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A review of positron emission tomography studies exploring the dopaminergic system in substance use with a focus on tobacco as a co-variat

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Abstract

With the evolving sensitivity of positron emission tomography (PET) and the emergence of novel radiotracers, greater insight has been gained into the dopaminergic system as it relates to substance use. In this review, we summarize PET investigations from the last ten years that explore the dopaminergic system in tobacco, alcohol, stimulant, opiates and cannabis addiction. In light of the prevalence of substance co-use, this review will also explore tobacco and other substance abuse co-morbidity on the dopaminergic system across study samples in the reviewed literature. In non-dependence, increased DA transmission following acute stimulant administration is a robust and consistent observation, but is less detectable following acute alcohol and tobacco, where it likely represents a conditioned effect mediating reward expectation. Chronic drug exposure is generally associated with a hypo-functioning pre-synaptic dopamine system and lower D2/D3 receptor availability relative to healthy controls. Emerging evidence also shows that stimulant use disorders in particular may also be associated with greater D3 receptor availability relative to controls. A defined role for the dopaminergic system in cannabis and opiate use has yet to be elucidated. Future work is also needed to delineate the potential interactive effects of tobacco and substance co-use on the dopaminergic system.

Keywords

PET; tobacco; nicotine; dopamine; addiction

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Introduction

A wealth of animal and earlier human studies provide support for a critical role of the mesolimbic dopaminergic system in the incentive salience of drugs of abuse [1]. Acute administration of drugs of abuse elicits increases in dopamine (DA) preferentially in the limbic areas of the brain. With chronic administration and the onset of substance use disorder (SUD), dopaminergic alterations including lower D2-type (D2/D3) receptor availability and decreased pre-synaptic activity are evident (See Volkow *et al.* [2] for previous review).

Over the last ten years, the use of the specialized technology of Positron Emission Tomography (PET) imaging has generated a more nuanced understanding of the precise role of DA in addiction processes. Using ligands labeled with positron-emitting isotopes (e.g. carbon-11), PET enables the non-invasive imaging of important dopaminergic targets including DA receptors and molecules involved in the physiological functioning of the system (i.e. DA pre-synaptic reuptake through dopamine transporters (DAT), DA pre-synaptic vesicular transport and DA transmitter synthesis [3]). A PET parameter commonly used to quantify the availability of receptors in the brain is binding potential (BP), which simultaneously reflects the receptor density available for binding and the affinity of the radiolabeled ligand ("tracer") for the receptor [4]. To bind to the DA neuroreceptors, the tracers compete with endogenous DA in the brain [5]; such competition enables the measurement of acute changes in DA neurotransmitter levels in response to drug challenges. A reduction in BP in a drug challenge scan condition, as compared to a baseline scan condition, is commonly interpreted as increases in extracellular DA. This conceptualization has acquired support through micro-dialysis studies in animals [5, 6].

The present review summarizes current PET data exploring the effects of acute and chronic use of tobacco, alcohol, stimulant, opiate and cannabis use on the DA system. As substance co-use, particularly co-morbid tobacco dependence with other SUDs, is a prevalent pattern [7], there is a need to better understand potential interactive drug effects on the brain. To this aim, we will also examine the influence of tobacco as a co-variate in the reviewed literature.

The Dopaminergic System in Tobacco Use and Dependence

Acute Challenge

The literature is inconsistent as to whether tobacco evokes DA release in humans to an extent measurable using PET. While in tobacco dependent samples, smoking a cigarette containing nicotine decreased [^{11}C]-raclopride binding (~8 to 10%) in the nucleus accumbens compared to smoking a de-nicotinized cigarette [8, 9], nicotine administration alone – without the sensory stimuli associated with cigarette smoking – did not significantly affect [^{11}C]-raclopride binding in minimally dependent smokers [10] or in non-smokers [11]. However, a significant decrease in striatal BP was noted in a subgroup of dependent smokers in the latter study [11]. This suggests that sensory cues may be necessary for a robust dopaminergic response to tobacco smoking. Indeed, cigarettes without the major addictive nicotine component were shown to significantly increase DA levels in the striatum by Domino *et al.*, highlighting the specific importance of cue-related effects [12]. The

tobacco-induced DA response may therefore represent a conditioned response of reward expectation developed through extensive smoking history. Of note, as carriers of the minor AG/GG allele of the *OPRM1* mu opioid gene receptor evidence more robust decreases in [^{11}C]-raclopride binding in the caudate and ventral putamen following a regular cigarette than individuals with the more commonly occurring AA allele ([13]; see also [14]), potential genetic heterogeneity in the aforementioned study samples may have also influenced the results.

However, there are inherent challenges to imaging the dopaminergic response to acute drug administration, particularly with drugs that act indirectly on DA terminals. With substances such as tobacco and alcohol (reviewed below), acute administration in animals results in transient increases in DA, considered moderate in magnitude relative to stimulants [15, 16]. As described by Morris *et al.*, detection of this transient response may be influenced by methodological variability, including the duration between smoking and scan acquisition, dose and mode of drug administration (presence/absence of sensory cues, number of cigarettes smoked, etc.), and analysis techniques [3]. To address some of these limitations, a novel PET image analysis approach incorporating a time-varying component to capture transient changes in DA throughout the scan, was recently developed [17]. This technique showed sex-differences in the dopaminergic response, whereby smoking a cigarette rapidly and consistently elicited right ventral striatal DA release in male smokers and dorsal striatal DA release in female smokers [18].

Finally, earlier PET work may have been limited by the ability of the commonly used D2/D3 antagonist radiotracer [^{11}C]-raclopride, to detect modest tobacco-induced changes in DA. Novel tracers such as the D2/D3 agonist [^{11}C]-(+)-PHNO allow for exploration of the DA system with greater sensitivity [19, 20]. [^{11}C]-(+)-PHNO also allows for the imaging of extra-striatal sites considered D3-rich [21], with potential to explore the drug induced DA activity at the D3 and D2 receptor separately [22]. Using [^{11}C]-(+)-PHNO in moderately dependent smokers (mean of 13 cigarettes/day), smoking a regular cigarette elicited a 12% decrease in tracer binding relative to a non-smoking condition within the ventral striatum ([23]; Figure 1) and a 15% decrease in the D3-rich region of the ventral pallidum, corroborating growing preclinical work suggesting a crucial role for both the D3 receptor and ventral pallidum in drug motivation [24, 25]. Similarly, through the use of [^{11}C]-FLB457 (FLB), a PET radiotracer with higher signal-to-noise ratio than [^{11}C]-raclopride, [26], a 12% decrease in tracer binding following a regular cigarette was observed within the cingulate gyrus of dependent smokers, reflecting increased cortical DA. Such increased phasic levels of DA within the human cortical regions in response to tobacco may underlie the ‘pro-cognitive’ effects of the drug [27].

Taken together, the tobacco-induced striatal dopamine response appears most robust among heavier smokers.

Neurochemical Changes Associated with Tobacco Use

Chronic tobacco use is linked to a hypo-functioning dopaminergic system. Using the [^{11}C]-SCH23390 D1 antagonist tracer, lower D1 receptor availability in the bilateral ventral striatum was reported in a pilot study of highly dependent smokers (mean of 25 cigarettes/

day; [28]) and significantly lower striatal D2/D3 availability in smokers compared to non-smokers has also been observed [29, 30]. The latter effect was not seen in Scott *et al.*'s study [8], perhaps due to a small sample size. Preliminary evidence favors sex differences in D2/D3 striatal availability where, unlike females, only dependent male smokers showed lower [^{18}F]-Fallypride binding in the striatum compared to non-smoking males [31].

