Decoding the Structure of Abuse Potential for New Psychoactive Substances: Structure–Activity Relationships for Abuse-Related Effects of 4-Substituted Methcathinone Analogs

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Abstract Many cathinone analogs act as substrates or inhibitors at dopamine, norepinephrine, and serotonin transporters (DAT, NET, SERT, respectively). Drug selectivity at DAT vs. SERT is a key determinant of abuse potential for monoamine transporter substrates and inhibitors, such that potency at DAT > SERT is associated with high abuse potential, whereas potency at DAT < SERT is associated with low abuse potential. Quantitative structure–activity relationship (QSAR) studies with a series of 4-substituted methcathinone analogs identified volume of the 4-position substituent on the methcathinone phenyl ring as one structural determinant of both DAT vs. SERT selectivity and abuse-related behavioral effects in an intracranial self-stimulation procedure in rats. Subsequent modeling studies implicated specific amino acids in DAT and SERT that might interact with 4-substituent volume to determine effects produced by this series of cathinone analogs. These studies illustrate use of QSAR analysis to investigate pharmacology of cathinones and function of monoamine transporters.

Keywords Dopamine transporter • Flephedrone • Intracranial self-stimulation • Mephedrone • Methcathinone • Methedrone • Microdialysis • Serotonin transporter • Structure–activity relationship

Contents

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1 Introduction

Synthetic cathinone analogs are new members of an old family of drugs with high abuse potential [1, 2]. Most drugs in this family share a common effectiveness to either traverse or block dopamine transporters (DAT) and ultimately to increase extracellular dopamine (DA) levels in key brain reward structures such as the nucleus accumbens. In addition to their effects on the DAT, many drugs in this family also act on two related transporter proteins, the norepinephrine transporter (NET) and serotonin transporter (SERT), to modulate extracellular levels of their respective monoamine neurotransmitters norepinephrine (NE) and serotonin (5HT). A growing body of evidence supports the general hypothesis that abuse potential of drugs in this family is determined by their relative selectivity to act at DAT vs. SERT. As a prelude to discussing the relationship between structure and abuse potential of novel methcathinone analogs, this chapter will begin by reviewing evidence that implicates DAT/SERT selectivity as a determinant of abuse potential. This evidence provides a framework for interpreting effects of new psychoactive substances.

2 Amphetamine, MDMA, and Fenfluramine as Prototype Monoamine Releasers

2.1 Neurochemical Effects

The drugs amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and fenfluramine illustrate the range of effects that can be produced by drugs with different profiles of selectivity for DAT vs. SERT. All three drugs can traverse monoamine transporters and trigger a series of intracellular events that promote monoamine neurotransmitter release [3–6]. As a group, these drugs are sometimes called "transporter substrates," because like the endogenous neurotransmitters, they can pass from the extracellular space through the transporter channel to the intracellular space. They are also often called "monoamine releasers," because one consequence of their transport is the release of monoamine neurotransmitter stored in synaptic terminals. Although these drugs share a similar general mechanism of action as transporter substrates and monoamine releasers, they differ in their

Table 1 EC_{50} values (nM ± SD) for (+)amphetamine, (+)MDMA, and (±)fenfluramine to promote monoamine release from rat brain synaptosomes

Drug	EC_{50} values		DAT vs. SERT Selectivity[a]
	DA release	5HT release	
(+)Amphetamine[b]	25 ± 4	1765 ± 94	71
(+)MDMA[c]	142 ± 4	74 ± 3	0.52
(±)Fenfluramine[b]	>10,000	79 ± 12	<0.01

