not reflect what is seen in the human studies. This suggests caution in extrapolating from preclinical models, and may explain the failure to develop treatments for stimulant addiction based on them. There are a number of potential explanations for this inconsistency, including differences in the dosing regimens and durations used in preclinical models relative to human usage patterns. Nevertheless, this discrepancy suggests we need to develop new preclinical models that reproduce the dopaminergic changes seen in the human condition.

Our findings are also striking in showing reductions in both presynaptic and post-synaptic aspects of the dopaminergic system, suggesting a generalised down-regulation. One potential explanation for the reduction in dopamine release and transporter availability (seen in amphetamine/methamphetamine users only) could be a loss of dopamine neurons per se or damage to the dopaminergic terminals. There is evidence that both cocaine and amphetamines induce apoptosis as indexed by activation of caspases, loss of mitochondrial potential, cytochrome c release and oxidative stress. [82] However, in addition to this, amphetamine and methamphetamine induce dopaminergic neuron damage through the formation of quinones and free radicals. [83-85]

Moreover, preclinical models with methamphetamine have shown evidence of dopamine terminal damage that recovers with detoxification [86,87]. Furthermore, in humans, dopamine transporters recover with detoxification in methamphetamine abusers (reviewed in [5,35]), which was interpreted to indicate that dopamine neurons were not lost. Moreover in the only post-mortem study we could find, which was in methamphetamine abusers, there was evidence of reduction in dopamine transporters but not of dopamine neuronal loss [88]. However preliminary evidence from two recent epidemiological studies that methamphetamine abuse might increase the risk for Parkinson's disease [89,90] suggests that in some cases its abuse might accelerate age-associated dopamine neuronal degeneration [91].

It has been suggested that repeated drug use causes tolerance by various mechanisms including dopamine receptor alterations, changes in second messenger systems and altered regulation of dopamine neuron function.[92-94] Thus, it is possible that the dopaminergic differences noted in our meta-analysis could be due to the development of tolerance through one or more of these mechanisms. Pre-clinical studies have also demonstrated that dopaminergic synaptic transmission is modulated by glutamatergic and GABAergic neurons[94]. Neuroimaging studies investigating these interactions are needed to determine if this is the case in humans.

Two alternative basic models are possible to account for both our pre and post-synaptic findings. The first is that repeated stimulant use results in adaptive changes in the dopamine system that lead to reduced firing of dopamine neurons, potentially similar to the depolarisation blockade that is seen after a period of repeated firing [95], and consequently reduced dopamine synthesis and release. In this context, reduced transporter levels may be compensatory in response to reduced tonic dopamine levels in the synapse. The reduction in D2/3 receptor levels is less easy to understand in the context of the presynaptic reductions. However, D2/3 receptors undergo internalisation following activation by dopamine, and this would reduce radiotracer binding, at least to a number of the tracers used in the studies in our analyses [96,97]. Thus the reduction in D2/3 receptor availability could reflect a compensatory increase in internalized D2/3 receptors, which would reduce the

number of D2/3 receptors available to bind to dopamine. Repeated exposure may lead to loss of these internalized receptors and long-term transcriptional changes that reduce receptor availability. The second model is that reductions underlie the pathoetiology of stimulant misuse, and precede its onset. Thus individuals at risk of stimulant misuse may have reductions in dopamine release, transporter levels and D2/3 receptor levels secondary to genetic and/or environmental risk factors. Reductions in D2/3 receptor levels and reduced release of dopamine to stimulants could mean an individual is less sensitive to the effects of taking a stimulant, leading to escalating use. However, it is less easy to see how reduced dopamine transporter levels fit with this model, as they would be anticipated to prolong the effects of stimulants. Longitudinal studies on the effects of stimulant drugs on patients with attention deficit disorder showed downregulation of dopamine release with chronic exposure [98], which indicates that some of the changes are driven by chronic drug exposures. Finally, a hybrid model may best account for our findings. Evidence suggests that reduced D2/3 receptor levels may precede and predispose to the onset of stimulant misuse but also show further reductions during stimulant use [99], and similar effects may be seen with dopamine release and transporter levels. It is interesting to note in our meta-analysis that dopaminergic alterations are marked even in the studies of several months abstinence, with evidence suggesting that dopamine receptor density and release are down-regulated even after 9 months of abstinence [35]. This suggests that effects may persist, with implications for understanding relapse. Our findings also support the opponent process model [100].

This highlights one fundamental issue raised by our meta-analysis, namely that current findings do not address the temporal relationship between down-regulation in the dopamine system and phase of addiction. Future longitudinal human PET studies as well as preclinical studies that investigate changes in the dopamine system prior to and during stimulant misuse-, and following abstinence are needed to test these models (Box 1; supplementary material). In addition this will help to identify biomarkers to guide treatment and predict outcomes.

Clinical implications of our findings

Our data identify a number of clear targets for treatment interventions. D2/3 receptors stand-out, given our finding of a large effect size reduction. This is further supported by studies in cocaine and in methamphetamine users showing that lower dopamine D2/3 receptor availability at baseline predicts relapse following treatment [19][21]. Our data also provide a rationale for the development of drugs that target the presynaptic dopaminergic system to restore tonic striatal dopamine release, which is necessary for the function of the striato-cortical indirect pathway (a key system disrupted in addiction) [5]. This is supported by recent preclinical evidence showing that administration of the dopamine precursor L-DOPA restored the aberrant dopaminergic signalling in a cocaine addiction animal model [6,100], and preliminary clinical evidence that inhibiting dopamine reuptake (e.g., with bupropion, modafinil, or mazindol), or inhibiting dopamine metabolism (e.g., with selegiline or disulfiram) might hold some promise in the treatment for stimulant addiction [101]. Strategies to upregulate striatal D2 receptors have been shown in animal models to protect against compulsive stimulant drug intake [102] and, thus, interventions that lead to D2 up-regulation, such as physical activity as recently shown in a preliminary study in methamphetamine abusers [103], merit further investigation. A number of dopaminergic treatments have been tried to treat stimulant use disorder with limited success to date.[104-106] Our data suggest some potential explanations for the lack of success and identify a number of clear targets for treatment interventions. D2/3 receptors stand-out, given our finding of a large effect size reduction. This is further supported by studies in cocaine and in methamphetamine users showing that lower dopamine D2/3 receptor availability at baseline predicts relapse following treatment [19][21]. Our findings may also explain why strategies to block dopamine neurotransmission, for example using dopamine receptor antagonists[107] , have largely been disappointing to date as dopamine receptor levels are already low, and suggest that strategies to increase dopamine receptor levels or sensitivity could have potential.

Conclusions

There is robust evidence for down-regulated presynaptic and post-synaptic dopamine function in

stimulant addiction with large effect sizes. These findings suggest that drug development should

target the restoration of dopaminergic function as a target for the treatment of stimulant addiction.

Acknowledgments Statement

Conflict of Interest Disclosure

Author Contributions:

Dr Ashok and Dr Mizuno had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ashok, Howes
Acquisition, analysis, or interpretation of data: All authors
Drafting of the manuscript: All authors
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Statistical analysis: Ashok
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Study supervision: Howes and Volkow

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Figure 1: Studies of dopamine release in stimulant users.

The forest plot shows the effect sizes estimated using a random effects model and 95% confidence intervals of the percentage change in the difference of change in D2/3 binding after challenge. There was an overall decrease in dopamine release in stimulant users relative to controls with a large to very large effect size (-0.84 [95% CI, -1.08 - -0.60], $p<0.001$) Schrantee et al. 2015 and Wang et al. 2012 studied amphetamine-like stimulant users. The remaining studies included cocaine users.

Figure 2: Studies of dopamine transporter availability.

The forest plot shows the effects sizes estimated using a random effects model and 95% confidence intervals of the difference between amphetamine/methamphetamine users and controls. There was an overall decrease in the dopamine transporter availability in methamphetamine users relative to controls (-0.91 [95% CI, -1.5 - -0.32], $p <0.01$)

Figure 3: Studies of dopamine receptor availability.

