

Pharmacology of “bath salts” and their newly-emerging analogs



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Presentation Outline

- “Bath salts” contain synthetic cathinones
- Monoamine transporters and stimulant drugs
- SAR for “next generation” analogs of MDPV
- SAR for “next generation” analogs of mephedrone
- Summary & conclusions

So-called “bath salts” products contain synthetic cathinones

- “Bath salts” are psychoactive synthetic cathinones sold as non-drug products (e.g., “plant food”, “potpourri”)
- Marketed as “legal high”, “legal meth” or “legal cocaine”
- Labeled-“not for human consumption” as means to skirt regulatory control



Bath salts products are readily available online and in retail shops

- Sold on Internet websites
- Sold in gas stations, head shops, etc.
- Easier to obtain than cigarettes or beer
- Inexpensive (\$10 to \$25) – sold in ¼ gm, ½ gm and 1 gm product containers
- Anonymous purchase

Bath salts powders are self-administered by various routes

- White, off-white or beige powders
- Usually less than 1 gm packages
- Injected, insufflated (snorted), or ingested



Synthetic cathinones can produce life-threatening medical side-effects

- No clinical data are available from controlled studies
- Human testing is being done by “street” users who purchase products
- Agitation, psychosis, and violent behaviors are side-effects



Bath salts overdose and “excited delirium”

- ExD is a life-threatening syndrome associated with dopaminergic stimulants
- Constellation of symptoms identify ExD
 - Delirium and agitation
 - Sustained hyperthermia
 - Rhabdomyolysis and kidney failure
- Many reports of ExD in patients admitted to EDs under the influence of bath salts

Kesha et al., 2013; Penders et al., 2013

Mephedrone, methylone and MDPV were found in bath salts during 2010-2012 in the U.S.

- Fourteen different bath salt products were purchased and analyzed by GC/MS
- **Mephedrone** (3), **Methylone** (6), **MDPV** (9), caffeine (2) were found
- 3 products had more than one cathinone
- Apparently different recipes - based on simple volunteer reports (more “energy” or more “mellow”)

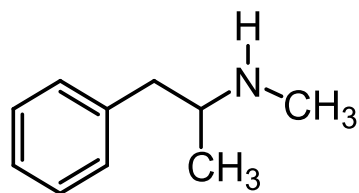
Spiller et al., 2011

MDPV is the main cathinone present in blood and urine from bath salts ED cases in U.S.

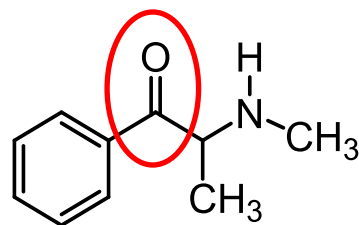
- For 18 ED patients – blood or urine levels of cathinones were assayed (GC/MS)
 - **Only MDPV was detected in biological fluids**
 - *US findings differ from European findings*
- Blood levels of MDPV ranged – 24 ng/ml to 241 ng/ml (mean 58 ng/ml)
- Urine levels of MDPV ranged – 34 ng/ml to 1386 ng/ml (mean 856 ng/ml)

Spiller et al., 2011

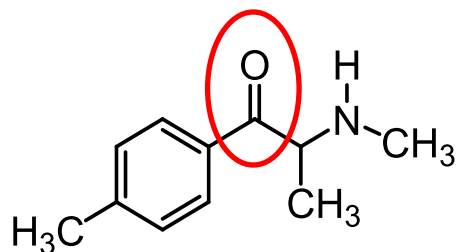
Due to health risks, components of bath salts have been rendered illegal by DEA Scheduling



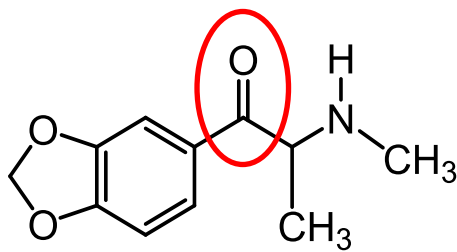
Methamphetamine



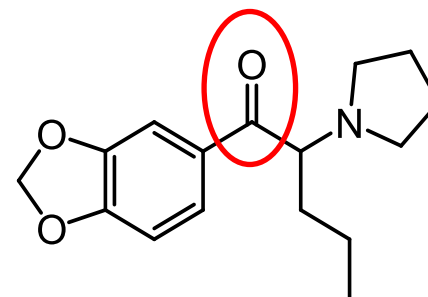
Methcathinone



4-Methylmethcathinone
(Mephedrone)



3,4-Methylenedioxymethcathinone
(Methylone)

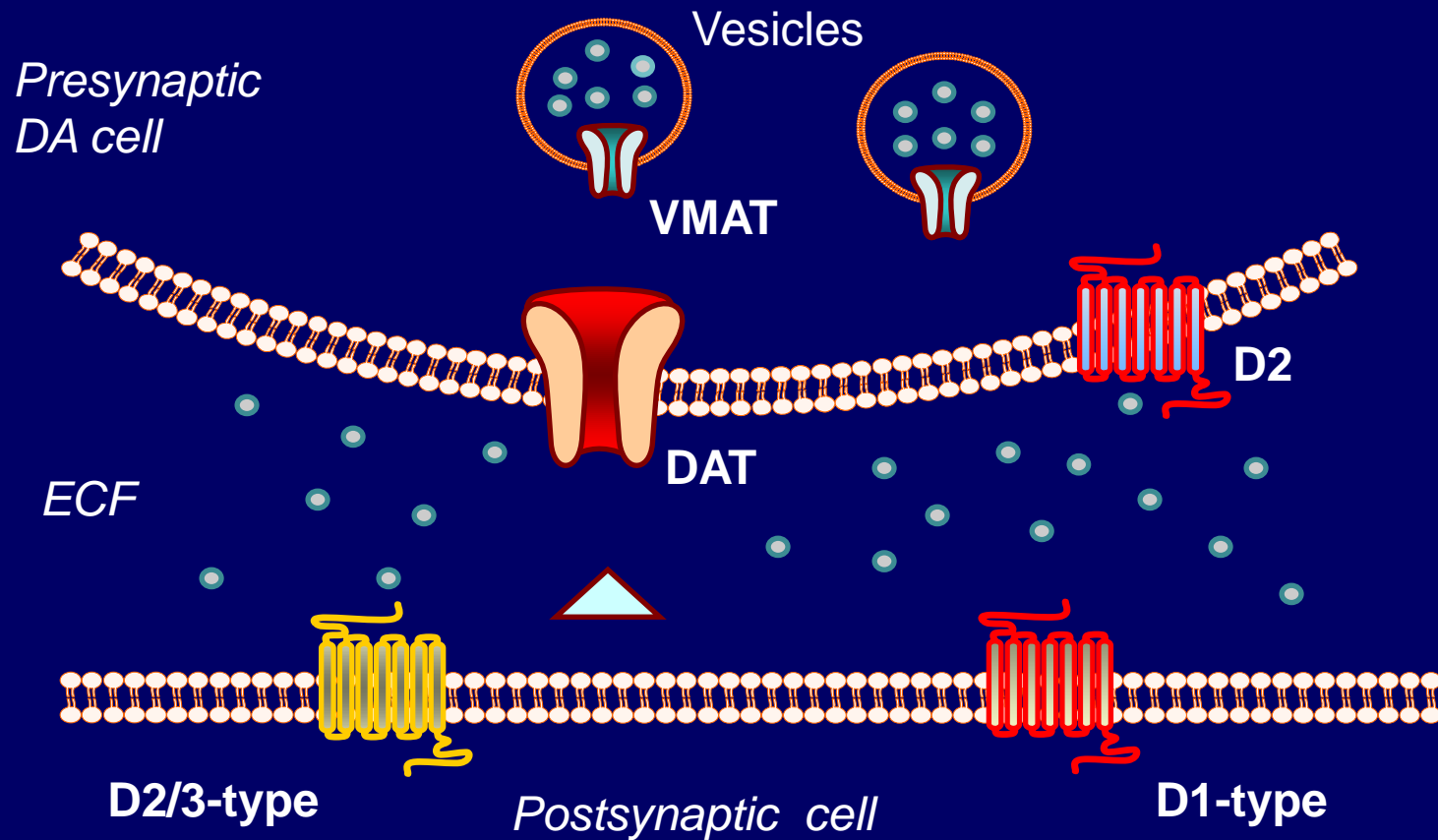


3,4-Methylenedioxypyrovalerone
(MDPV)

