

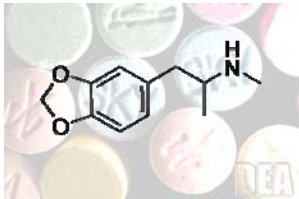
Un nou Mecanisme d'acció per a l'èxtasi

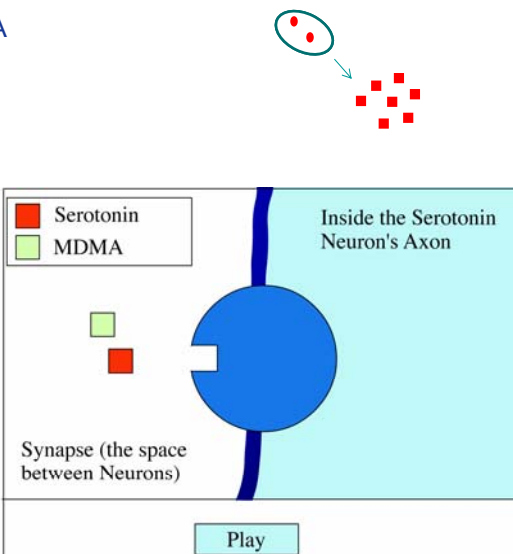
Dra Elena Escubedo



MDMA: Mecanisme

- Alliberament SA >> DA > NA



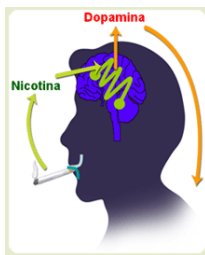



MDMA: èxtasi



DOPAMINA

SEROTONINA

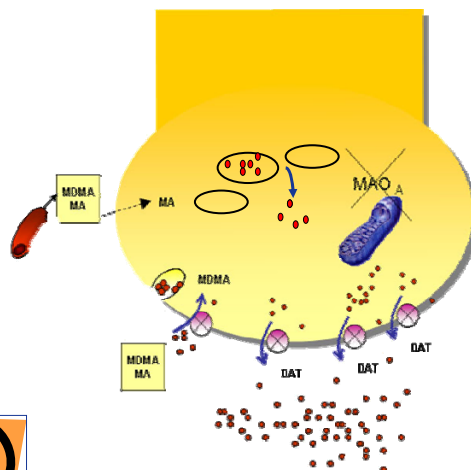


La SA regula l'estat d'ànim, la són, el dolor, les emocions, la gana i altres comportaments

EFFECTES MDMA

AGUT, efecte bifàsic:

- ❖ Augment ràpid de la conc extracel·lular de DA i sobre tot 5-HT
- ❖ Bloq de la seva recaptació (DA, 5-HT)
- ❖ Inh enzim limitant de la seva síntesi
- ❖ Ràpid depleció dels nivells endògens de NT.

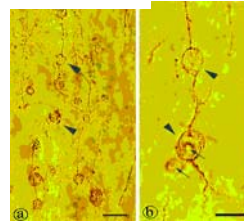
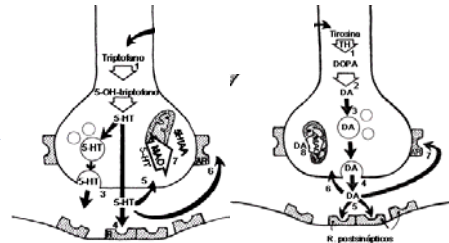


NEUROTOXICITAT

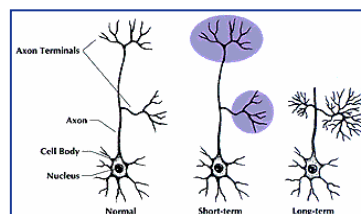
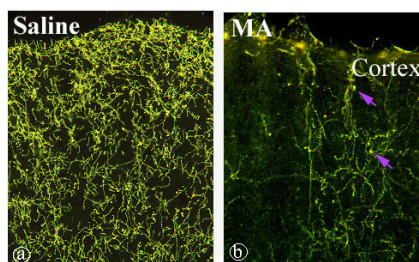
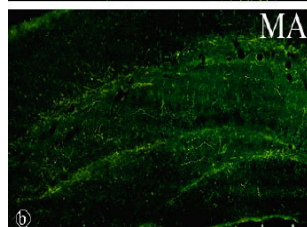
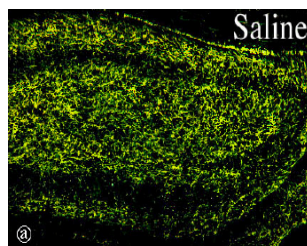
CRÒNIC:

Pèrdua de terminals SA-DA

- ↓ 5-HT, àcid 5-hidroxiindolacètic o DA
- ↓ triptofan hidroxilasa / tirosina hidroxilasa
- ↓ llocs de recaptació (SERT o DAT)
- alteracions morfològiques de les neurones: varicositats als axons i destrucció del terminal.



NEUROTOXICITAT

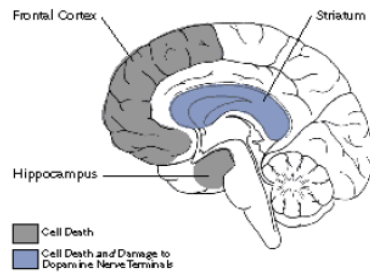


NEUROTOXITAT

MDMA

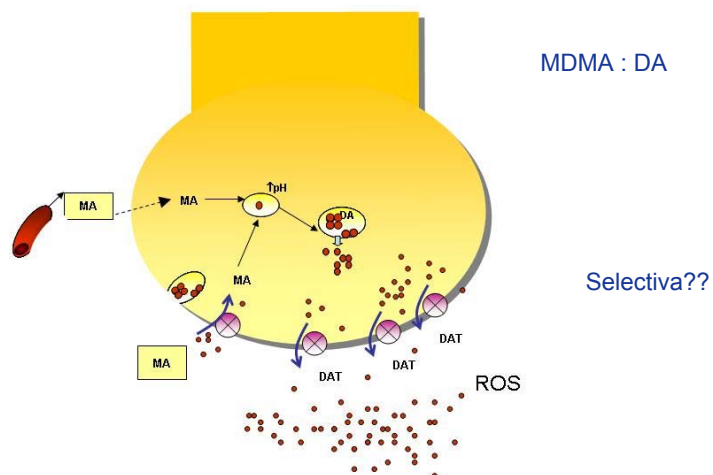


5-HT Ctx i Hpc

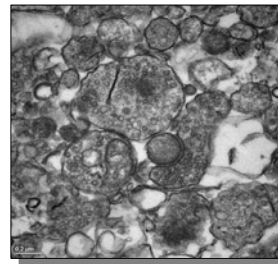
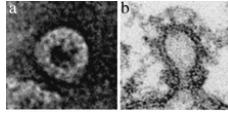
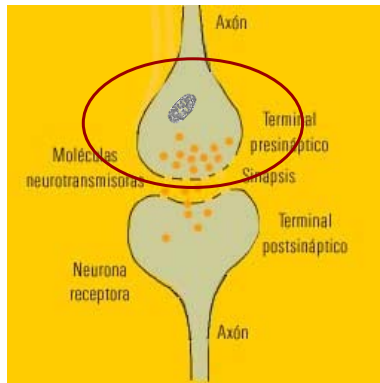


DA
Còrtex i estriat
5-HT
Ctx, Str, Hpc

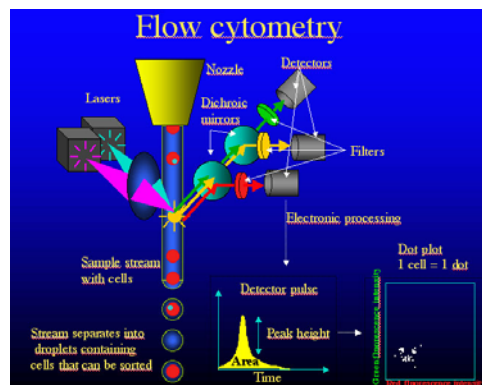
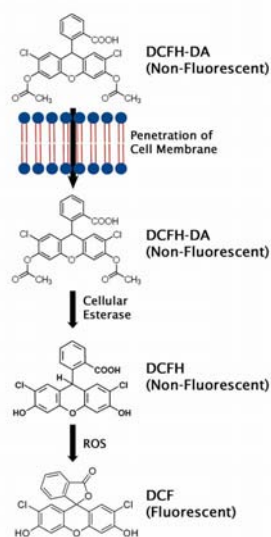
TEORIES NEUROTOXICITAT: ROS