The forest plot shows the effect sizes estimated using a random effects model and 95% confidence intervals of D2/3 receptor binding potentials. There was an overall decrease in dopamine receptor availability compared to controls (-0.76 [95% CI, -0.92 - -0.60], $p<0.001$).

Figure 4: A summary of dopaminergic alterations in stimulant users

Summary of the synaptic location of the major dopaminergic findings from our meta-analyses and findings from studies of other aspects of the dopamine system.
1.   Based on study by Wu et al. 1997
2.   Based on study by Boileau et al. 2008 & 2015
3.   Based on study by Narendran et al. 2012 and Johanson et al. 2006
4.   Meta-analysis finding with effect size of -0.81 [95% CI, -1.34 - -0.29], $p<0.01$
5.   Meta-analysis finding with effect size of -0.91 [95% CI, -1.50 - -0.32], $p<0.01$
6.   Meta-analysis finding with effect size of -0.71 [95% CI, -0.91 - -0.52], $p<0.001$

Supplementary methods

Search strategy

The Pubmed, EMBASE and PsycINFO databases were searched from inception date to May 14, 2016 for relevant papers without language restrictions. The electronic searches using EMBASE and PsycINFO were carried out together using Ovid. The following keywords were used for cocaine use: "(Positron Emission Tomography OR PET OR Single photon emission tomography OR SPET OR Single Photon Emission Computed Tomography OR SPECT) AND (dopamine OR dopamine release OR dopamine synthesis OR dopamine availability OR dopamine transporter OR dopamine reuptake OR dopamine receptor) AND (cocaine OR cocaine abuse OR cocaine dependence)". Furthermore the following keywords were used for amphetamine-like substance use: "(Positron Emission Tomography OR PET OR Single Photon Emission Tomography OR SPET OR Single Photon Emission Computed Tomography OR SPECT) AND (dopamine OR dopamine release OR dopamine synthesis OR dopamine availability OR dopamine transporter OR dopamine reuptake OR dopamine receptor) AND (amphetamine OR amphetamine abuse OR amphetamine dependence OR methamphetamine OR methamphetamine abuse OR methamphetamine dependence OR methylphenidate OR methylphenidate abuse OR methylphenidate dependence)". In addition the reference lists in the included studies and relevant review papers were screened to search for additional studies.

Inclusion and exclusion criteria

The inclusion criteria were: 1) original molecular imaging studies that indexed dopamine receptors, dopamine transporters and/or dopamine release or synthesis; 2) in patients with a diagnosis of cocaine or amphetamine/ methamphetamine dependence or abuse and 3)

reported data for the whole striatum or a striatal sub-region. We excluded studies which did not have a healthy control group or that included subjects with CNS co-morbidity. For studies with an overlap in participants, we included the study with the largest sample size without potentially missing any subjects and excluded the smaller study from the meta-analysis to avoid duplication of subjects.

Outcome measures

Our primary outcome was the effect size for the difference in the dopaminergic index for the whole striatum between the stimulant user and control groups. Some studies only reported values for striatal subdivisions. Where this was the case, we averaged the striatal subdivision values to estimate the value for the whole striatum as described in other imaging meta-analyses [1,2].

Meta-analysis

The statstodo software was used for the estimation of pooled standard deviation (http://statstodo.com/ComMeans_Pgm.php). Plot digitizer software was used to extract the data from studies where data was available only in a plot format (http://plotdigitizer.sourceforge.net/). The statistical analysis of the extracted data was conducted using the R statistical programming language version 3.2.2 with the 'metafor' package. One study reported data on dopamine receptor availability with both [11C] raclopride and 11C-(+)-4-propyl-9-hydroxynaphthoxazine ([11C]-(+)-PHNO). We included the [11C] raclopride data for the main analysis to maintain consistency with the other studies, more of which had also used this tracer than had used [11C] PHNO. However we also discuss the [11C] PHNO findings where relevant. A minimum of five studies was required to conduct a meta-analysis as findings from meta-analyses with small numbers of studies may

be less stable. Two independent authors (A.H.A. and Y.M.) conducted the electronic search and assessed studies for eligibility, while data extraction was carried out by A.H.A. and results of analyses confirmed by Y.M. and O.D.H.

Supplementary results:

Dopamine release

Heterogeneity and sensitivity analyses

Methylphenidate challenge was used to index dopamine release in 5 studies while one study each used intravenous amphetamine and dexamphetamine challenge. The I^2 value was 0 % (95% CI, 0%-70%), indicating low heterogeneity. The regression test for funnel plot asymmetry was not significant (t = 0.69, df = 5, p = 0.52), suggesting publication bias is unlikely. In addition, the trim-and-fill analysis indicated that there were no missing studies on the funnel plot (Supplementary figure 3). The summary effect size reached significance in all cases in the leave-one-out analysis, with summary effect sizes varying from −0.78 to −0.90 (all p<0.001).

Among the 7 studies investigating dopamine release, two studies used oral methylphenidate challenge while the others used intravenous challenge. Since only two studies used the oral challenge, we could not perform sub-analysis to investigate effect of route of administration. However, it appears that an oral challenge produces less dopamine release [effect size: *-0.4[3] and -0.57[4]*] compared to an iv challenge, although further studies are needed to directly test this.

Dopamine transporter

Heterogeneity and sensitivity analyses

The I^2 value was 84 % (95% CI, 69%- 94%), indicating high heterogeneity between studies. The regression test for funnel plot asymmetry was significant (t =-2.5, df = 10, p = 0.03). In addition, visual inspection of the funnel plot revealed asymmetry, indicating possible publication bias. The trim-and-fill analysis indicate two missing studies on the right side of the funnel plot (Supplementary figure 4). However, the results remained significant after

correcting for putatively missing studies [effect size= -0.68, (95%CI, - 1.3 - - 0.09), p=0.02].

The summary effect size reached significance in all cases in the leave-one-out analysis, with

summary effect sizes (SMD) varying from -0.77 to -1.07 (all p<0.01).

Dopamine receptor availability

Heterogeneity and sensitivity analyses

The I^2 value was 0 % (95% CI, 0 %- 57 %), indicating heterogeneity was low. The regression

test for funnel plot asymmetry was not significant (t = -1.1, df = 17, p = 0.27). However, a

visual inspection of the funnel plot revealed asymmetry, indicating possible publication bias.

The trim-and-fill analysis indicated that there were potentially five missing studies on the

funnel plot (Supplementary figure 5). Nevertheless, the summary effect size remained large

and highly significant after correcting for these putatively missing studies (corrected effect

size: −0.66 [95% CI, −0.83 - −0.5]; z: -8; p<0.001). The summary effect size reached

significance in all cases in the leave-one-out analysis, with summary effect sizes varying

from SMD = −0.72 to −0.8 (all p<0.001). To investigate the effect of radiotrace on the

outcome measure, we performed sub-analysis of studies which used [11C] raclopride. The

results remained significant with an effect size of -0.69 [95% CI, -0.9—0.48; p<0.001].

Supplementary discussion:

Moreover, where a value was not given for the whole striatum, we derived this by averaging the data for sub-regions. These variations may add noise to the findings. Thus the effect sizes calculated from these studies should be considered an estimate. It should also be noted that there are differences in dopaminergic indices between striatal sub-regions. For example D3 receptor levels are higher in ventral than dorsal striatum. [5,6] Most of the radiotracers used to measure D2-like receptor levels have similar affinities for both D2 and D3 receptors. Thus the reduction in whole striatal D2/3 receptor levels we report could potentially mask sub-regional increases in D3 receptors or differences in the ratio of D2 to D3 receptors. Unfortunately there were insufficient studies reporting data from sub-regions for us to investigate sub-regional dopaminergic changes and only two studies in cocaine users used [11C]-PHNO, a tracer that does show significant selectivity for D3 over D2 receptors.[7,8] Interestingly, in contrast to our overall findings, the two studies that used [11C] PHNO did not show significant differences in BP_{ND} between stimulant users and controls in the whole striatum. [5,6] One explanation could be that an increase in D3 in ventral striatum is off set by a reduction in D2 receptors in dorsal striatum. However, neither study reported a significant difference in ventral striatum with [11C]-PHNO. Moreover, studies which used [11C] raclopride demonstrated reduced ventral as well as whole striatal D2/3 availability.[9-13]. Taken together, these lines of evidence suggest our findings in the whole striatum are not explained by opposing sub-regional differences. Nevertheless, the absence of marked differences in the [11C]-PHNO studies is intriguing and warrants further investigation.