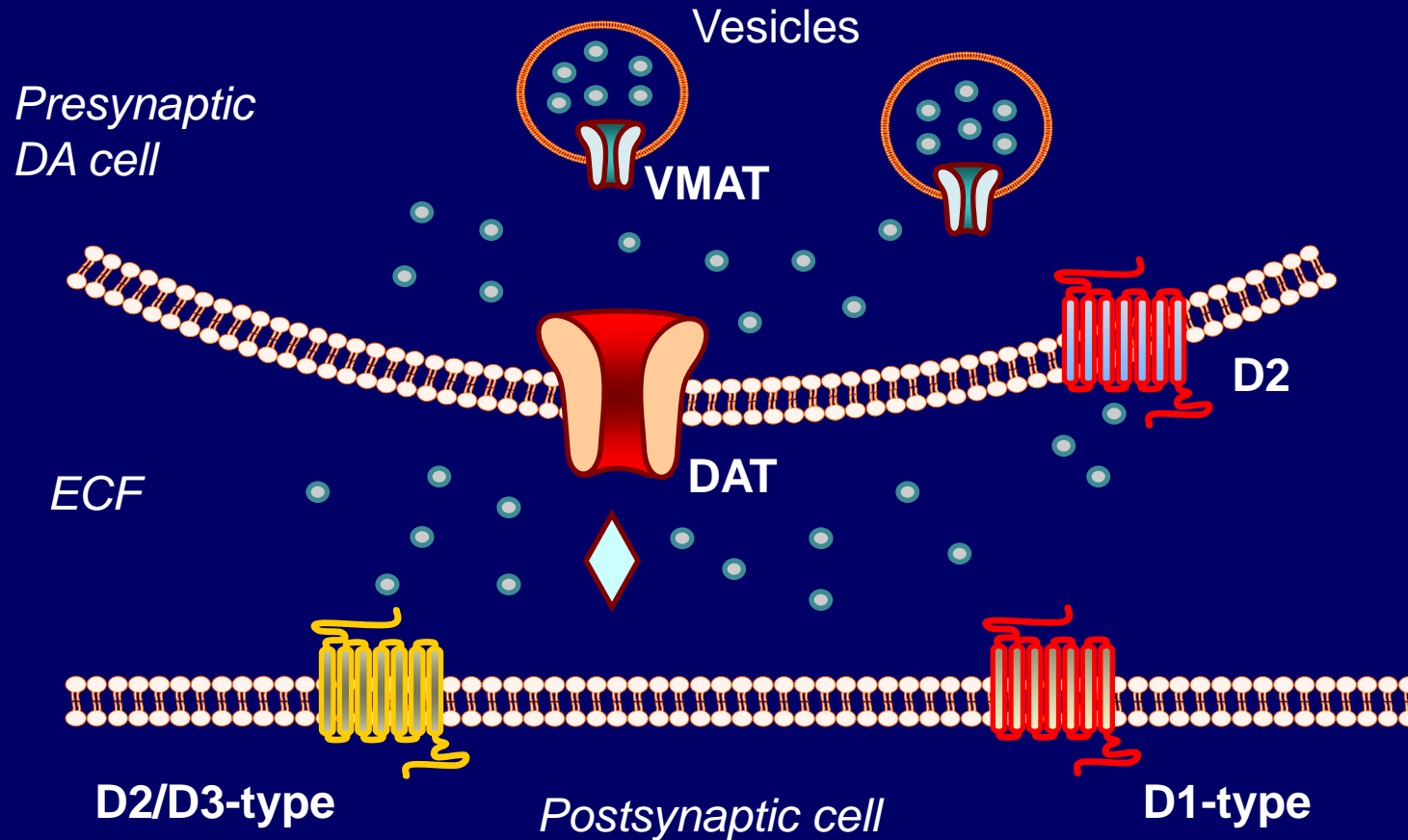
Therapeutic and abused stimulant drugs interact with monoamine transporters

- Monoamine transporters (i.e., NET, DAT & SERT) are membrane proteins that mediate uptake of previously released NE, DA and 5-HT
- Transporter drugs can be divided into two types:
 - Transporter blockers inhibit transmitter reuptake
 - Transporter substrates (i.e., releasers) enter cells and cause non-exocytotic transmitter release
- Transporter blockers and substrates both increase synaptic concentrations of amine transmitters

▲ Cocaine is a DAT blocker that prevents uptake of extracellular DA back into neurons



◆ Amphetamine is a DAT substrate that releases intracellular DA into the ECF



There are important differences between transporter substrates versus blockers

- Substrates, but not blockers, induce transporter-mediated inward Na^+ currents (i.e., depolarization)
- Substrates reverse the direction of transporter flux to cause transmitter efflux (i.e., release)
- Substrates can cause long-term deficits in neuronal function (i.e., neurotoxicity)

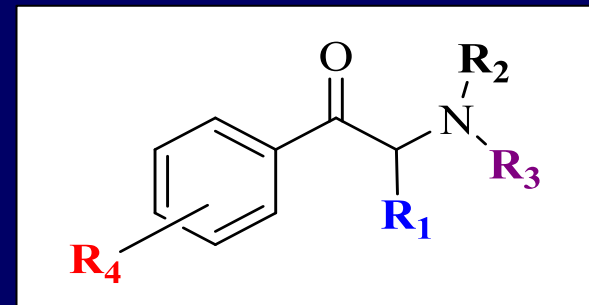
We reported that bath salts cathinones interact with monoamine transporters

- MDPV is a potent DAT/NET-selective transporter **blocker** (i.e., cocaine-like), *Baumann et al., Neuropsychopharmacology* 38:552; 2013
- Mephedrone and methylone are non-selective transporter **substrates** (i.e., amphetamine-like), *Baumann et al., Neuropsychopharmacology* 37:1192; 2012
- There are differences in pharmacology between synthetic cathinones and their amphetamine counterparts

New cathinone analogs have emerged since the scheduling of MDPV, mephedrone & methylone

- MDPV-related cathinones (pyrrolidinophenones)
 - e.g., α -PVP, α -PBP, α -PPP
- Mephedrone-related cathinones (4-methyl cathinones)
 - e.g., 4-MEC, 4-MePPP
- Methylone-related cathinones (3,4-methylenedioxy cathinones)
 - e.g., butylone, pentylone

New “replacement” cathinones are structurally- related to scheduled drugs



Common Name	R1	R2	R3	R4
Methcathinone	CH3	CH3	H	H
Ethcathinone	CH2CH3	CH3	H	H
Mephedrone (4MMC)	CH3	CH3	H	4-CH3
4-MEC	CH3	CH2CH3	H	4-CH3
4-MePPP	CH3	PYRROLIDINYL		4-CH3
Methylone (MDMC)	CH3	CH3	H	3,4-METHYLENEDIOXY
Butylone	CH2CH3	CH3	H	3,4-METHYLENEDIOXY
Pentylone	CH2CH2CH3	CH3	H	3,4-METHYLENEDIOXY
MDPV	CH2CH2CH3	PYRROLIDINYL		3,4-METHYLENEDIOXY
α -PVP	CH2CH2CH3	PYRROLIDINYL		H
α -PBP	CH2CH3	PYRROLIDINYL		H
α -PPP	CH3	PYRROLIDINYL		H

Boos et al., 2013

Newer products make claims that banned cathinones are not present

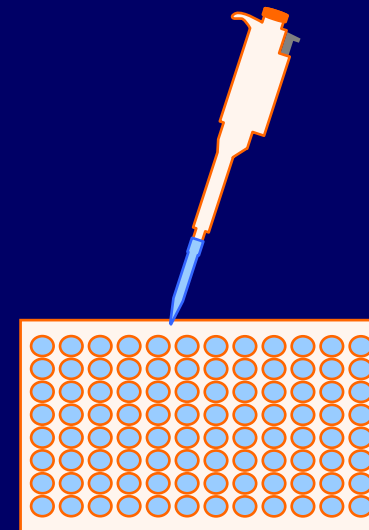


We evaluated actions of new drugs at monoamine transporters based on two key considerations

- **1) Molecular mechanism of action**
 - *Is the drug a transporter blocker or substrate?*
 - Substrates display a number of unique features...
 - Substrates enter cells; evoke inward depolarizing currents; induce reverse transport; release transmitters from a non-vesicular pool; disrupt vesicular storage of transmitters
- **2) Selectivity across transporters**
 - *What is the potency of the drug at DAT/NET versus SERT?*
 - Drugs selective for DAT/NET are self-administered by animals and humans, whereas drugs selective for SERT are not

