Parkinsons-2015 Meeting. October 11-13 , Frankfurt , Germany

The impact of DA D2 heteroreceptor complexes and their receptor-receptor interactions on Parkinson's disease and its treatment. Focus on the A2A-D2-mGlu5 and NTS1-D2 heterocomplexes

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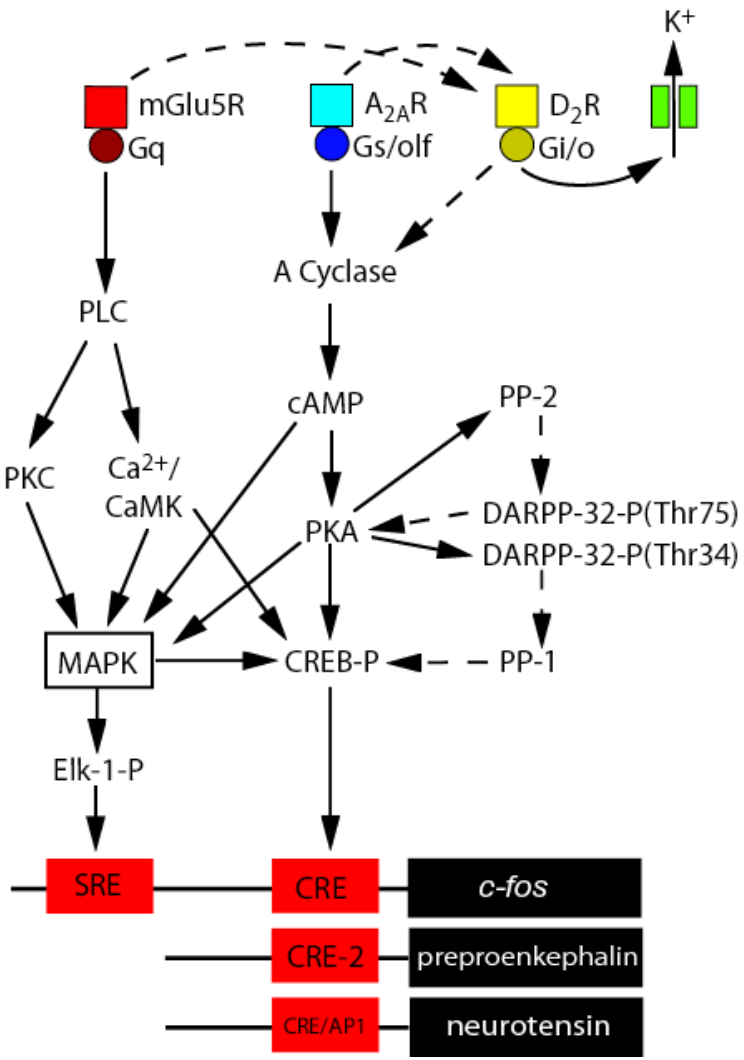
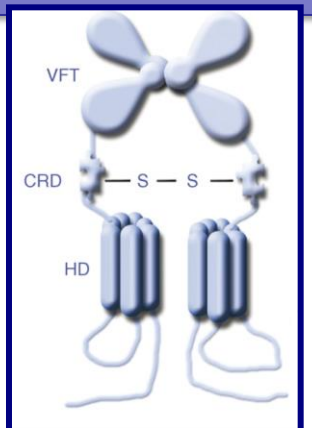
Heteroreceptor complexes formed between mGluR5, D2R and A2AR

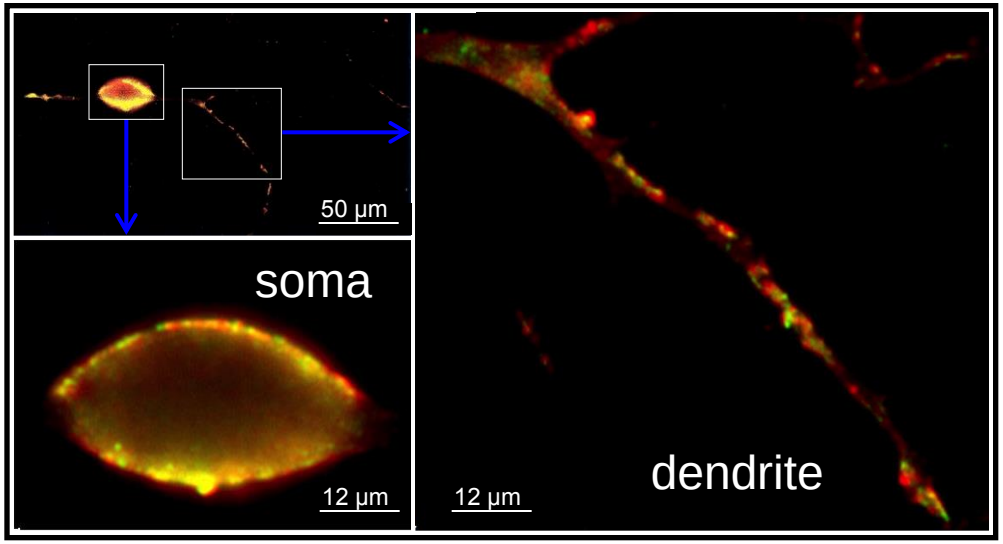


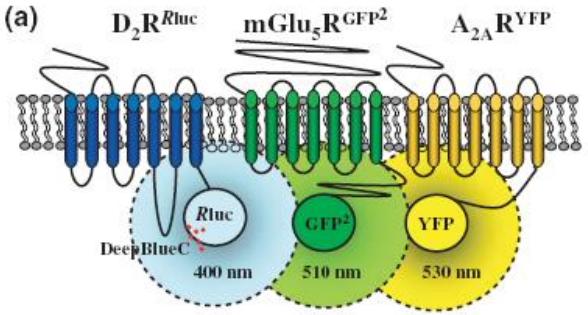
Signaling	Potential relevance PD
A2AR-**mGluR5 synergize to reduce D2R recognition and Gi/o coupling and signaling** *Popoli et al.2001,Fuxe et al.2003,Agnati et al.2003,.* Interactions **also at the level of the signaling cascades: MAPK and CREB**-P. A2AR and mGluR5 agonists, NAMs synergistically increase GABA release in ventral pallidum *Diaz-Cabiale et al.2002*	mGluR5 antagonists and negative allosteric modulators may significantly target the mGluR5 protomer. They **increase locomotion and exert antiparkinsonian actions and antidyskinetic actions especially combined with A2A antagonists**. *Coccurello et al.2004,Kachroo et al. 2005 Schwarzschild et al.2006,Vallano et al.2013*



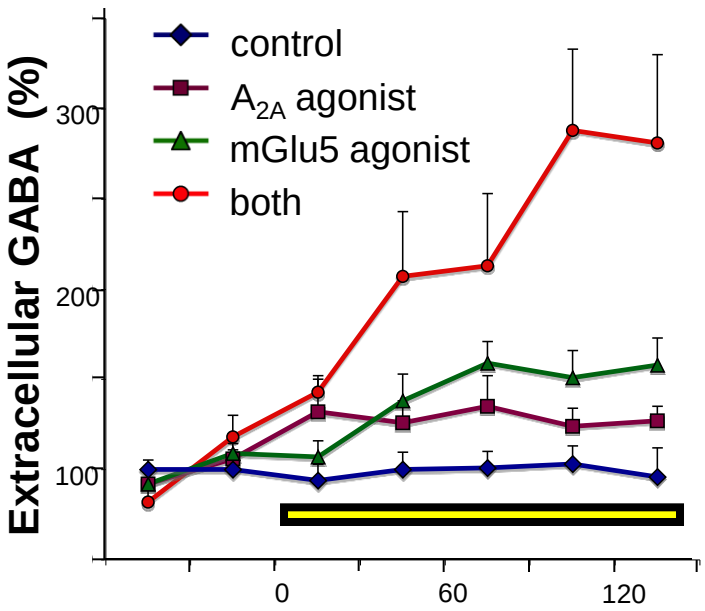
Fuxe et al.1984, Popoli et al.2001, Ferre et al.2002, Fuxe et al.2003, Cabello et al.2009

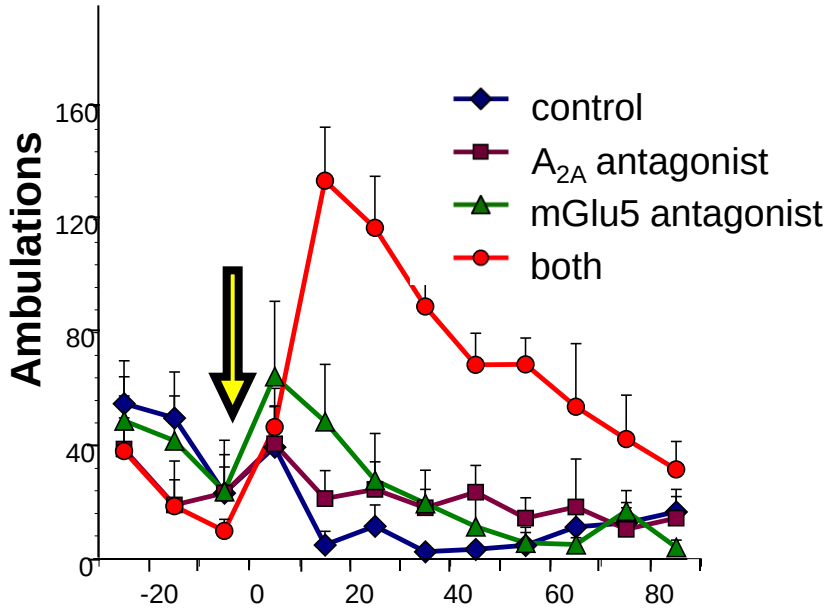
Neuronal A_{2A} and mGlu5 immunoreactivity