SINAPTOSOMES PURIFICATS

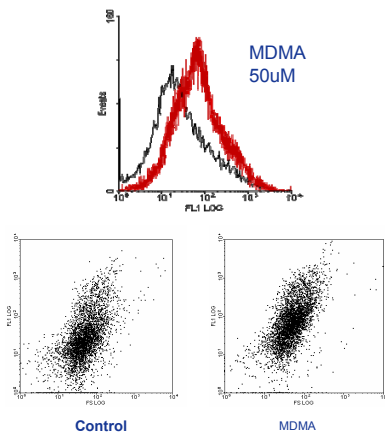


FLUORESCÈNCIA

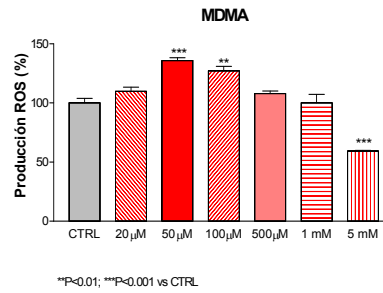


http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html

FORMACIÓ DE RADICALS LLIURES D'O

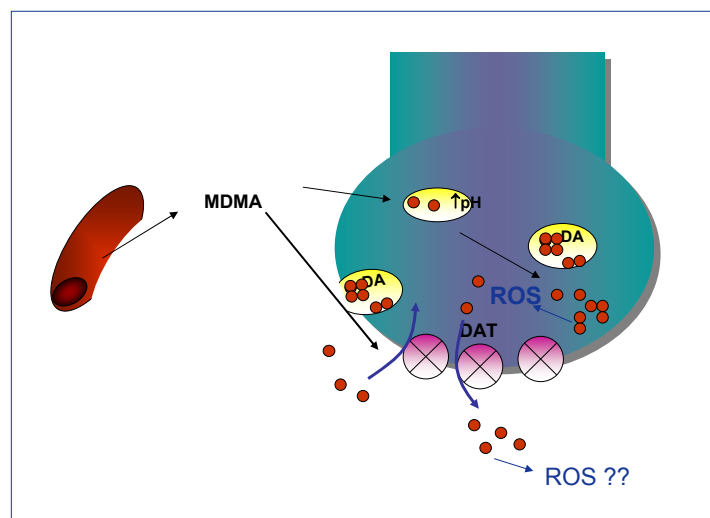


Producció de ROS dependent de la concentració d'MDMA

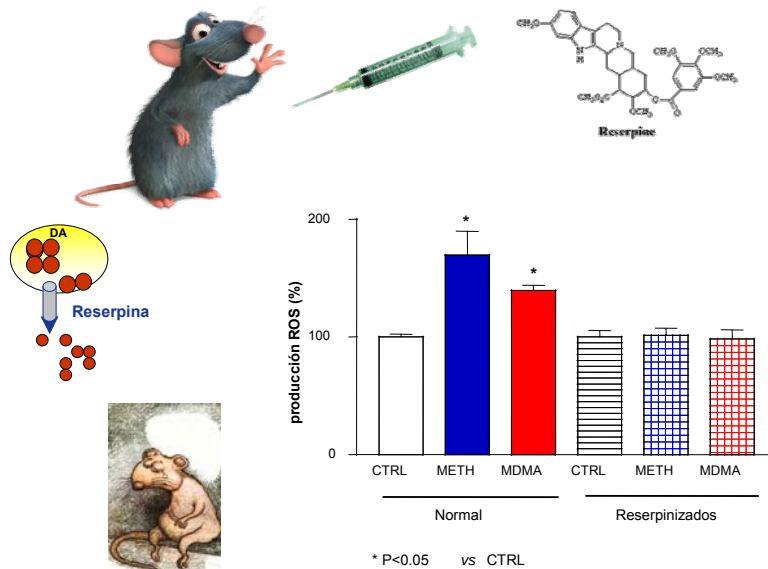


30 min - 2 h. 500-900 sinpt/sec 1 min (~ 50000)

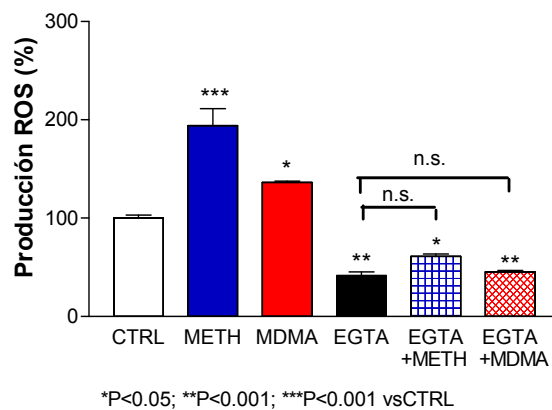
RADICALS LLIURES INTRANEURONALS



ROS induïts per MDMA



Paper del Calci



PARTICIPACIÓ DEL RECEPTOR NICOTÍNIC



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European Journal of Pharmacology 460 (2003) 9–17



www.elsevier.com/locate/ejphar

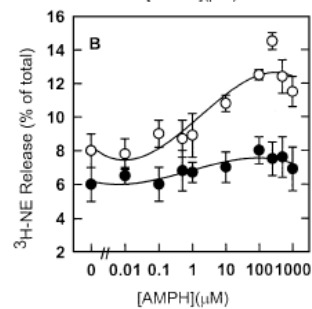
Amphetamine enhances Ca^{2+} entry and catecholamine release via nicotinic receptor activation in bovine adrenal chromaffin cells

Pei-Shan Liu^{a,*}, Chwen-Tzy Liaw^a, Meng-Kai Lin^a, Song-Huah Shin^a,
Lung-Sen Kao^b, Lih-Fang Lin^c

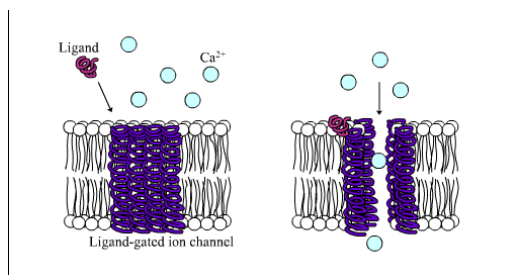
^aDepartment of Microbiology, Szechow University, Shiklin, Taipei, Taiwan, ROC

^bDepartment of Life Science, National Yang-Ming University, Taipei, Taiwan, ROC

^cNational Narcotics Bureau, Department of Health, Executive Yuan, Taipei, Taiwan, ROC



EL RECEPTOR NICOTÍNIC



Ionotròpics:
Canals RD o LLD

- **TETRAMÈRICS.** Canals acoplats a receptors ionotròpics de GLU
- **TRIMÈRICS.** Canals dependents d'ATP.
- **PENTAMÈRICS**
 - Canals aniònics (GABA_AR , GlyR)
 - Canals catiònics ($5\text{-HT}_3\text{R}$, nAChR)

RECEPTOR NICOTÍNIC

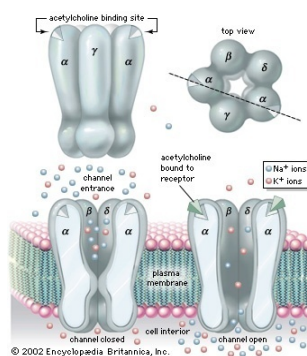
Neuronal-type					Muscle-type
I	II	III			IV
$\alpha 9, \alpha 10$	$\alpha 7, \alpha 8$	1	2	3	$\alpha 1, \beta 1, \delta, \gamma, \epsilon$
		$\alpha 2, \alpha 3, \alpha 4, \alpha 6$	$\beta 2, \beta 4$	$\beta 3, \alpha 5$	