Supplementary figure 1. Flowchart showing the inclusion of studies for the meta-analysis on dopaminergic function in cocaine users

Identification	Records identified through database searching
	Records after duplicates removed
Screening	Records screened → Records excluded
Eligibility	Full-text articles assessed for eligibility → Full-text articles excluded for the following reasons Reviews, editorials, commentaries (n=12) No healthy control group (n=10)
	Full-text articles remaining (n=10) ← Additional articles identified through hand-
Included	Studies included in qualitative synthesis → Insufficient number of studies for the quantitative synthesis (meta-analysis) Dopamine synthesis (n=1) Vesicular monoamine transporter (n=1) D1 receptor (n=1)
	Studies included in quantitative synthesis (meta-analysis) (n=15)[*1] Dopamine release: 5

[*1] Dopamine release studies also reported on D2/3 availability.

Supplementary figure 2. Flowchart showing the inclusion of studies for the meta-analysis on dopaminergic function in amphetamine-like stimulant users
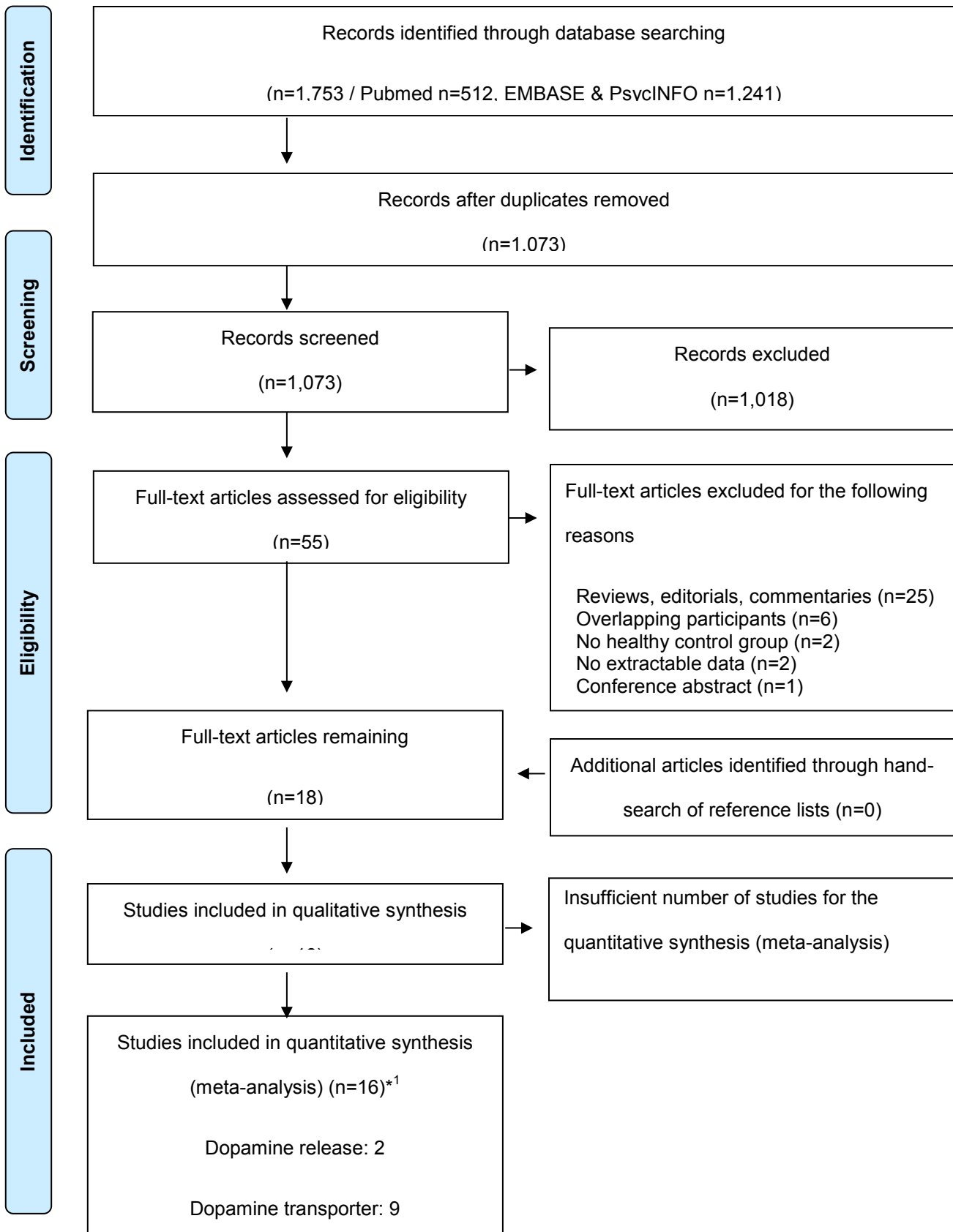
*[1] Dopamine release studies also reported on D2/3 availability.

Supplementary figure 3: Funnel plot of dopamine release studies in stimulant users
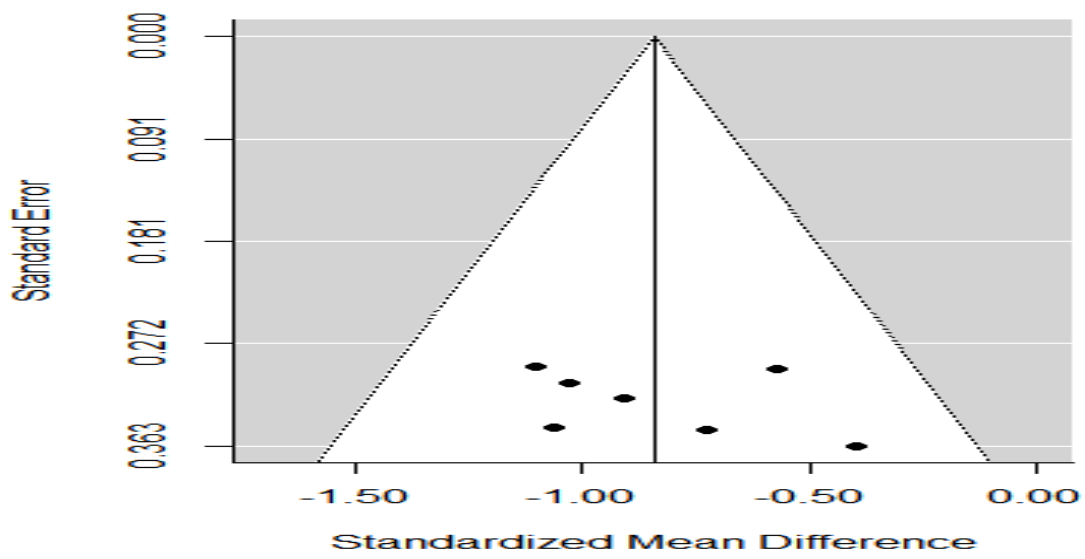
Supplementary figure 4: Funnel plot of dopamine transporter studies in stimulant users

