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

Nigro-striatal DA neurons in the world of volume transmission and hetero-receptor complexes . Relevance for Parkinson's disease and its treatment

K. Fuxe, LF.Agnati and D. Borroto-Escuela

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Karolinska Institutet (Sweden)

Dasiel Borroto-Escuela
Anders Jansson
Malin Höistad
Börje Bjelke Jan Kehr

University of Málaga (Spain)

Adelaida de la Calle Jose Narvaez
Alicia Rivera Zaida Diaz-Cabiale

Univ of Complutense and Barcelona (Spain)

Alberto del Arco Rafael Franco
Francisco Mora Francisco Ciruela

New York University (USA)

Charles Nicholson
Kevin Chen
Margerat Rice

Univ of Modena and Palermo

Luigi Agnati Diego Guidolin
Susanna Genedani
Giuseppina Leo –
Natale Belluardo, Giuseppa Mudo

University of Ottawa (Canada)

Kirsten Jacobsen
Barbro Tinner-Staines
William Staines

Charles University (Czech Republic)

Eva Syková ,Tomas Mazel

University of Ferrara (Italy)

Sergio Tanganelli, Luca Ferraro

FROM THE DEPARTMENT OF HISTOLOGY,
KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

EVIDENCE FOR THE EXISTENCE OF MONOAMINE NEURONS IN THE CENTRAL NERVOUS SYSTEM

BY

KJELL FUXE

This thesis is a summary of the following papers:

I. Dahlström, A. and Fuxe, K.: I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* (1964), *62*, Suppl. 232.

II. Dahlström, A. and Fuxe, K.: II. Experimentally induced changes in the intraneuronal amine levels of bulbo-spinal neuron systems. *Acta physiol. scand.* (1965) *64*, Suppl. 247.

III. Fuxe, K.: III. The monoamine nerve terminal. *Z. Zellforsch.* (1965), *65*, 573–596.

IV. Fuxe, K.: IV. The distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.* (1965), *64*, Suppl. 247.

References to these papers will be made by the roman numerals.