[a]Selectivity calculated as SERT EC_{50}/DAT EC_{50}
[b]Rothman et al. [7]
[c]Setola et al. [8]

relative potencies at DAT and SERT. For example, Table 1 shows the relative in vitro potency of each drug to promote monoamine release via DAT or SERT from rat brain synaptosomes loaded with radiolabeled monoamine [7, 8]. By this metric, (+)amphetamine is DAT selective, (±)fenfluramine is SERT selective, and (+)MDMA displays similar potencies to act at both transporters. (Note: The potency of each compound is slightly greater to act at NET than DAT, but effects at NET are not addressed further here because other evidence suggests a minimal role for NE in abuse potential.) These in vitro neurochemical effects mirror effects of these drugs on brain neurochemistry in vivo. For example, Fig. 1 shows the effects of behaviorally active doses of (+)amphetamine and (±)fenfluramine on extracellular DA and 5HT levels measured in nucleus accumbens of rats using in vivo microdialysis [9]. (+)Amphetamine selectively increases DA levels, whereas (±)fenfluramine selectively increases 5HT levels. By contrast, MDMA increases both DA and 5HT levels in rat nucleus accumbens as assessed by in vivo microdialysis ([10]; Lazenka MF, Suyama JA, Banks ML, Negus SS, unpublished results).

2.2 Abuse-Related Behavioral Effects

These in vitro and in vivo neurochemical effects of amphetamine, MDMA, and fenfluramine also correspond to expression of abuse-related behavioral effects by these drugs. Drug self-administration procedures are the most widely used preclinical procedures to assess abuse potential [11–13], and in these procedures, laboratory animals emit an operant response (e.g., pressing a lever) to receive a dose of drug (e.g., by intravenous infusion). Thus, animals in drug self-administration procedures engage in drug-taking behaviors that are analogous to the drug-taking behaviors displayed by human drug abusers. A drug is considered to produce "reinforcing effects" and to function as a "reinforcer" in a drug self-administration procedure if subjects respond at higher rates for delivery of some dose of drug than they respond for delivery of vehicle, and drugs that function as reinforcers in animals often function as drugs of abuse in humans. Evidence from drug self-administration procedures indicates that amphetamine produces stronger

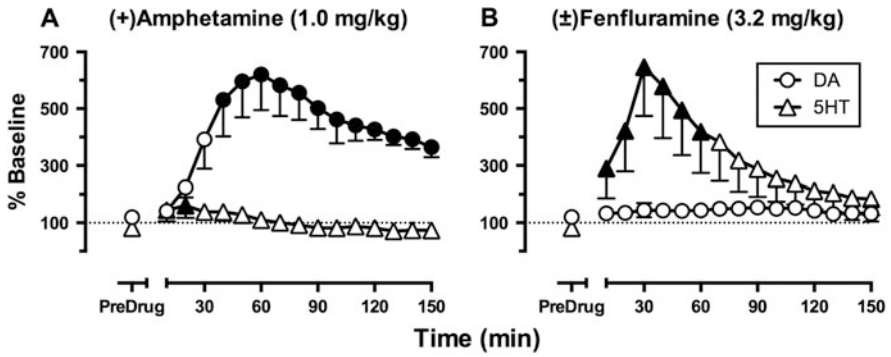

Fig. 1 (+)Amphetamine selectively increases DA > 5HT levels (**a**), and (±)fenfluramine significantly increases 5HT > DA levels (**b**), in rat nucleus accumbens as measured by in vivo microdialysis. *Abscissae*: Time relative to IP drug administration in min. *Ordinates*: Percent baseline levels of DA and 5HT. Points show mean ± SEM for 5–7 rats, and filled points show a significant difference from the "PreDrug" point ($p < 0.05$). Adapted from Suyama et al. [9]

reinforcing effects than MDMA, and fenfluramine does not produce reinforcing effects [14–16].