However, DA synthesis capacity, a marker of pre-synaptic function measured with a radiolabeled DA precursor ([^{18}F]-DOPA), was found not to significantly differ between a dependent and non-smoking sample [32]. As smokers in this study were minimally dependent (average 8 cigarettes/day) [32], it is possible that dysregulation of synthesis capacity occurs only with heavier use. Indeed, in a heavier smoking sample (average 17 cigarettes/day), Leroy *et al.* reported lower dopamine transporter (DAT) availability in smokers compared to healthy controls in both striatal and extra-striatal (midbrain, anterior cingulate) regions [33]. As DAT levels are regulated by DA homeostasis, decreased DAT availability may reflect lower pre-synaptic DA levels associated with chronic tobacco use [33]. In support, Brody *et al.* found that a 3-week period of smoking abstinence resulted in decreased [^{11}C]-raclopride availability in the ventral striatum compared to baseline in dependent smokers, possibly suggestive of an increase (or normalization) of intra-synaptic DA levels following smoking cessation. Importantly, one existing caveat of the PET competition model is that lower receptor availability can reflect either decreased receptor density or greater synaptic endogenous DA. If basal DA is depressed in tobacco dependence as suggested by Brody *et al.* [34], then lower D2/D3 receptor availability reported in the aforementioned studies [29, 30] is interpretable as lower receptor density.

The Dopaminergic System in Alcohol Use and Dependence

Acute Challenge

The current literature on the effects of acute alcohol in social drinkers is largely mixed. Using a controlled intravenous infusion paradigm to circumvent variability in alcohol pharmacokinetics across participants ('alcohol clamp'), Yoder *et al.* found that infused ethanol targeting BACs from 0.06g% to 0.08g% led to large variability in the magnitude and direction of the DA response, and overall non-significant changes in striatal DA [35, 36]. While these negative findings must be interpreted in the context of very small sample sizes and methodological variabilities (i.e. differences in infusion time), they may underscore the importance of sensory cues to the alcohol-induced DA response. Indeed, two larger studies by Oberlin *et al.*, elegantly designed to disassociate alcohol cues from alcohol intoxication, revealed that sensory properties alone sufficiently elicit detectable increases in ventral striatal DA (~ 5%) [37], with infused alcohol (intoxication) seemingly playing an additive role in this response [38].

Studies using oral administration of alcohol therefore assess DA release in response to cues as well as intoxication. Broadly, the results from such studies suggest that acute alcohol can indeed elicit detectable elevations in striatal DA, although the response is most robust in certain subgroups of drinkers. Setiawan *et al.* proposed that the acute release of DA is a marker of risk for dependence: only social drinkers with 'risky' phenotypes (greater trait impulsivity, greater subjective 'high' from alcohol) evidenced increased ventral striatal DA

(ΔBP = ~−9%) following a high dose of oral alcohol [39]. In a similar vein, Urban *et al.* demonstrated more robust and consistent striatal DA release in the subgroup of male heavy drinkers compared to female heavy drinkers following a similarly high dose of oral alcohol [40]. In both cases however, the magnitude of drug-induced change in DA level may have been confounded by the use of a fruit juice placebo as a basal scan [3]. Indeed, previous work highlights that expectation to receive alcohol influences the dopaminergic response, with negative prediction error (expecting alcohol but receiving placebo only) actually leading to *decreased* DA [41].

Notably, to minimize this influence of placebo (and therefore expectation), the largest intravenous alcohol study by Yoder *et al.* used a baseline scan without infusion and explicitly informed participants of the study condition [42]. Intravenous infusion to a BAC level of 0.08g% led to significant decreases in ventral striatal tracer binding in the subgroup of non-treatment seeking dependent drinkers, but not in social drinkers [42]. Similar to acute tobacco, the collective results suggest that the dopaminergic response to acute alcohol is most observable in a state of dependence, and may be conditioned through a history of heavy drinking. However, a pre-existing phenotype of neural sensitivity to alcohol-induced DA transmission in certain subgroups of drinkers is also conceivable [39, 43].

Neurochemical Changes Associated with Alcohol Use

Again similar to tobacco, chronic high-risk drinking has generally been associated with a hypo-functioning dopaminergic system. Relative to controls, recently detoxified individuals with alcohol dependence displayed lower D2/D3 receptor [^{11}C]-raclopride binding in the ventral striatum [44] and in the whole striatum, with the lowest binding seen dorsally [45]. However, this effect was not observed using [^{11}C]-(+)-PHNO [46] or [^{18}F]-Fallypride [47]. If harmful drinking-induced alterations in receptor availability have the capacity to resolve following detoxification, the duration of abstinence may impact study findings; more longitudinal studies are needed to confirm the possibility of receptor normalization following prolonged abstinence. Indeed, Rominger *et al.* found increased striatal D2/D3 availability following 1 year of abstinence, but only in a very small subgroup of drinkers [47]. Instead, lower [^{18}F]-Fallypride binding in extra-striatal regions including the thalamus and insula was seen in this study [47]. The results from Erritzoe *et al.* also implicate the involvement of extra-striatal regions, as higher D3 receptor levels (using [^{11}C]-(+)-PHNO) was observed in the hypothalamus of detoxified participants relative to controls [46].

Finally, harmful alcohol consumption has also been associated with blunted pre-synaptic DA transmission, largely in the ventral striatum, as probed with acute stimulant challenges [44, 45]. Narendran *et al.* observed similar blunting of pre-synaptic DA activity following an amphetamine challenge across the cortex of their sample of recently abstinent alcohol dependent participants [48]. However, whether altered DA synthesis capacity in participants with alcohol use disorders (AUDs) contributes to this reduced transmission is unclear, as work using [^{18}F]-DOPA has failed to detect differences between patients and controls in the ventral striatum [49, 50].

Influence of Tobacco Co-Dependence

Three of the nine acute administration studies reviewed permitted regular cigarette smoking in their socially drinking samples (Table 1). Urban *et al.* and Setiawan *et al.* performed specific analyses in subgroups of drinkers who smoke and revealed that the main outcome of change in tracer binding did not significantly differ from their non-smoking counterparts [39, 40]. While separate analysis was not performed by Yoder *et al.*, they appropriately identified nicotine withdrawal as a potential confound of the alcohol-induced change in binding outcome and addressed this by placing nicotine patches on the subset of participants who smoked [42].

All of the aforementioned studies in alcohol dependent samples reported smoking co-morbidity, and attempted to control for this potential confound either by matching the number of smokers (and level of tobacco dependence) between study groups or through statistical analysis. No significant effect of smoking on the alcohol specific study outcome of tracer binding was reported however.

To our knowledge, Albrecht *et al.* is the only PET study to directly explore the potential interactive effect of alcohol and tobacco dependence on the striatal dopaminergic system [51]. D2/D3 receptor availability was similar across alcohol dependent smokers, social drinking smokers and social drinking non-smokers, suggestive of a lack of interactive effect. Notably, smokers had significantly lower striatal receptor availability compared to non-smokers [51], independent of drinking status. However, the authors propose that an alcohol dependent non-smoking subgroup would be necessary to confirm whether chronic smoking is the sole driver of the observed group differences. Moreover, the alcohol dependent subgroup was comprised of non-treatment seeking individuals of potentially lower dependence severity in contrast to much of the prior work which included treatment-seeking patients.