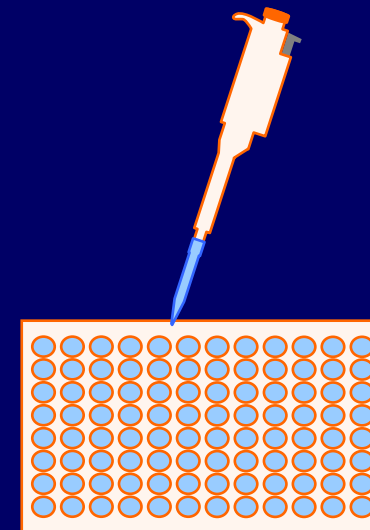
We examined the effects of new cathinone analogs on transporter-mediated uptake

- Synaptosomes are prepared from rat brain tissue (*Rothman et al., 2003*)
- **Uptake of [^3H]DA by DAT and [^3H]5-HT by SERT are measured by standard methods**
- [^3H]Transmitter and test drug are added to synaptosomes simultaneously
- Assays terminated by vacuum filtration, and retained radioactivity is counted



We examined the effects of new cathinone analogs on transporter-mediated release

- Synaptosomes are prepared from rat brain tissue (*Rothman et al., 2003*)
- [^3H]MPP $^+$ and [^3H]5-HT are radiolabeled substrates for DAT and SERT, respectively
- **Synaptosomes are preloaded with [^3H]substrate, then drugs are added to evoke release**
- Assays terminated by vacuum filtration, and retained radioactivity is counted

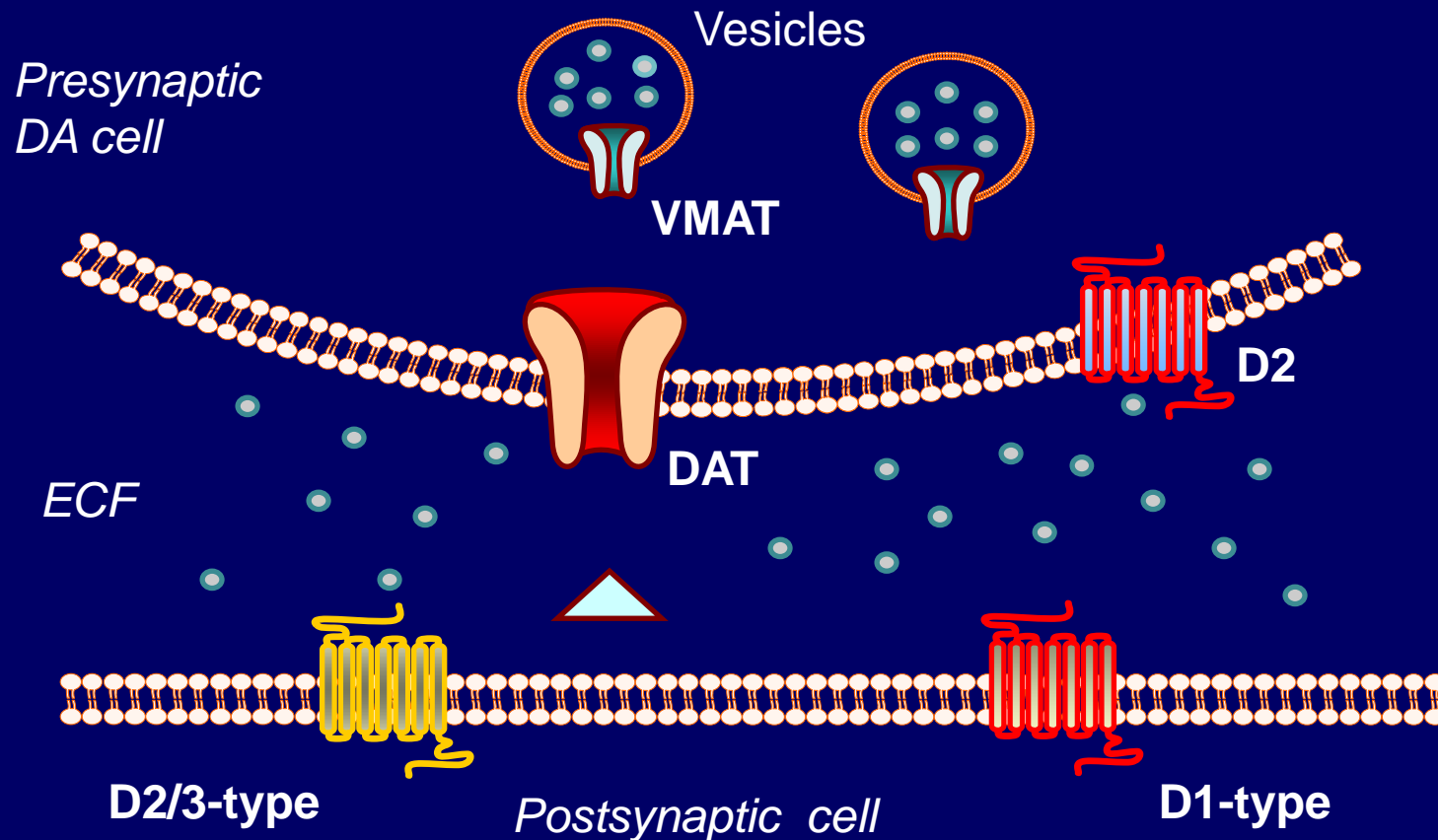


We examined in vivo effects of new cathinone analogs in rodent models

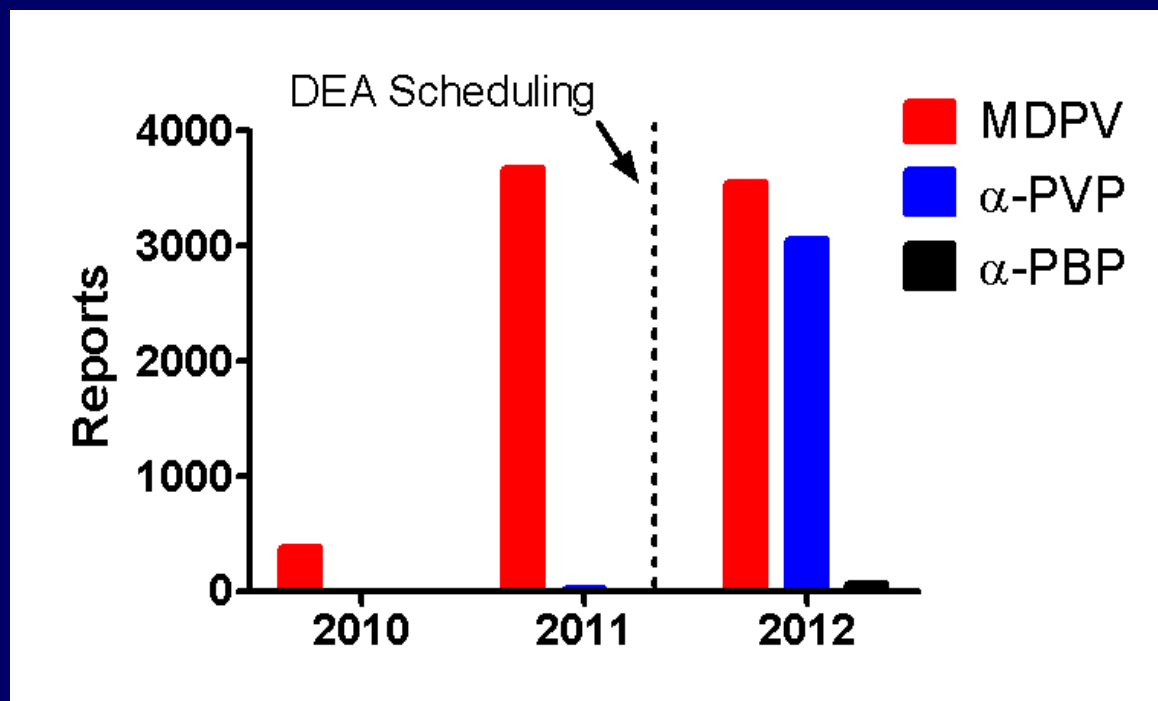
- Effects of MDPV-related analogs were evaluated in **locomotor activity** tests
 - Dose-dependent effects in male mice
 - Antagonist experiments with SCH23390
- Effects of mephedrone-related analogs were examined using **in vivo microdialysis**
 - Rats with probes in the n. accumbens
 - DA and 5-HT quantified by HPLC-ECD