NEURONAL-TYPE

Homomèrics: 5α $\alpha 7 \alpha 7$
 $\alpha 7 \alpha 7$

Heteromèrics: $2\alpha : 3\beta$

$\beta 2 \beta 2$
 $\alpha 4 \beta 2 \alpha 4$

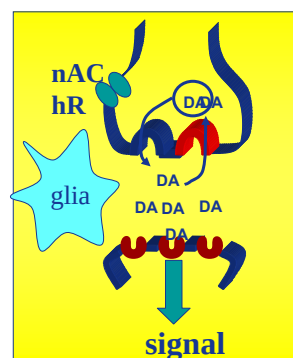
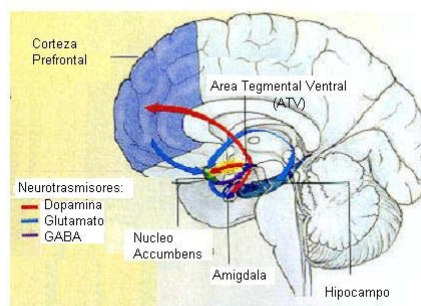


MUSCLE-TYPE

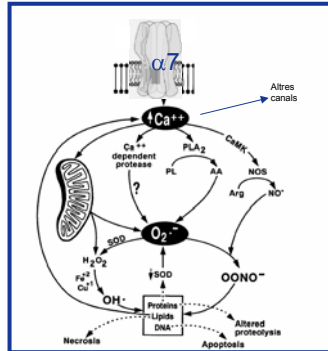
$2\alpha : \beta : \gamma : \delta / \epsilon$

nAChR en les vies dopaminèrgiques

- ❖ Les vies dopaminèrgiques mesolímbiques i nigroestriats expressen múltiples subtipus de receptors nicotínics ($\alpha 3, \alpha 7$ i $\alpha 4\beta 2$).
- ❖ D'aquesta manera, els nAChRs exerceixen múltiples influències per si sols i sobre la senyalització de la DA.

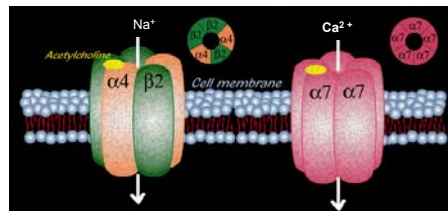


Permeabilitat al calci

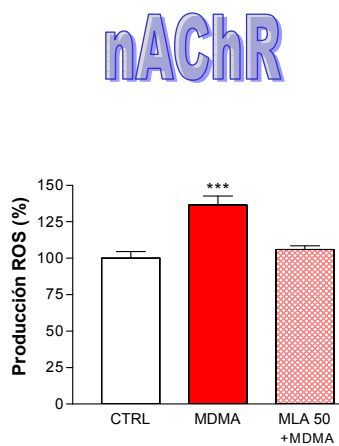


Ca / Na:

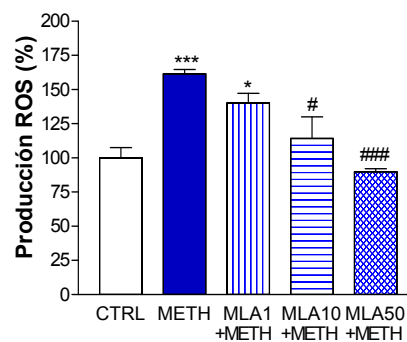
- ~ 0,1 per al múscul
- ~ 2.0 per heteromèrics i
- ≥ 10 per homomèrics $\alpha 7$.



Producció sinaptosomal de ROS



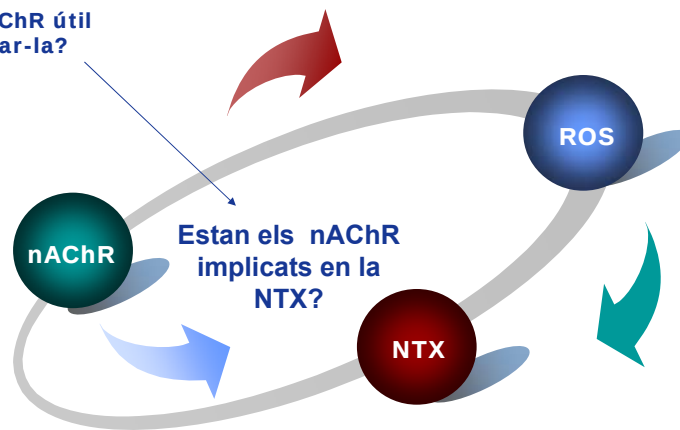
P<0.01, *P<0.001 vs CTRL; ##P<0.01 vs MDMA



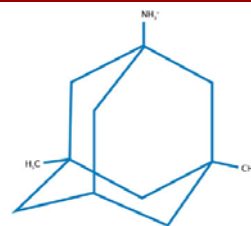
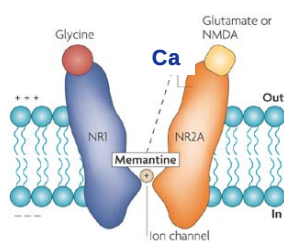
*P<0.05, ***P<0.001vs CTRL; #P<0.05, ###P<0.001 vs Meth

HIPÒTESI

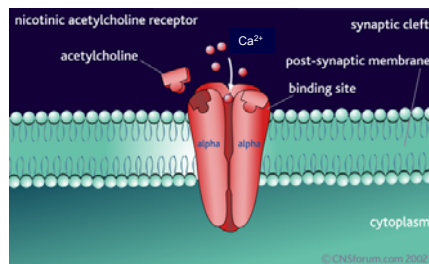
Bloq nAChR útil
per evitar-la?



MEMANTINA



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Nature Reviews | Drug Discovery



ALZHEIMER

$\alpha 7$ en la NTX de l'MDMA



Rates DA:
SF (5ml/kg)
MEM (5 mg/kg) IP
MDMA (18 mg/kg) SC
MEM (30 min abans) + MDMA