The Dopaminergic System in Stimulant Use and Dependence

Acute Challenge

Earlier PET imaging literature in non-dependent individuals have consistently demonstrated significant decreases in [^{11}C]-raclopride binding following either intravenous or oral administration of a stimulant (i.e., cocaine, amphetamine, methylphenidate, methamphetamine), relative to placebo or baseline. Indeed, the ability of stimulants to reliably elicit large reductions in tracer binding has been well substantiated such that acute stimulant challenges are commonly used to probe the functional response of the dopaminergic system across various psychopathologies [5]. Recent work corroborates large reductions in striatal [^{11}C]-raclopride binding following acute stimulant administration in non-dependent individuals [52], although some variability has been noted (i.e. as a function of sex [53] and genetic predisposition to addiction [54]).

In contrast, active and detoxified stimulant dependent individuals consistently demonstrate significantly attenuated stimulant-induced DA transmission in the striatum relative to controls [55–57]. No appreciable change in DA levels has also been demonstrated [58], with greater blunting associated with relapse [57] and choice of cocaine administration over a

competing reward of greater value [56]. Despite such hypo-functioning transmission probed by direct pharmacological action, similarly dependent samples responded to stimulant cue exposure, relative to neutral cue exposure, with large reductions in striatal binding [59, 60]. Thus, like alcohol and tobacco, cues conditioned to stimulant use elicit acute increases in DA, likely to reflect expectation of reward.

However, a large study by Volkow *et al.* did not find that cocaine cues concomitantly delivered with active cocaine produced enhanced DA increase relative to the administration of active cocaine alone [58]. Notably, the former studies by design assessed reward expectation [59, 60], while the Volkow *et al.* trial measured co-occurring expectation and receipt – a condition that may have dampened the transient DA transmission elicited by expectation [58].

Neurochemical Changes Associated with Stimulant Use

In line with blunted drug-induced transmission of DA in the striatum in chronic stimulant use, recent evidence reveals alterations in pre-synaptic DAT levels and in vesicular monoamine transporter (VMAT) levels, a pre-synaptic vesicular protein used as a marker of neuronal terminal integrity. Specifically, in methamphetamine dependent individuals either partially remitted or abstinent for a moderate duration (~ 1 year), significantly lower DAT levels were evidenced in both the caudate and putamen of the striatum relative to healthy controls [61, 62]. In the former study, lower VMAT levels were also seen across the striatum (albeit, minimally at 10%) in stimulant users who reported an average time of last drug use of 3 years [61]. Paradoxically, Boileau *et al.* noted *higher* VMAT2 tracer binding in two separate samples of acutely abstinent methamphetamine dependent individuals compared to controls [63, 64]. These VMAT2 levels did not differ from controls with longer periods of abstinence [64]. While differences in abstinence length between the studies may have contributed to these seemingly divergent results, Boileau *et al.* have instead argued that at low levels, the tracer used to probe VMAT2 competes with endogenous intra-vesicular DA; greater binding thereby reflects decreased vesicular DA resulting from chronic methamphetamine use [63]. Similarly, cocaine dependent individuals (≥2 weeks abstinent) also evidenced lower VMAT2 tracer binding across all striatal sub-regions compared to controls [65], while an acutely abstinent (<10 days) cocaine dependent sample did not differ from their healthy comparators [64]. Regardless, these data in culmination provide strong support for depressed pre-synaptic dopaminergic function in stimulant dependence.

While one recent study reported no detectable differences in D1 receptor availability in dependent individuals relative to controls within any of the striatal, cortical and subcortical regions explored [66], lower D2/D3 receptor availability (via [^{11}C]-raclopride) has been consistently shown across the striatum in both acutely detoxified and active (non-detoxified) cocaine dependent individuals [55, 56, 58, 67]. Similarly, lower D2/D3 availability probed with [^{18}F]-Fallypride is also seen largely in the caudate and putamen in methamphetamine dependence [68]. In the case of stimulant dependence, lower striatal D2/D3 receptor availability may reflect a down-regulation of receptors as administration of Alpha-methyl-p-tyrosine (AMPT), an acute DA depleting agent, resulted in less increase (from baseline) in striatal [^{11}C]-raclopride binding in cocaine dependent individuals compared to healthy

controls [67]. Notably, work using the D3-preferring [^{11}C]-(+)-PHNO ligand in cocaine and methamphetamine dependent samples evidenced significantly higher binding within extra-striatal regions (substantia nigra (Figure 2; [69–71]), hypothalamus and amygdala [71]) relative to controls, although concurrent lower D2/D3 receptor availability in the striatum (as suggested using antagonist tracers [^{11}C]-raclopride and [^{18}F]-Fallypride) was inconsistently seen in these studies [69, 71]. While simultaneous up-regulation of D3 and down-regulation of D2 receptors has been proposed to explain this observation [70], a secondary scan following the administration of either a selective D2 and D3 antagonist is needed to confirm this hypothesis [46].

Influence of Tobacco Co-Dependence

Tobacco co-use was reported in 18 of the 21 studies reviewed (Table 1), with the majority of these studies attempting to match subgroups on smoking status, or performing specific analyses to delineate the influence of tobacco in the main study outcomes. Aside from Martinez *et al.*, where smokers relative to non-smokers demonstrated marginally greater D1 receptor availability in the ventral striatum [66], results generally indicate that tobacco smoking does not explain the observed alterations in the functioning of the dopaminergic system in stimulant dependent individuals. However, investigations aimed specifically at investigating how co-morbid tobacco and stimulant dependence affects the dopaminergic circuits, relative to each substance alone, are presently lacking.

The Dopaminergic System in Opiate Use and Dependence

Acute Challenge

Intravenous diamorphine (heroin) or subcutaneous hydromorphone challenges have failed to affect striatal [^{11}C]-raclopride binding despite marked increases in subjective reports of intoxication, rush and/or high in opioid-dependent individuals [72, 73]. Further, no significant correlations between visual analog scale scores for “high” and tracer binding in the striatum were observed nor did expectation of heroin reward elicit DA release [73]. Three major methodological details may have influenced the results of the aforementioned studies. First, participants were retained on substitution therapy. Participants were therefore not in acute withdrawal as either methadone or buprenorphine was prescribed at varying dosages, and experimental sessions were timed to occur after the substitute medication dose was due (i.e. >24 hour after last dose). It is also possible that the neural response to these pharmacological challenges would differ in long-term opioid users not maintained on substitution therapy. Second, the small sample sizes (≤10) of both investigations along with the limited number of studies in this population hinder interpretation of results. Finally, opioid-induced DA changes may be too small to be detected using [^{11}C]-raclopride.

Neurochemical Changes Associated with Opiate Use

In a 2012 [^{11}C]-raclopride study by Martinez *et al.*, a recently detoxified (~2–3 weeks of abstinence) heroin-dependent group had significantly lower D2/D3 receptor availability than healthy controls in the bilateral limbic striatum, anterior/posterior caudate and anterior/posterior putamen. Relative to controls, heroin dependence was additionally associated with blunted pre-synaptic DA release in response to a methylphenidate challenge. Notably,

neither of these measures correlated with the choice to self-administer heroin [74]. In another investigation, Shi *et al.* employed [^{11}C]-CFT to evaluate striatal DA transporter (DAT) availability in heroin dependent individuals who were either on methadone maintenance therapy (n=10) or former heroin users abstinent for a minimum of 6 months (n=11), and healthy volunteers [75]. Both methadone-treated dependents and former users had lower DAT uptake function than healthy controls in the bilateral caudate. The methadone group also had lower DAT availability in the bilateral putamen relative to controls. This region also exhibited a statistical difference between methadone and abstinent groups. Notably there was no correlation between craving for heroin and DAT uptake function in the abstinent or methadone treated groups. In sum, these imaging studies reveal that dopaminergic system is impaired in heroin dependence, with subjects with opioid use disorders having decreased D2/3 receptor availability, decreased pre-synaptic DA release to psychostimulants and decreased striatal DAT uptake function, relative to healthy controls. Whether these neuroadaptations are causative or consequential of opiate use disorder mandates further investigation. Prolonged drug withdrawal may initiate normalization of disrupted DA function in opioid dependent individuals [75]. Notably however, the validity of these studies is compromised by small sample size, which influences both the power of the study as well as the ability to isolate potential gender differences. Study-selective limitations were also apparent. Despite the literature showing an association between age and [^{11}C] CFT binding, the control group in the Shi study was younger than the comparison group, presenting age as a possible confound. Differences in laterality were not examined in the region of interest analysis by Martinez *et al.*