▲ MDPV is a DAT blocker that prevents the uptake of extracellular DA back into neurons

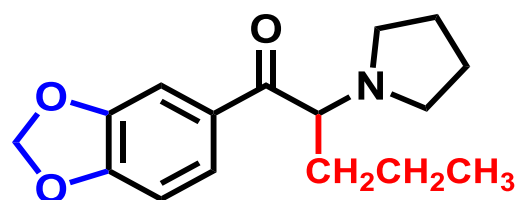


New pyrrolidinophenones (PPs) have appeared in the marketplace since MDPV scheduling

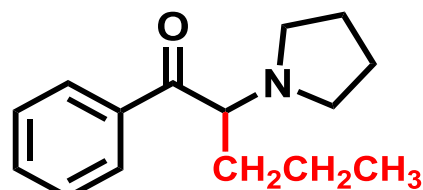


National Forensic Lab Info System, 2013

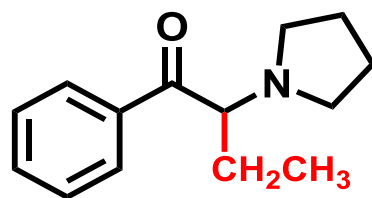
New PPs lack 3,4-methylenedioxy ring-substitution and have shorter α -carbon chain length



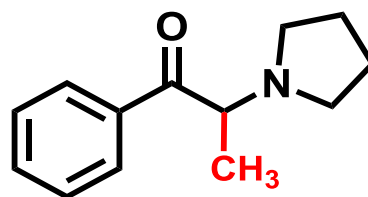
MDPV



α -PVP

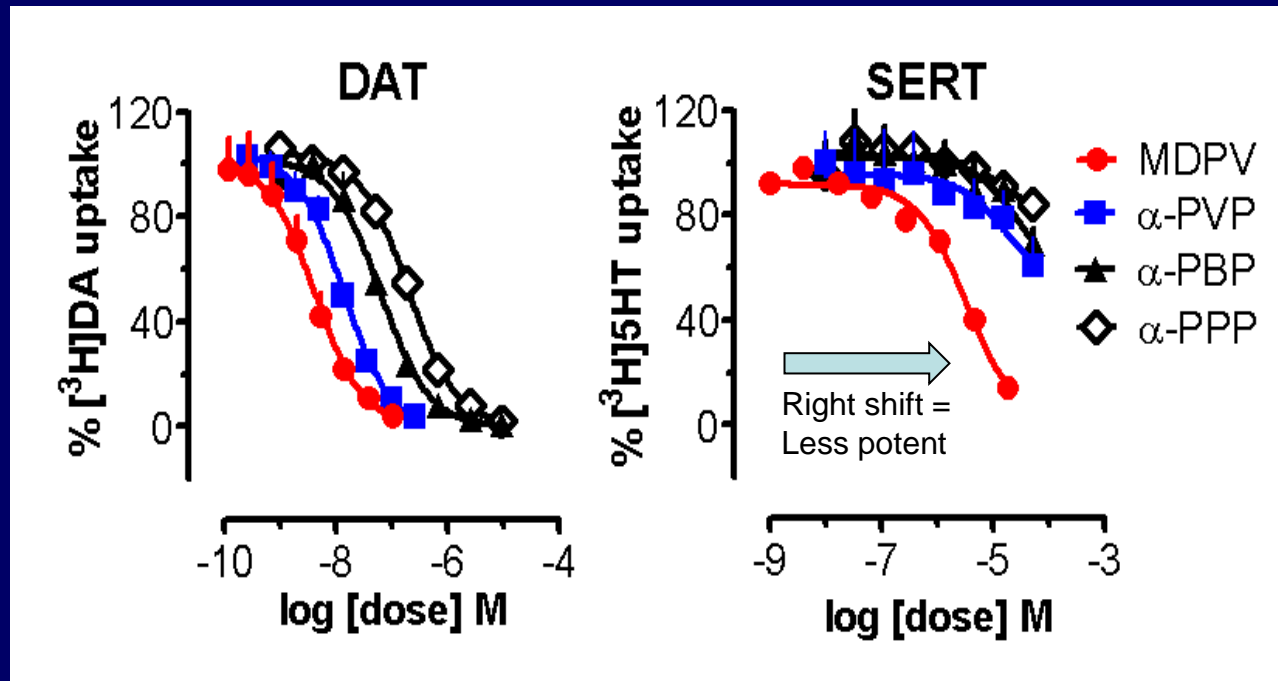


α -PBP



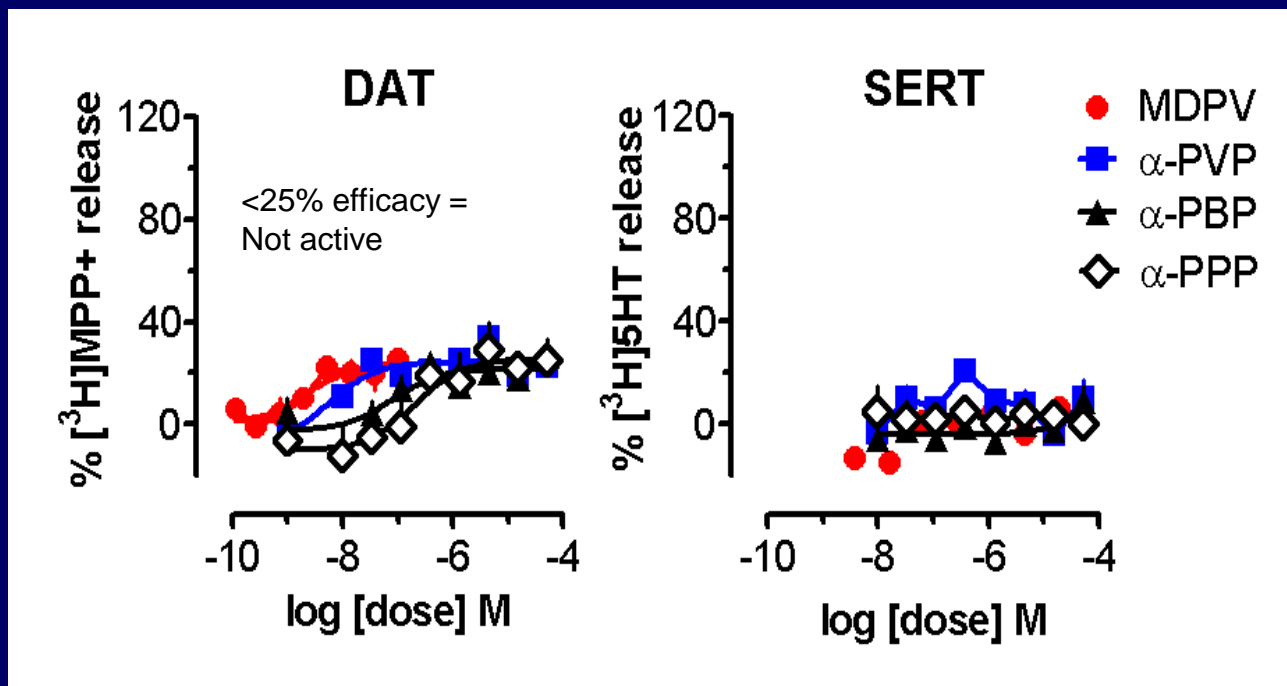
α -PPP

New PPs are DAT-selective uptake blockers; shorter α -carbon chain decreases potency