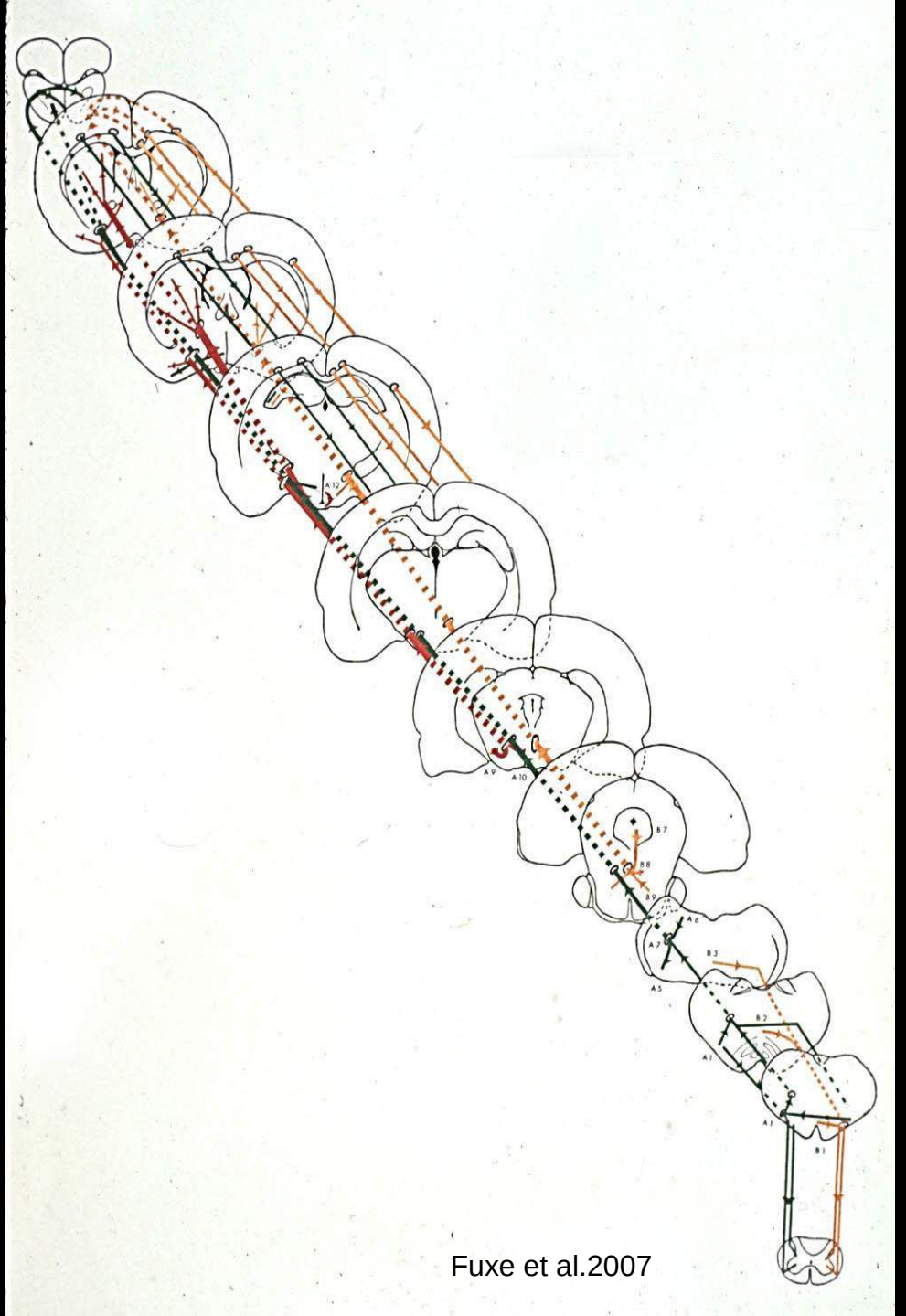
Professor N-Å Hillarp

1965

Schematic description of the DA, NA and 5-HT pathways from 1965

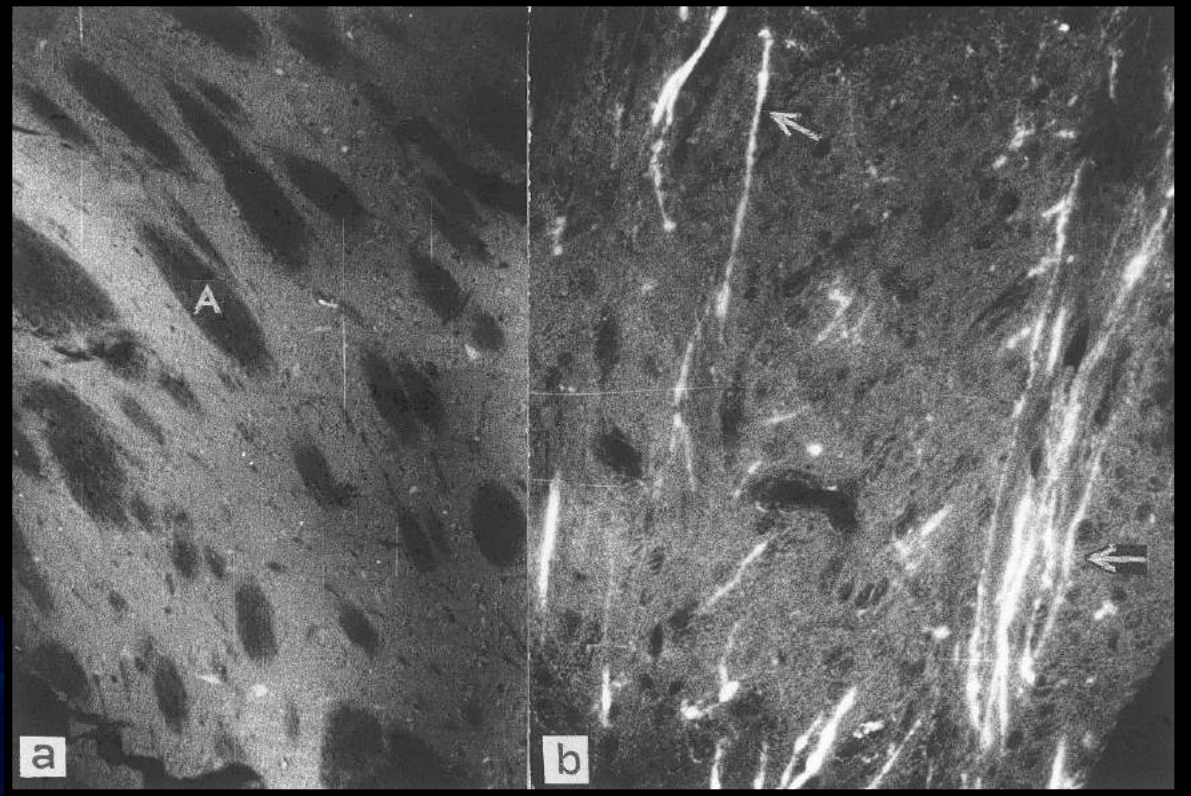
Prepared by Dahlström and Fuxe in 1965 and presented by Fuxe at the second CA Symposium in Milano in July 4-9,1965

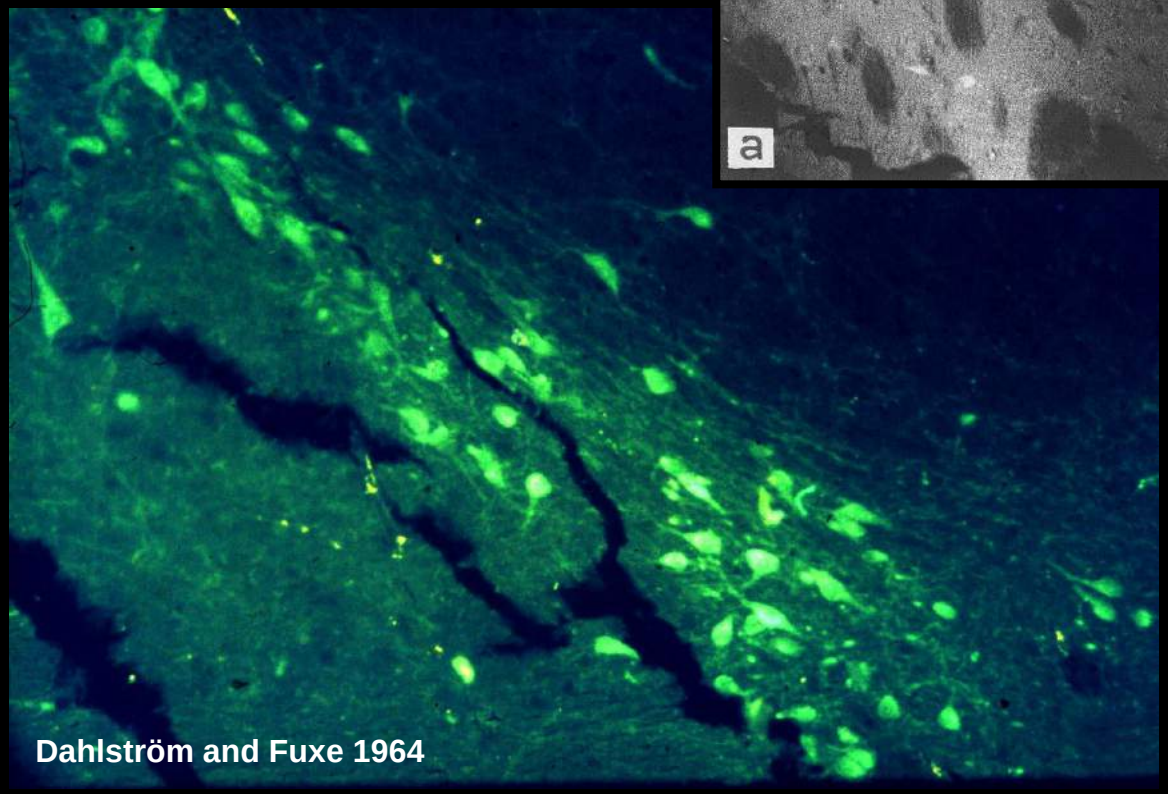
Based on papers in the Fuxe thesis (1965)
Fuxe 1965a,b
Dahlström and Fuxe 1964,1965
and the papers by Fuxe 1963,1964, and
Anden,Carlsson,Dahlström,Fuxe,Hillarp and Larsson 1964