Cabello et al.2009

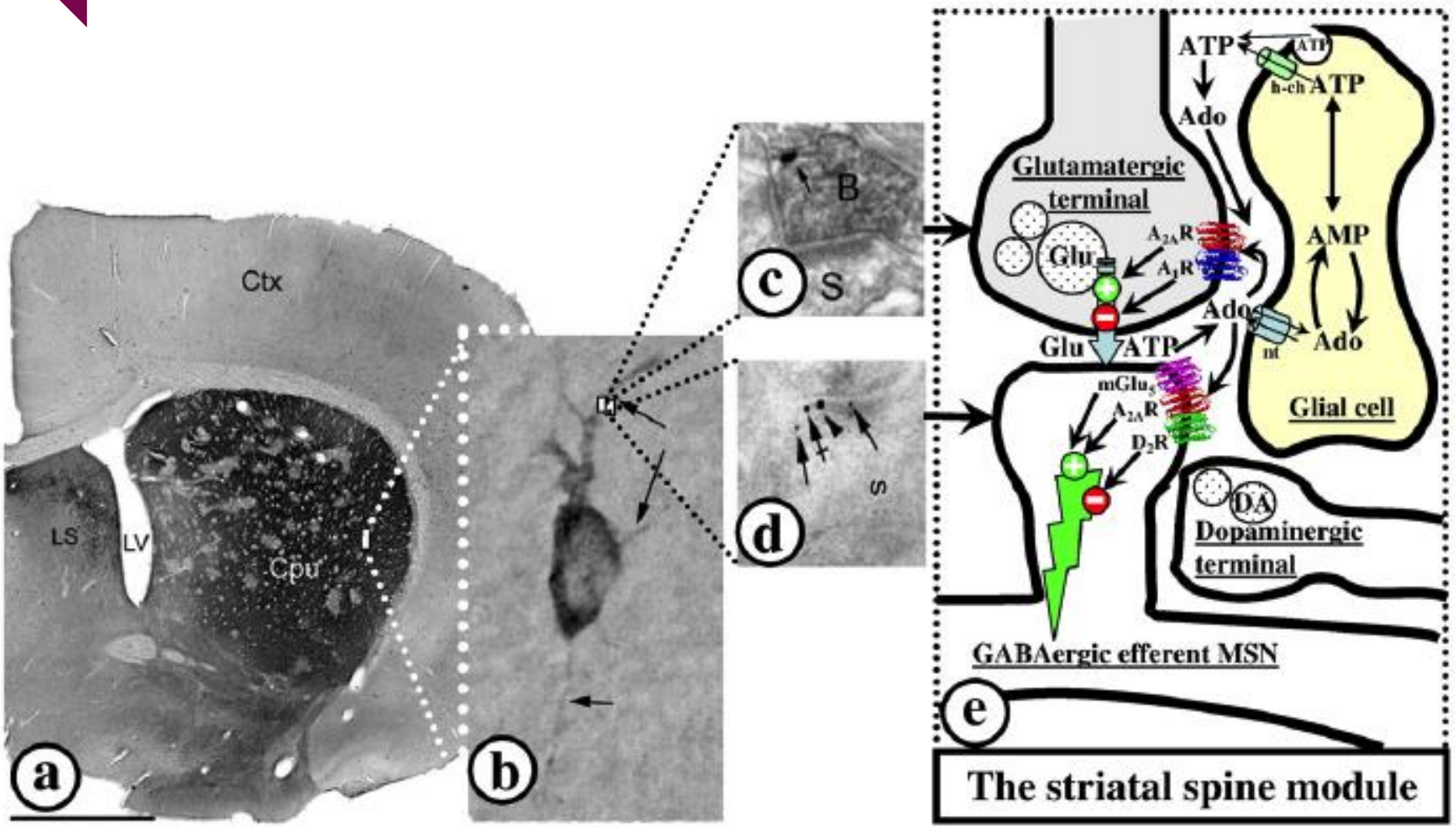




Schwarzschild,Agnati,Fuxe ,Morelli 2006

Adenosine and extrasynaptic glutamate as important VT signals

striatal local circuit of the striato-pallidal GABA neurons identified through immunoreactivity of an $A_{2A}R$ interacting protein NECAB2



Ciruela et al.2011

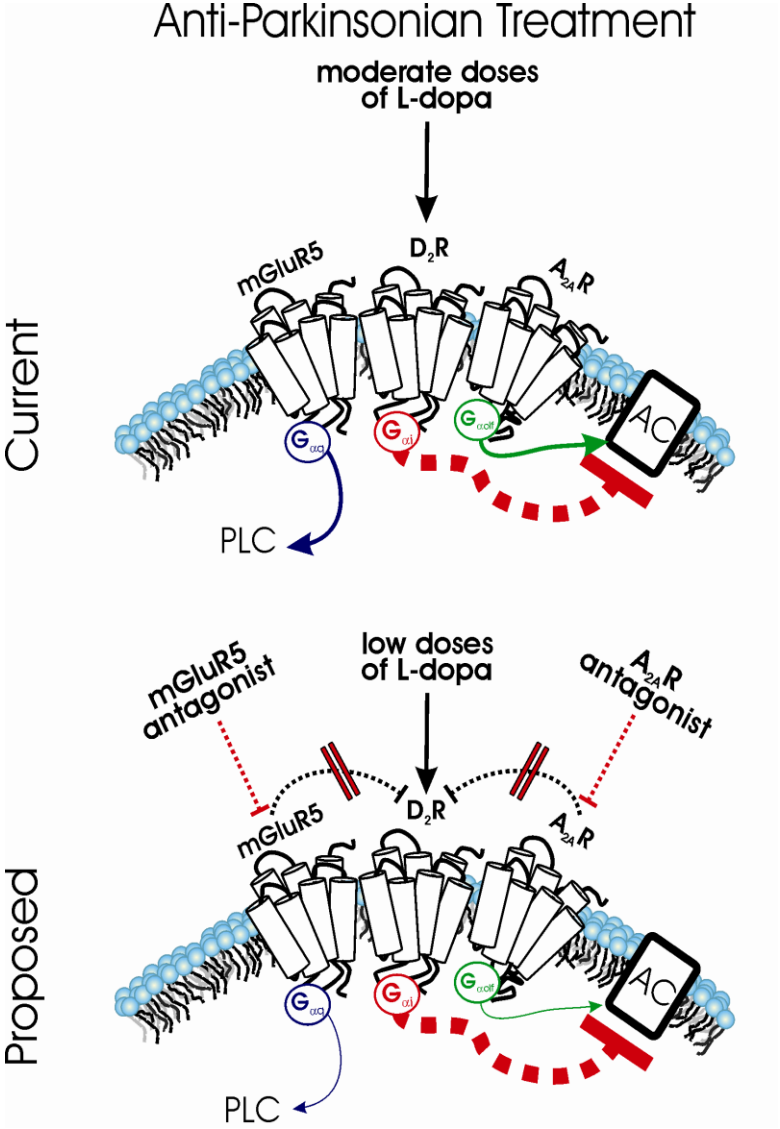
Conclusions

Integration of synaptic and volume transmission strongly involves receptor-receptor interactions in heteroreceptor complexes in the plasma membrane

Heteroreceptor complexes represent a new fundamental principle in molecular medicine for integration of transmitter signals enabling diversity and bias of the receptor protomers and novel strategies for treatment of Parkinson's disease. DA receptor agonists can target many D1 and D2 heteroreceptor complexes

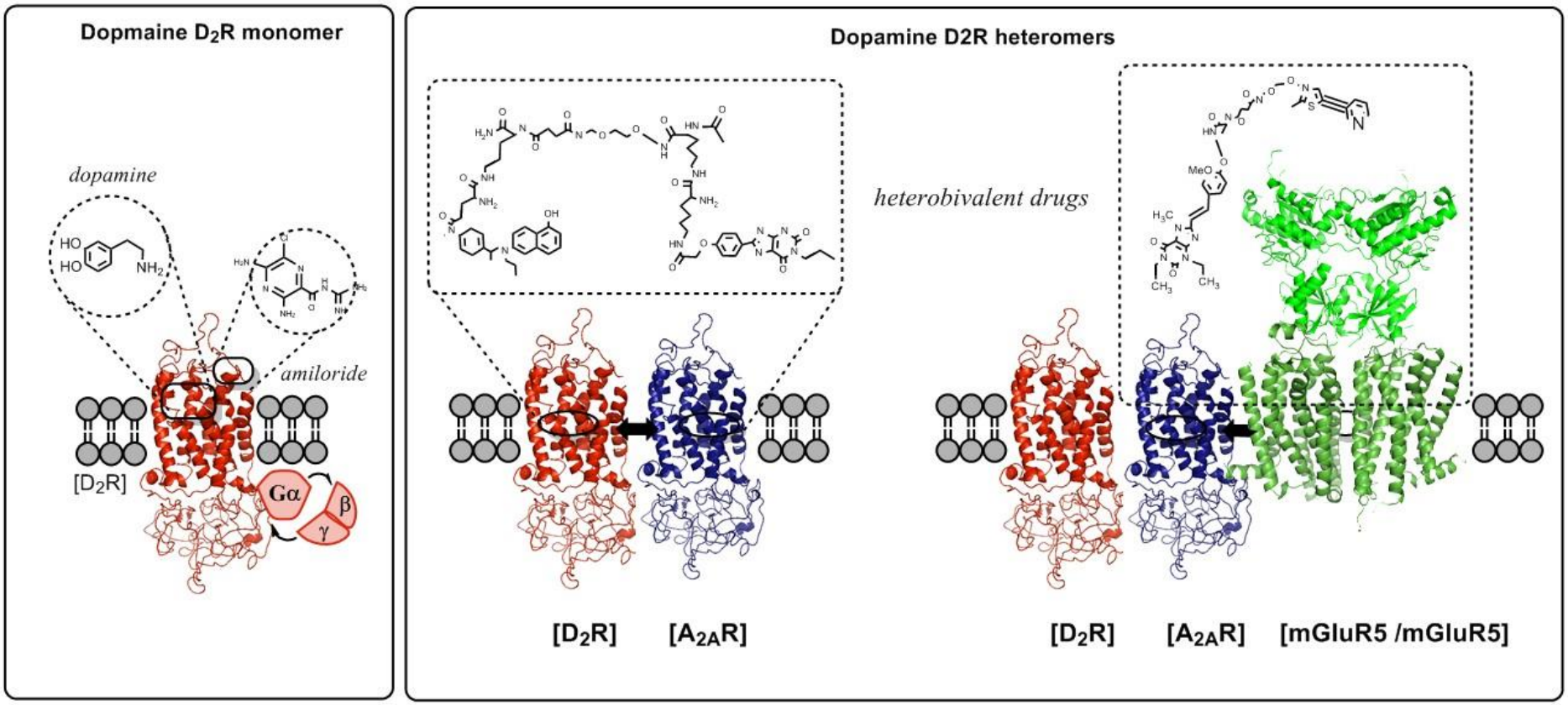
Proposal: *Heterobivalent compounds with A2A and mGluR5 antagonist/NAM pharmacophors and/or small molecules with combined A2A and MGluR5 antagonist activity to be explored as novel antiparkinsonian drugs for early and chronic treatment of PD.*

They can remove the brake on D2R signaling that develops with disease Progression, enhanced by chronic L-DOPA and D2 agonist treatment.