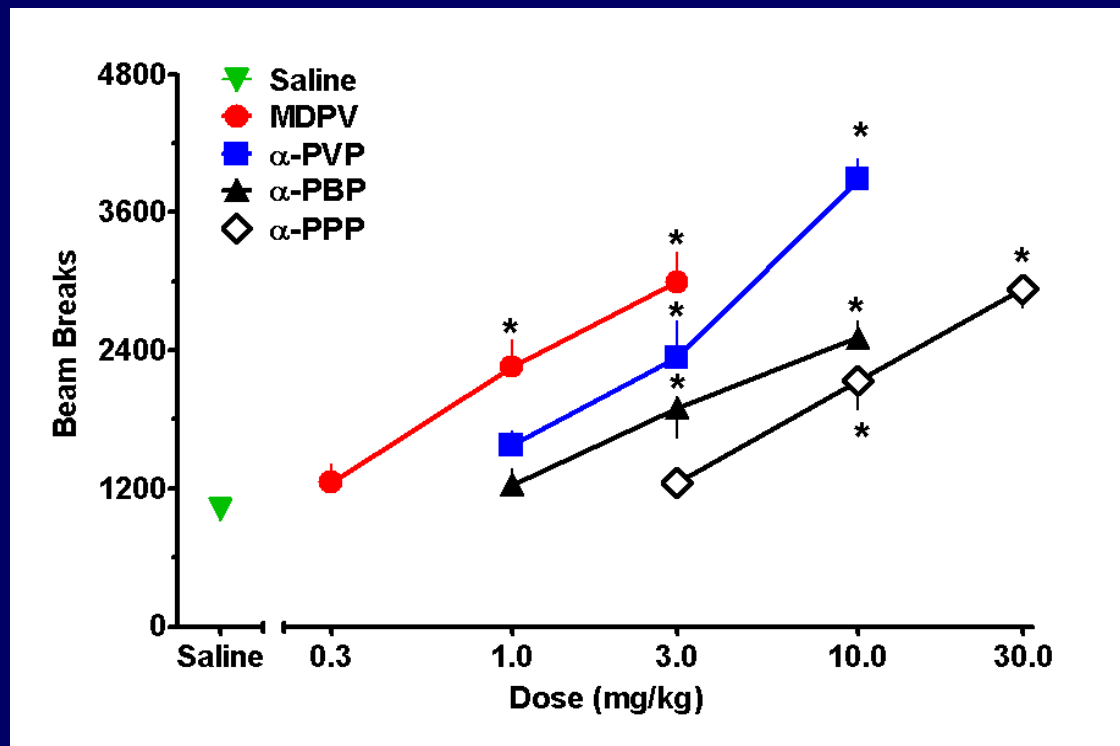
Marusich et al., 2014

New PPs are devoid of substrate activity at monoamine transporters



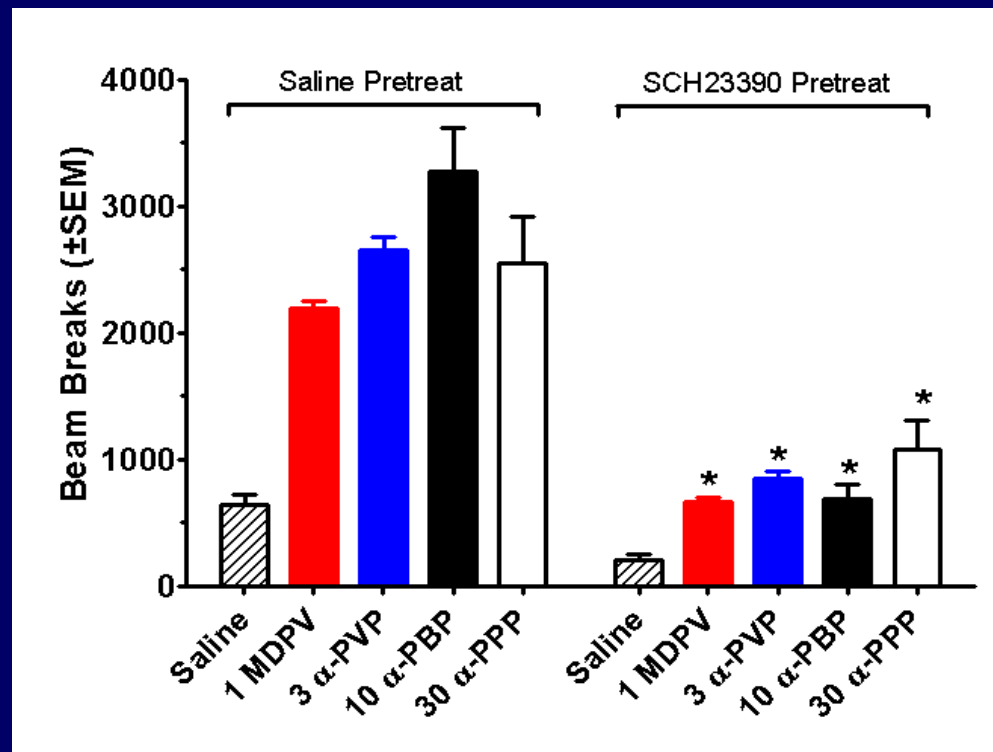
Marusich et al., 2014

New PPs are locomotor stimulants in mice; in vivo potency parallels activity at DAT