○ indicate potentially missing studies

Supplementary figure 5: Funnel plot of dopamine D2/3 receptor studies in stimulant users

o indicate potentially missing studies

Standardized Mean Difference

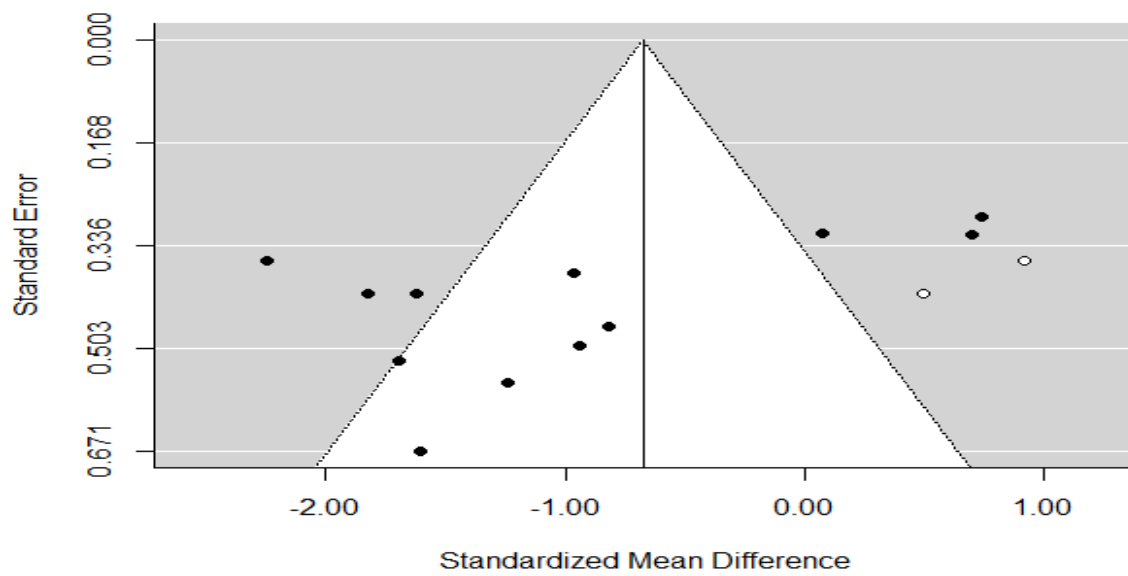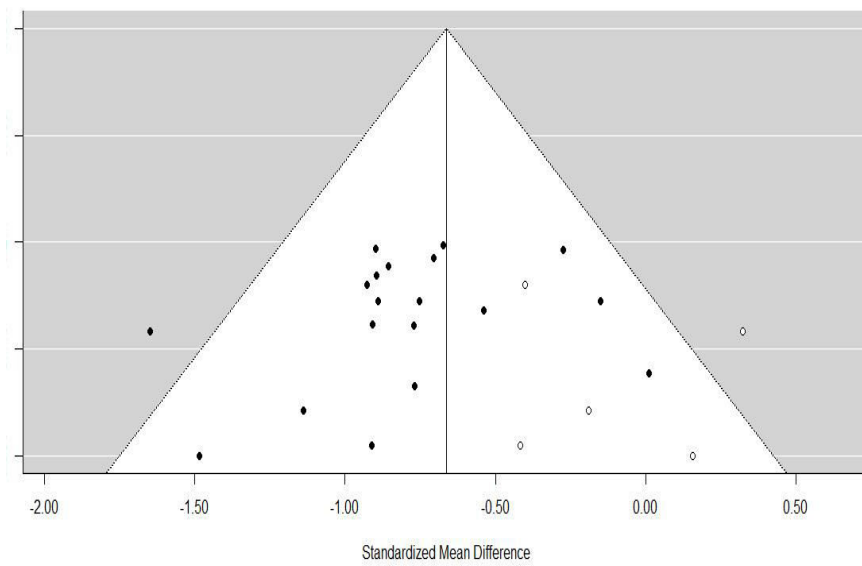| Author/year	Patients/ Controls, n	Dopamine release paradigm	Diagnosis	Duration of abstinence	Co-morbid substance abuse	Abstinence confirmed by	Region of interest	Tracer	Reference region	Measure	Results in patients compared to controls
Cocaine users											
Volkow et al. 1997[14]	20/23	i.v. MP challenge	DSM-IV, cocaine dependence	3-6 wk	Nicotine and caffeine	Toxicological drug screens before scan	Striatum, thalamus	[11C] raclopride	Cerebellum	$\Delta B_{max}/ K_d$	↓
Volkow et al. 2014[13]	43/19	i.v. MP challenge with concomitant cocaine cue-video	DSM-IV, cocaine dependence	Mean(SD) = 5(5) d	Nicotine	Not specified	Striatum prefrontal cortex	[11C] raclopride	Cerebellum	ΔBP_{ND}	↓
Volkow et al. 2005[15]	21/15	i.v. MP challenge	DSM-IV, cocaine dependence	Maximum 1 mo, mean(SD) = 14(7) d	Nicotine and caffeine	Supervised admission	Striatum	[11C] raclopride	Cerebellum	$\Delta B_{max}/ K_d$	↓
Martinez et al. 2007[11]	24/24	i.v. amphetamine challenge	DSM-IV, cocaine dependence	Minimum 14 d	Nicotine	Supervised admission	Striatum	[11C] raclopride	Cerebellum	ΔV_3	↓
Martinez et al. 2011[9]	25/24	Oral MP challenge	DSM-IV, cocaine dependence	Minimum 14 d	Nicotine	Supervised admission or repeated urine drug screen	Striatum	[11C] raclopride	Cerebellum	ΔBP_{ND}	↓
Amphetamine-like stimulant users											
Schrantee et al. 2015[16]	16/19	i.v. dAMPH challenge	dAMPH users with ≥30 lifetime and ≥10 past year exposures	Minimum 1 wk	Nicotine	Multi-drug screen on a urine sample before scan	Striatum	[123I] IBZM	Occipital cortex	BP_{ND} ratio	↓
Wang et al. 2012[3]	16/15	Oral MP challenge	DSM-IV, MA dependence	Minimum 2 wk	Nicotine	Urine screening tests	Striatum	[11C] raclopride	Cerebellum	ΔBP_{ND}	↓
Volkow et al. 2015[17] [*1]	16/15	Oral MP challenge	DSM-IV, MA abuse or dependence	Minimum 2 wk	Nicotine	Urine screening tests	Striatum	[11C] raclopride	Cerebellum	BP_{ND} ratio	↓

Supplementary table 1: Molecular imaging studies on dopamine release in stimulant users compared to healthy controls

Abbreviations: BP, binding potential; d, days; dAMPH, dexamphetamine; i.v., intravenous; MA, methamphetamine; mo, months; MP, methylphenidate; SD, standard deviation; wk, weeks

*1 Excluded from the meta-analysis on dopamine release due to overlapping participants with Wang et al. 2012.

Supplementary table 2: Molecular imaging studies on other presynaptic dopaminergic indices in stimulant users

Dopaminergic measure	Author/year	Patients/ Controls, n	Diagnosis	Duration of abstinence	Abstinence confirmed by	Co-morbid substance abuse	Region of interest	Tracer	Measure	Results in patients compared to controls
					Cocaine users					
Dopamine synthesis capacity	Wu et al. 1997[18]	11/8	DSM-III-R, cocaine dependence	Less than 30 d	Frequent random urine drug screens during admission	Not mentioned	Striatum	[18]-6-FDOPA	K_i	↓
Vesicular Monoamine Transporter 2	Narendran et al. 2012[19]	12/12	DSM-IV, cocaine dependence	Minimum 2 wk	Witnessed urine sampling 3 times a week for 2 weeks in outpatient setting and 2 day admission	Nicotine	Striatum	[11C]DTBZ	BP_{ND}	↓
					Amphetamine-like stimulant users					
Vesicular Monoamine Transporter 2	Boileau et al. 2008[20]	16/14	DSM-IV, MA dependence or abuse	Recently withdrawn, mean(SD) = 19(24) d	Urine sample for drug toxicology	Nicotine	Striatum	[11C]DTBZ	BP_{ND}	↑
	Boileau et al. 2015[21]	28/22	DSM-IV, MA dependence or abuse	2 scans, mean(SD) = 2.6(2.0) and 9.9(2.3) d	Urine and blood samples for drug toxicology	Nicotine	Striatum	[11C]DTBZ	BP_{ND}	↑
	Johanson et al. 2006[22]	16/18	DSM-IV, past MA dependence	Minimum 3 mo	Urine drug screen	Nicotine, past abuse of other drugs were allowed	Striatum	[11C]DTBZ	BP	↓