A related preclinical procedure, known as intracranial self-stimulation (ICSS), will be referenced extensively in this chapter [17]. As in drug self-administration, laboratory animals in ICSS procedures emit an operant response to receive a reinforcer; however, in ICSS, the reinforcer is not drug delivery, but instead is the delivery of electrical stimulation to a brain reward area via a surgically implanted microelectrode. In one common type of the ICSS procedure, the amount of electrical brain stimulation is varied during each behavioral session by manipulating the frequency of electrical pulses, and increasing frequencies of brain stimulation maintain increasing rates of ICSS responding. Figure 2a shows a photograph of a rat in an ICSS procedure, and Fig. 2b shows the sigmoidal plot that relates brain stimulation frequency to ICSS rate. Thus, low frequencies of brain stimulation maintain low rates of ICSS, whereas higher frequencies maintain high rates of ICSS. Once subjects are trained in this procedure, drugs can be administered before daily behavioral sessions, and abuse potential can be inferred from the profile of drug effects on the ICSS frequency-rate curve. For example, Fig. 3 shows the effects of (+)amphetamine, (+)MDMA, and (±)fenfluramine on ICSS in rats [18]. (+)Amphetamine produces leftward and upward shifts in the ICSS frequency-rate curve (Fig. 3a) and a dose-dependent increase in the total number of stimulations delivered across all brain stimulation frequencies (Fig. 3b). This drug-induced increase in responding is described as "facilitation of ICSS," and drugs that facilitate ICSS also usually function as reinforcers in preclinical drug self-administration procedures and display high abuse liability in humans. Accordingly, facilitation of ICSS can be viewed as a behavioral index of a drug's abuse potential. In contrast to (+)amphetamine, (±)fenfluramine produces only dose-dependent decreases in ICSS (Fig. 3e, f), and drugs that only depress ICSS usually fail to

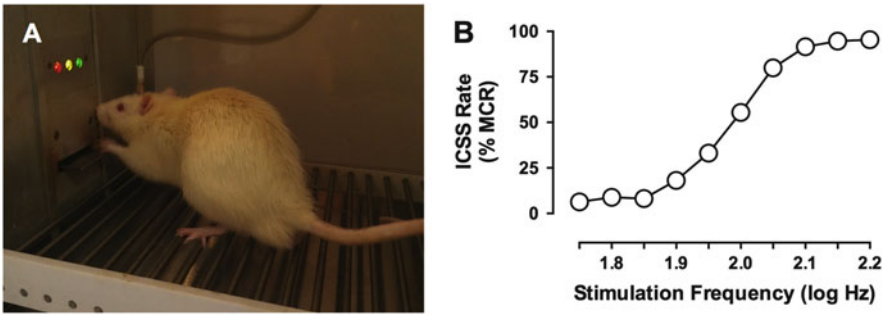

Fig. 2 Photograph of a rat engaged in intracranial self-stimulation (ICSS) (**a**), and example of a baseline frequency-rate curve from the ICSS procedure (**b**). In this ICSS procedure, responding on a lever results in the delivery of electrical brain stimulation delivered via a microelectrode surgically implanted into a brain reward area. In (**a**), a cable connects the electrode mounted on the subject's skull to a stimulator located outside the picture. In (**b**), the abscissa shows the frequency in log Hz of the electrical pulses delivered during each stimulation delivery, and the ordinate shows the ICSS rate expressed as percent maximum control rate (%MCR), which normalizes ICSS rate measurements within each subject. Low frequencies of brain stimulation maintain low ICSS rates, whereas high ICSS rates maintain high ICSS rates. Adapted from Negus and Miller [17]

function as reinforcers in preclinical drug self-administration procedures and lack abuse liability in humans. Lastly, (+)MDMA produces a mixed profile of effects that includes both facilitation of low ICSS rates maintained by low brain stimulation frequencies and depression of high ICSS rates maintained by high brain stimulation frequencies (Fig. 3c). As a result of this mixed-effect profile, MDMA produces a lower maximal stimulation of total ICSS than amphetamine (Fig. 3d). Drugs that produce this mixed profile of ICSS facilitation and depression often function as relatively weak or unreliable reinforcers in preclinical drug self-administration procedures and display relatively modest abuse liability in humans.