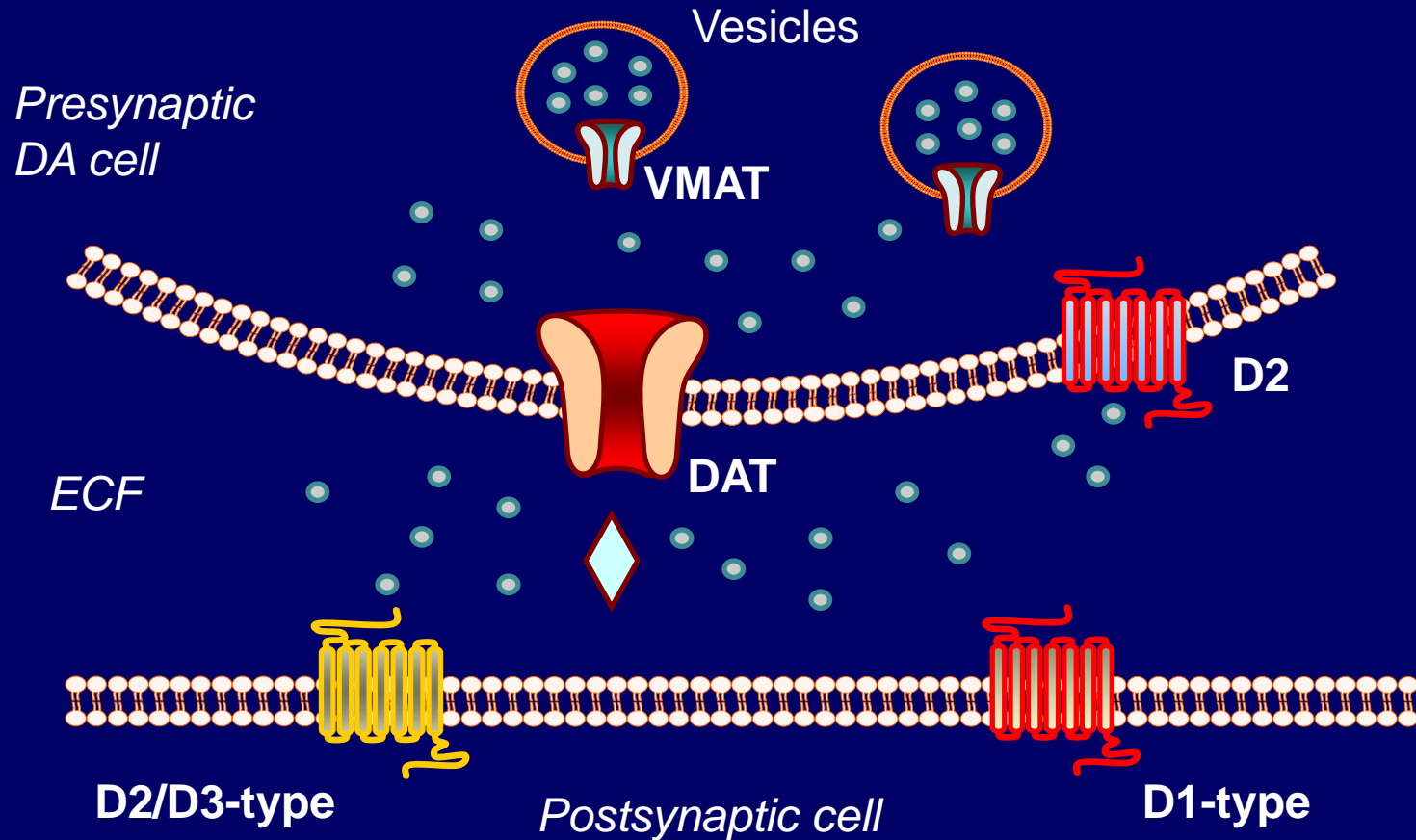
Marusich et al., 2014

Locomotor effects of PPs are reduced by the D1 antagonist SCH23390

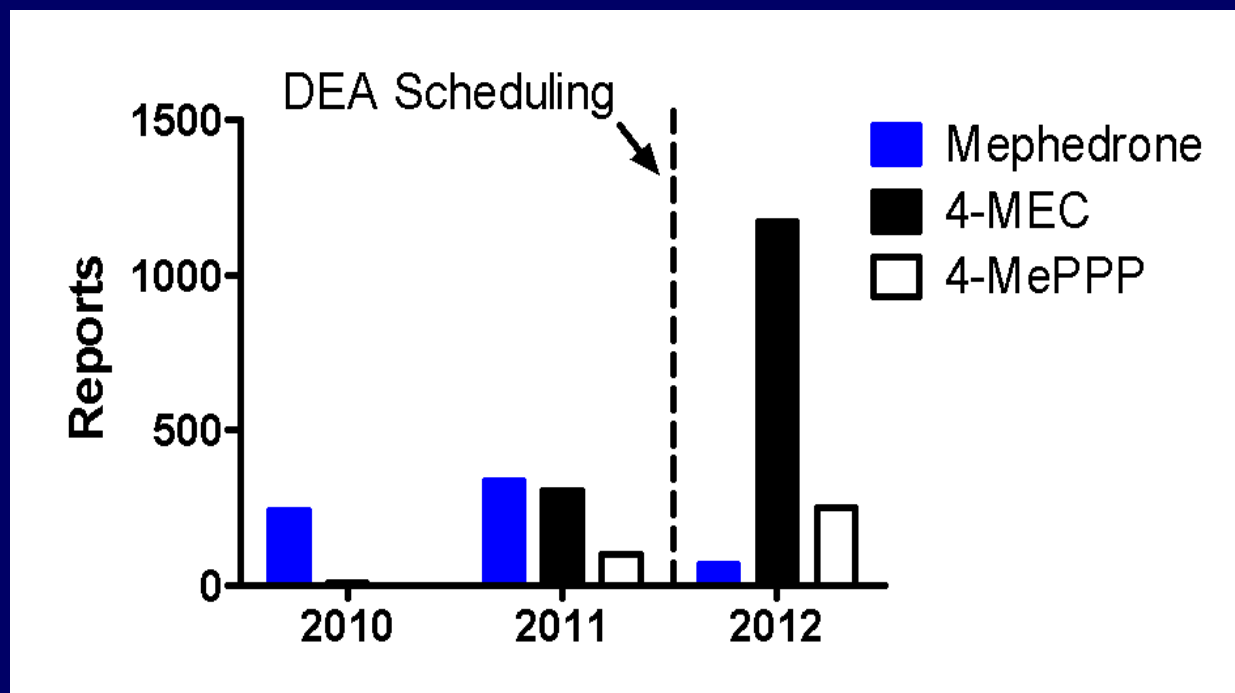


Marusich et al., 2014

◆ Mephedrone is a DAT substrate that releases intracellular DA into the ECF

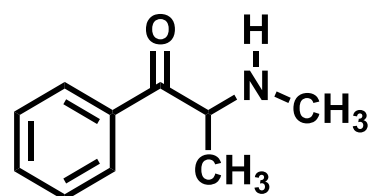


New 4-methyl cathinone analogs have appeared in the marketplace since mephedrone scheduling

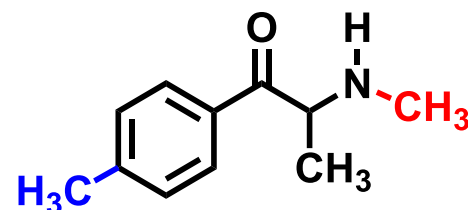


National Forensic Lab Info System, 2013

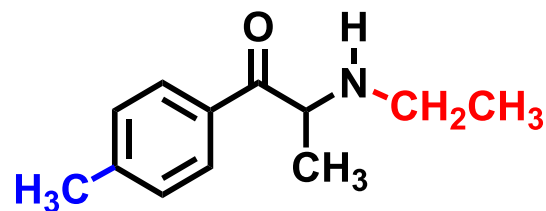
New 4-methyl cathinone analogs have longer *N*-alkyl chain length