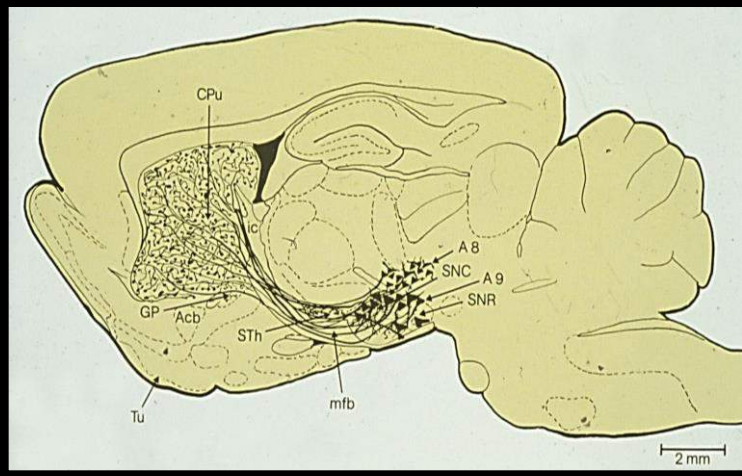
Fuxe et al.2007

Anden et al.1964; Anden, Dahlström, Fuxe and Larsson 1965
Am J Anat 116:329-334





Dahlström and Fuxe 1964



Fuxe, Agnati, Kalia, Goldstein, Andersson and Härfstrand 1985,

Karolinska Institutet

Life Sciences Vol. 3, pp. 523-530, 1964. Pergamon Press, Inc. Printed in the United States.