2.3 Correlation Between Neurochemical and Behavioral Effects

Figure 4a shows a correlation between maximal ICSS facilitation (defined as the maximum increase in total ICSS as in Fig. 3d–f) and DAT vs. SERT selectivity (defined as shown in Table 1) for (+)amphetamine, (+)MDMA, (±)fenfluramine, and 7 other monoamine releasers [18]. Figure 4b shows a correlation between maximal ICSS facilitation in rats and maximal reinforcing effects in a nonhuman primate drug self-administration procedure for most of these same drugs [18]. These significant correlations provide one source of evidence to suggest that ICSS can be useful both (1) as a behavioral correlate to neurochemical drug effects and (2) as a complement to drug self-administration procedures for preclinical

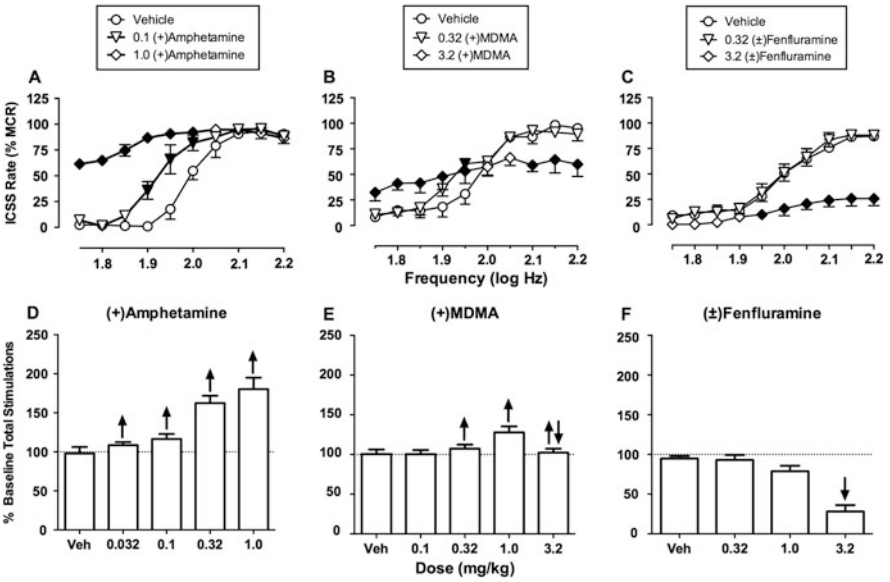

Fig. 3 (+)Amphetamine, (+)MDMA, and (±)fenfluramine produce qualitatively different effects on ICSS in rats. Top panels **a**–**c** show effects of selected drug doses on full frequency-rate curves. *Abscissae*: brain stimulation frequency in log Hz. *Ordinates*: ICSS rate expressed as %MCR. Filled points indicated a significant difference from "Vehicle" ($p < 0.05$). Bottom panels **e** and **f** show a summary measure of total ICSS across all 10 frequencies of brain stimulation. *Abscissae*: Drug dose in mg/kg. *Ordinates*: Total ICSS expressed as a percentage of the baseline number of total stimulations delivered in the absence of any treatment. *Upward/downward arrows* indicate a significant increase/decrease in ICSS for at least one brain stimulation frequency in the full frequency-rate curves as shown in Panels **a**–**c**. The maximum increase in total ICSS produced by any drug dose was used for correlations shown in Fig. 4. Adapted from Bauer et al. [18]

assessment of the abuse potential of monoamine releasers. Moreover, these results also provide evidence to suggest that drug selectivity to act at DAT vs. SERT is a significant determinant of abuse-related behavioral effects for monoamine releasers. Of course, one ultimate goal of these preclinical neurochemical and behavioral studies is to predict abuse potential of novel drugs in humans. The risk of abuse by humans is a difficult endpoint to quantify, in part because definitions of abuse include not only the extent of drug use, but also the degree of harm caused by that use [17, 19]. However, with these caveats in mind, abuse liability is generally considered highest for amphetamine and lower for MDMA, and fenfluramine is considered to have little or no abuse liability.