Influence of Tobacco Co-Dependence

As indicated in Table 1, the majority of study participants were tobacco smokers [72–74], and none of the investigations prescribed changes in smoking habits within their design. Martinez *et al.* are the exclusive group to specifically report no statistical difference in the average cigarettes smoked daily between their control and heroin-dependent groups while Shi *et al* did not collect any information on the smoking status of their opioid-dependent and control populations. Most of these investigations failed to conduct specific analyses linking tobacco use to any of the main outcomes. However, Daglish *et al.* argue the dopaminergic response to the acute opiate challenge was unlikely influenced by smoking status as participants were not in nicotine withdrawal [72].

The Dopaminergic System in Cannabis Use and Dependence

Acute Challenge

The impact of an acute THC challenge on brain DA release in healthy cannabis users is inconclusive. In a study by Stokes *et al.*, 10 mg of oral THC (equivalent to one cannabis cigarette) had no appreciable effect on [^{11}C]-raclopride binding (relative to placebo) despite a significant behavioral response in 13 non-dependent recreational cannabis users [76]. In contrast, Bossong *et al.* found that a THC challenge through a Volcano vaporizer did yield a decrease in [^{11}C]-raclopride binding in the ventral striatum and the dorsal putamen, in 7 mild cannabis users [77]. Pooling and reanalyzing the imaging data from the aforementioned studies revealed that THC administration induced a significant reduction in [^{11}C]-raclopride

binding exclusively in the limbic striatum [78]. While the oral THC study was originally underpowered to detect a surge in DA neurotransmission, the possibility that the method of THC delivery impacted the results cannot be dismissed as oral consumption has previously been described to have slower absorption and delayed psychological effects, compared to smoking. Indeed, the validity of combining these 2 datasets with varying routes of THC administration is debatable [78]. In fact, contrary to the above results [77, 78], Kuepper *et al.* using [^{18}F]-fallypride PET failed to detect DA release post inhalation of THC (8mg) with the Volcano vaporizer in healthy controls with minimal cannabis use [79]. THC-mediated striatal DA release was instead only observed in cannabis-cognizant individuals with psychotic disorders and their unrelated first degree relative, with the effect dominant in the caudate region. In these populations, statistical significance was maintained post correction for gender, nicotine and alcohol use, yet no associations between THC-driven DA release and THC-induced changes in visual analog scale were observed [79]. Overall, the effect of an acute cannabis challenge on brain dopamine release in healthy cannabis users remains inconclusive. In general, small sample size combined with the heterogeneity of study design (e.g. the variable time lag between THC administration and PET data acquisition, non-randomized drug administration, etc.) yields non-negligible confounds.

Neurochemical Changes Associated with Cannabis Use

A consistent finding is that chronic marijuana users show no significant differences in striatal D2/D3 receptor density relative to non-cannabis using controls [80–84]. This finding was observed across studies, in spite of varying degrees of cannabis consumption and different periods of cannabis abstinence prior to testing (from < 20.6 hours to 12 weeks) in the chronic users [80–84]. It was also not modulated by age or cigarette smoking status [82]. Additionally, while [^{18}F]-FDG-PET showed decreased cerebral glucose metabolism in the right OFC, bilateral putamen and precuneus in cannabis users, no association with D2/D3 receptor availability was identified [81]. Notable observations regarding these studies include the following: (1) inconsistent collection of information about the potency of cannabis used may have impacted the data, (2) prolonged abstinence may have corrected cannabis-induced alterations in dopaminergic neurotransmission, and (3) small sample size may have biased the results.

Preliminary evidence suggests that pre-synaptic DA synthesis capacity may be decreased in chronic cannabis users [85]. Using [^{18}F]-DOPA, Bloomfield *et al.* revealed reductions in striatal DA synthesis capacity in cannabis users who consumed the drug at least weekly, relative to healthy controls. Subsequent analysis further revealed differences in the limbic and associative striatal subregions, between the groups. Indeed, when the marijuana user group (n=19) was sub-classified into dependents (n=10) and non-dependents (n=9) and compared, a statistical reduction in DA synthesis capacity was exclusively present in the dependents [85]. Also, DA synthesis capacity correlated positively with the age of onset of cannabis use, and negatively with higher levels of cannabis consumption [86]. Yet, results from studies exploring pre-synaptic dopaminergic transmission as probed by acute stimulant challenges are conflicting. While the dopaminergic response to an amphetamine challenge in abstinent mild cannabis dependents was shown to be analogous to that seen in matched controls in one study [83], another investigation found significantly blunted ventral striatal

methylphenidate-induced dopaminergic activity (reduced decreases in distribution volumes of [^{11}C]-raclopride) in dependent cannabis users compared to controls [84]. Taken collectively, these studies suggest that a history of chronic cannabis use may produce alterations in the brain dopamine system, an effect that may not necessarily be driven by abnormalities in D2/3 receptor levels [87]. Nonetheless, at this time, it would appear that the severity of damage caused by long-term cannabis use may be of decreased magnitude relative to other drugs of abuse [88].

Influence of Tobacco Co-Dependence

Tobacco co-use in study participants was notable (Table 1), yet this information was not consistently collected. When smoking co-occurrence was examined, smokers were asked to refrain from smoking for a minimum of 2 hours before study commencement [82, 84, 89], and efforts were made to evaluate the impact of smoking on the outcome measure. Evidence suggests that some alterations to the dopaminergic system observed in chronic cannabis users may be partly attributable to smoking co-morbidity. Specifically, in the Stokes *et al.* study, re-categorization of their sample into smokers versus non-smokers revealed that baseline limbic striatal binding values were 10% lower in the nicotine smokers, irrespective of cannabis smoking status [82]. These data further corroborate the suggestion that regular long-term cannabis exposure in itself is not associated with neuroadaptive changes in D2/D3 receptor levels. However, in another investigation exploring DAT availability using [^{11}C]-PE21, cannabis smokers with tobacco dependence consistently showed lower striatal, midbrain and cortical DAT levels (−1% to −9%) compared to those with only tobacco dependence [33]. Although this difference was not statistically significant, it highlights the potential of an additional effect of cannabis, above and beyond chronic tobacco use, on deficits in pre-synaptic function.