A2A and mGlu5 antagonists can target many A2A and mGlu5 heteroreceptor complexes, respectively

Fuxe et al.2008



Borroto-Escuela and Fuxe 2015

Adenosine A2A receptor containing heteroreceptor complexes

1. The adenosine A1 and A2A heteroreceptor complex and the control of glutamate release in the central nervous system (Ciruela et al.2006)

2. A2A and dopamine D2 heteroreceptor complex (Fuxe et al.2003,Kamiya et al.2003)

3. A2A-D3 heteroreceptor complexes (Torvinen et al.2004)

4. A2A and mGlu5 heteroreceptor complexes including A2A-D2-mGlu5 higher order heteroreceptor complexes (Fuxe et al.1984,Popoli et al.2001 Ferre et al.2002,Fuxe et al.2003,Cabello et al.2009). Hypothesis**: A brake on D2R signaling develops in PD due to A2A-mGluR5 dominance**

5. A2AR and cannabinoid CB1 receptor (CB1R) heteroreceptor complexes including A2A-CB1-D2 higher order heteroreceptor complexes (Carriba et al.2007,2008; Marcellino et al.2008)

Acta Physiol Scand 1987, 131, 625–626

AGNATI, L.F., FUXE, K., BENFENATI, F. & BATTISTINI, N. 1983. Neurotensin *in vitro* markedly reduces the affinity in subcortical limbic [^{3}H]N-propylnorapomorphine binding sites. *Acta Physiol Scand* 119, 459–461.

Neurotensin reduces the affinity of D-2 dopamine receptors in rat striatal membranes

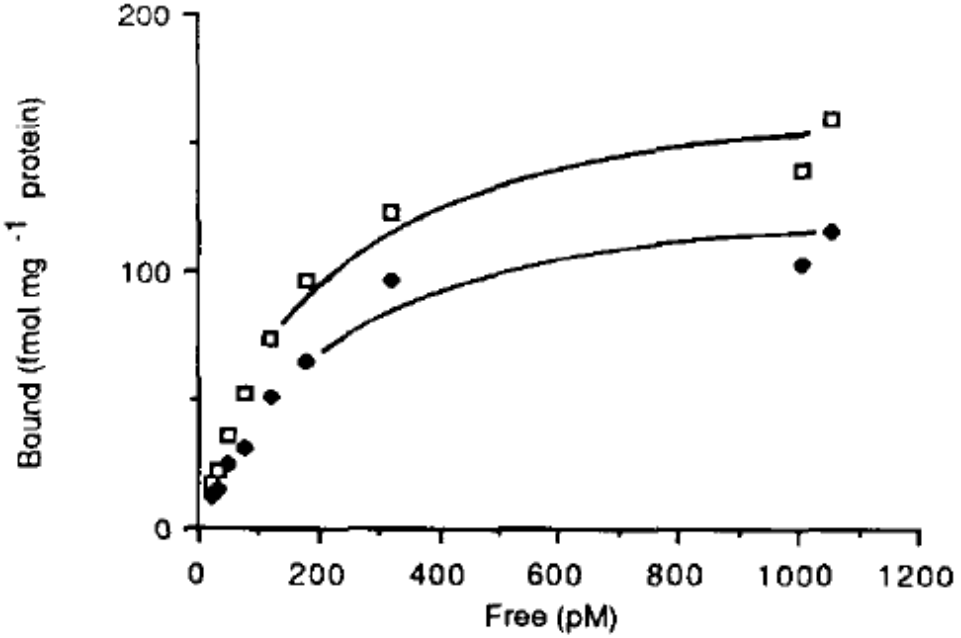
G. VON EULER and K. FUXE
Department of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden

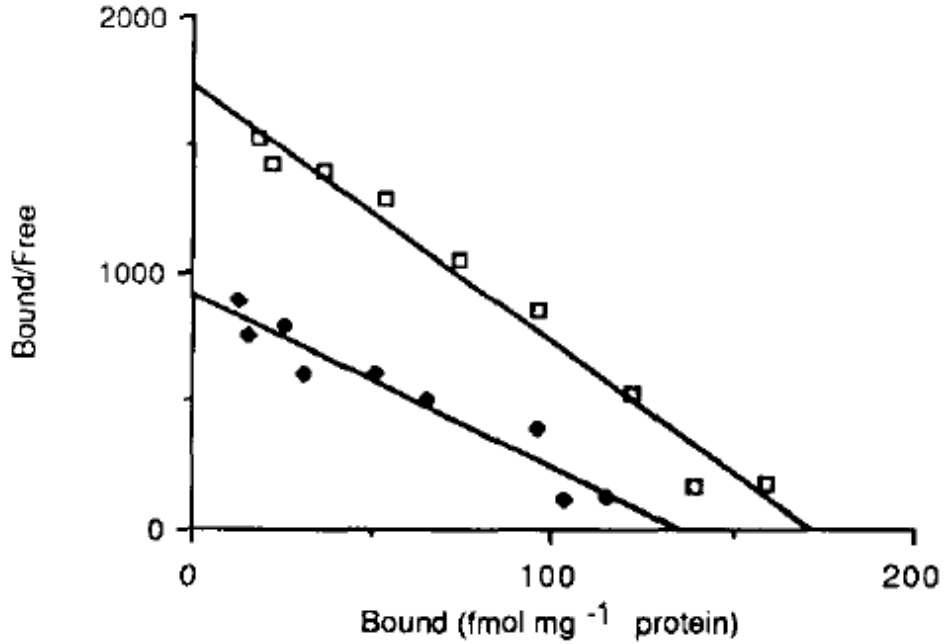
Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5591–5595, June 1992
Neurobiology

Evidence for a substrate of neuronal plasticity based on pre- and postsynaptic neurotensin–dopamine receptor interactions in the neostriatum

(microdialysis/D_2 receptors/dopamine release/γ-aminobutyric acid release)

KJELL FUXE*†, WILLIAM T. O'CONNOR‡, TIZIANA ANTONELLI‡, PETER G. OSBORNE‡, SERGIO TANGANELLI*, LUIGI F. AGNATI§, AND URBAN UNGERSTEDT‡





Neurotensin reduces the affinity of D-2 dopamine receptors in rat striatal membranes

K_d = 98 pM, control

Kd = 149 pM , NT 10nM

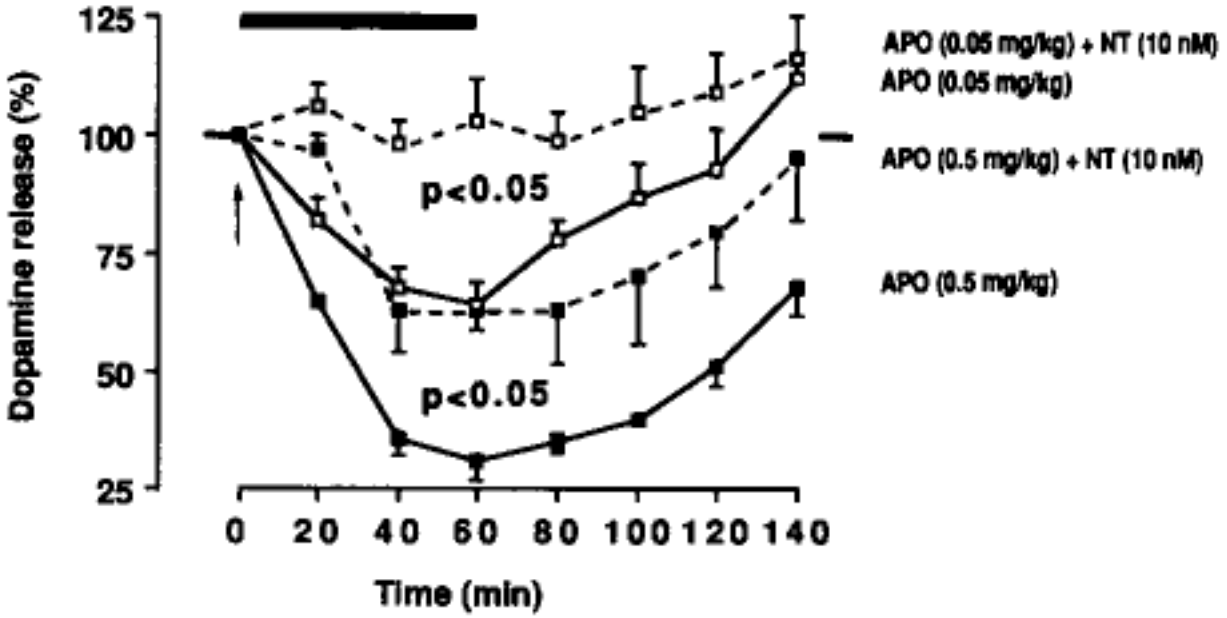
Agnati,Fuxe et al.1983,Von Euler and Fuxe 1987

Brain Research, 502 (1989) 319–324
Elsevier

BRES 14976

Neurotensin counteracts apomorphine-induced inhibition of dopamine release as studied by microdialysis in rat neostriatum

S. Tanganelli[1], G. von Euler[1], K. Fuxe[1], L.F. Agnati[2] and U. Ungerstedt[3]



Ann N Y Acad Sci. 1992;668:186-204

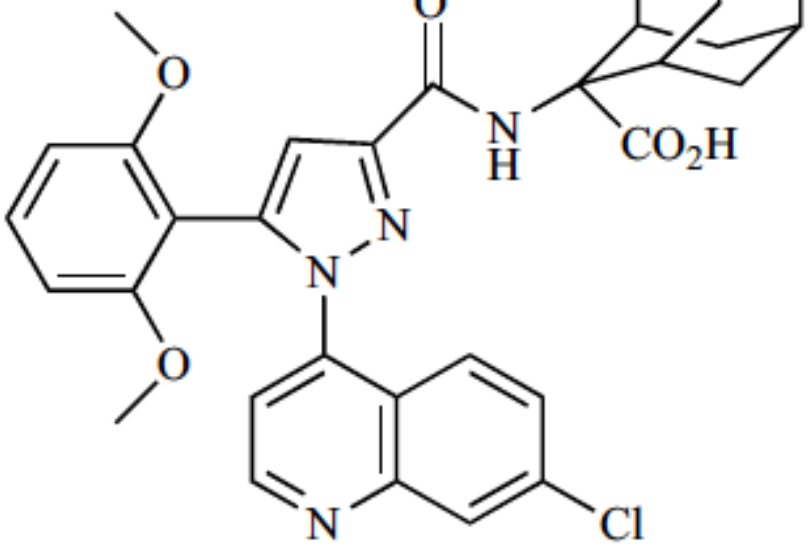
Intramembrane Interactions between Neurotensin Receptors and Dopamine D_2 Receptors as a Major Mechanism for the Neuroleptic-like Action of Neurotensin[a]