DEMONSTRATION AND MAPPING OUT OF NIGRO-NEOSTRIATAL DOPAMINE NEURONS

N.-E. Andén, A. Carlsson, A. Dahlström, K. Fuxe,

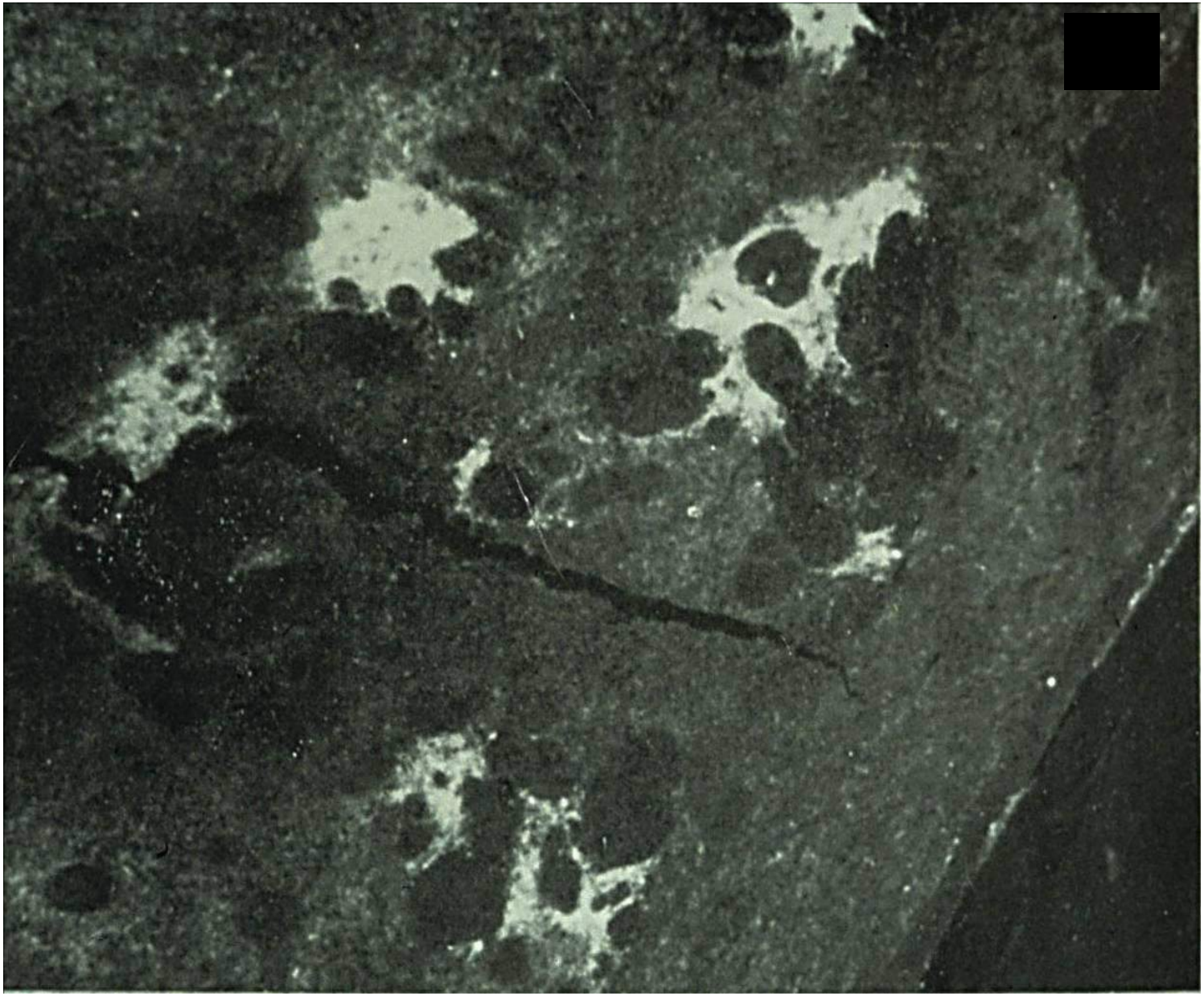
N.-Å. Hillarp and K. Larsson

Departments of Pharmacology and Psychology, University of Göteborg and

Department of Histology, Karolinska Institutet, Stockholm, Sweden

(Received 1 May 1964)

Islands of neostriatal DA nerve terminals (postnatal)



Olson, Seiger and Fuxe 1972, Brain Res 44, 283-288, first reported by Fuxe (1970)



Professor Menek Goldstein

Psychopharmacology: A Generation of Progress, edited by M. A. Lipton, A. DiMascio, and K. F. Killam. Raven Press, New York © 1978.

Mapping Out Central Catecholamine Neurons: Immunohistochemical Studies on Catecholamine-Synthesizing Enzymes

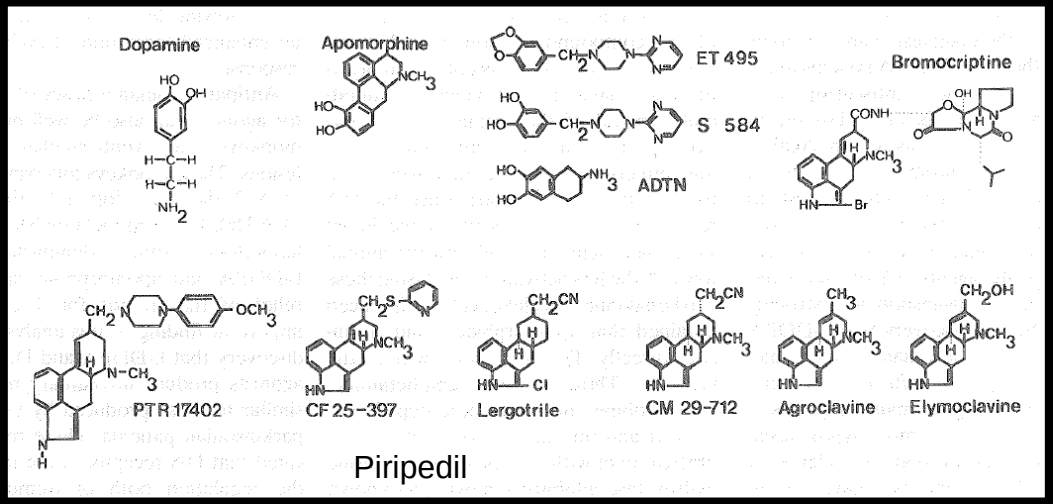
*K. Fuxe, *T. Hökfelt, *L. F. Agnati, *O. Johannson, †M. Goldstein, ‡M. Perez de la Mora, ‡L. Possani, ‡R. Tapia, §L. Teran, and ‖R. Palacios