Abbreviations: BP, binding potential; d, days; MA, methamphetamine; mo, months; SD, standard deviation; wk, weeks

Supplementary table 3: Molecular imaging studies on dopamine transporter availability in stimulant users compared to healthy controls

Author/year	Patients/ Controls, n	Diagnosis	Duration of abstinence	Abstinence confirmed by	Co-morbid substance abuse	Region of interest	Reference region	Tracer	Measure	Results in patients compared to controls
					Cocaine users					
Volkow et al. 1996[23] [*1]	12/20	DSM-III-R, cocaine dependence	3-6 wk	Random urine samples at least twice a week during admission	Nicotine and caffeine	Basal ganglia, thalamus, occipital cortex	Cerebellum	[11C]Cocaine	Distribution volume ratio	↔
Wang et al. 1997[24]	20/20	DSM-IV, cocaine dependence	Active users, mean(SD) = 5(8) d	Pre-scan urine tests ensured the absence of psychoactive drugs except cocaine	Not specified	Striatum	Cerebellum	[11C]Cocaine	Distribution volume ratio	↔
Crits-Christoph et al. 2008[25]	21/21	DSM-IV, cocaine dependence	Minimum 1 d, mean(SD) = 7.5(9.7) d	History, urine drug screen for other substance use	Nicotine and cannabis	Striatum	Elliptical ROI was placed on two consecutive slices of the supratentorial uptake	99mTc TRODAT-1.	Distribution volume ratio	↑
Malison et al. 1998[26]	28/24	DSM-III-R, cocaine dependence	Maximum of 96 hours	Urine laboratory test to rule out other illicit drug use	Not specified	Striatum	Occipital cortex	[123I]b-CIT	Distribution volume ratio	↑
					Amphetamine-like stimulant users					
Johanson et al. 2006[22]	16/18	DSM-IV, past MA dependence	Minimum 3 mo	Urine drug screen	Nicotine, past abuse of other drugs were allowed	Striatum	Occipital cortex	[11C] Methylphenidate	BP	↓
McCann et al. 1998[27]	6/10	MA users (diagnostic criteria not	Minimum 2 wk	Blood and urine drug screens before scan	No restrictions	Striatum	Cerebellum	[11C]WIN-35,428	BP	↓

		specified)								
McCann et al. 2008[28]	7/16	DSM-IV, MA users	Minimum 2 wk	Urine drug screen	No restrictions	Striatum	Cerebellum	[11C]WIN-35,428	BP	↓
Sekine et al. 2001[29]	11/9	DSM-IV, MA users	Minimum 7 d, range 7 d-1.5 years	Urinary drug screening test	Occasional nicotine and alcohol	Striatum, nucleus accumbens, prefrontal cortex	Cerebellar cortex	[11C]WIN-35,428	BP	↓
Volkow et al. 2001[30]	15/18	DSM-IV, MA dependence	Minimum 2 wk, mean(SD) = 5.9(9.0) mo, range 0.5-36.0 mo	Urine toxicology screen	Nicotine	Striatum	Cerebellum	[11C]d-threo-methylphenidate	B_{max}/K_d	↓
Volkow et al. 2015[17]	16/15	DSM-IV, MA abuse or dependence	Minimum 2 wk, mean(SD) = 102(49) d	Urine screening tests	Nicotine	Striatum	Cerebellum	[11C]cocaine	BP_{ND}	↓ caudate, putamen and ventral striatum
Chou et al. 2007[31]	7/7	DSM-IV, MA abuse	Two scans (baseline and after 2 wk abstinence)	Drug urine analysis used to exclude polysubstance abuse	Nicotine	Striatum	Occipital region	Tc-99m TRODAT	SUR	↓ baseline, partial recovery after two wk
Schouw et al. 2013[32]	8/10	Recreational dAMPH users, more than 40 occasions of previous use	Minimum 2 wk, mean(SD) = 1.1(1.3) mo	Urine drug screening	No caffeine on scan day	Striatum	Occipital cortex	[123I]FP-CIT	Binding ratio: striatum to occipital cortex	↓

Yuan et al. 2014[33]	25/25	DSM-IV-TR, MA dependence	Three scans (24-48 hours, 2 wk, and 4 wk abstinence	Urine drug screen	None	Striatum	Occipital cortex	99mTc-TRODAT-1	SUR	↓
Volkow et al. 2001[34] *2	12/11	DSM-IV, MA dependence	Mean(SD) = 64 ± 40 d	Urine drug screen	Nicotine and caffeine	Striatum	Cerebellum	[11C]d-threo-methylphenidate	B_{max}/K_d	↓
Sekine et al. 2003[35] *3	11/9	MA users (diagnostic criteria not specified)	Minimum 7 d abstinence	Not specified	Not specified	Orbitofrontal cortex, prefrontal cortex, amygdala	Cerebellum	[11C]WIN 35,428	BP	↓
Yuan et al. 2015[36] *4	25/21	DSM-IV, MA dependence	2 wk	Urine drug screen	None	Striatum	Occipital cortex	99mTc-TRODAT-1	SUR	↓

Abbreviations: BP, binding potential; d, days; dAMPH, dexamphetamine; MA, methamphetamine; mo, months; ROI, region of interest; SD, standard deviation; SUR, specific uptake ratio; wk, weeks
*1 Excluded from the meta-analysis on dopamine transporter availability due to overlapping participants with Wang et al 1997.
*2 Excluded from the meta-analysis on dopamine transporter availability due to overlapping participants with Volkow et al. 2001.
*3 Excluded from the meta-analysis on dopamine transporter availability due to overlapping participants with Sekine et al. 2001.
*4 Excluded from the meta-analysis on dopamine transporter availability due to overlapping participants with Yuan et al. 2014.

Supplementary table 4: Molecular imaging studies on baseline D2/3 availability in stimulant users compared to healthy controls

Author/year	Patients/ Controls, n	Diagnosis	Duration of abstinence	Abstinence confirmed by	Co-morbid substance abuse	Region of interest	Reference region	Tracer	Measure	Results in patients compared to controls
					Cocaine users					
Martinez et al. 2011[9]	25/24	DSM-IV, cocaine dependence	Minimum 14 d	Supervised admission or repeated urine drug screen	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Martinez et al. 2009[10]	15/15	DSM-IV, cocaine dependence	Minimum 14 d	Admitted for 14 d and underwent random urine toxicology tests	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Volkow et al. 1993[37]	20/20	DSM-III-R, cocaine dependence	Minimum 8 d	Random urine samples at least twice a wk during hospitalization	Nicotine and caffeine	Striatum	Cerebellum	[18F]N-methylspiroperidol	Ratio index	↓
Volkow et al. 1996[23]	12/20	DSM-III-R, cocaine dependence	3-6 wk	Random urine samples at least twice a wk during hospitalization	Nicotine and caffeine	Basal ganglia, cortex, thalamus	Cerebellum	[18F]N-methylspiroperidol	Distribution volume ratio	↓
Volkow et al. 1997[14]	20/23	DSM-IV, cocaine dependence	3-6 wk	Toxicological drug screens before scan	Nicotine and caffeine	Striatum, thalamus	Cerebellum	[11C]raclopride	B_{max}/K_d	↓
Payer et al. 2014[6]	15/15	DSM-IV, cocaine dependence	Mean (SD) 50.1(64.4) d; range 7-240 d	Urine drug screen	Nicotine, caffeine and cannabis	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Volkow et al. 2014[13]	43/19	DSM-IV, cocaine dependence	Mean(SD) = 5(5) d	Not specified	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Matuskey et al. 2014[5]	10/10	DSM-IV, cocaine dependence	Mean(SD) = 7(4) d	Urine drug screen	Nicotine, caffeine and cannabis	Amygdala, hypothalamus, striatum, thalamus	Cerebellum	[11C](+)PHNO	BP_{ND}	↑ amygdala, hypothalamus and substantia nigra; ↔ striatum