Commentary

Collectively, the large body of work using PET imaging over the last decade suggests that while DA remains a central neurotransmitter in drug use and dependence, its role is more complex than initially conceptualized [90]. The recent literature corroborates the well-established stimulant-induced promotion of DA levels in striatal and extra-striatal regions, and highlights that tobacco and alcohol lead to less consistent DA elevations, most likely representing a conditioned response through heavier use. It should be noted that striatal DA increases following acute opiate and cannabis use are not strongly supported. However, a limited number of studies have been performed and this lack of dopamine response may reflect the experimental conditions of these studies. In a similar vein, the literature also corroborates neuroadaptive changes related to a general hypo-functioning dopaminergic system characterized by decreased D2-type receptor availability, reduced DAT functioning and reduced DA synthesis capacity in stimulant dependence, and to some extent in alcohol, tobacco and opiate dependence. An important recent finding to emerge across the different SUDs is an *upregulation* in D3 receptor availability in extra-striatal areas (hypothalamus, substantia nigra, ventral pallidum). Such an upregulation is in line with animal findings [91–93] and alterations observed in post-mortem studies of chronic drug users, and signals the potential importance of this site as a target for addiction treatment [94]. However, larger

longitudinal studies are needed to elucidate whether the observed differences in the dopaminergic system seen in dependence either precedes or directly results from chronic use.

In the reviewed literature presented in Table 1, there was considerable tobacco co-occurrence in study samples, paralleling the high prevalence of cigarette smoking co-morbidity in other SUDs [7]. As discussed in a recent review by Hillmer *et al.*, failure to effectively control for the influence of co-occurring smoking in study participants may serve as a major confound. This review indicated that the vast majority of the recent investigations reported the smoking status of their sample, but with only some effort to control for group differences in smoking status and patterns of use through a matched control group. While matched samples circumvent the issue of disproportionate smoking in one group driving observed group differences in DA function, little can be said about the sub-population of substance users who smoke versus those who do not. Such an approach is critical to identifying important neurobiological markers specific to comorbid tobacco and substance use, which may inform the development of more targeted treatments. The few studies that incorporated specific analyses to determine whether the main outcomes differed as a function of tobacco smoking (i.e. through inclusion of smoking status as a covariate) – largely in the alcohol and stimulant literature –found that smoking status was unrelated to the observed acute and chronic drug effects. Notably, we only identified a few investigations specifically designed to explore the influence of chronic tobacco use comorbidity on DA function within alcohol users and cannabis users [33, 82]; evidence that lower D2/D3 availability and DAT function are largely driven by tobacco use was strongest for cannabis, further supporting a less critical role of DA in cannabis use disorder. More investigations of similar design to the aforementioned studies are needed to help elucidate the relative contribution of individual drugs to the neuroadaptations of the dopaminergic system in polydrug use. Importantly, we are unaware of any human imaging studies aimed at investigating the combined effects of *acute* nicotine and other substance administration on dopamine levels. Future studies with such an aim would further elucidate the mechanisms by which substance co-use is maintained (i.e., through an additive effect on dopamine release relative to each substance alone).

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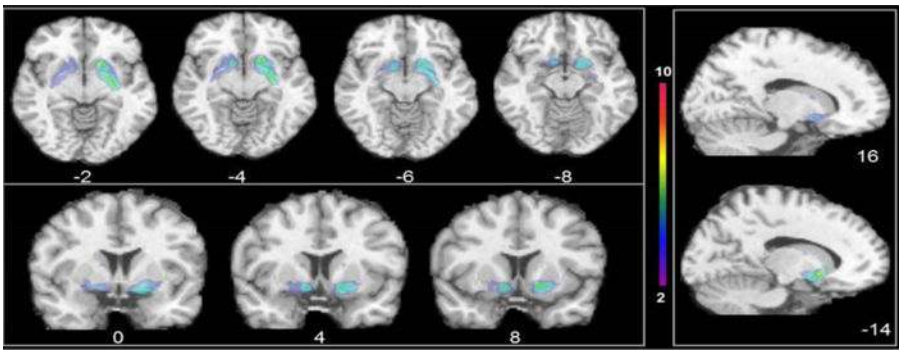

Figure 1.

T-statistical overlaid average T1 MRI showing clusters of significantly reduced [^{11}C]-(+)-PHNO BP_{ND} after smoking a cigarette. Greatest decreases in [^{11}C]-(+)-PHNO BP_{ND} cluster in the ventral part of the striatum and in the area that corresponds to the ventral pallidum $p < 0.05$. Adapted from [23]

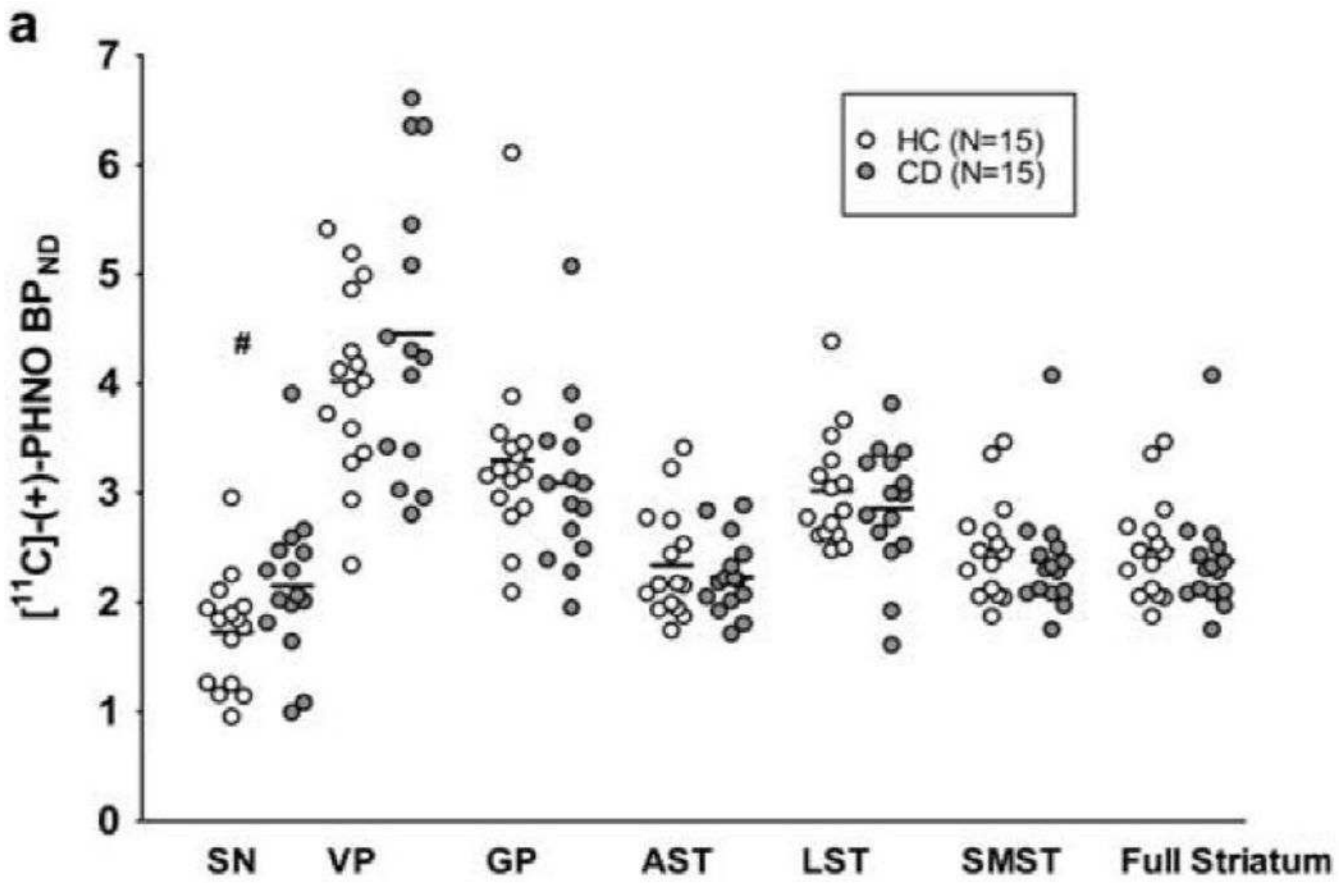

Figure 2.