K. FUXE,[b,c] G. VON EULER,[b] L. F. AGNATI,[d]
E. MERLO PICH,[d] W. T. O'CONNOR,[b]
S. TANGANELLI,[e] X. M. LI,[b] B. TINNER,[b]
A. CINTRA,[b] C. CARANI,[d] AND F. BENFENATI[d]

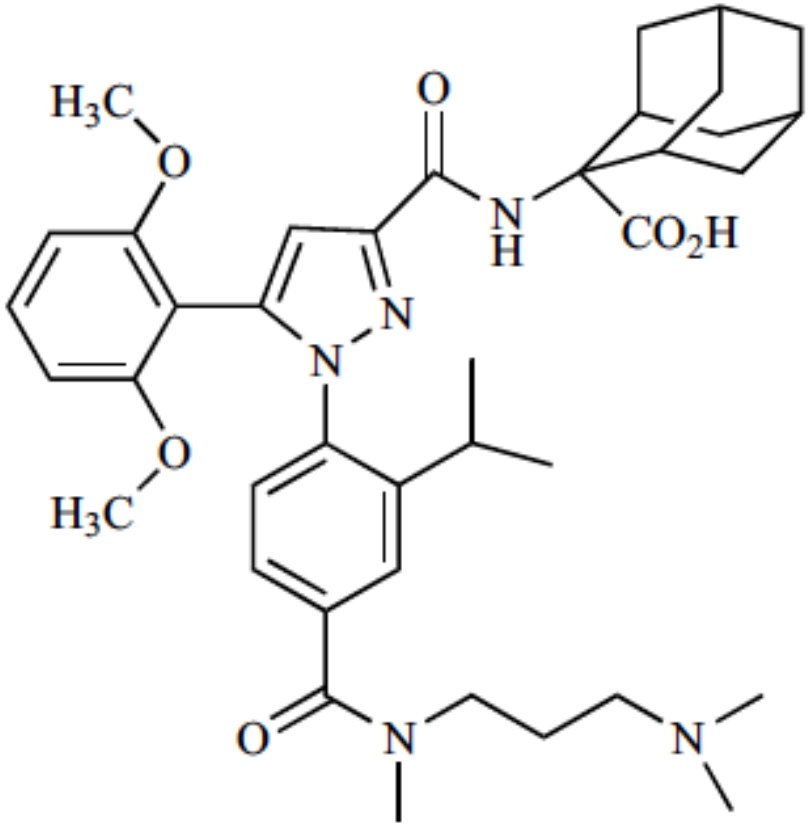
Neuromedin N is a potent modulator of dopamine D2 receptor agonist binding in rat neostriatal membranes. Li XM, Finnman UB, von Euler G, Hedlund PB, Fuxe K. Neurosci Lett. 1993 Jun 11;155(2):121-4.

The C-terminal neurotensin-(8-13) fragment potently modulates rat neostriatal dopamine D2 receptors.Li XM, Von Euler G, Hedlund PB, Finnman UB, Fuxe K. Eur J Pharmacol. 1993 Mar 30;234(1):125-8.

NT receptor antagonists

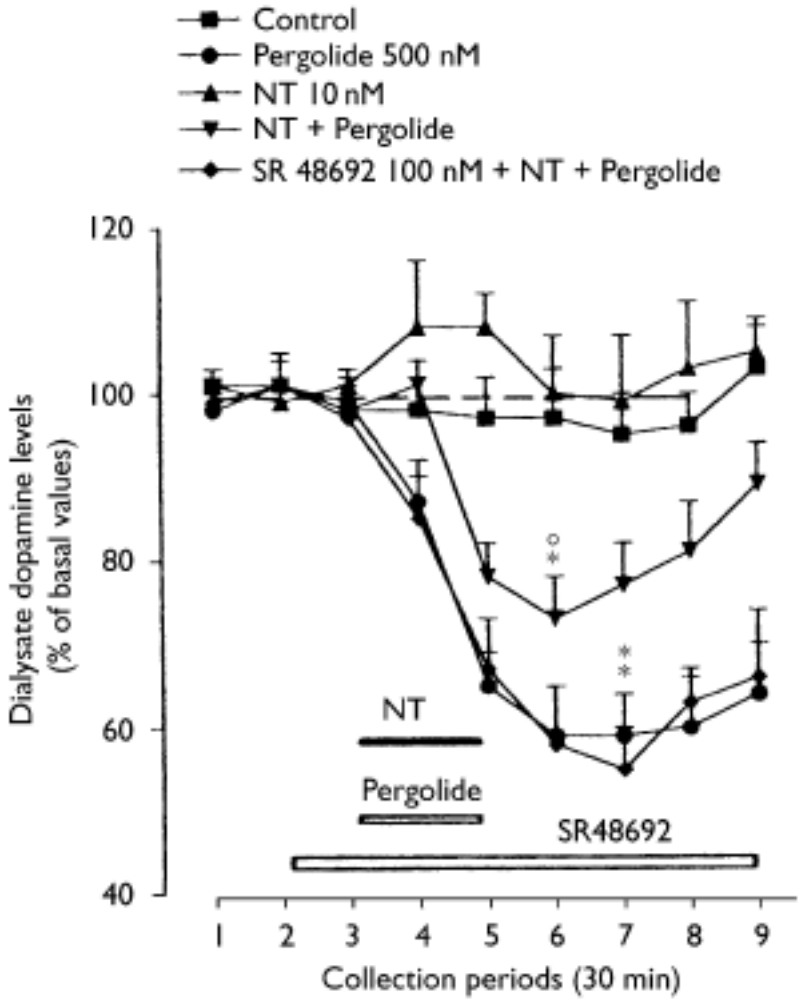


SR48692



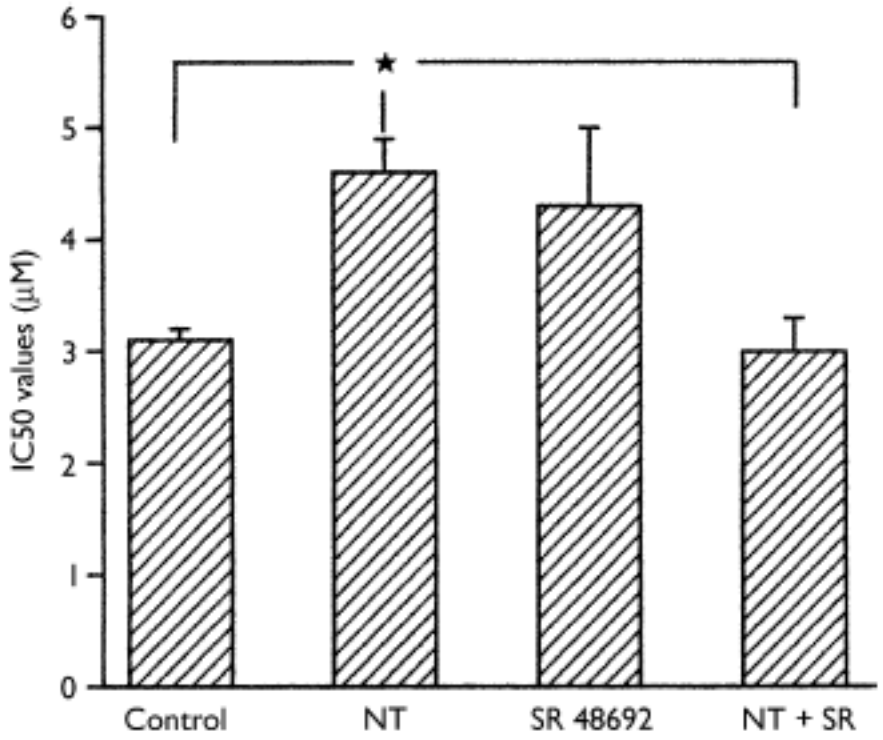
SR142948A

Effects of intrastriatal perfusion of NT alone, pergolide with or without NT, or the NT antagonist SR48692 on striatal DA release in the awake rat