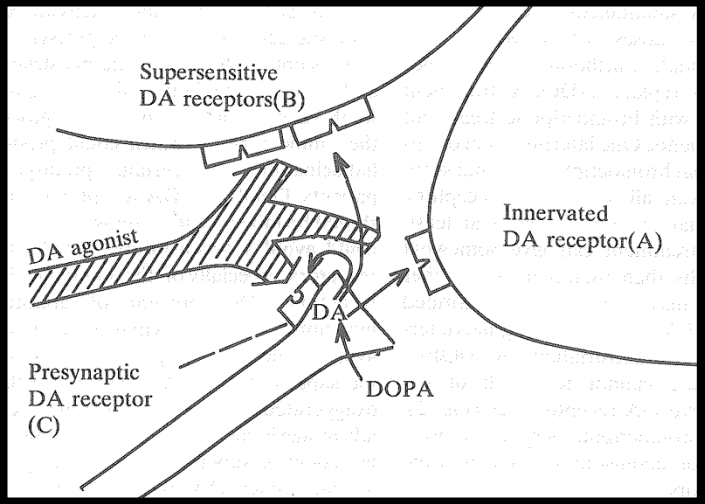
TINS – January 1979

Neuroviews

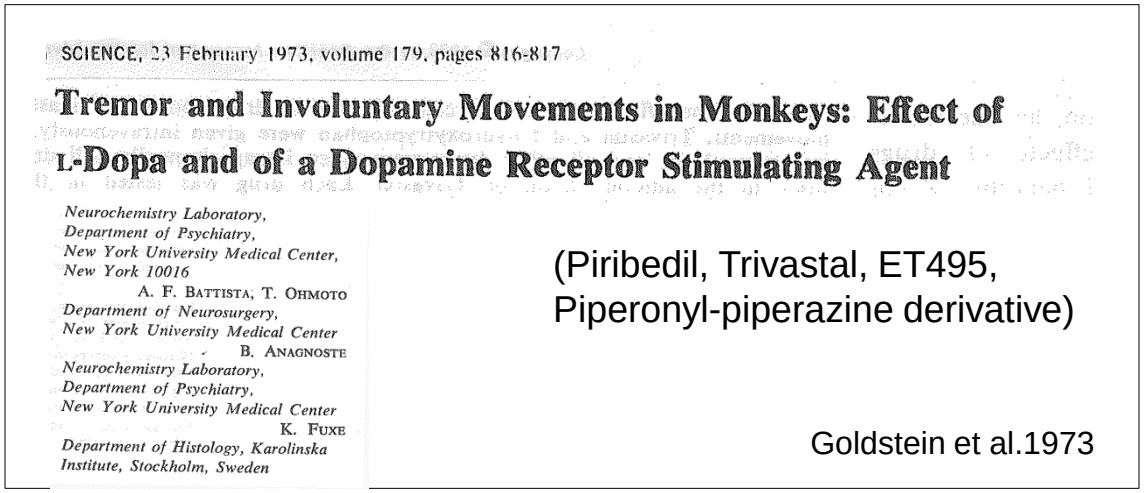
Dopamine receptor agonists in brain research and as therapeutic agents

Kjell Fuxe





DA receptor agonist actions of ET495 and 2-Br-alpha-ergocryptine



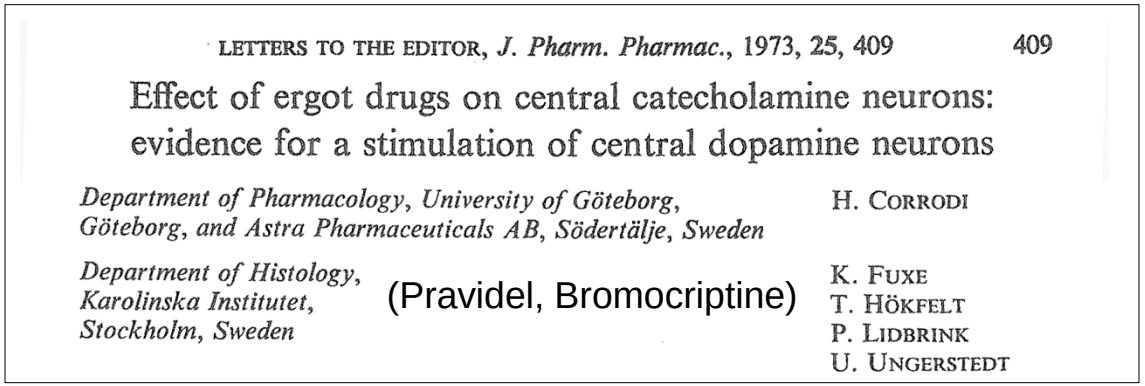
SCIENCE, 23 February 1973, volume 179, pages 816-817

Tremor and Involuntary Movements in Monkeys: Effect of L-Dopa and of a Dopamine Receptor Stimulating Agent

Neurochemistry Laboratory, Department of Psychiatry, New York University Medical Center, New York 10016
A. F. BATTISTA, T. OHMOTO
Department of Neurosurgery, New York University Medical Center
B. ANAGNOSTE
Neurochemistry Laboratory, Department of Psychiatry, New York University Medical Center
K. FUXE
Department of Histology, Karolinska Institute, Stockholm, Sweden

(Piribedil, Trivastal, ET495, Piperonyl-piperazine derivative)

Goldstein et al.1973



LETTERS TO THE EDITOR, *J. Pharm. Pharmac.*, 1973, 25, 409 409

Effect of ergot drugs on central catecholamine neurons: evidence for a stimulation of central dopamine neurons

Department of Pharmacology, University of Göteborg, Göteborg, and Astra Pharmaceuticals AB, Södertälje, Sweden H. CORRODI

Department of Histology, Karolinska Institutet, Stockholm, Sweden K. FUXE, T. HÖKFELT, P. LIDBRINK, U. UNGERSTEDT

(Pravidel, Bromocriptine)

cyclic peptide group of ergoline derivatives (ergopeptides).

Evidence for a new type of dopamine receptor stimulating agent.
Corrodi H, Fuxe K, Ungerstedt U.
J Pharm Pharmacol. **1971** 23 :989-91