Narendran et al. 2011 [12]	10/10	DSM-IV, cocaine dependence	Minimum 2 wk	Witnessed urine sampling 3 times a wk for 2 wk in outpatient setting and 2 d admission	Nicotine	Striatum	Cerebellum	[11C] raclopride	BP_{ND}	↓
Martinez et al. 2007 [11]	24/24	DSM-IV, cocaine dependence	Minimum 14 d	Supervised admission	Nicotine	Striatum	Cerebellum	[11C] raclopride	BP (ml/g)	↓
Volkow et al. 1990 [38]	10/10	DSM-III-R, cocaine abuse	Minimum 2 d, range 2-30 d	Not specified	Nicotine and caffeine	Striatum	Cerebellum	[18F]N-methylspiroperidol	Ratio Index: striatum to cerebellum	↓ in detoxified for one wk or less ↔ after one mo
Volkow et al. 2005 [15]	21/15	DSM-IV, cocaine dependence	Maximum 1 mo, mean(SD) = 14(7) d	Supervised admission	Nicotine and caffeine	Striatum	Cerebellum	[11C]raclopride	B_{max}/K_d	↓
Payer et al. 2014 [6] *1	15/15	DSM-IV, cocaine dependence	Mean(SD) = 50.1(64.4) d; range 7-240 d	Urine drug screen	Nicotine, caffeine and cannabis	Striatum	Cerebellum	[11C](+)PHNO	BP_{ND}	↔
Martinez et al. 2004 [39] *2	17/17	DSM-IV, cocaine abuse or dependence	19-21 d	Random urine testing during admission	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP	↓
Wiers et al. 2016a [40] *3	38/42	Cocaine abusers (criteria not specified)	Mean(SD) = 5(5) d (From Volkow et al. 2014)	Not specified	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Wiers et al. 2016b [41] *4	24/21	DSM-IV, cocaine dependence	Mean(SD) = 5(5) d (From Volkow et al. 2014)	Not specified	Nicotine	Striatum	Cerebellum	[11C]raclopride	BP_{ND}	↓
Amphetamine-like stimulant users										
Ballard et al. 2015 [42]	27/27	DSM-IV, MA dependence	4-7 d	Supervised admission and urine test on scan day	Nicotine and cannabis	Striatum	Cerebellum	[18F]fallypride	BP_{ND}	↓
Boileau et al. 2012 [43]	16/16	DSM-IV, MA abuse	Mean(SD) =	Urine drug screen on	Nicotine	Substantia	Cerebellar	[11C]-PHNO	BP_{ND}	↑

Study	n	Criteria	Abstinence	Verification	Other drug	Brain regions	Reference	Radiotracer	Outcome	Finding
		or dependence	18.5(20.5) d, range 6-90 d	scan day		nigra, globus pallidus, ventral pallidum, striatum	cortex			Substantia nigra, globus pallidus, ventral pallidum ↓ striatum
Iyo et al. 1993[44]	6/10	DSM-III-R, history of MA abuse and psychotic symptoms	Minimum 1 mo, range 2 mo-3 years	Not specified	Occasional alcohol	Striatum, frontal cortex	Cerebellum	[11C]N-Methylspiperone	K'	↔ in striatum, ↓ striatum to frontal cortex ratio
Volkow et al. 2001[45]	15/20	DSM-IV, MA dependence	Minimum 2 wk, range 2 wk-35 mo	Urine toxicology screen	Nicotine	Striatum	Cerebellum	[11C]raclopride	B_{max}/K_d	↓
Wang et al. 2012[3]	16/15	DSM-IV, MA dependence	Minimum 2 wk	Urine screening tests	Nicotine	Striatum	Cerebellum	[11C] Raclopride	BP_{ND}	↓
Okita et al. 2016[46]	27/20	DSM-IV, MA dependence	Mean(SD) = 6.9(2.24) d	Urine testing	Nicotine and marijuana	Amygdala, striatum	Cerebellum	[18F] fallypride	BP_{ND}	↔ in amygdala, ↓ in striatum
Schrantee et al. 2015[16]	16/19	dAMPH users with ≥30 lifetime and ≥10 past year exposures	Minimum 1 wk	Nicotine	Multi-drug screen on a urine sample before scan	Striatum	Occipital cortex	[123I] IBZM	BP_{ND}	↓
Lee et al. 2009[47] [*5]	22/30	DSM-IV, current MA dependence	4-10 d	Residing at a research center during study	Nicotine	Striatum	Cerebellum	[18F]fallypride	BP_{ND}	↓
Ballard et al. 2015[48] [*6]	18/18	DSM-IV, MA dependence	4-7 d	Supervised admission	Nicotine and caffeine	Striatum	Cerebellum	[18F] fallypride	BP_{ND}	↓
Okita et al 2015[49] [*7]	23/17	DSM-IV, MA dependence	Mean(SD) = 7.2(3.11) d, range: 4-15	Nicotine, cannabis and alcohol	Admission and urine screening	Striatum	Cerebellum	[18F] Fallypride	BP_{ND}	↓

				d								

Abbreviations: BP, binding potential; d, days; dAMPH, dexamphetamine; MA, methamphetamine; mo, months; N/A, not applicable; SD, standard deviation; SUR, specific uptake ratio; wk, weeks

*1 [11C] PHNO data was excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with [11C] raclopride data.

*2 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Martinez et al. 2007.

*3 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Volkow et al. 2014.

*4 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Volkow et al. 2014.

*5 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Ballard et al. 2015.

*6 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Ballard et al. 2015.

*7 Excluded from the meta-analysis on dopamine receptor availability due to overlapping participants with Lee et al. 2009.

Preclinical research
- Develop preclinical models that reflect the chronic usage seen in human users and measure levels of dopamine receptors, transporters and release prior to and following chronic use in longitudinal studies
- Use translational methods such as imaging so findings can be directly related to human studies
- Evaluate dosing regimens (both doses and patterns) that reflect human usage
- Develop genetic or other models that reproduce the magnitude of lower dopamine receptor, transporter and release levels seen in human users and determine if these increase the risk of the animals self-administering stimulants
- Determine if reduced dopamine release, transporter and receptor levels seen in chronic stimulant use are reversible with abstinence

Clinical research
- Determine if the reduced dopaminergic activity is a pre-existing vulnerability factor or a direct result of repeated drug exposure
- Determine the dopaminergic changes over the course of stimulant use and its reversibility with abstinence.
- Determine dopaminergic activity in extra-striatal regions in stimulant addiction.
- Address the possible confounding factors which may affect dopamine signalling such as disrupted sleep patterns, obesity and co-morbid substance use (particularly of tobacco)
- Determine the interaction of the dopaminergic system with the glutamatergic and GABAergic systems.