HPC

24 hores

7 dies

fixació [3 H] PK 11195

fixació [3 H] Paroxetina

fixació [3 H] Paroxetina

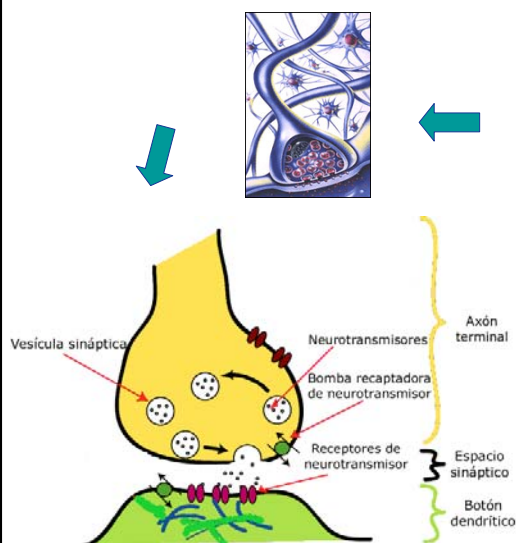
Proliferació glial

Pèrdua de terminals serotoninèrgics HPC

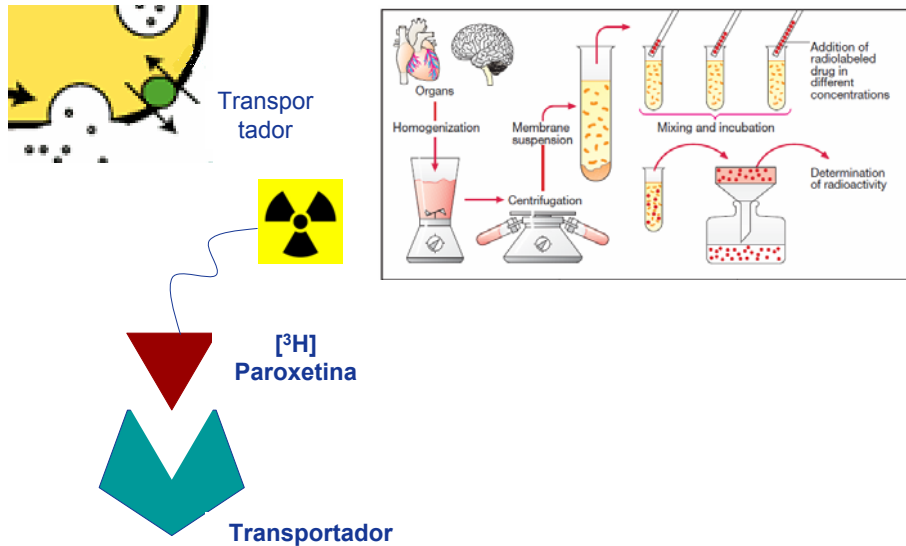
WB SOD

Estrès oxidatiu

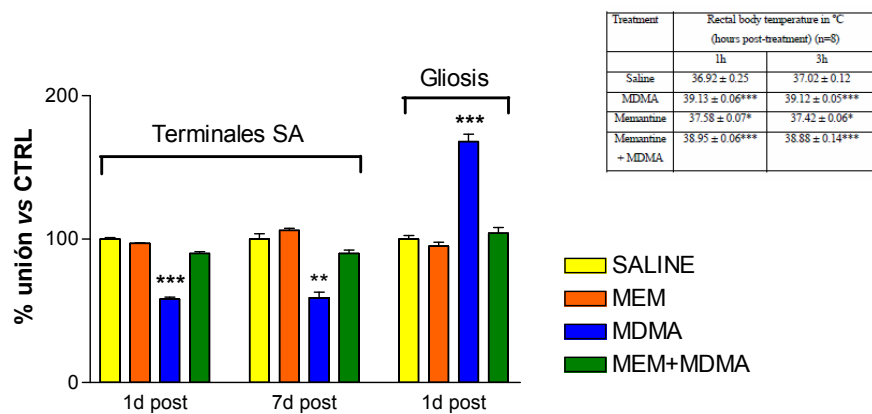
TERMINAL - TRANSPORTADOR



MARCATGE TRANSPORTADOR

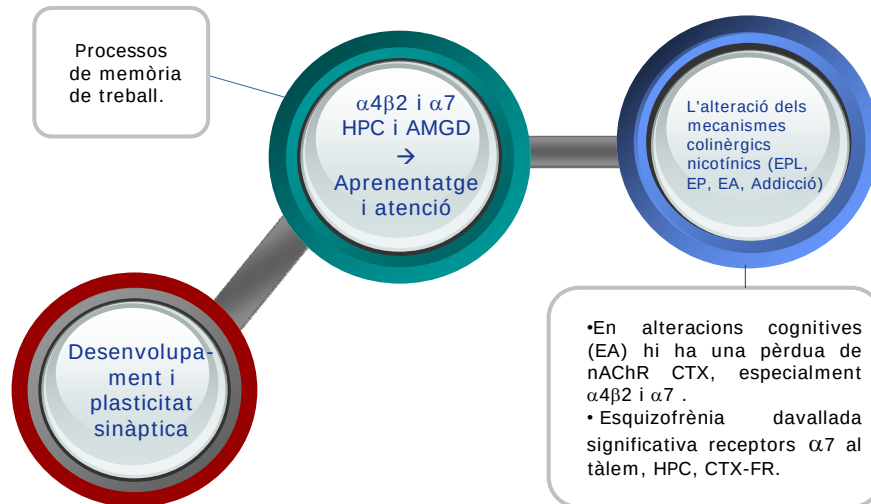


MDMA - MEMANTINA



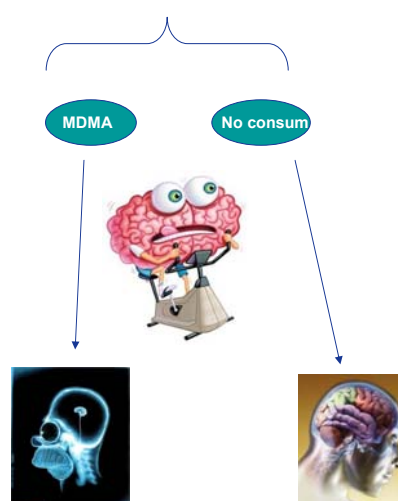
La MEM prevé la pèrdua de terminals SA induïda per l'MDMA

PAPER DELS nAChR

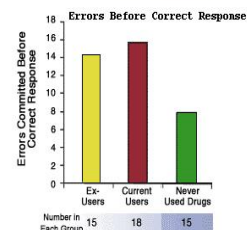
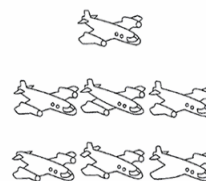


Alteració cognitiva

Nivells normals de DA STR



Matching Familiar Figures Test



nAChR 7

Estan els nAChR7 implicats en la alteració cognitiva?

Consumidors d'èxtasi



Memòria / aprenentatge

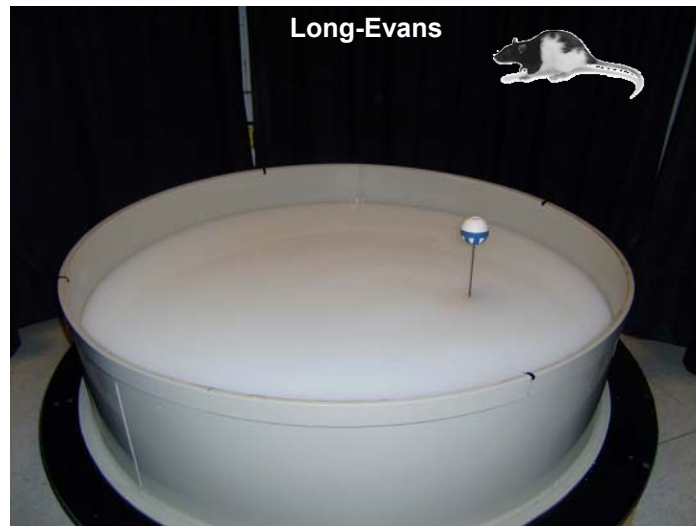


SF
MDMA
Memantina
M + M

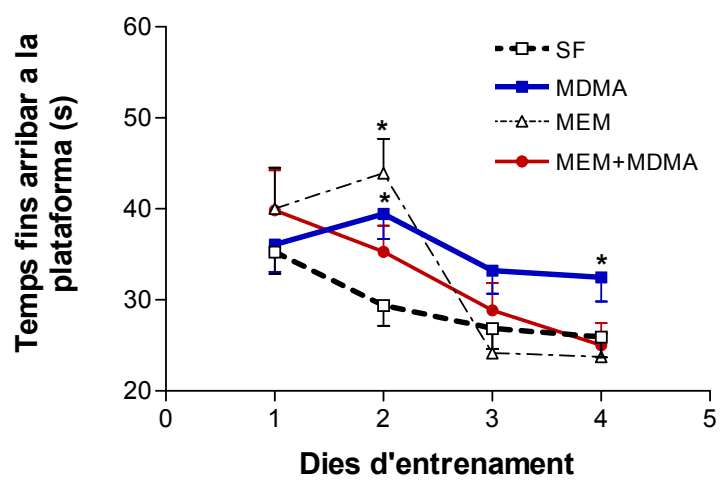
3 dies post-
tractament.
Assaig de
Familiarització i
de Prova