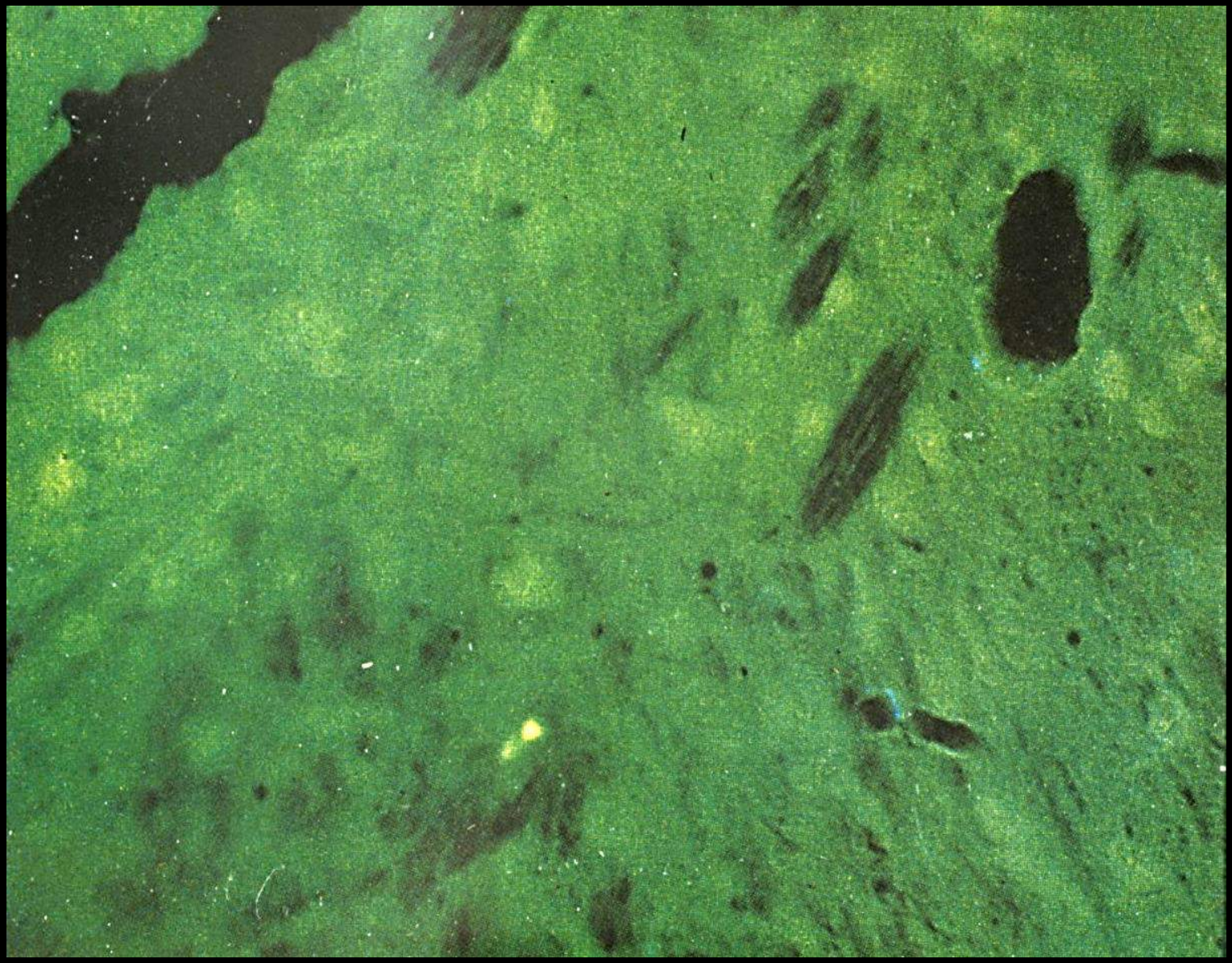
ET495 and brain catecholamine mechanisms: evidence for stimulation of dopamine receptors.

Corrodi H, Farnebo LO, Fuxe K, Hamberger B, Ungerstedt U.
Eur J Pharmacol. **1972** 20 :195-204

Ergocornine and 2-Br-alpha-ergocryptine. Evidence for prolonged dopamine receptor stimulation.
Fuxe K, Corrodi H, Hökfelt T, Lidbrink P, Ungerstedt U.
Med Biol. **1974** 52 :121-32

Appearance of extraneuronal greenish DA fluorescence among midbrain DA nerve cells after amphetamine in reserpine-nialamide-L-dopa treated rats

Amphetamine: enhancement of DA release (Carlsson,Fuxe et al.1966) and reversal of DA uptake



Fuxe and Ungerstedt 1970

Volume Transmission (VT)

A widespread mode of intercellular communication that occurs in the extracellular fluid and in the cerebrospinal fluid (CSF) of the brain with VT signals moving from source to target cells via energy gradients leading to diffusion and convection (flow). Source: all cells in CNS

channels : diffuse ; privacy : reserved ; safety : low

Connectivity : dynamic .Several forms of VT.