Box 1: Future research direction

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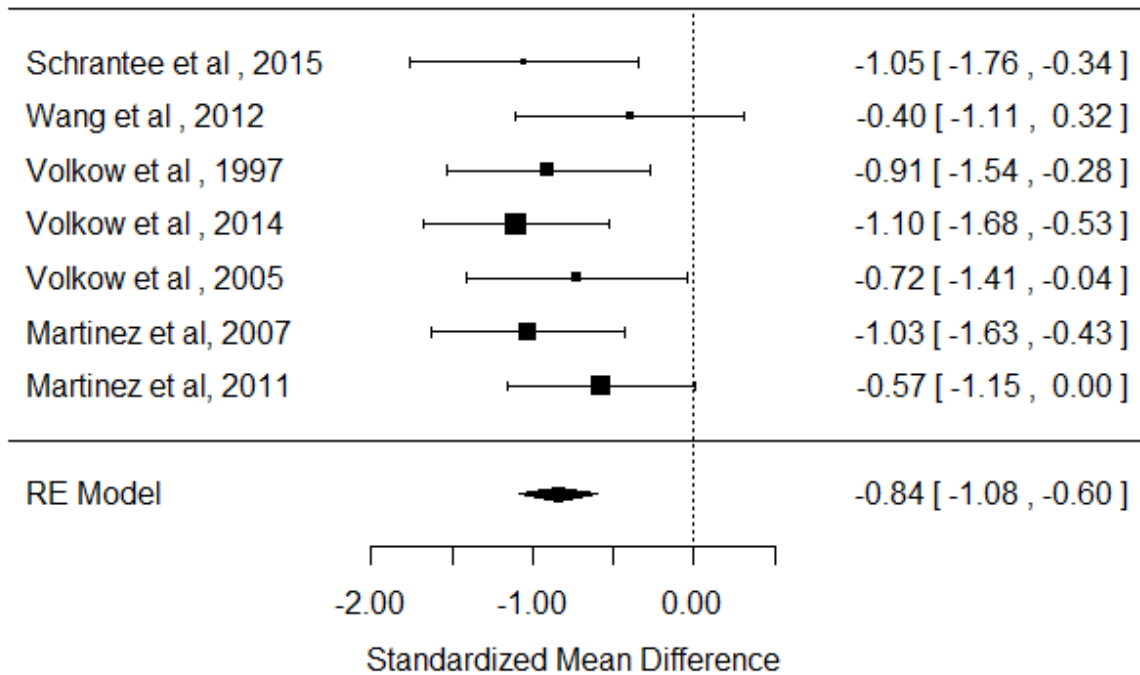
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Figure 1: Studies of dopamine release in stimulant users. The forest plot shows the effect sizes estimated using a random effects model and 95% confidence intervals of the percentage change in the difference of change in D2/3 binding after challenge. There was an overall decrease in dopamine release in stimulant users relative to controls with a large to very large effect size (−0.84 [95% CI, −1.08 - −0.60], p<0.001)

Schrantee et al. 2015 and Wang et al. 2012 studied amphetamine-like stimulant users. The remaining studies included cocaine users.
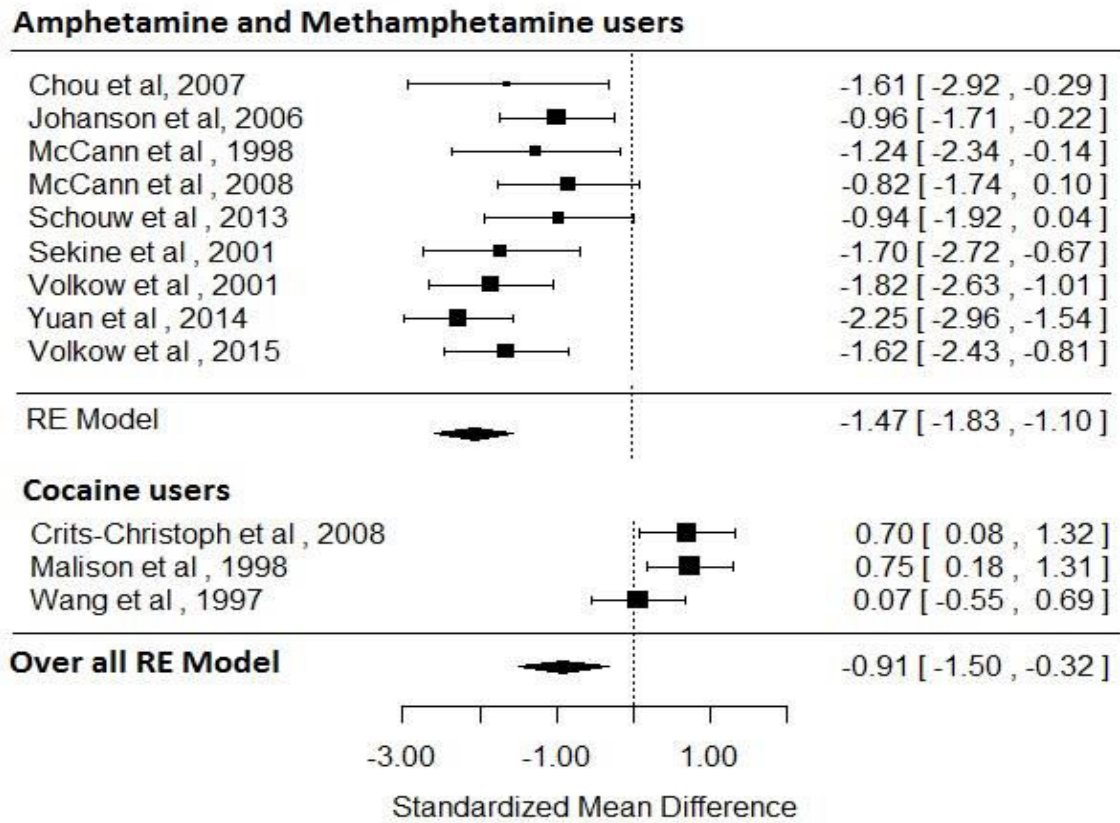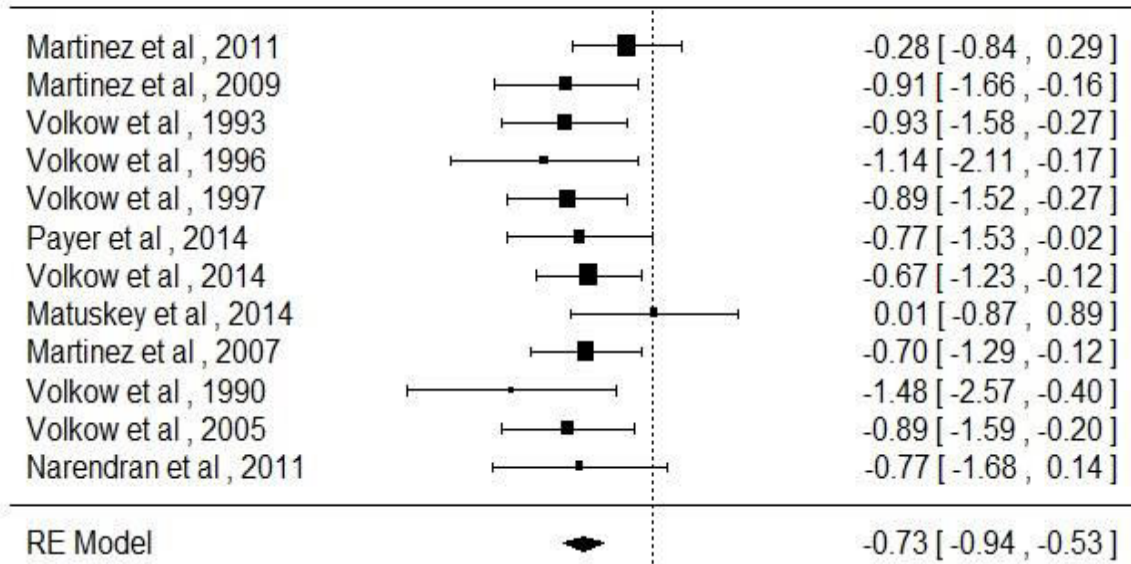
Figure 2: Studies of dopamine transporter availability. The forest plot shows the effects sizes estimated using a random effects model and 95% confidence intervals of the difference between amphetamine/methamphetamine users and controls. There was an overall decrease in the dopamine transporter availability in methamphetamine users relative to controls (−0.91 [95% CI, −1.5 - −0.32], p <0.01)

Amphetamine and Methamphetamine users

Study	SMD [95% CI]
Chou et al, 2007	-1.61 [-2.92 , -0.29]
Johanson et al, 2006	-0.96 [-1.71 , -0.22]
McCann et al , 1998	-1.24 [-2.34 , -0.14]
McCann et al , 2008	-0.82 [-1.74 , 0.10]
Schouw et al , 2013	-0.94 [-1.92 , 0.04]
Sekine et al , 2001	-1.70 [-2.72 , -0.67]
Volkow et al , 2001	-1.82 [-2.63 , -1.01]
Yuan et al , 2014	-2.25 [-2.96 , -1.54]
Volkow et al , 2015	-1.62 [-2.43 , -0.81]
RE Model	-1.47 [-1.83 , -1.10]