Als 15 dies
post-
tractament
laberint aquàtic
de Morris

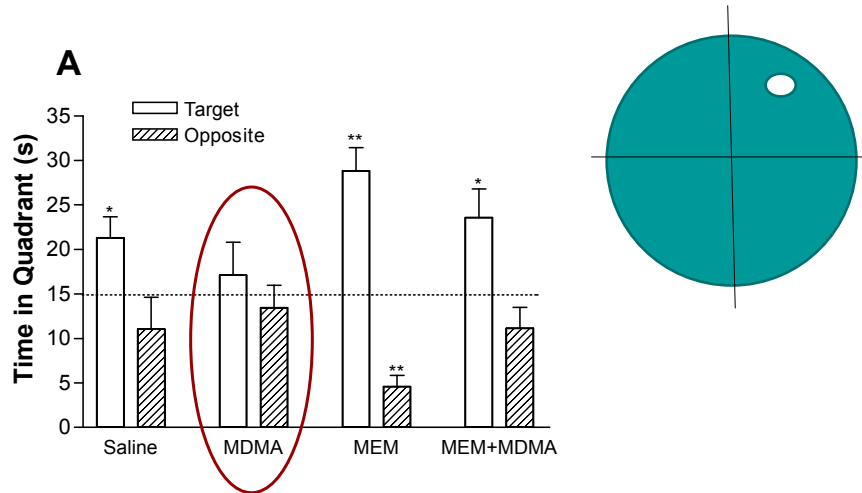
Laberint aquàtic de Morris



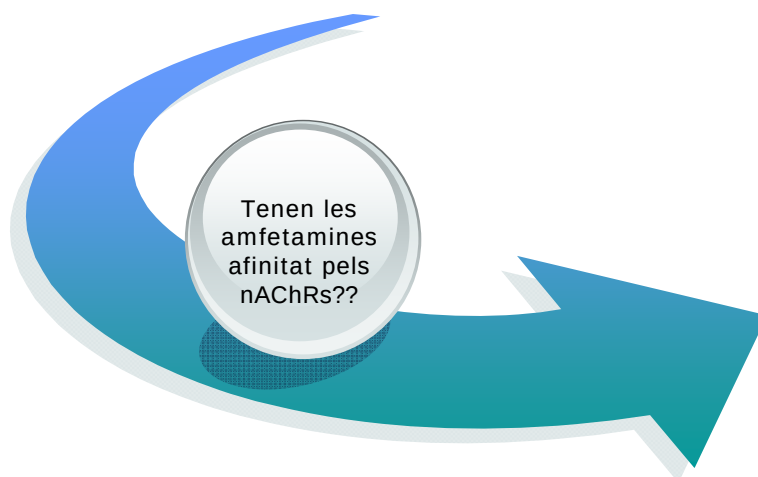
Laberint aquàtic de Morris



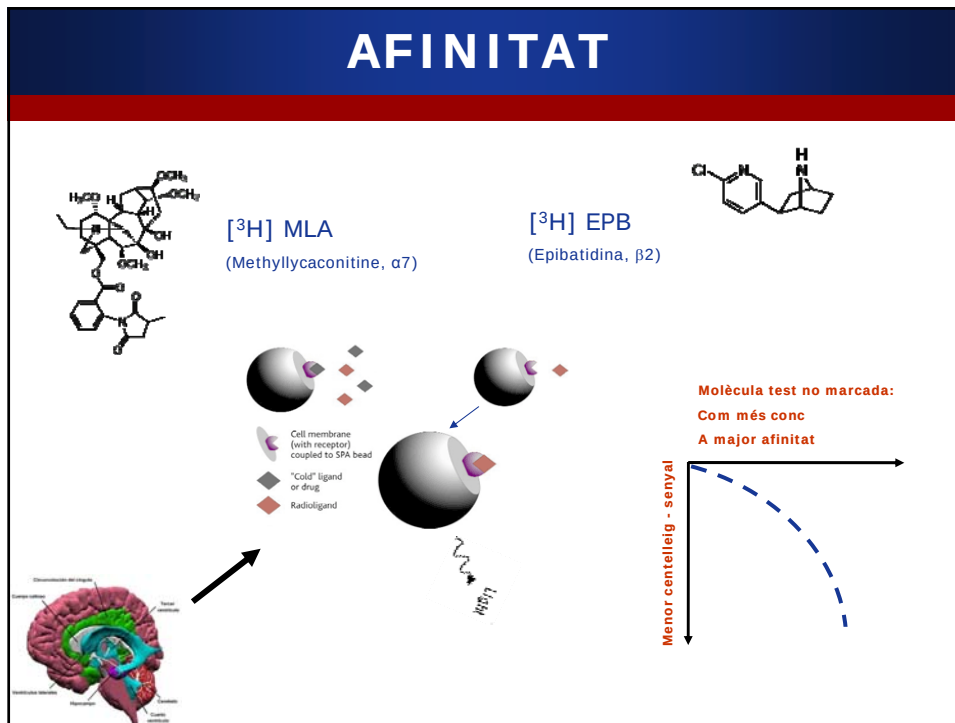
Laberint aquàtic de Morris



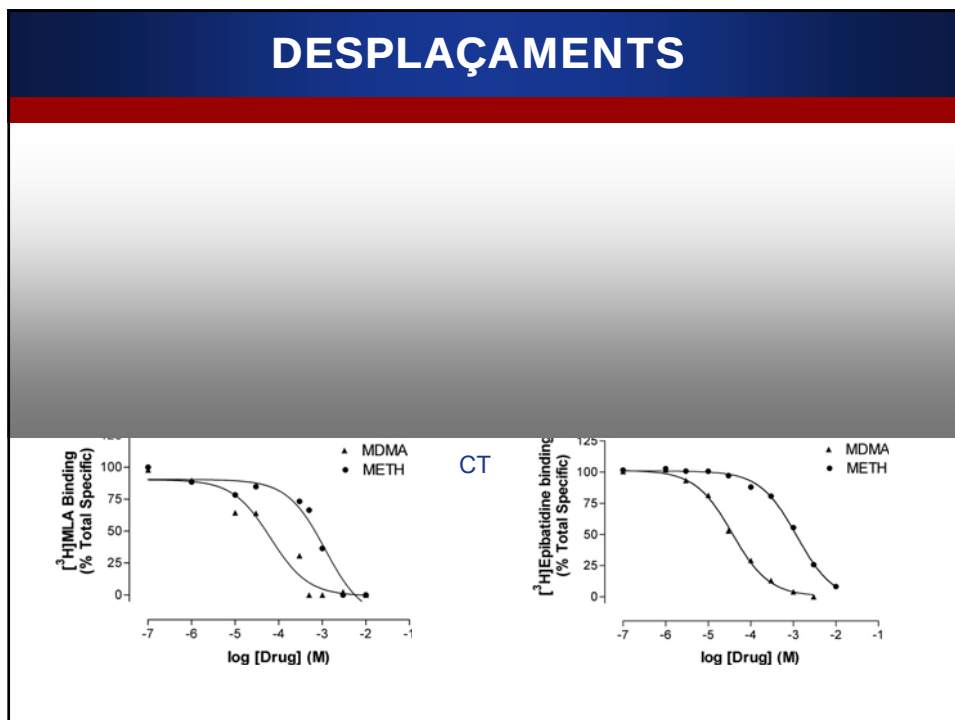
SEGÜENT OBJECTIU



AFINITAT



DESPLAÇAMENTS



AFINITATS

K_i values of METH and MDMA against [3 H]MLA binding in PC 12 cells and mouse brain

Cheng-Prusoff eq.