Wiring Transmission

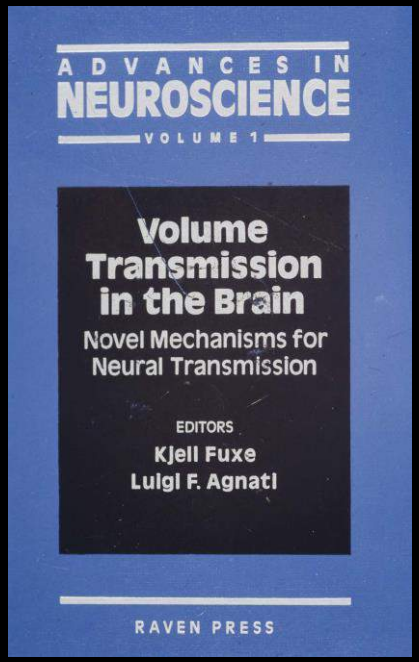
Prototype : synaptic transmission

Channels : private ; privacy : reserved ; safety : high

Connectivity : static and/or dynamic

One subtype : gap junctions

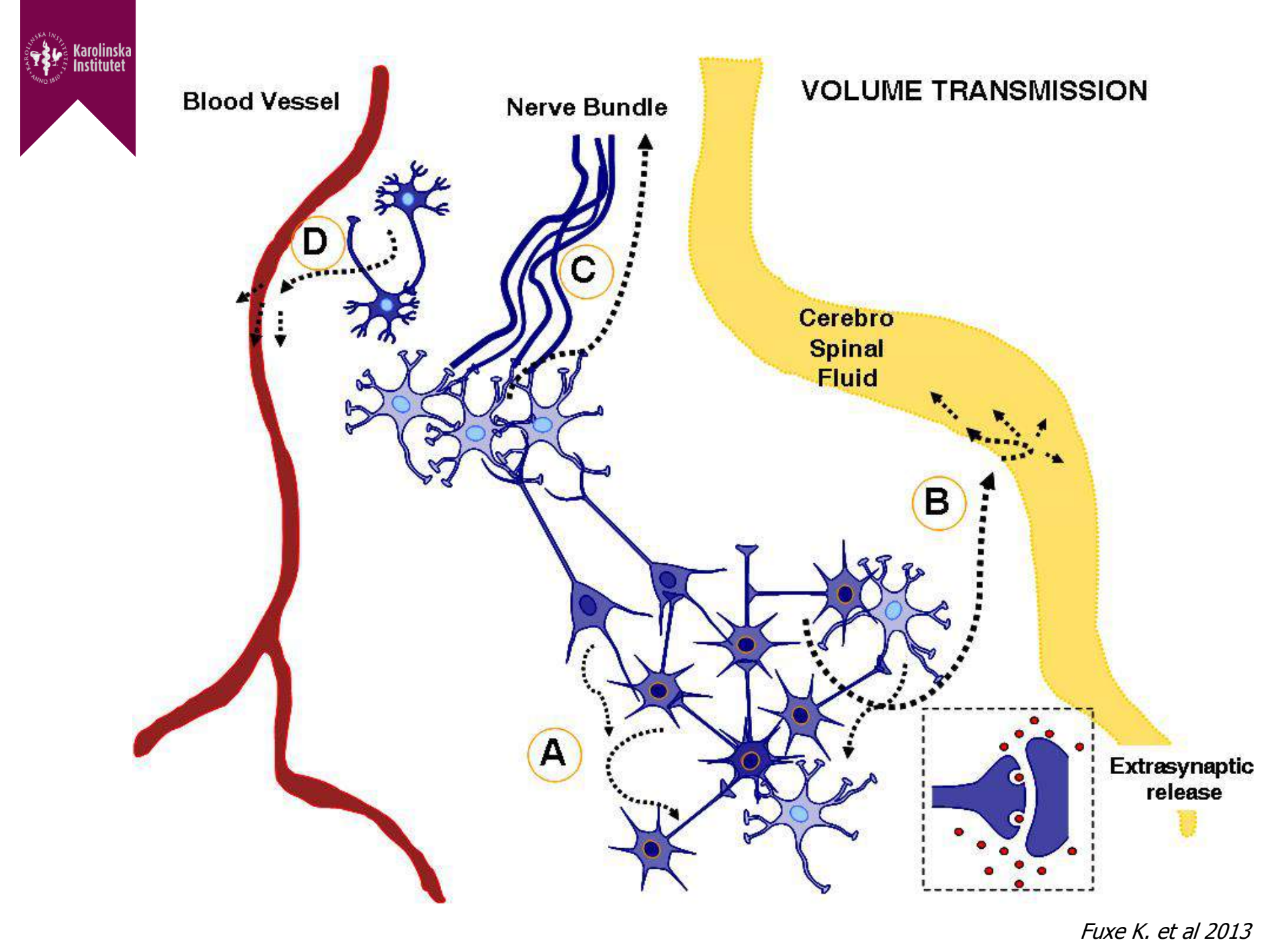
Agnati, Fuxe et al. 1986 ;Fuxe and Agnati 1988, 1991,2009



Volume Transmission (VT): Extrasynaptic and Widespread (diffuse) Communication in the extracellular space via extracellular channel plexa and in the CSF

Channel	Main Features
Local extracellular channel plexa mainly in the um range (**extrasynaptic VT**)	Extracellular fluid transmission linked to synaptic transmission. Through extrasynaptic release and synapse spillover involving a role of local field potentials, the ions and transmitters diffuse at the local circuit level to influence nerve cells , glial cells and endothelial cells, mainly via targeting local receptors
Extracellular fluid pathways mainly along paravascular and paraaxonal extracellular channels. **Long distance VT**, also above 1 mm Transmitter-receptor mismatches	Extracellular fluid transmission; long-distance diffusion and flow of signals in the ECF involving mainly peptides and proteins targeting distant receptors . Flow is generated by temperature and pressure gradients
Cerebrospinal fluid pathways. **CSF VT** Long distance migration,	CSF transmission; long-distance transmission of signals in the CSF, facilitated by flow generated by the arterial pulse