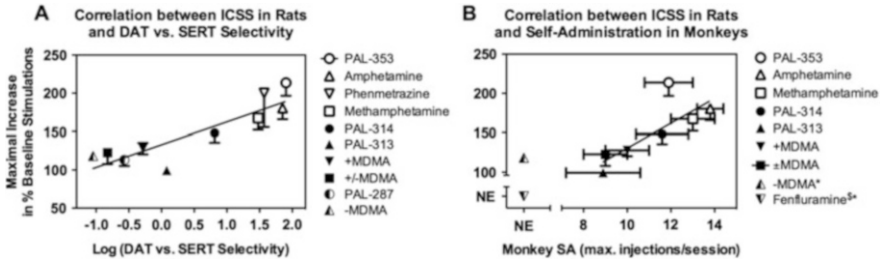

Fig. 4 Drug-induced facilitation of ICSS in rats correlates with both (**a**) DAT vs. SERT selectivity as determined from in vitro studies of monoamine release in rat brain synaptosomes as shown in Table 1 ($r = 0.89$, $p < 0.001$), or (**b**) maximum self-administration produced by any dose of each drug in a progressive-ratio assay of drug self-administration in rhesus monkeys ($r = 0.80$, $p = 0.032$). Error bars show SEM. Adapted from Bauer et al. [18]

3 Quantitative Structure–Activity Relationships for Para-Substituted Methcathinone Analogs

The results summarized above suggest a strong relationship for monoamine releasers between:

(1) in vitro neurochemical effects determined by measures of selectivity to promote monoamine release via DAT vs. SERT in rat brain synaptosomes,
(2) in vivo neurochemical effects determined by microdialysis measures of selectivity to release DA vs. 5HT in nucleus accumbens, and
(3) abuse-related behavioral effects in an ICSS procedure

These results also provide a framework for assessment of new psychoactive substances, and as one example, we conducted quantitative structure–activity relationship (QSAR) analysis for a series of seven racemic methcathinone analogs with different substitutions at the *para* (or 4-) position on the phenyl ring (Fig. 5) [9, 20, 21]. For the purposes of these studies, drugs were named using the convention "4-R MCAT," and the series included the parent compound methcathinone (MCAT) as well as the recently scheduled analogs flephedrone (4-F MCAT) and mephedrone (4-OCH_3 MCAT) and the other halogenated analogs brephedrone (4-Br-MCAT) and clephedrone (4-Cl-MCAT). Substituents were selected with respect to the three structural attributes as shown in Table 2: (1) steric bulk of the substituent in three-dimensional space, quantified here by volume (Vol); (2) electron-withdrawing capacity of the substituent (σ_p); and (3) lipophilicity of the substituent (π_p). A goal of the study was to evaluate the correlation between the structural attributes of these substituents and the functional effects of the associated drugs (also shown in Table 2) to produce neurochemical effects in in vitro and in vivo assays of monoamine release and abuse-related behavioral effects in the ICSS procedure.

Figure 6 shows the results of these QSAR analyses. There were two main findings. First, as discussed above, there were significant positive correlations

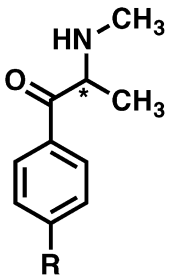

Fig. 5 Structure of 4-R methcathinone analogs used for QSAR analysis. Seven compounds were synthesized and evaluated with different 4-R substituents to vary structural parameters as shown in Table 2. *Asterisk* indicates position of the chiral carbon

Table 2 Structural and functional attributes of 4-substituted methcathinone (4-R MCAT) analogs used in quantitative structure–activity response (QSAR) analysis