Cocaine users

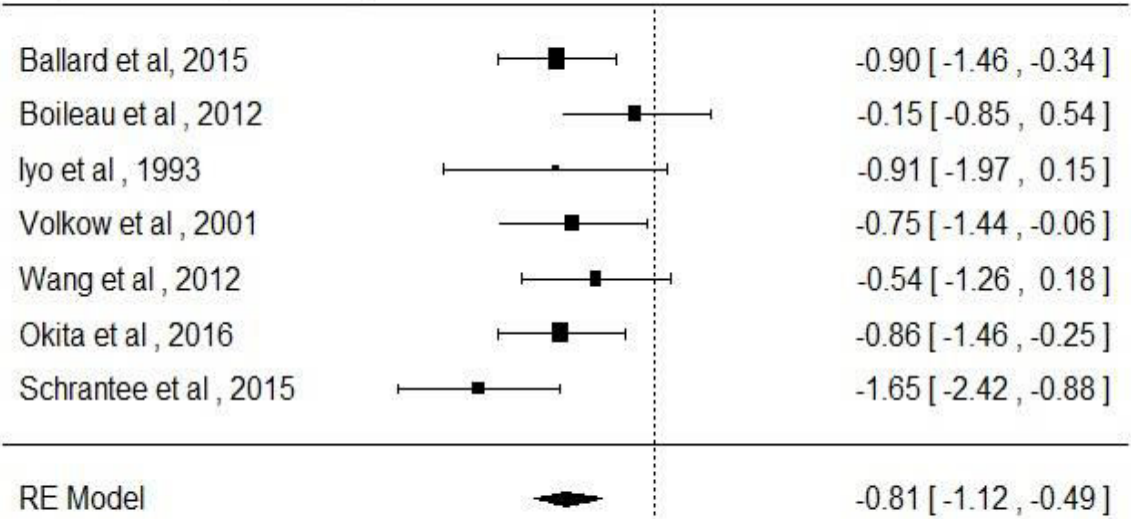| Study	SMD [95% CI]
Crits-Christoph et al , 2008	0.70 [0.08 , 1.32]
Malison et al , 1998	0.75 [0.18 , 1.31]
Wang et al , 1997	0.07 [-0.55 , 0.69]
Over all RE Model	-0.91 [-1.50 , -0.32]

-3.00   -1.00   1.00

Standardized Mean Difference

Figure 3: Studies of dopamine receptor availability. The forest plot shows the effect sizes estimated using a random effects model and 95% confidence intervals of D2/3 receptor binding potentials. There was an overall decrease in dopamine receptor availability compared to controls (−0.76 [95% CI, −0.92 - −0.60], p<0.001).
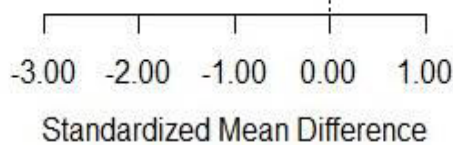

Cocaine users

Martinez et al , 2011	-0.28 [-0.84 , 0.29]
Martinez et al , 2009	-0.91 [-1.66 , -0.16]
Volkow et al , 1993	-0.93 [-1.58 , -0.27]
Volkow et al , 1996	-1.14 [-2.11 , -0.17]
Volkow et al , 1997	-0.89 [-1.52 , -0.27]
Payer et al , 2014	-0.77 [-1.53 , -0.02]
Volkow et al , 2014	-0.67 [-1.23 , -0.12]
Matuskey et al , 2014	0.01 [-0.87 , 0.89]
Martinez et al , 2007	-0.70 [-1.29 , -0.12]
Volkow et al , 1990	-1.48 [-2.57 , -0.40]
Volkow et al , 2005	-0.89 [-1.59 , -0.20]
Narendran et al , 2011	-0.77 [-1.68 , 0.14]
RE Model	-0.73 [-0.94 , -0.53]

Amphetamine/ Methamphetamine users

Ballard et al, 2015	-0.90 [-1.46 , -0.34]
Boileau et al , 2012	-0.15 [-0.85 , 0.54]
Iyo et al , 1993	-0.91 [-1.97 , 0.15]
Volkow et al , 2001	-0.75 [-1.44 , -0.06]
Wang et al , 2012	-0.54 [-1.26 , 0.18]
Okita et al , 2016	-0.86 [-1.46 , -0.25]
Schrantee et al , 2015	-1.65 [-2.42 , -0.88]
RE Model	-0.81 [-1.12 , -0.49]

Stimulant users

RE Model	-0.76 [-0.92 , -0.60]

-3.00 -2.00 -1.00 0.00 1.00

Standardized Mean Difference
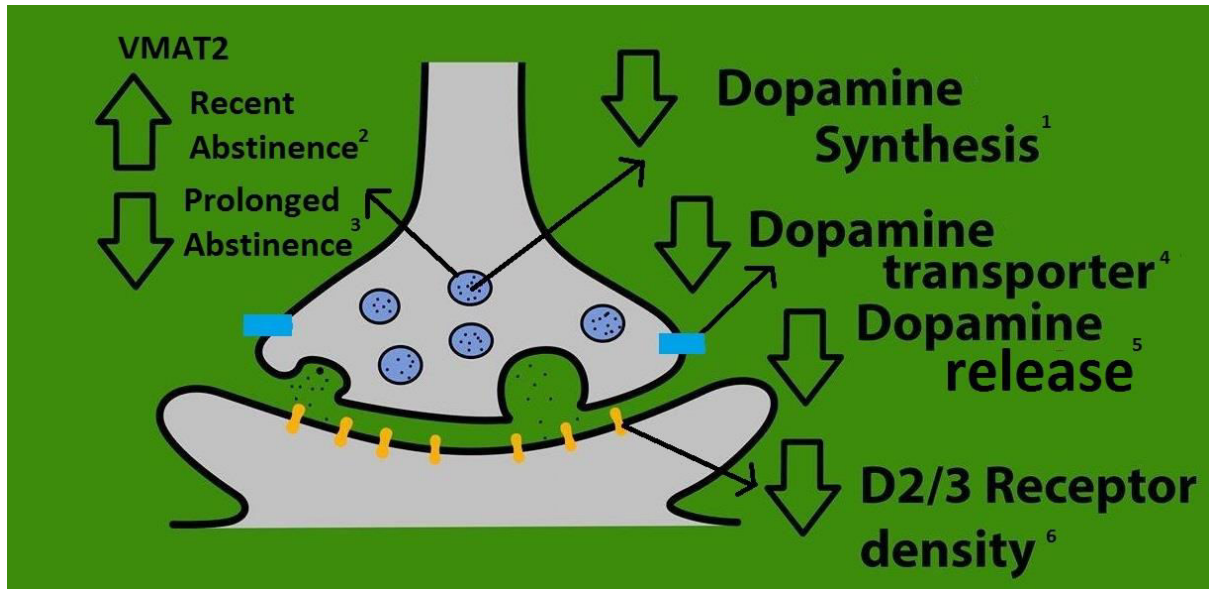
Figure 4: A summary of dopaminergic alterations in stimulant users

Summary of the synaptic location of the major dopaminergic findings from our meta-analyses and findings from studies of other aspects of the dopamine system.

1.   Based on study by Wu et al. 1997

2.   Based on study by Boileau et al. 2008 & 2015

3.   Based on study by Narendran et al. 2012 and Johanson et al. 2006

4.   Meta-analysis finding with effect size of −0.81 [95% CI, −1.34 - −0.29], p<0.01

5.   Meta-analysis finding with effect size of −0.91 [95% CI, −1.50 - −0.32], p<0.01

6.   Meta-analysis finding with effect size of −0.71 [95% CI, −0.91 - −0.52], p<0.001