DRD_3 upregulation in cocaine dependent subjects. Individual binding potential (BP_{ND}) values across regions of interest for each PET tracer in cocaine-dependent (CD) and healthy control (HC) subjects. (a) [^{11}C]-PHNO binding (a measure of DRD_3 in the substantia nigra and ventral pallidum) is elevated in the substantia nigra of CD (SN). Ventral pallidum (VP), another D3-rich region, also showed this pattern, but this effect was not statistically significant. No group differences were found in globus pallidus (GP), striatal subregions, or whole striatum. Reproduced with permission from [70]

Table 1

Smoking co-occurrence in study samples of reviewed literature

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
Yoder 2005 [35]	9 (8 males, 1 female) healthy social drinkers	Not reported.	Not reported.
Yoder 2007 [36]	13 (11 males, 2 females) healthy social drinkers	Sample indicated to be non-smoking	N/A
Oberlin 2013 [37]	49 male social to heavy drinkers	Regular habitual smoking was an exclusion criterion. However, two subjects reported smoking up to one cigarette or cigar per week.	N/A
Oberlin 2015 [38]	26 male heavy drinkers	Regular habitual smoking was an exclusion criterion. However, two subjects reported smoking up to one cigarette or cigar per week.	N/A
Setiawan 2013 [39]	26 (18 males, 8 females) healthy social drinkers categorized into 2 groups: 1) high risk drinkers (n=13) vs. low risk drinkers (n=13)	6/13 low risk individuals were smokers vs. 4/13 in the high risk group. The number of current smokers in each risk group did not significantly differ.	Subjects were asked to abstain from cigarettes on PET scan days. Statistical analyses were conducted with and without smokers. Results without the 10 current smokers were similar/in the same direction as the results with the entire sample included.
Urban 2010 [40]	21 social drinkers (11 males, 10 females)	3/11 males were smokers vs. 1 female smoker. All smokers were indicated to smoke <10 cigarettes per day.	Subjects were asked to refrain from smoking tobacco for the 2 hours prior to the PET scan. The main study outcome of change in binding was compared between smokers (n=4) and non-smokers (n=17). Smoking status was also included as a covariate in main study outcome analysis. Results indicated that smokers and non-smokers did not significantly differ in outcome of change in binding (ΔBP), and that smoking revealed no effect in main analysis.
Yoder 2009 [41]	8 (5 males, 3 females) healthy social drinkers	Not reported.	Not reported.
Yoder 2016 [42]	24 (18 males, 6 females) social drinkers vs. 21 (18 males, 3 females) non-treatment seeking alcohol dependent individuals	12/24 social drinkers were smokers vs. 18/21 alcohol dependent individuals. There were a significantly greater number of smokers in the alcohol dependent group.	Subjects were not required to remain abstinent from nicotine prior to the study. However, they were not allowed to smoke cigarettes during the study day. Trans-dermal nicotine patches (TNP) were placed on cigarette smokers shortly after arrival on study days; dose was based on self-report of cigarettes smoked/day.
Ramchand-ani 2011 [43]	Healthy male social drinkers recruited into 2 groups: 1) individuals homozygous for the major 118A allele (AA group, n=16) of the OPRM1 polymorphism and 2) individuals carrying one or two copies of the variant 118G allele (AG group, n=12) of the OPRM1 polymorphism.	Participants were non-smokers who had never smoked, or had quit at least a year prior to enrolling in the study.	N/A
Volkow 2007 [44]	20 male detoxed alcohol dependent individuals (at least 30 days in withdrawal) vs. 20 male healthy controls	16/20 alcohol dependent participants were smokers vs. 3/20 smokers in healthy control group.	Receptor availability was descriptively compared for smokers and nonsmokers in each of the groups separately, and relationship between smoking histories and stimulant-induced change in

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
			receptor availability explored. Results revealed no significant difference in D2/D3 receptor availability between smokers and non-smokers in either the healthy control or alcohol dependent group, although sample may be small for conclusive results. Smoking history did not correlate with change in receptor availability in whole alcohol dependent group, but did reveal significant correlation in subgroup of alcohol dependent individuals who smoke.
Martinez 2005 [45]	15 (13 males, 2 females) detoxified alcohol-dependent participants (scans occurring 2 weeks after 3 week detox) vs. 15 (12 males, 3 females) healthy controls	9/15 alcohol dependent participants were smokers (13 cigarettes per day) vs. 7/15 smokers in healthy control group (11 cigarettes per day).	Study groups were matched for cigarette smoking. Participants were allowed to smoke during the study, although they were instructed to abstain from smoking on PET scan days. No indication of separate analysis done.
Erritzoe 2014 [46]	16 male detoxified (minimum of 4 weeks) alcohol-dependent patients vs. 13 age matched male healthy controls	11/16 alcohol dependent participants were smokers (17 cigarettes per day) vs. 10/13 smokers in healthy control group (11 cigarettes per day). Both groups had comparable numbers of smokers, ex -smokers and never smokers; however, alcohol dependent smokers smoked significantly more and were significantly more dependent than controls	Sample was divided into current smokers (n=20), former smokers (n=5) and never-smokers (n=3) and differences in regional binding was compared between groups. The currently smoking sample was further divided into two groups of high vs. low dependence, and differences in regional binding were compared between groups. Results indicated no significant differences in regional binding of tracer between groups in any of the regions of interest.
Rominger 2012 [47]	17 male acutely abstinent alcohol dependent participants vs. 14 male healthy controls. The two groups were compared at baseline, after 2 weeks of withdrawal and at 1 year of abstinence	14/17 alcohol dependent individuals were dependent smokers vs. 0/14 smokers in the control group	The relationship between main study outcome (tracer binding/BP) and Fagerstrom index of nicotine dependence was explored. Smoking was also noted to be included as a covariate to explore group differences in main study outcomes. Results indicated no significant correlations between tracer binding and marker of nicotine dependence; smoking was noted not to alter observed differences between alcohol dependent group and healthy control group.
Narendran 2014 [48]	21 (16 male, 5 females) alcohol dependent individuals (minimum of 14 days abstinent) vs. 21 (16 male, 5 females) healthy controls	12/21 alcohol dependent individuals were regular smokers vs. 12/21 regular smokers in healthy control group.	Groups were matched for smoking status and the number of cigarettes smoked per day.
Deserno 2015 [49]	13 recently detoxified alcohol-dependent males vs. 14 male healthy controls	8/13 alcohol dependent participants were smokers vs. and 6/14 smokers in healthy control group. The groups did not statistically differ in the number of smokers.	Unclear if smoking was included as a co-variate in the voxel-wise PET analysis of difference in dopamine synthesis capacity between groups. However, smoking status was included as a covariate in analysis of fMRI regional activation, and the analysis exploring relationship between dopamine synthesis capacity and regional activation. Results indicated that smoking status did not change observed effects.
Heinz 2005 [50]	12 male alcohol dependent individuals (mean of 36 days abstinent) vs. 12 healthy controls	Presence of smoking mentioned, but no further details provided.	The relationship of smoking status and main outcome of FDOPA net influx explored. Results indicated no