		Structural attributes[a]			Neurochemical selectivity[b]		Behavior[c]
Drug	R	Vol	σ_p	π_p	In vitro	In vivo	Maximal ICSS
MCAT (methcathinone)	-H	150.36	0	0	309	12.56	191.9
4-F MCAT (flephedrone)	-F	153.78	0.06	0.14	15.4	1.24	156.3
4-Cl MCAT (clephedrone)	-Cl	164.43	0.23	0.71	3.40	1.23	114.9
4-CH_3 MCAT (mephedrone)	-CH_3	166.89	−0.17	0.56	2.41	0.62	102.5
4-Br MCAT (brephedrone)	-Br	169.43	0.23	0.86	1.01	0.89	118
4-OCH_3 MCAT (methedrone)	-OCH_3	175.01	−0.27	−0.02	0.24	0.32	110.9
4-CF_3 MCAT	-CF_3	178.40	0.54	0.88	0.07	Not determined	90.9

Drugs are listed in order of increasing volume of the 4-substituent

[a]Reported in Bonano et al. [20]; Sakloth et al. [21]

[b]In vitro selectivity calculated as effective concentration to produce a 50% increase (EC_{50}) in monoamine release via SERT ÷ EC_{50} to increase monoamine release via DAT from rat brain synaptosomes [20]. In vivo selectivity calculated as effective dose to produce a 250% increase (ED_{250}) to increase 5HT levels ÷ ED_{250} to increase DA levels in rat nucleus accumbens as assessed by in vivo microdialysis [9]

[c]Maximal facilitation of ICSS as determined in a behavioral assay of ICSS [20]

for all functional measures (Fig. 6d). Specifically, the in vitro and in vivo measures of drug selectivity to promote monoamine release via DAT vs. SERT correlated with each other and with the measure of abuse-related behavioral effects in the ICSS procedure. These correlations support the propositions that (a) in vitro measures of neurochemical selectivity at DAT vs. SERT in rat brain synaptosomes are

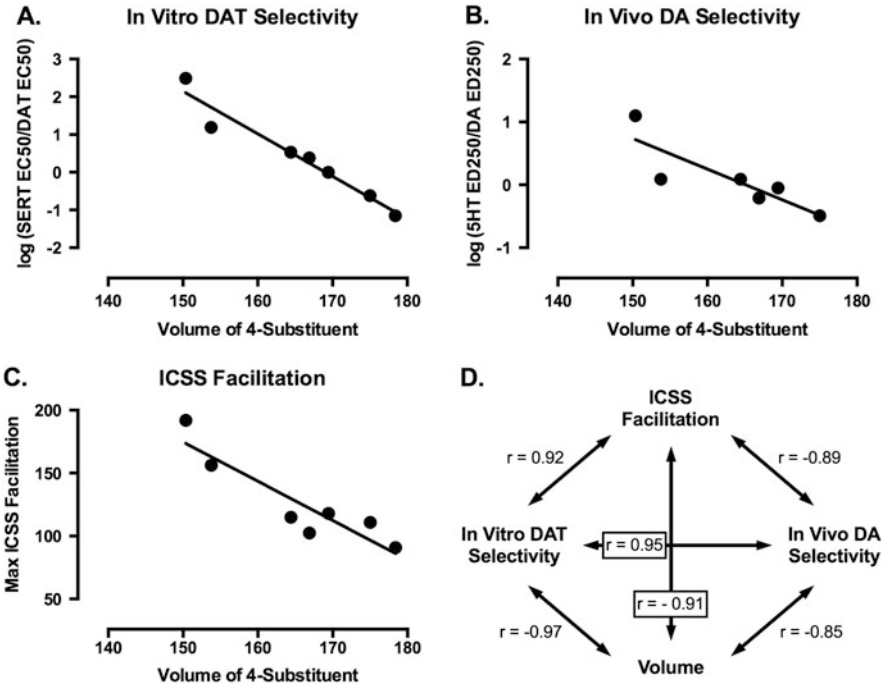

Fig. 6 Correlations between volume of the 4-substituent and (**a**) in vitro selectivity to promote monoamine release via DAT vs. SERT in rat brain synaptosomes, (**b**) in vivo selectivity to increase extracellular DA vs. 5HT levels in rat nucleus accumbens, and (**c**) in vivo effectiveness to produce abuse-related facilitation of ICSS. (**d**) Matrix of correlations between 4-substituent volume and each of the three functional endpoints. Volume correlated negatively with all functional measures, and all functional measures correlated positively with each other. All correlations were significant ($p < 0.05$)