$$K_i = \frac{IC_{50}}{1 + \frac{[D]}{K_d}}$$

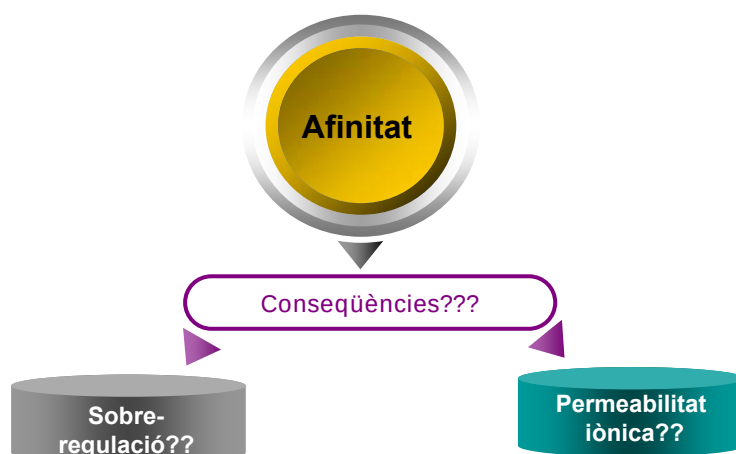
Drugs	PC 12 cells		Mouse brain	
	K_i (μ M)	n_H	K_i (μ M)	n_H
METH	283 $\pm$ 109	1.20 $\pm$ 0.15	369.77 $\pm$ 95.61	0.29 $\pm$ 0.08**
MDMA	15.35 $\pm$ 1.03	1.35 $\pm$ 0.11	9.50 $\pm$ 0,71	0,85 $\pm$ 0,06

K_i values of METH and MDMA against [3 H]epibatidine binding in PC 12 cells and mouse brain

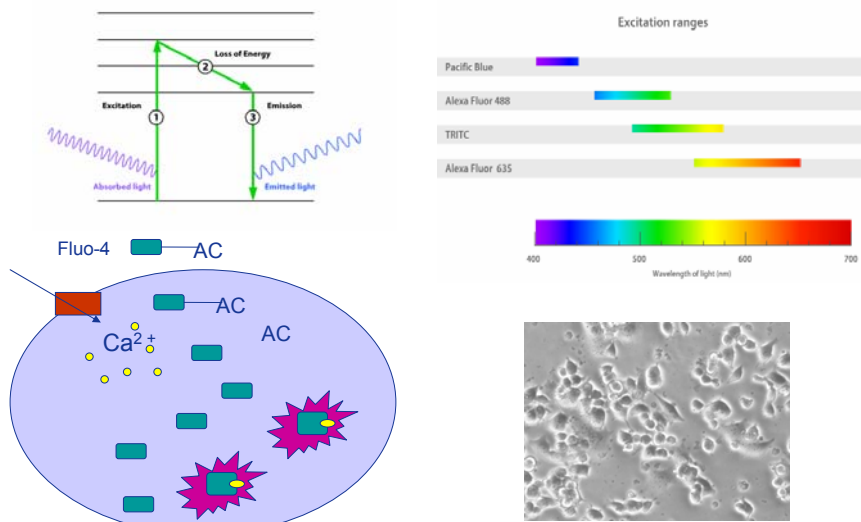
Drugs	PC 12 cells		Mouse brain	
	K_i (μ M)	n_H	K_i (μ M)	n_H
METH	155.36 $\pm$ 5.36	0.76 $\pm$ 0.11	23.90 $\pm$ 2.65	1.27 $\pm$ 0.28
MDMA	25.71 $\pm$ 3.13	0.92 $\pm$ 0.04	0.76 $\pm$ 0.11	0.83 $\pm$ 0.12

(0,61 μ M vs SERT)

Conseqüències farmacodinàmiques

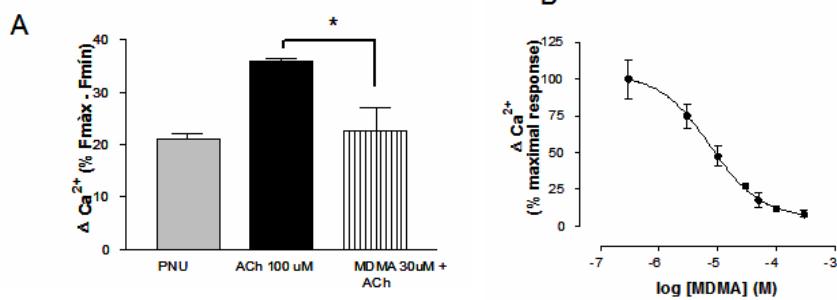


Mesura de la incorporació de Ca^{2+}



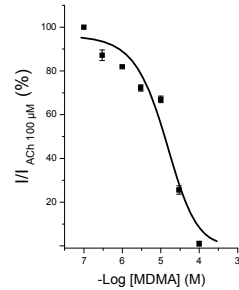
Inhibició de ACh

$\text{IC}_{50} = 10.42 \mu\text{M}$



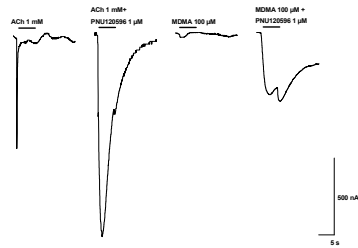
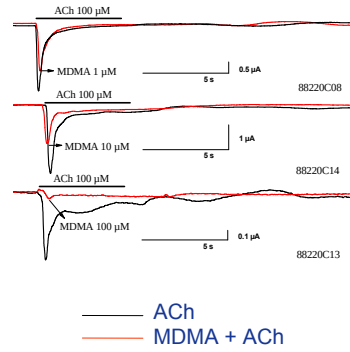
$K_{\text{binding}} : 9.5 \mu\text{M}$

Oòcits de Xenopus RNAm $\alpha 7$ humà



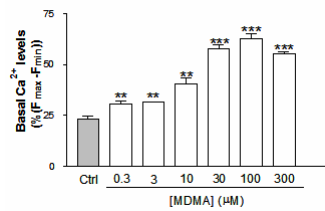
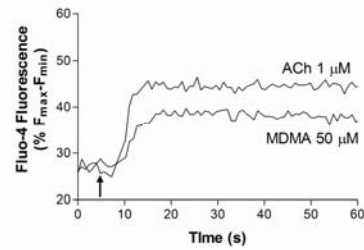
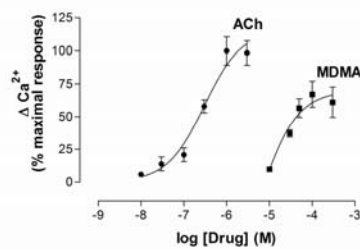
IC_{50} de $17\ \mu M$