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
			significant correlations between the two variables.
Albrecht 2013 [51]	34 non-treatment seeking alcohol dependent smokers (27 males, 7 females) vs. 21 social drinking smokers (18 males, 3 females) vs. 26 social drinking non-smokers (16 males, 10 females)	This study investigated alcohol and smoking co-occurrence specifically. All participants in the 'non-treatment seeking alcohol dependent smokers' (n=34) and 'social drinking smokers' (n=21) groups were regular smokers. These two subgroups of smokers did not differ in dependence levels.	ANOVA was used to compare differences in tracer binding across the three groups (2 groups of smokers with differing alcohol dependence status and 1 group of non-smokers).
		Stimulants	
Reference	Study sample	Smoking co-occurrence in sample	Details of controlling for co-occurrence in main study outcome
Oswald 2005 [52]	16 non-dependent healthy males and females	Sample was indicated to be non-smoking.	N/A
Munro 2006 [53]	43 non-dependent healthy males (n=28) and females (n=15)	Sample was indicated to be non-smoking. Current smoking was an exclusion criterion.	N/A
Casey 2014 [54]	16 (6 males, 10 females) non-dependent, family history positive individuals with current occasional psychostimulant use (FH+ exposed group) vs. 15 (9 males, 6 females) non-dependent, family history negative with current occasional psychostimulant use (FH− exposed group) vs. 17 (10 males, 7 females) healthy controls who are psychostimulant naive (FH – non-exposed group)	14/16 of the FH+ exposed group and 13/15 of the FH− exposed groups reported lifetime tobacco smoking. For healthy controls, occasional tobacco was not an exclusion criterion, although quantification of use is not provided for this group. FH+ exposed and FH− exposed groups differed only in the age of onset of tobacco use.	All smoking variables entered as co-variates into ANOVA of main study outcome (change in tracer binding). Text indicates that group differences remained after controlling for tobacco (and other drug) co-use.
Volkow 2005 [55]	21 male cocaine dependent inpatients (tested within one month of last cocaine hit) vs. 15 healthy male controls	Nicotine dependence indicated to not be an exclusion criterion.	Not reported.
Martinez 2007 [56]	24 (19 males, 5 females) non-treatment seeking cocaine dependent inpatients (minimum of 14 days abstinent) vs. 24 (19 males, 5 females) healthy controls	The healthy comparison group smoked an average of 12 cigarettes per day (SD=6), comprised of 6 non-smokers and 3 ex-smokers. The cocaine-dependent group smoked an average of 11 cigarettes per day (SD=4) and comprised of 4 non-smokers and 3 ex-smokers.	Groups were matched for smoking (avg. cigarettes smoked per day, number of non-smokers, and number of ex-smokers).
Wang 2012 [57]	16 (13 males, 3 females) treatment seeking methamphetamine abusers (minimum of 2 weeks detoxified) vs. 15 healthy controls (13 males, 2 females)	All methamphetamine abusers were cigarette smokers (n=16) vs. 4/15 in the control group. There were a significantly greater proportion of smokers in the methamphetamine using group. This group also demonstrated heavier smoking patterns.	The relationship between smoking status and study outcomes (baseline receptor availability and methamphetamine induced change in tracer binding) and was assessed with Pearson's correlations. Results indicated no significant associations.
Volkow 2014 [58]	43 male cocaine dependent individuals (active users not detoxified) vs. 19 male healthy controls	24/43 dependent participants were smokers vs. 3/15 of the healthy controls. There were a significantly greater proportion of smokers in the cocaine using group than control group.	Change in tracer binding induced by methylphenidate compared between cocaine users who were smokers and cocaine users who were non-smokers. The results indicated no significant difference.

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
Volkow 2006 [59]	18 (17 males, 1 female) cocaine dependent individuals who were active users for the past 6 months	15 participants were cigarette smokers	Not reported.
Wong 2006 [60]	19 (16 males, 3 females) individuals meeting criteria for stimulant abuse (non-treatment seeking). Participants were subsequently categorized into 'cravers' (n=11) and 'non-cravers' (n=8) based on self-report of craving following cue exposure.	18/19 participants had histories of nicotine dependence. These participants were considered regular smokers with an average of 6.57 cigarettes smoked per day. There were no significant differences in smoking severity (number of cigarettes smoked per day) between cravers and non-cravers.	Participants were asked to refrain from nicotine for least 6 h prior to the start of the brain imaging procedures on the study day.
Johanson 2006 [61]	16 methamphetamine dependent individuals (11 males, 5 females) in early partial remission (no use of methamphetamine for minimum of 3 months; average time of last use was 3 years) vs. 18 healthy controls (12 males, 6 females)	11/16 methamphetamine dependent individuals were smokers vs. 2/18 smokers in control group	Not specific analysis reported. Notably, smoking was allowed up to the time that sessions began.
McCann 2008 [62]	7 methamphetamine dependent individuals (4 males, 3 females) and 7 individuals with Parkinson's Disease (2 males, 5 females) vs. 16 healthy controls (12 males, 4 females)	5/7 stimulant dependent individuals had tobacco/nicotine exposure vs. 4/16 of the healthy controls	Not reported.
Boileau 2008 [63]	16 active methamphetamine dependent individuals (11 males, 5 females) vs. 14 healthy controls (11 males, 3 females)	9 methamphetamine dependent individuals were smokers vs. 5 healthy controls. There was a statistical trend towards greater smokers in the stimulant dependent group (p=0.07)	Not reported.
Boileau 2016 [64]	1st PET scan (2–3 days abstinent): 28 (12 males, 16 females) methamphetamine dependent individuals vs. 9 (3 males, 6 females) cocaine dependent individuals vs. 22 (12 males, 10 females) healthy controls 2nd PET scan (10 days abstinent): 17 methamphetamine users vs. 8 cocaine users vs. 9 healthy controls	24/28 methamphetamine users and 7/9 cocaine users vs. 4/22 healthy controls were smokers. The number of smokers in each of the drug groups differed significantly from number of smokers in the control group (proportion of smokers in each drug group did not differ).	ANOVA of main outcome was adjusted for number of cigarettes smoked per day. Results indicated that the main finding of higher DTBZ binding in the methamphetamine group within the striatum did not change.
Narendran 2012 [65]	12 (8 males, 4 females) cocaine dependent individuals (14 days abstinent) vs. 12 (8 males, 4 females) healthy controls	7/12 dependent subjects were smokers vs. 7/12 healthy controls.	Groups were matched for number of smokers.
Martinez 2009 [66]	25 (19 males, 6 females) non-treatment seeking cocaine dependent individuals (abstinent for 14 days) vs. 23 (19 males, 4 females) healthy controls	1/25 cocaine dependent individuals were smokers vs. 16/23 healthy controls.	Groups were matched for smoking status and number of cigarettes smoked. Smoking status added as group factor in 2-way ANOVA with tracer binding as outcome variable. Smoking revealed a main effect; tracer binding in the VST was higher in smokers than non-smokers at trend level (p=0.078)
Martinez 2009 [67]	15 (13 males, 2 females) cocaine dependent individuals (abstinent for 14 days) vs. 15 healthy controls (13 males, 2 females)	13/15 cocaine dependent individuals were smokers (average 11 cigarettes per day) vs. 12/15 healthy control (average 10 cigarettes per day).	Groups were matched for number of smokers and number of cigarettes smoked.
Lee 2009 [68]	22 (13 males, 9 females) methamphetamine dependent	19/22 dependent subjects were smokers vs. 11/30 healthy	The effect of smoking on tracer binding was examined only in the