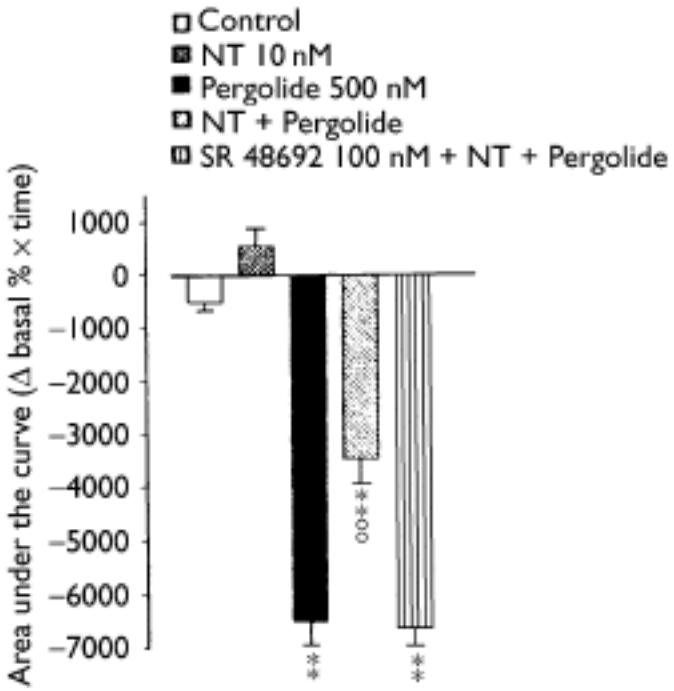


Diaz-Cabiale et al.2002

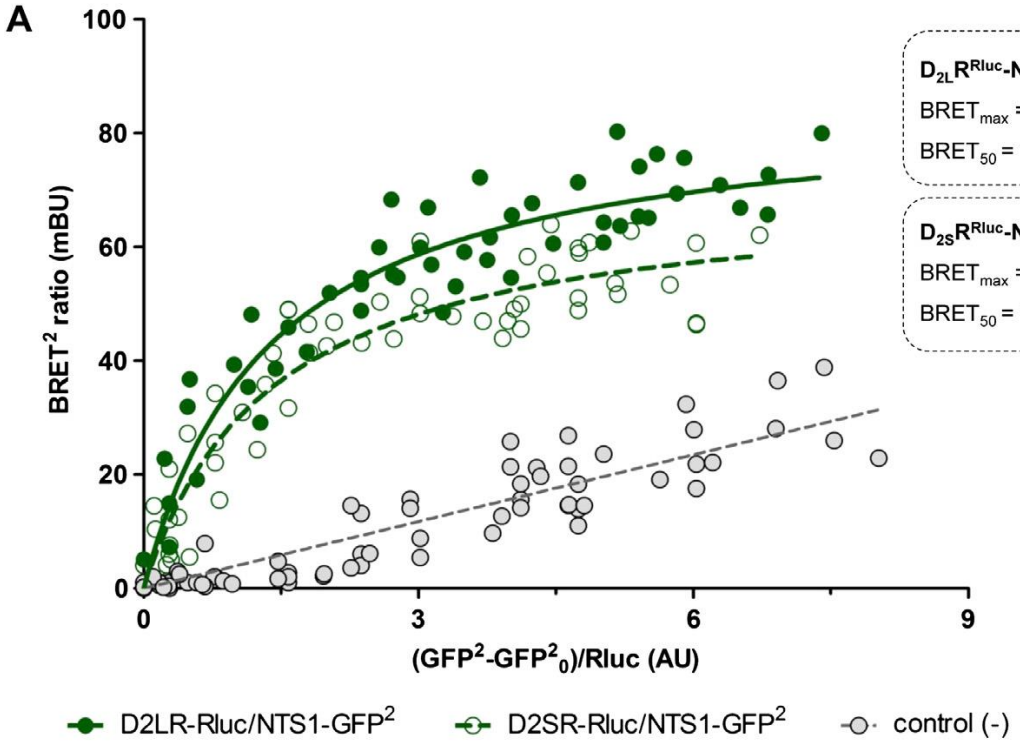
Effects of NT (10 nM) and the NT antagonist SR48692 (100 nM) and in combination on D_2 receptor binding in competitive-inhibition experiments with [^{125}I]-iodosulpiride vs DA in striatal sections

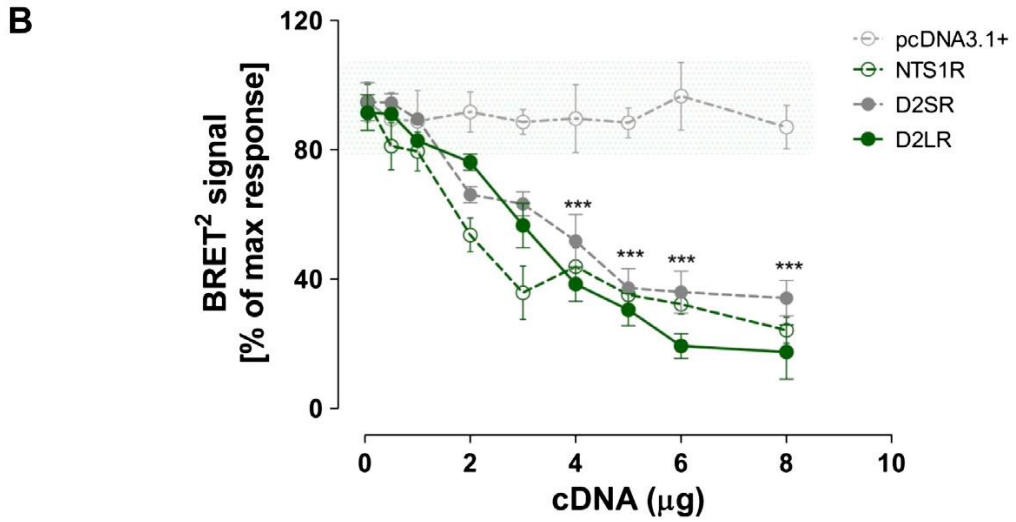


Histograms of the areas under the curves in the microdialysis exp.



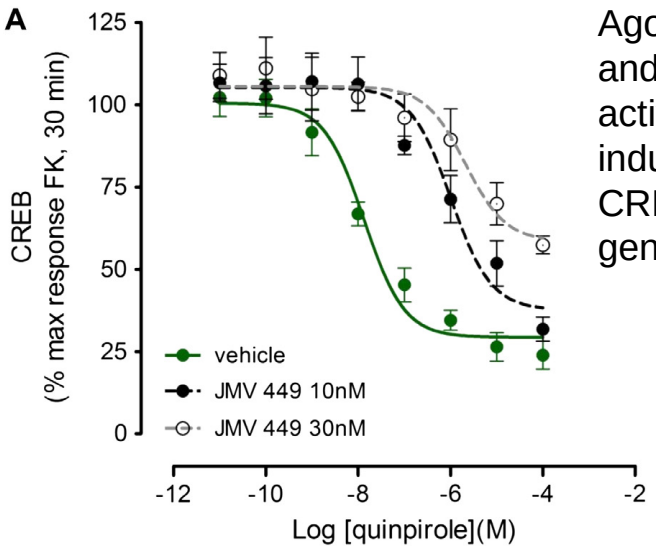
Diaz-Cabiale et al.2002



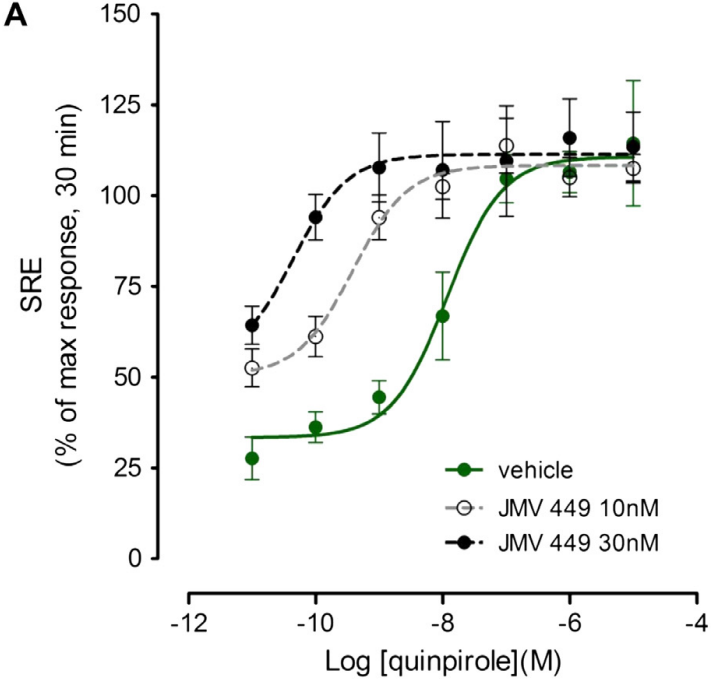


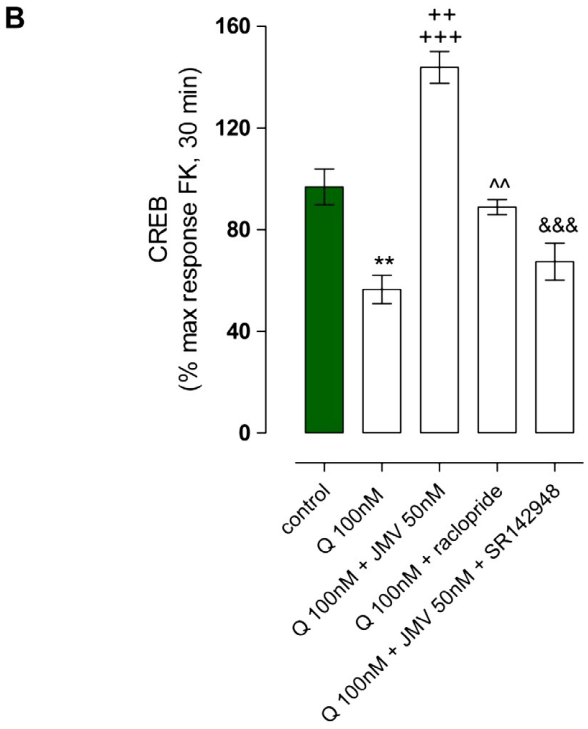
BRET^2 studies on D2R and NTS1R heteromerization in HEK293T cells

Borroto-Escuela et al.2013



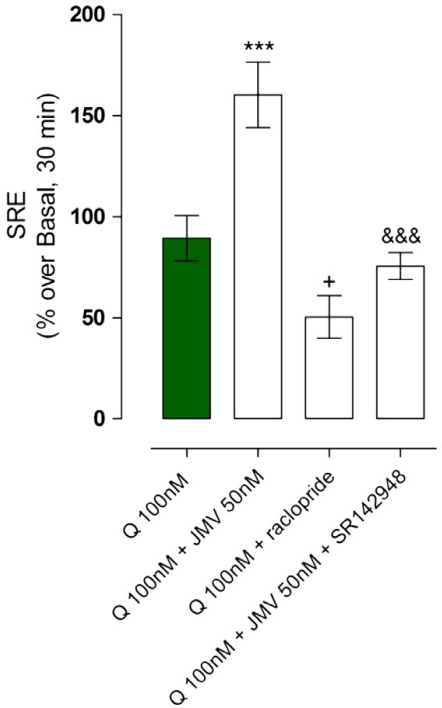
Agonist-induced D2LR and NTS1R receptor activation in a forskolin-induced CRE-luciferase reporter gene assay.