predictive of in vivo neurochemical selectivity to promote DA vs. 5HT release, and (b) these measures of neurochemical selectivity are predictive of abuse-related behavioral effects. It is also important to note that drug effects on ICSS did not correlate reliably with potency of drugs to act at DAT alone in vitro or to release DA alone in vivo (data not shown). This indicates that expression of abuse-related behavioral effects results from an integration of DAT- and SERT-mediated effects, and it provides a rationale for QSAR studies that consider structural determinants of drugs at both transporters rather than at DAT alone.

The second main finding of the QSAR studies was that each of the three functional measures (in vitro DAT selectivity, in vivo DA selectivity, and ICSS effects) correlated negatively with volume of the 4-position substituent (Fig. 6), but none of the functional measures correlated with either the electronic or lipophilic attributes of the 4-substituent (data not shown). These results suggest that steric bulk of the 4-substituent plays a more important role than either electronic or lipophilic attributes in governing each drug's interaction with DAT and SERT.

More specifically, larger 4-substituent volumes were associated with declining DAT potencies but increasing SERT potencies, suggesting that DAT has limited tolerance for bulk at the 4-position, whereas SERT prefers larger substituents at this location, yielding a net loss in DAT vs. SERT selectivity as 4-substituent volume increases. On the basis of these observations, molecular modeling was conducted with homology models of human DAT and SERT (hDAT and hSERT, respectively) based on the *Drosophila melanogaster* DAT (dDAT) to identify the characteristics of substrate-binding pockets that might account for the differential selectivities of 4-R MCAT analogs at DAT and SERT. These results suggested two determinants of 4-R MCAT selectivity. First, docking studies indicated that hDAT contains a relatively large serine residue (S149) in the substrate-binding pocket at the site that interacts with the 4-substituent of MCAT analogs, whereas hSERT contains a smaller alanine residue (A169) at the homologous location. The larger S149 amino acid in hDAT limits the volume of the 4-substituent that can be accommodated, resulting in a preference by hDAT for 4-R MCAT analogs with small 4-substituents (e.g., 4-H for MCAT itself). Conversely, the smaller A169 amino acid in hSERT allows more space in the substrate-binding pocket for larger 4-substituents. Although the A169 amino acid in the docking pocket renders hSERT more tolerant than DAT of larger 4-substituents, it did not explain why hSERT displays a preference for larger 4-substituents. To address this issue, Hydropathic INTeraction (HINT) analysis was conducted, and this suggested a second determinant of 4-R MCAT selectivity. Specifically, HINT analysis indicated that the substrate-binding pocket of hSERT displayed a preference for relatively larger 4-substituents due in part to hydrophobic interactions between transporter and substrate. Overall, these studies indicated that hDAT prefers smaller 4-substituents, whereas SERT prefers larger 4-substituents. Figure 7 shows a simplified diagram to summarize these conclusions and their implications for abuse potential.

4 Stereoselective Effects of Methcathinone and Mephedrone

The QSAR studies summarized above were conducted with racemic compounds, but more recent studies have identified an additional role for stereoselectivity as a determinant both of 4-R MCAT interactions with transporters and of ultimate expression of abuse-related effects [22, 23]. Specifically, methcathinone, methamphetamine, and many of their analogs possess a single chiral carbon atom (the α carbon signified by the asterisk in Fig. 5), and the *S* enantiomer of these compounds is typically more potent and/or effective than the *R* enantiomer to promote DA release via DAT [7, 24] or to produce abuse-related behavioral effects in assays of drug self-administration, drug discrimination, or ICSS [18, 25–27]. However, recent studies suggest a potentially more nuanced role for stereochemistry in