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
	individuals (4–10 days abstinent) vs. 30 (16 males, 14 females) healthy controls	controls. There were a significantly greater proportion of smokers in the dependent group.	control group as this had comparable numbers of smokers and non-smokers (smoking entered as covariate in MANOVA). Results indicated that binding did not differ between groups (the effect of smoking was non-significant).
Boileau 2012 [69]	16 (14 males, 2 females) methamphetamine dependent users of varying severity (at least 2 weeks abstinent) vs. 16 (12 males, 4 females) healthy controls	7/16 methamphetamine users were smokers vs. 1/16 healthy controls.	Cigarette smoking status was included as a covariate in ANOVA of main outcome (tracer binding). Cigarette smoking did not change main outcome of higher tracer regional binding in methamphetamine group vs. controls.
Payer 2014 [70]	15 (13 males, 2 females) cocaine dependent individuals (abstinent for a minimum of 10 days; 50 days abstinent on average) vs. 15 (13 males, 2 females) healthy controls	6/15 cocaine dependent individuals were smokers vs. 5/15 healthy controls.	Groups were matched for number of smokers. No specific analyses reported. Notably, smokers were asked to smoke till satiation up until scan to avoid nicotine withdrawal.
Matuskey 2014 [71]	10 (8 males, 2 females) cocaine dependent individuals (acutely abstinent; 7 days average of abstinence) vs. 10 (8 males, 2 females) healthy controls	11 cigarettes a day (on average) for cocaine dependent individuals vs. 0 cigarettes a day for healthy controls.	Relationship between smoking status and regional tracer binding in dependent group was explored. Results indicated that nicotine use did not correlate with tracer binding in regions of interest.
		Opiates	
Reference	Study sample	Smoking co-occurrence in sample	Details of controlling for co-occurrence in main study outcome
Daglish 2008 [72]	14 opioid dependent males maintained on methadone	All participants were noted to be current smokers.	Indicated that smoking status was not controlled for.
Watson 2014 [73]	10 opioid dependent males maintained on methadone or buprenorphine	All participants were noted to be current smokers.	Not reported.
Martinez 2012 [74]	16 heroin dependent individuals (14 men and 2 women) who underwent acute detoxification vs. 16 healthy controls	13/16 heroin dependent individuals were smokers (average of 11 cigarettes per day) vs. 14/16 healthy controls (average of 11 cigarettes per day). Groups did not significantly differ in smoking status and pattern of smoking.	Not reported.
Shi 2007 [75]	11 opioid dependent individuals (10 men and 1 women) in prolonged abstinence (6 months) vs. 10 opioid dependent individuals (7 men and 3 women) on methadone maintenance vs. 20 healthy controls (5 men and 5 women)	Not reported.	Indicated that smoking status was not controlled for.
		Cannabis	
Reference	Study sample	Smoking co-occurrence in sample	Details of controlling for co-occurrence in main study outcome
Stokes 2009 [76]	13 healthy controls (7 men and 5 women) with some previous experience using cannabis	Not reported.	Not reported.
Bossong 2009 [77]	7 healthy male controls with some previous experience using cannabis	Not reported.	Not reported.

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
Bossong 2015 [78]	Comprised of 14 healthy men and 5 healthy women from Stokes 2009 and Bossong 2009.	Not reported.	Not reported.
Kuepper 2013 [79]	8 individuals with psychotic disorder and experience smoking cannabis (Group A) vs. 7 unrelated relatives of Group A with no psychotic disorder and experience smoking cannabis (Group B) vs. 9 cannabis users with no psychotic disorder (Group C)	6/8 individuals in Group A were smokers vs. 6/7 individuals in Group B vs. 5/9 individuals in Group C. Groups did not differ in overall nicotine use (cigarettes per day).	Nicotine use was treated as covariate in ANOVA of group differences in tracer binding. Smokers were asked to abstain from nicotine for 4 hours prior to scan.
Albrecht 2013 [80]	10 male chronic cannabis users vs. 8 male healthy controls	2/8 healthy controls were smokers vs. 5/10 chronic cannabis users.	Text indicates that groups were matched for smoking status.
Sevy 2008 [81]	6 male cannabis dependent users in early full remission (15 weeks since last use) vs. 6 male healthy controls	5/6 cannabis dependent individuals were smokers vs. 1/6 healthy controls. There were a significantly greater proportion of smokers in the dependent group. However, smokers in study indicated were not dependent as classified by DSM-IV.	Smokers asked to refrain from smoking for at least 10 h before and during PET scan procedures. Not separate analysis otherwise reported.
Stokes 2012 [82]	10 individuals (6 men and 4 women) with a history of cannabis use (at least 50 times in lifetime) vs. 10 controls (9 men and 1 woman) with no history of cannabis use (less than 5 times lifetime use)	3/10 individuals with history of cannabis use were smokers vs. 2/10 healthy controls. Groups did not statistically differ in smoking status.	Smokers asked to refrain from smoking for at least 3 h before scan. Group effect of nicotine status on D2/D3 tracer binding explored. Moreover, correlation of nicotine status and tracer binding explored. Results indicated that nicotine status did not correlate with tracer binding values in any region of interest. However, limbic striatal tracer binding was significantly lower in the 5 smokers vs. 15 non-smokers.
Urban 2012 [83]	16 individuals (15 men and 1 woman) with either cannabis dependence (n=15) or cannabis abuse (n=1) vs. 16 healthy controls (14 men and 2 women)	2/16 cannabis using individuals were smokers vs. 1/16 healthy controls. Smokers in dependent group indicated having stopped smoking within 1 month of scanning procedures, and were not nicotine dependent at time of scanning.	Smoker in control group was recruited to match the 2 smokers in the dependence group.
Volkow 2014 [84]	24 marijuana users diagnosed with abuse or dependence (12 men and 12 women) vs. 24 healthy controls (12 men and 12 women)	10/24 marijuana users were smokers vs. 2/24 healthy controls. There were a significantly greater number of smokers in the marijuana using group.	Smokers asked to refrain from smoking for at least 2 h before study. Carbon monoxide (CO) levels used as a covariate in main ANCOVA. Moreover, correlation of tobacco craving and change in tracer binding following methylphenidate (MP) challenge explored. Text in manuscript indicates that there were no differences in MP-induced change in binding between marijuana users who smoked tobacco and those who did not. However, MP-induced change in binding was shown to be related to tobacco craving, such that greater decreases in binding was related to greater craving.
Bloomfield 2014 [85]	19 cannabis users (17 men and 2 women) vs. 19 healthy controls (17 men and 2 women)	15/19 cannabis users were smokers vs. 8/9 smokers in healthy control group. There were a significantly greater proportion of smokers in the cannabis using group.	Smokers asked to refrain from smoking for at least 2 h before scan. Level of tobacco smoking entered as covariate in main ANCOVA. Moreover, correlation of level of tobacco smoking with dopamine synthesis capacity explored. Results

Alcohol			
Reference	**Study Sample**	**Smoking Co-occurrence in Sample**	**Details of controlling for co-occurrence**
			indicated that across the whole sample, tobacco smokers did not differ from non-smokers in synthesis capacity. Within the whole sample, striatal synthesis capacity did not correlate with amount of cigarette smoking in tobacco users.
Leroy 2012 [33]	11 healthy non-smoking men vs. 14 tobacco-dependent men (TS) vs. 13 cannabis and tobacco smoking men (CTS)	This study investigated cannabis and tobacco co-occurrence specifically. While all participants in the TS and CTS group were smokers, the CTS group had significantly lower FTND scores and daily tobacco consumption than TS group.	To avoid nicotine craving during the PET scan, tobacco smoking was allowed whenever smokers had the urge to do so. All smokers in the tobacco only group and a4 smokers in the cannabis and tobacco group smoked a tobacco cigarette just prior to the PET scan. Relationship between FTND and daily cigarette consumption patterns, and tracer binding were explored. Results indicated that main outcome of tracer binding did not correlate with cigarette consumption or FTND scores.