nAChR $\alpha 7$ humà

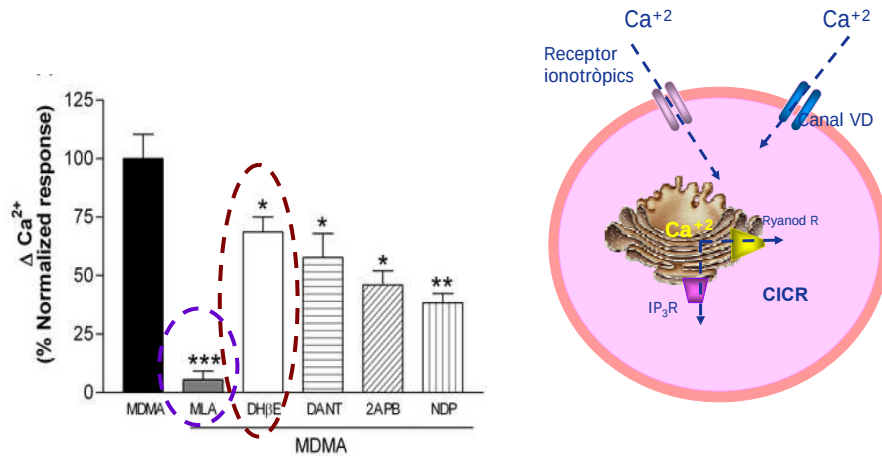


INCORPORACIÓ DE CALCI

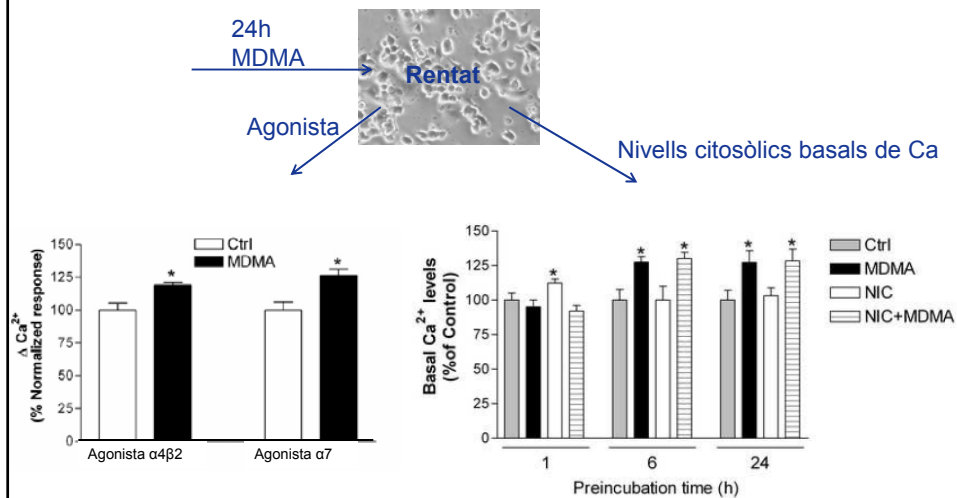
EC_{50} aprox $50\ \mu M$



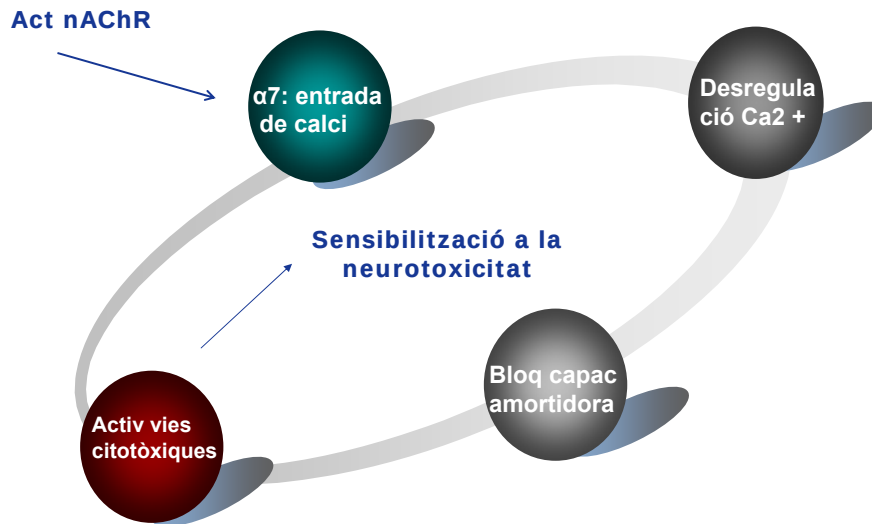
Efectes de l'activació nAChR



Potenciació Funcional

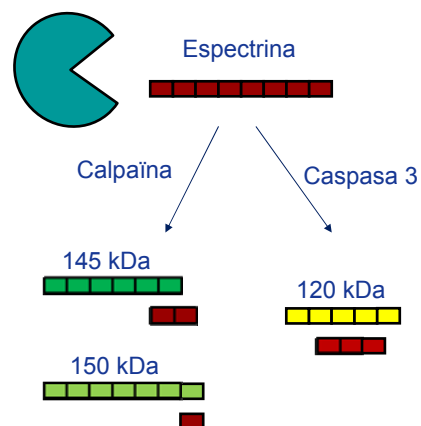
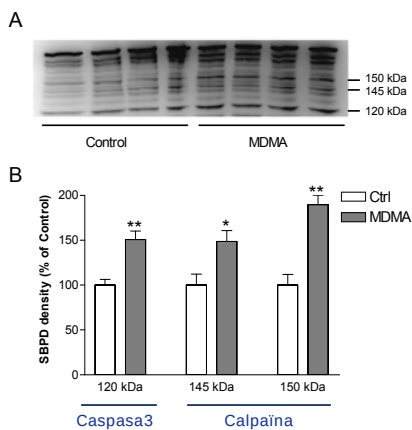


Efectes de l'activació nAChR

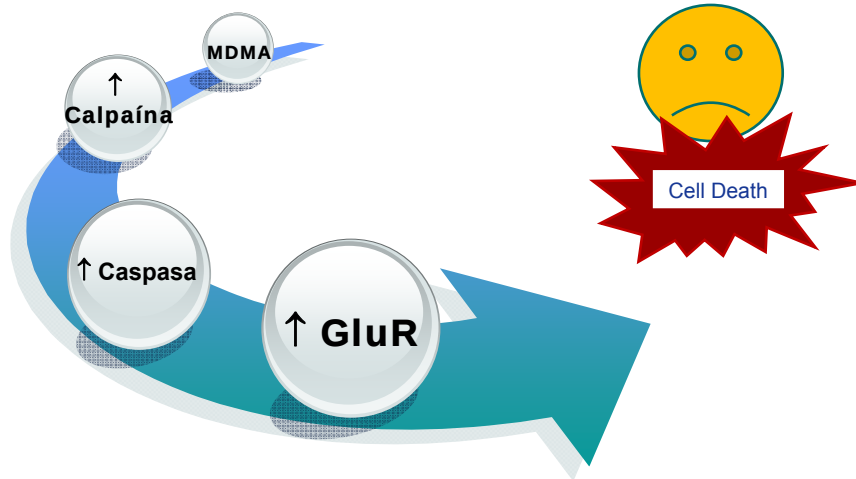


Calci i activació de proteases

En cultius de cèl·lules PC12 tractades amb MDMA durant 24 h



Hipersensibilització al GluR



Aproximació computacional a l'estudi de l'afinitat pels receptors nicotínics

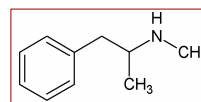
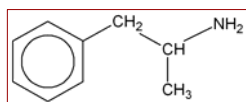
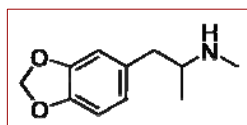


RELLEVÀNCIA FISIOLÒGICA

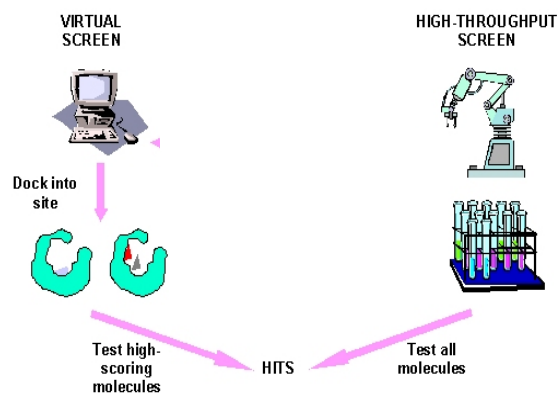
K_i values of METH and MDMA against [3 H]epibatidine binding

Drugs		Mouse brain	
		K_i (μ M)	n_H
METH	(0,61 μ M vs SERT)	23.90 ± 2.65	1.27 ± 0.28
MDMA		0.76 ± 0.11	0.83 ± 0.12