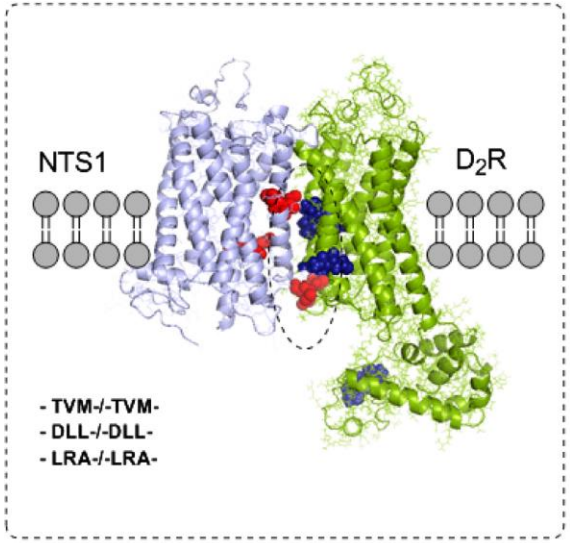


B

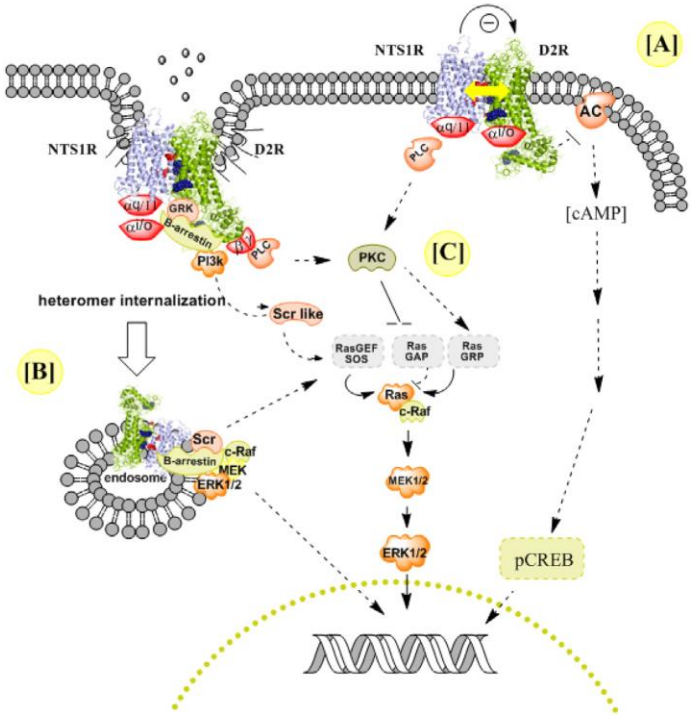
SRE-luciferase reporter gene response after agonist-induced D2LR and NTS1R receptor activation



Borroto-Escuela et al. 2013



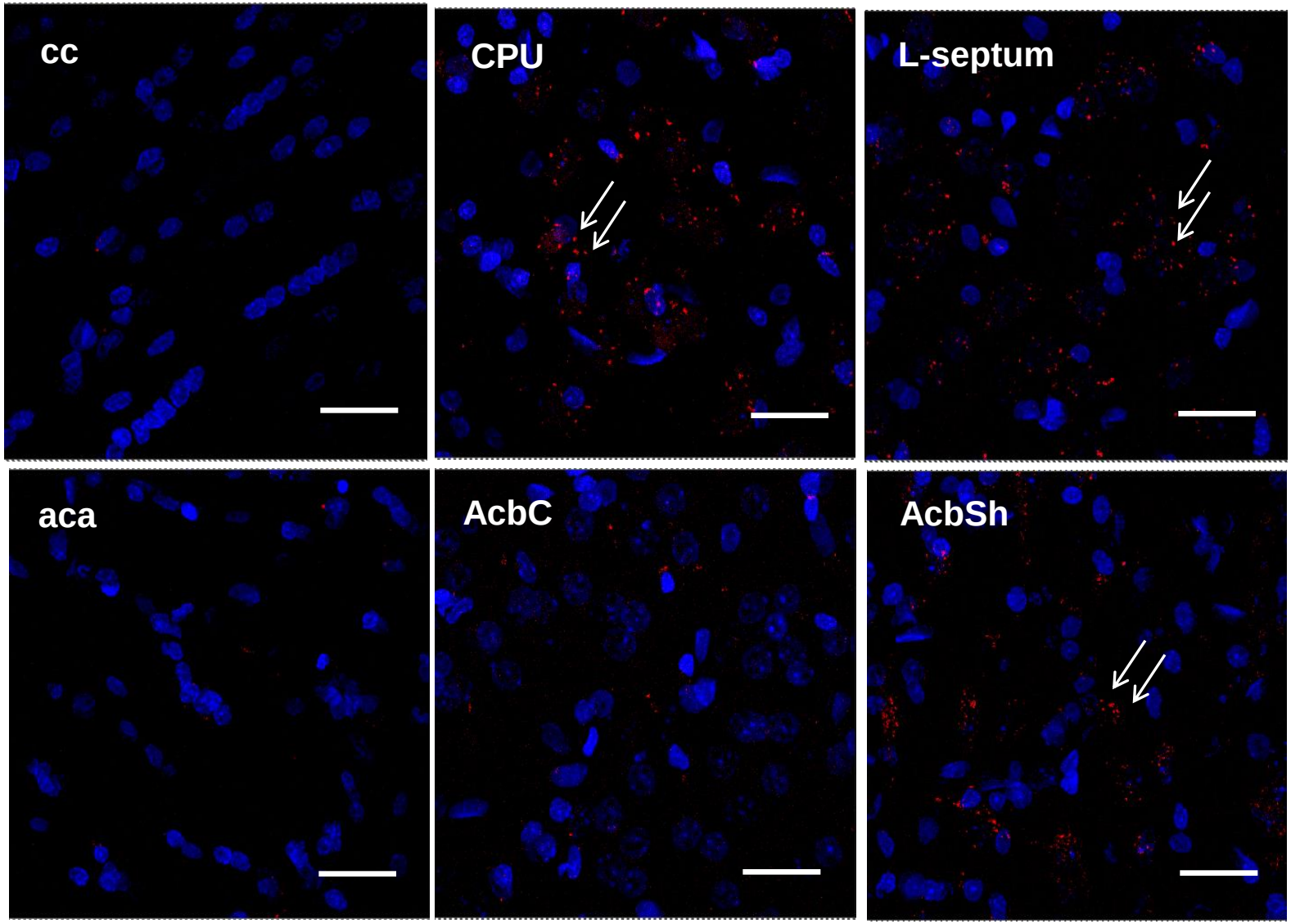
Three-dimensional molecular models of the D2LR and NTSR1 were built by means of the homology modeling program Accelrys Discovery Studio 2.5 (San Diego)



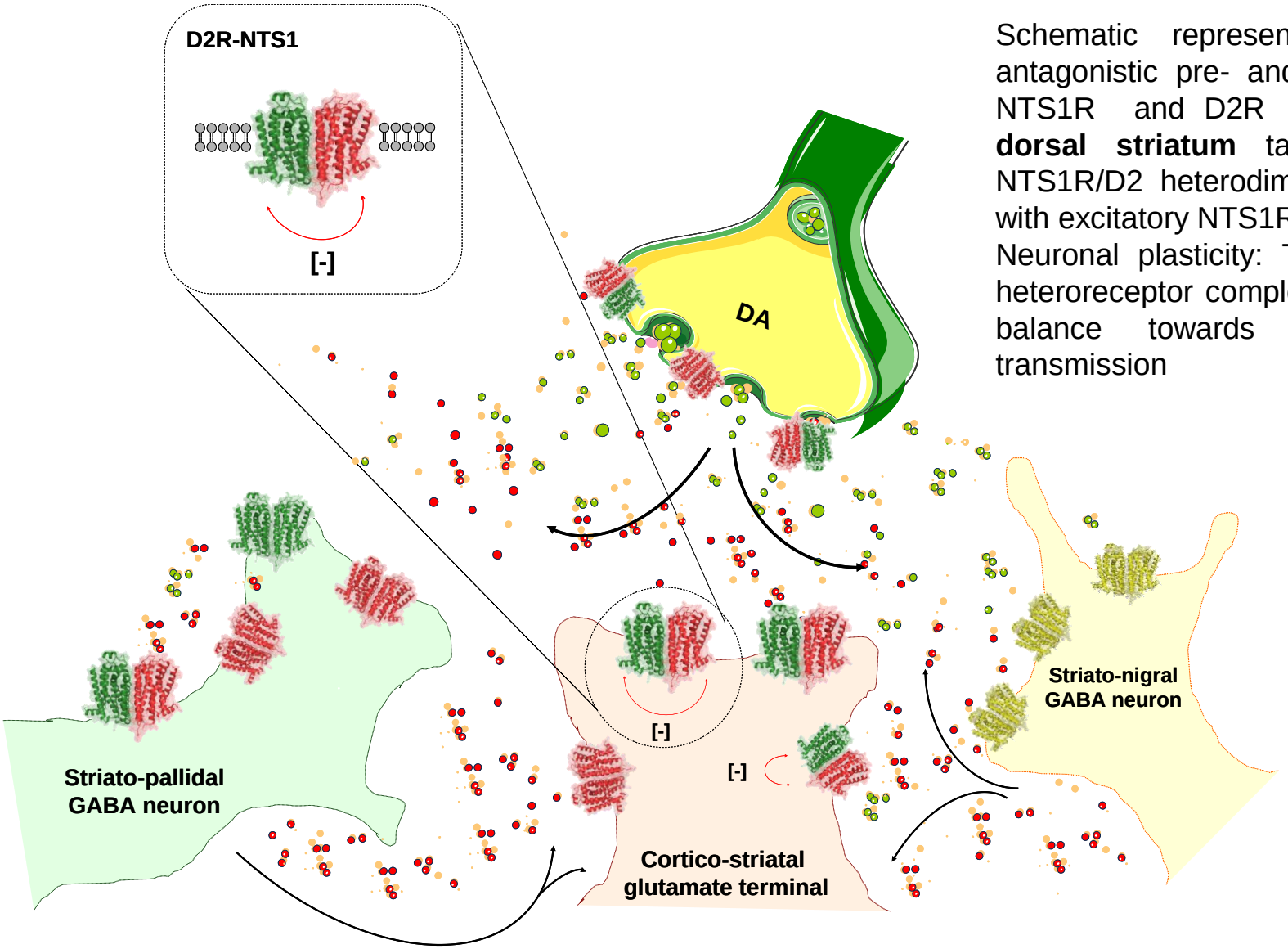
Schematic cross-talk signalling pathways of D2R and NTS1R. The D2R receptor is a GPCR which primarily produces cAMP inhibition via Gi/o proteins (A) but also PLC/PKC activation via beta/gamma G-protein subunits (C). In addition, G protein-coupled receptors couple to the MAPK pathway with pERK activation via both G proteins and b-arrestin recruitment (B). The latter often leads to desensitization and internalization of the GPCR. The NTS1R is a GPCR which primarily activates PLCb via Gq/11 proteins and thus also leads to pERK activation via PKC (C).