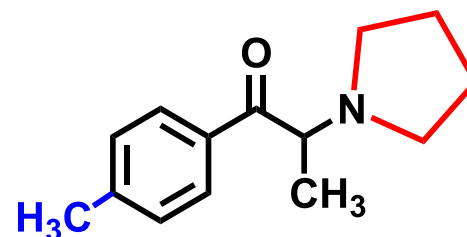
Methcathinone



Mephedrone

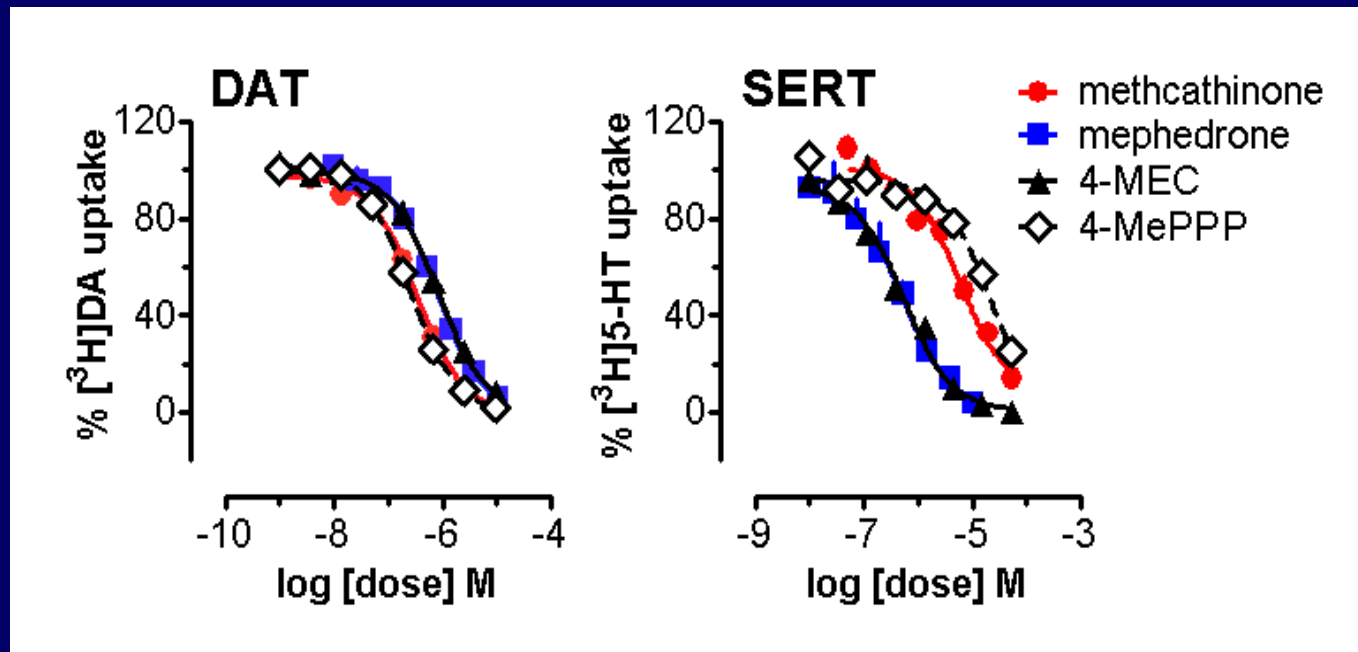


4-MEC



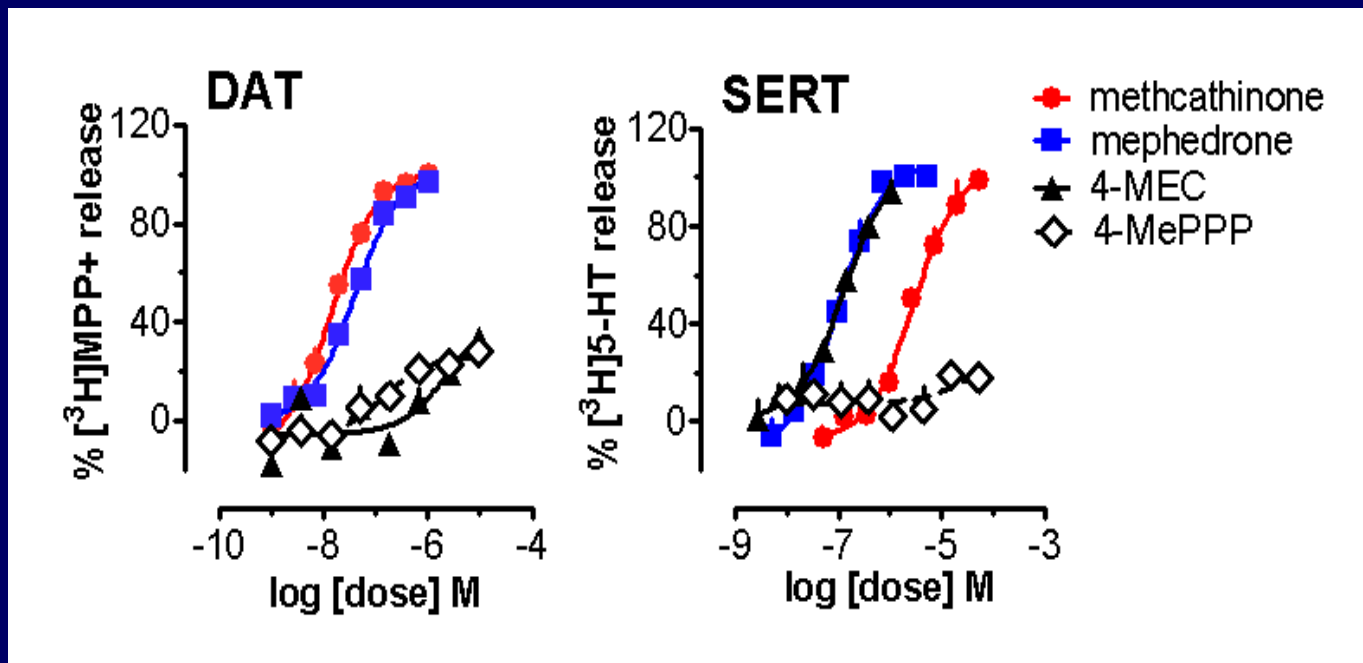
4-MePPP

4-MEC blocks transmitter uptake non-selectively while 4-MePPP is DAT-selective



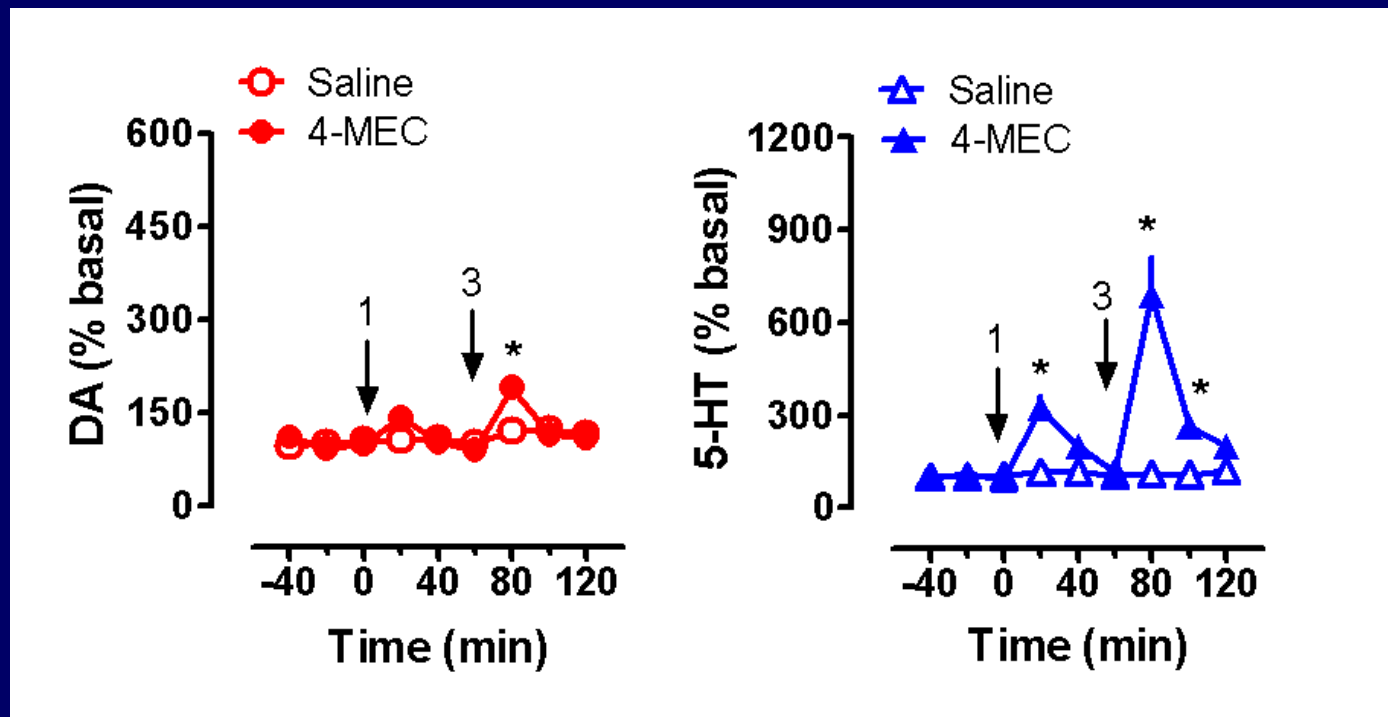
Partilla et al., 2014

Increasing *N*-alkyl chain length converts transporter substrates to blockers



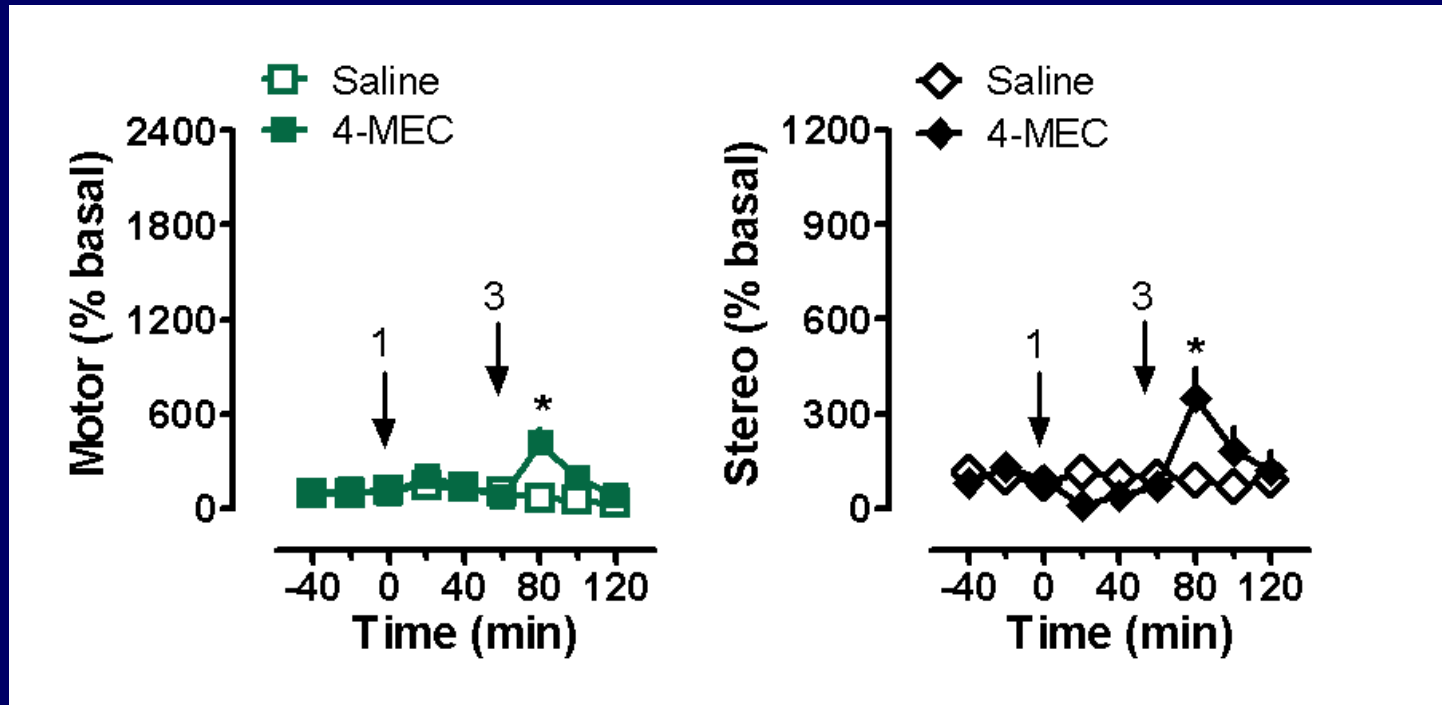
Partilla et al., 2014

4-MEC increases dialysate levels of 5-HT but not DA in rat nucleus accumbens



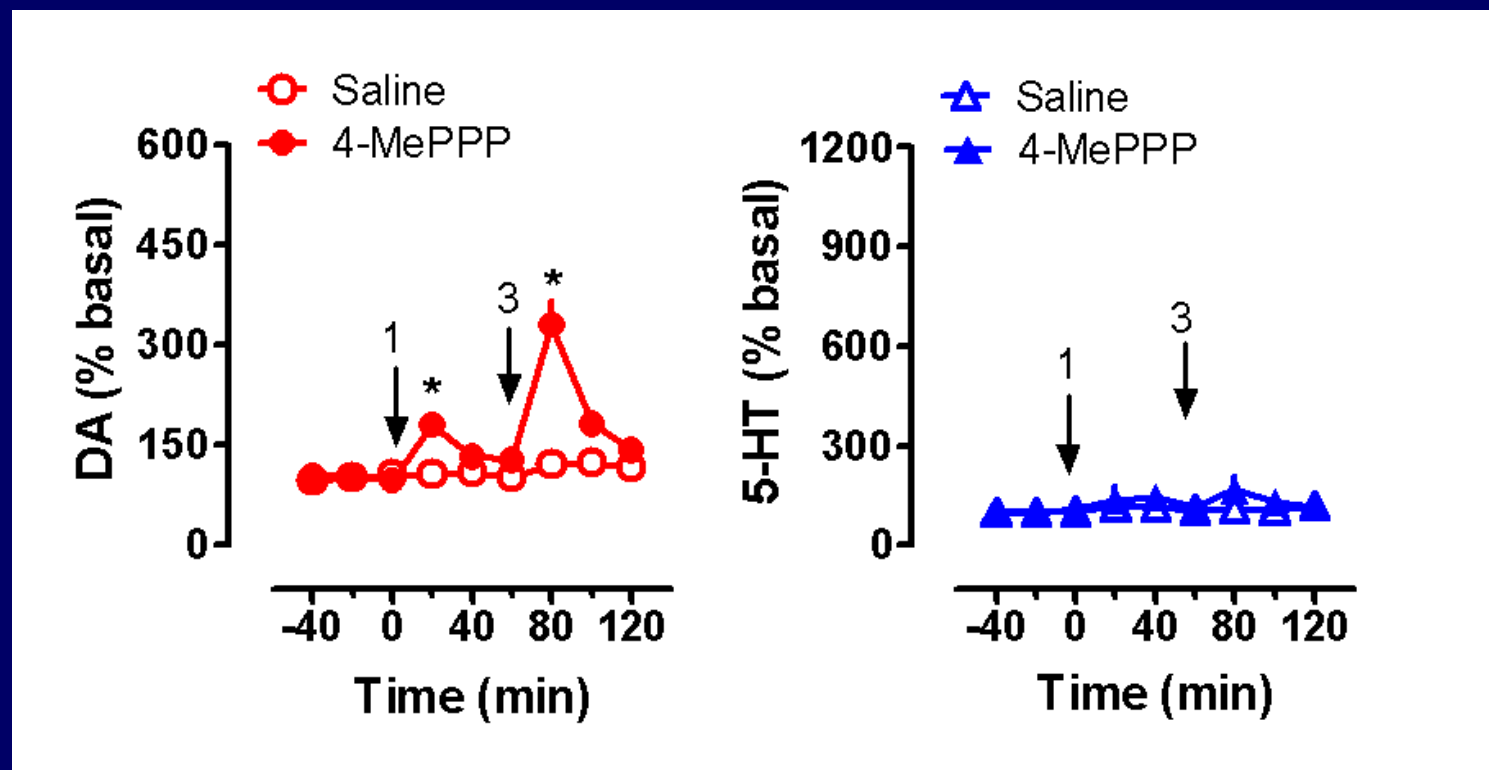
Saha et al., 2014

4-MEC does not cause robust locomotor activation in the rat



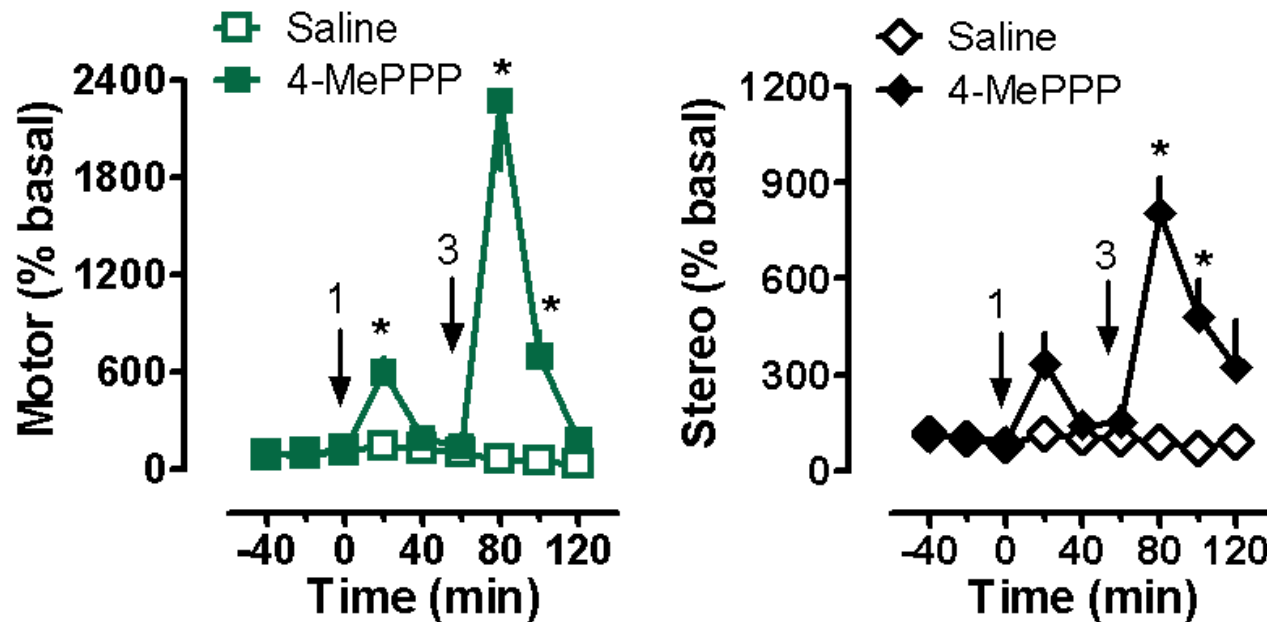
Saha et al., 2014

4-MePPP increases dialysate levels of DA but not 5-HT in rat nucleus accumbens



Saha et al., 2014

4-MePPP produces robust stimulation of locomotor activity in the rat



Saha et al., 2014

SAR for new cathinones reveal critical molecular determinants of drug-transporter interactions

- MDPV-related cathinones (pyrrolidinophenones)
 - All are DAT-selective transporter blockers
 - Potent locomotor stimulants in mice
- Mephedrone-related cathinones (4-methyl cathinones)
 - 4-MEC is a “hybrid” DAT blocker/SERT substrate
 - 4-MePPP is DAT-selective transporter blocker
 - 4-MePPP and 4-MEC increase extracellular dopamine in mesolimbic reward pathways

Collaborators

- NIDA IRP
 - John Partilla, BS; *[in vitro assays]*
 - Kurt Lehner, BS; Omar Bukhari, BS; *[microdialysis]*
 - Marilyn Huestis, PhD; *[PK studies]*
- Research Triangle Institute
 - Julie Marusich, PhD *[in vivo biology]*
- Medical University of Vienna
 - Harald Sitte, PhD *[in vitro assays]*
- NIDA HQ
 - Aidan Hampson, PhD; *[predictive toxicology]*