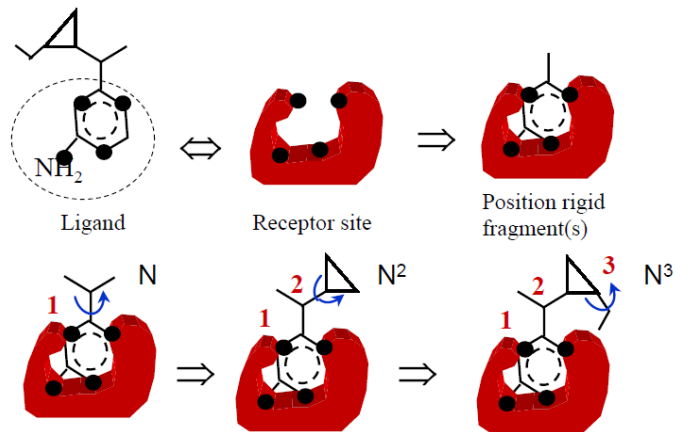
$\alpha 4\beta 2$: MDMA >>> AMF > MET



Cribat Virtual: Disseny basat en la proteïna diana



Cribat Virtual : acoplament-docking molecular



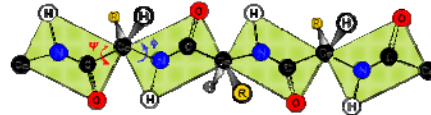
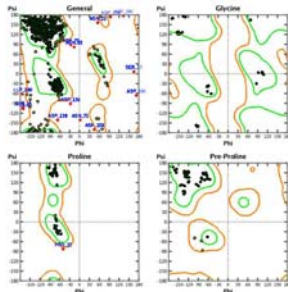
$$\Delta G_{unión} = \Delta H - T\Delta S \quad K_A = \exp\left(-\frac{\Delta G_{unión}}{RT}\right)$$

Cribat Virtual : acoplament-docking molecular

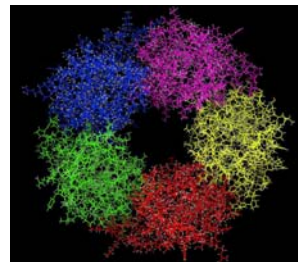


<http://www.youtube.com/watch?v=TTtrk0Ue-Cg&feature=related>

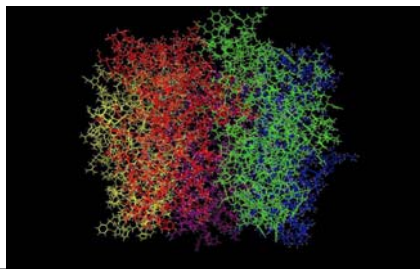
Modelització del receptor $\alpha 4\beta 2$ de rata



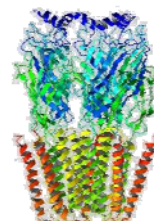
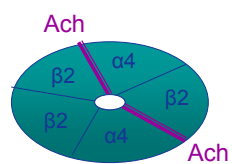
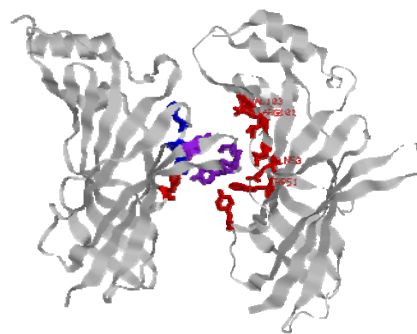
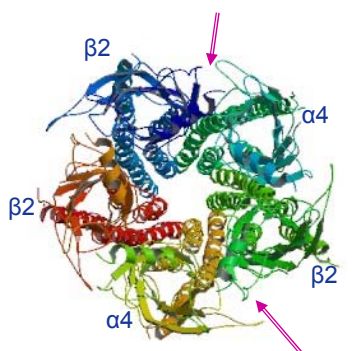
Estat de mínima energia



Model $\alpha 4\beta 2$ de rata (pdb: 1ole)

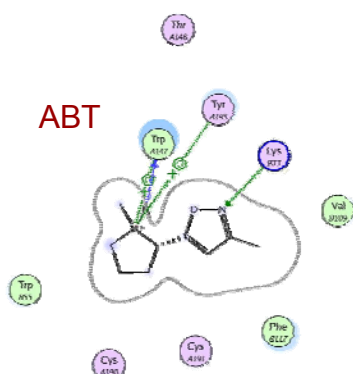


LLOC D'UNIÓ

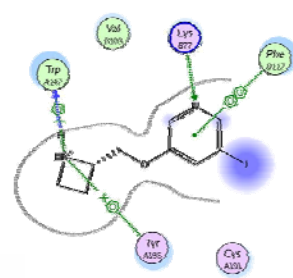
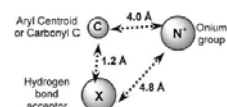


AGONISTES: Validació del mètode

ABT



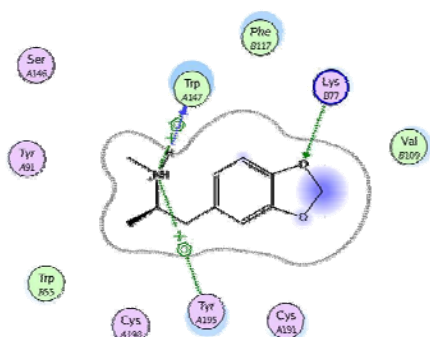
- polar
- acidic
- basic
- greasy
- proximity contour
- sidechain acceptor
- sidechain donor
- backbone acceptor
- backbone donor
- solvent residue
- metal complex
- solvent contact
- metal contact
- receptor exposure
- ligand exposure
- arene-arene
- arene-cation



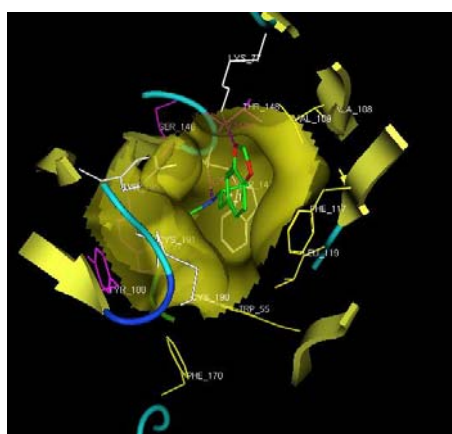
5IA

Acoplament Anfetamines - $\alpha 4\beta 2$ de rata

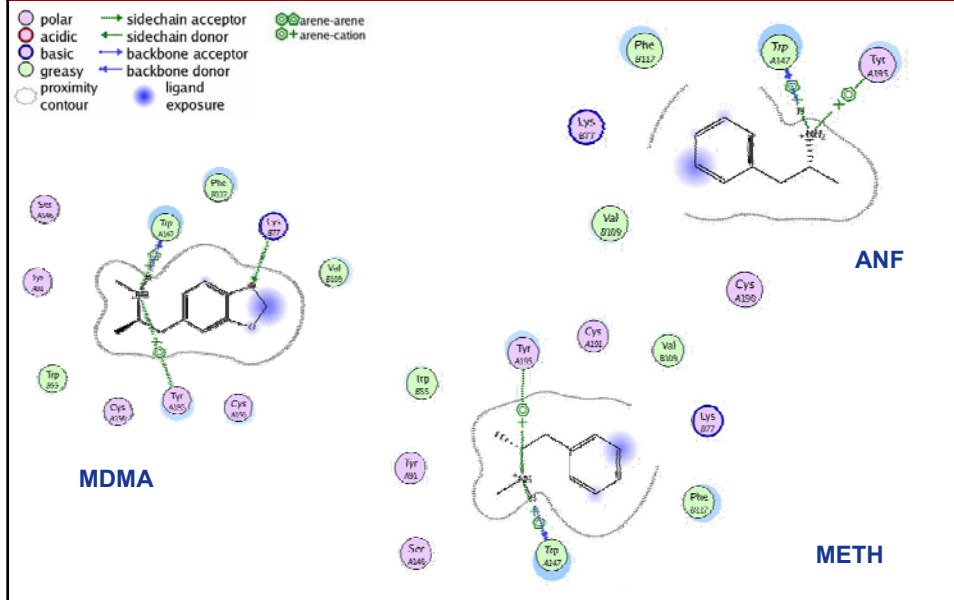
- polar
- acidic
- basic
- greasy
- proximity contour
- sidechain acceptor
- sidechain donor
- backbone acceptor
- backbone donor
- solvent residue
- metal complex
- solvent contact
- metal contact
- receptor exposure
- ligand exposure
- arene-arene
- arene-cation



MDMA



Acoplament Anfetamines - $\alpha 4\beta 2$ de rata



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