Borroto-Escuela et al.2013

Evidence for the existence of D2R-NTS1R heteroreceptor complexes in the CPU, AcbC and AcbSh region of the rat brain



Unpublished work in collaboration with Thorsten Schäfer and Kristina Friedland
These pictures have been taken by Borroto-Escuela 2015



Schematic representation of the antagonistic pre- and postjunctional NTS1R and D2R interactions in **dorsal striatum** taking place in NTS1R/D2 heterodimers in balance with excitatory NTS1R homodimers
Neuronal plasticity: The NTS1R-D2 heteroreceptor complex switches the balance towards D1 vs D2 transmission

Fuxe et al. 1992; Antonelli et al. 2007

ELSEVIER

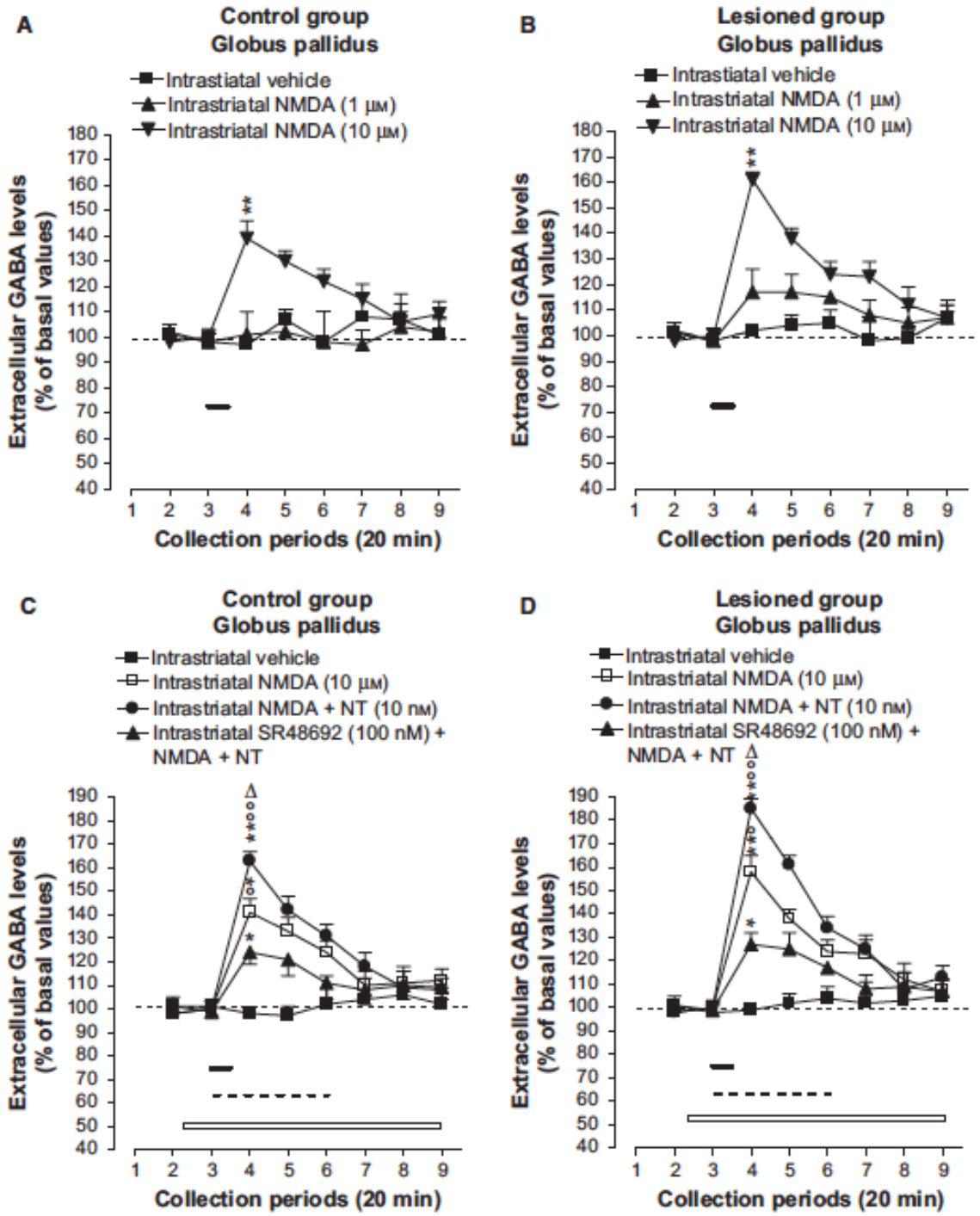
Progress in Neurobiology 83 (2007) 92–109

progress in Neurobiology

www.elsevier.com/locate/pneurobio

Neurotensin receptor mechanisms and its modulation of glutamate transmission in the brain
Relevance for neurodegenerative diseases and their treatment

T. Antonelli [a], K. Fuxe [b,*], M.C. Tomasini [a], E. Mazzoni [a], L.F. Agnati [c], S. Tanganelli [a], L. Ferraro [a]



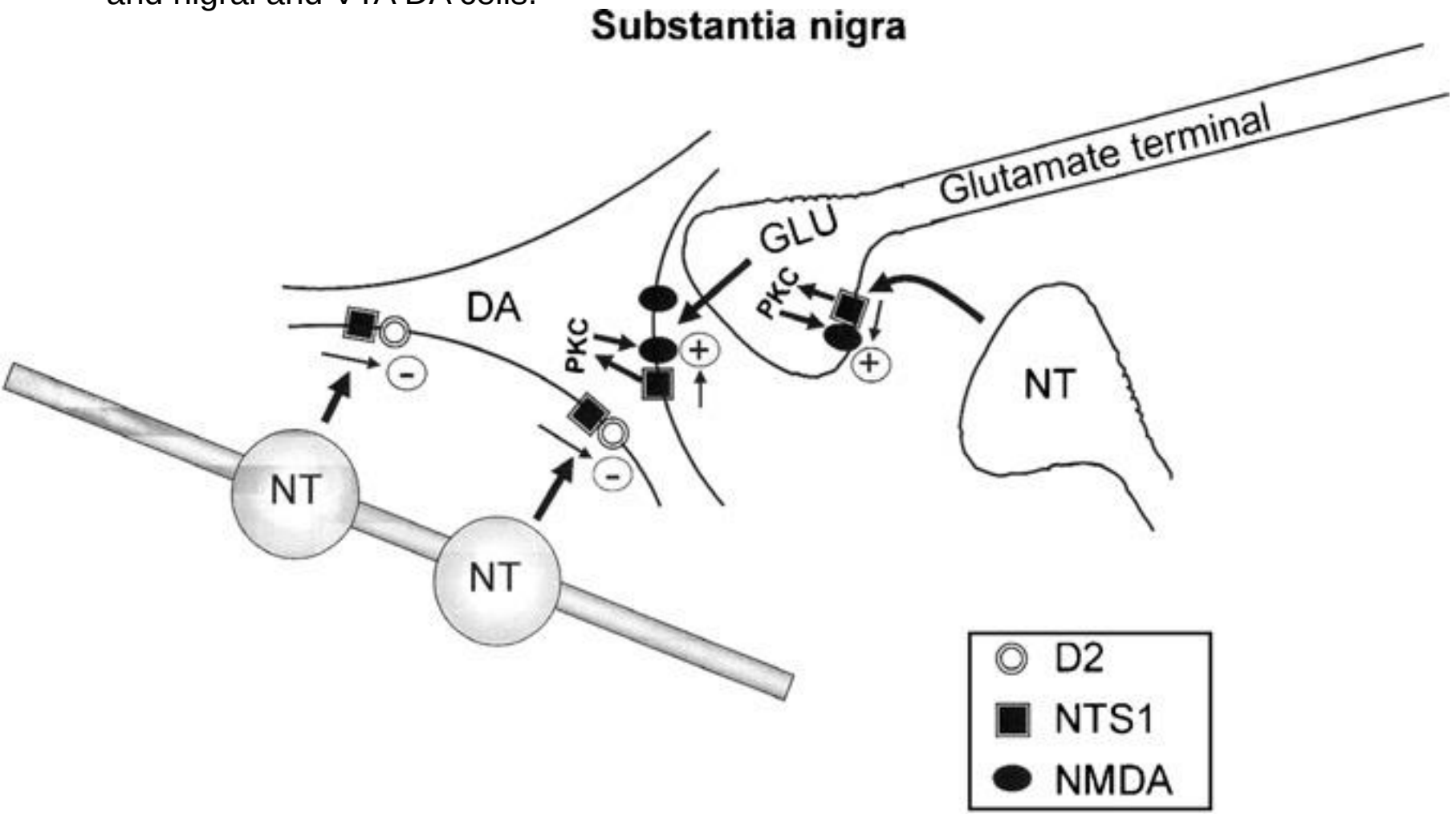
Effect of intrastriatal perfusion with NMDA alone or in combination with neurotensin (NT) or NT plus its receptor antagonist SR48692 on extracellular GABA levels from the ipsilateral globus pallidus of control (i.e. sham-operated) (A, C) and 6-OHDA lesioned (B, D) awake rats

Neurotensin increases endogenous glutamate release in the neostriatum of the awake rat.
Ferraro et al.1995 Synapse. 1995 ;20(4):362-4.