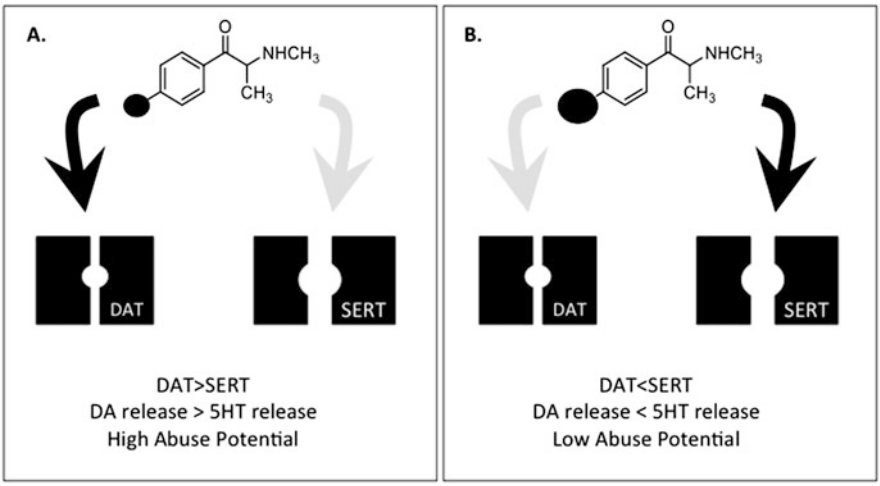

Fig. 7 QSAR and modeling studies suggest that DAT prefers small 4-substituents of 4-R MCAT analogs, whereas SERT prefers larger 4-substituents. (**a**) As a result of these structural differences in the transporters, 4-R MCAT analogs with small 4-substituents (e.g., MCAT) are more potent as substrates at DAT than SERT, leading to preferential DA release and strong abuse-related behavioral effects in vivo. (**b**) Conversely, 4-R MCAT analogs with larger 4-substituents are more potent as substrates for SERT than DAT, leading to preferential 5HT release and weak abuse-related behavioral effects in vivo

abuse-related effects of mephedrone (4-CH_3 MCAT) [22]. Specifically, the *R*(+) enantiomer of mephedrone is more effective than the *S*(−) enantiomer to produce locomotor activation, conditioned place preference, and facilitation of ICSS in rats [22]. Neurochemical evidence suggested that this apparent inversion of stereochemistry results from an unusual stereoselectivity not only in potency, but also in selectivity as a substrate at DAT vs. SERT. Thus, *R*(+)mephedrone was slightly more potent than its *S*(−) enantiomer to promote monoamine release via DAT but much less potent at SERT. As a result, the *R*(+) enantiomer displays a 50-fold greater selectivity than the *S*(−) enantiomer to promote monoamine release via DAT vs. SERT, and this stereoselectivity in neurochemical effects contributed to stereoselectivity in expression of abuse-related behavioral effects. It is unknown whether this stereoselectivity would also be apparent for other 4-R MCAT analogs, but a similar impact of stereochemistry was observed for isomers of 4-CH_3 cathinone [23]. Importantly, these results suggest that stereoselectivity at the chiral carbon at one end of the 4-R MCAT molecule can influence interactions of the 4-substituent at the other end of the molecule with its own portion of the DAT and SERT substrate-binding pockets.

5 Conclusions

Preclinical research with a wide range of monoamine transporter substrates has demonstrated that DAT > SERT selectivity is a strong determinant of abuse-related drug effects. Studies summarized in this chapter support this general proposition and extend it to a series of synthetic cathinone analogs. Furthermore, QSAR analyses suggest molecular mechanisms at the drug-transporter interface that may govern both neurochemical DAT/SERT selectivity and expression of abuse-related effects for one series of 4-R MCAT analogs. Specifically, these analyses suggest that volume of the 4-substituent functions as significant determinant of drug potency and selectivity, with DAT preferring smaller 4-substituents, whereas SERT prefers larger 4-substituents. These studies illustrate one application of QSAR analysis to investigate structural determinants of abuse-related drug effects.

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