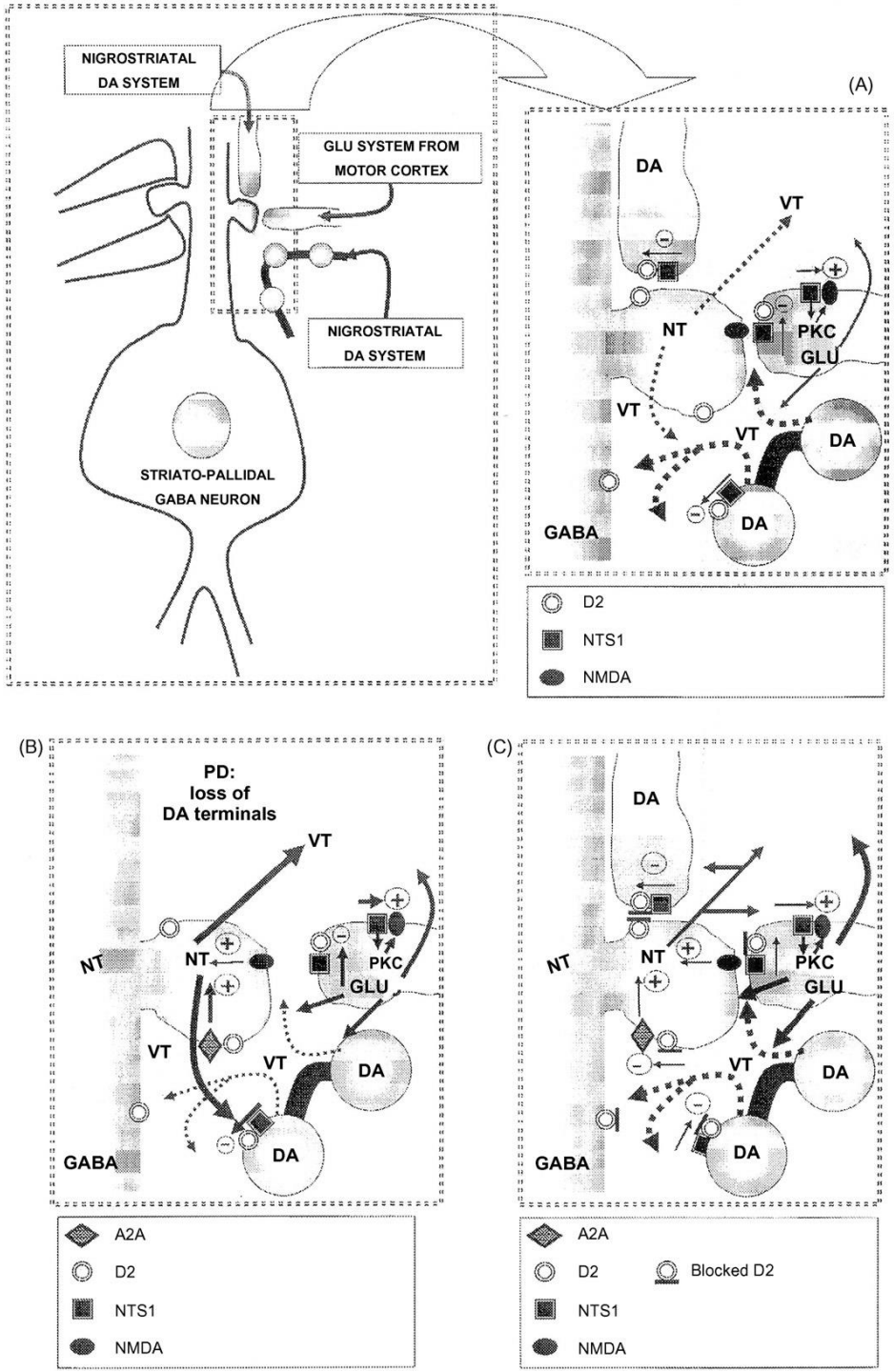
Ferraro et al.2012

Neurotensin (NT) mechanisms in the substantia nigra

Compensatory activation of NT release from nigral NT terminals in response to onset of Parkinson's disease. Increased dopamine (DA) cell firing by, e.g. activation of inhibitory NTS1/D2 autoreceptor interactions and of facilitatory NTS1/NMDA (receptor–receptor and/or cytoplasmic) interactions on glutamate (Glu) terminals and nigral and VTA DA cells.

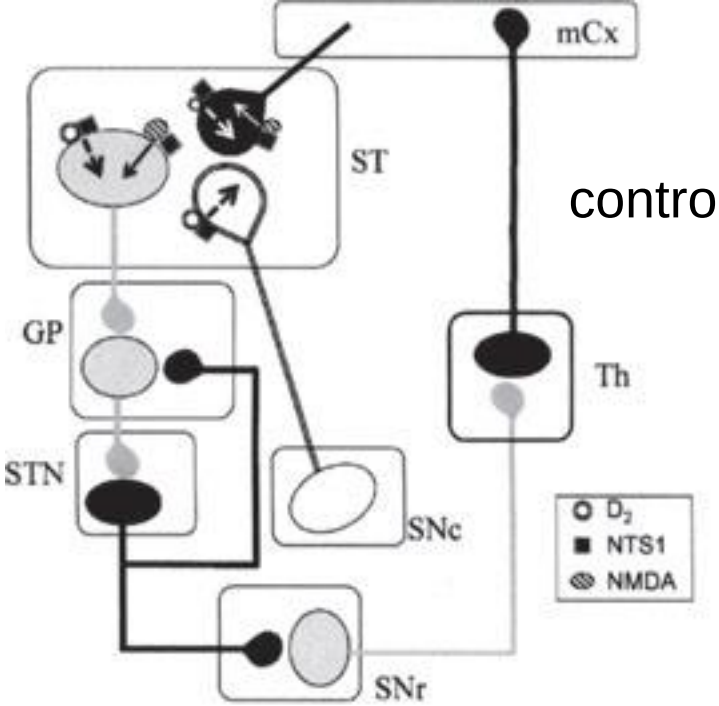


Antonelli et al.2007

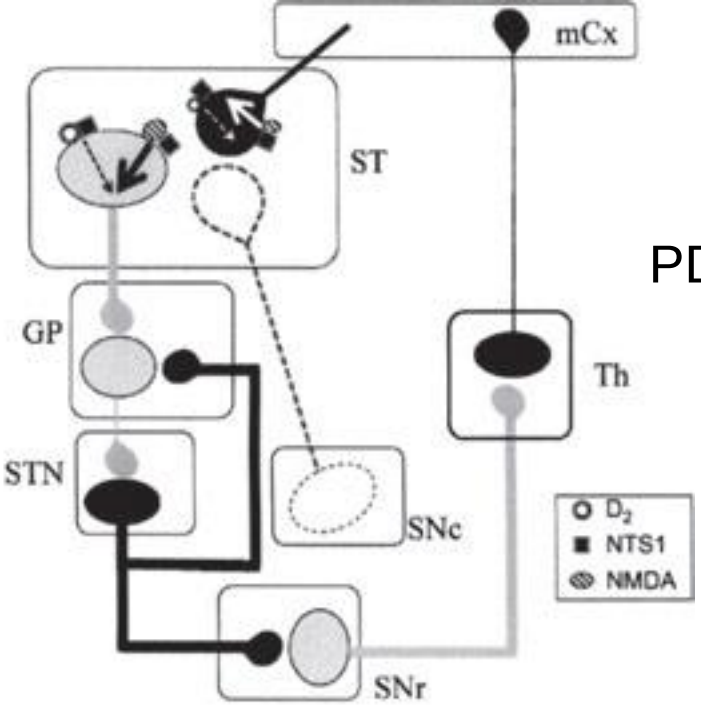


NTS1 containing dopamine (DA) and glutamate (GLU) nerve terminals in local circuits of the striato-pallidal GABA neurons showing the DA, GLU and neurotensin (NT) signalling in the **physiological state**. One DA and one glutamate synapse are indicated. Low NT tone and baseline release of DA and glutamate are indicated. The low density of NTS1 in the striato-pallidal GABA neurons is not indicated. VT, volume transmission.
(Panel B) In **Parkinson's disease,** loss of DA terminals followed by reduced D2 activity leads to an increased synthesis and release of NT from the dendrites and dendritic spines involving increased glutamate Release and increased adenosine A2A signalling due to reduced D2 inhibition of A2A signalling.
Panel C: Treatment with **D2 receptor antagonists.**

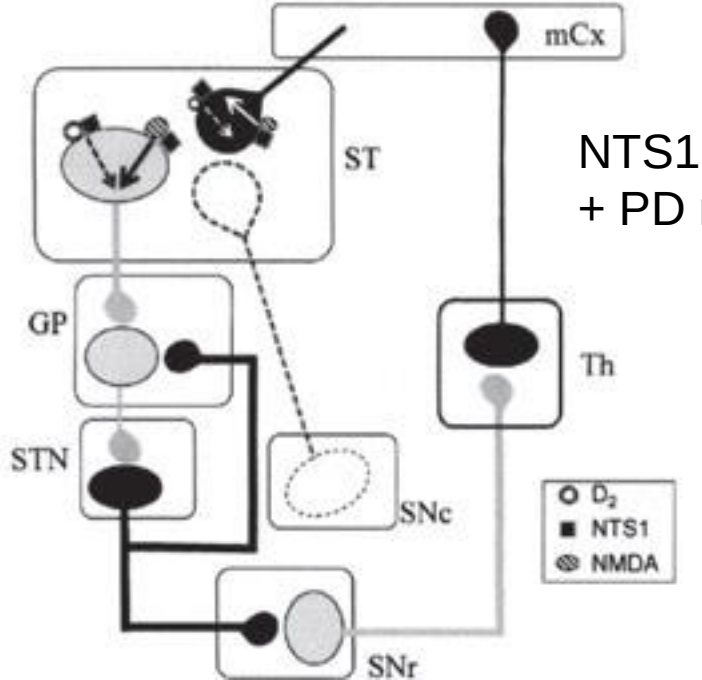
Antonelli et al.2007



control



PD model



NTS1 antagonist
+ PD model

Simplified block diagram of basal ganglia–thalamocortical neuronal circuitry, showing the indirect GABA pathways and illustrating NTS1 receptor activation and its effects on striatopallidal transmission through receptor–receptor interactions with striatal D2 and NMDA receptors in intact (A) and **unilaterally 6- OHDA-lesioned rats** (B).
The effects **of NTS1 antagonists** on the signalling of the NTS1 containing heteromers and associated changes in the activity of the striatopallidal GABA neurons in 6-OHDA-lesioned rats are also shown (C).

Ferraro et al. 2012

NT peptides and neurodegeneration

-

- NT : amplification of glutamate-induced neurotoxicity in mesencephalic dopamine and cortical neurons.

- Mechanisms : Enhancing NTS1-NMDA interactions and antagonistic NTS1/D2 R interactions in the cortico-striatal glutamate terminals, the nigral DA cell bodies and dendrites.

- Potential increases in NT levels in the basal ganglia in Parkinson's disease lead to the NTS1 enhancement of NTS1-NMDA receptor signaling and reduction of D2 autoreceptor signalling in the nigral DA cells may contribute to their neurodegeneration in PD

- The use of selective NTS1 antagonists together with conventional drug treatments could provide a novel therapeutic approach for symptomatic treatment of Parkinson's disease with potential neuroprotective actions.

Novel targets for antiparkinsonian drugs

- **A2A-D2 heteroreceptor complexes** with antagonistic A2A-D2 receptor
- -receptor interactions (mainly striato-pallidal GABA neurons)

- **A2A-D2-mGlu5 heteroreceptor complexes** where A2A and mGlu5 synergize
- to inhibit D2 receptor signaling (mainly striato-pallidal GABA neurons)

- **NTS1-D2 heteroreceptor complexes** with antagonistic NTS1-D2 receptor-receptor interactions (mainly cortico-striatal glutamate terminals, nigro-striatal DA neurons)

- **Putative NTS1-NMDA heteroreceptor complexes** with NTS1 enhancing NMDA receptor signaling (mainly cortico-striatal glutamate terminals, nigral DA nerve cells)

Combined targeting of different pathways in motor circuits can be of special value

Novel molecules: heterobivalent compounds; multitargeting compounds. A2A antagonists/mGlu5 antagonists/NTS1 antagonists targeting distinct D2 heteroreceptor complexes ?