Fuxe et al.2012, Agnati and Fuxe 2014



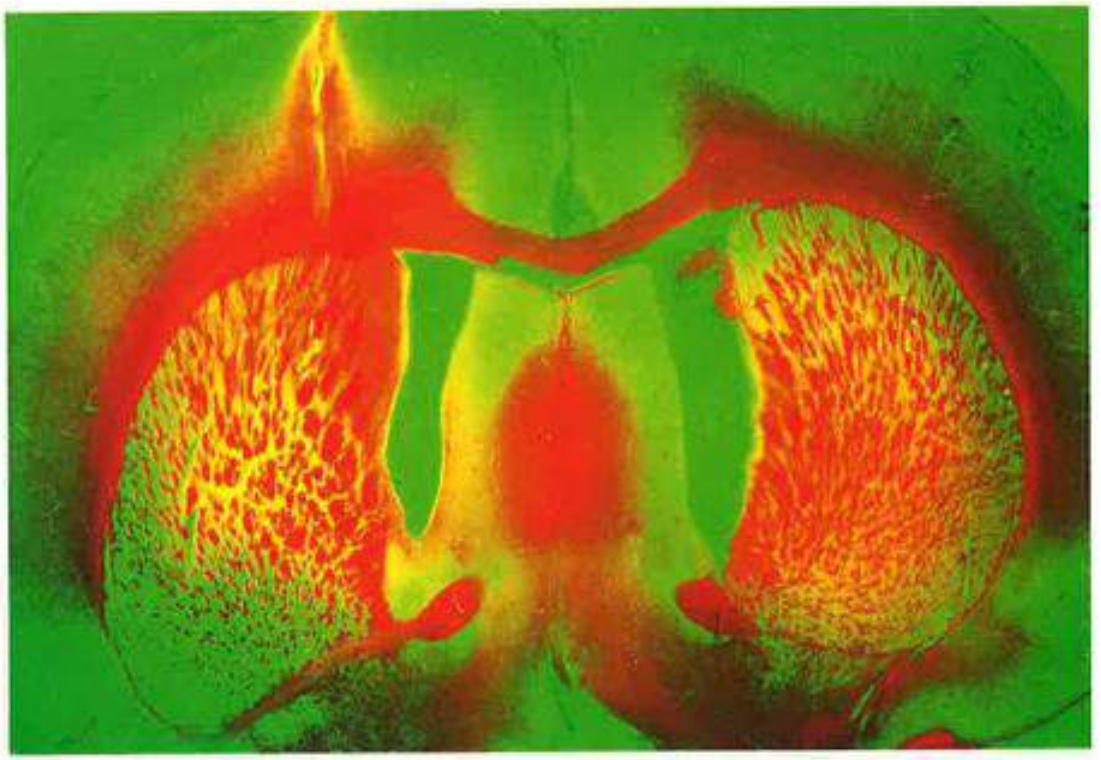
Fuxe K. et al 2013

Volume fraction: relative size of ECS compared to brain tissue volume
Tortuosity: increase in path length imposed by cells and extracellular matrix
Clearance: a constant that reflects the removal of a compound from ECS

NeuroReport 6, 1005–1009 (1995)

Long distance pathways of diffusion for dextran along fibre bundles in brain. Relevance for volume transmission

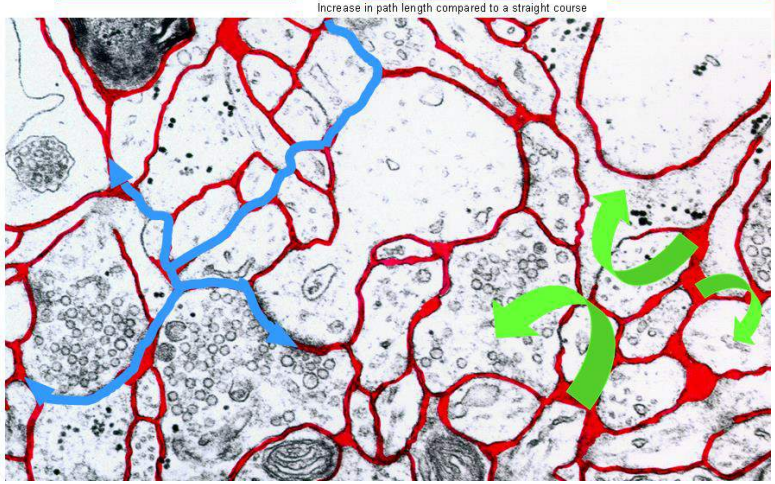
Börje Bjelke,[CA] Robert England,[1] Charles Nicholson,[2] Margaret E. Rice,[2] Jakob Lindberg,[1] Michele Zoli,[3] Luigi F. Agnati[3] and Kjell Fuxe

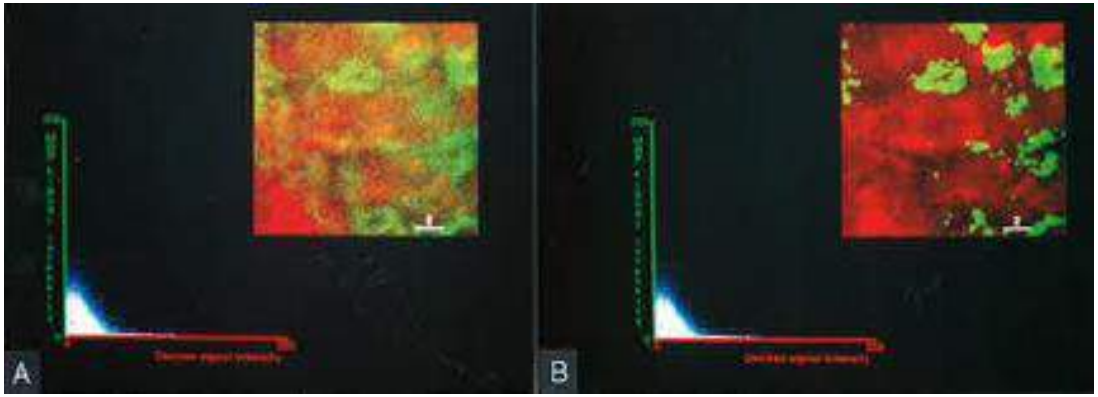


$D^*=D/\lambda^2$

α volume fraction λ tortuosity κ clearance

Increase in path length compared to a straight course

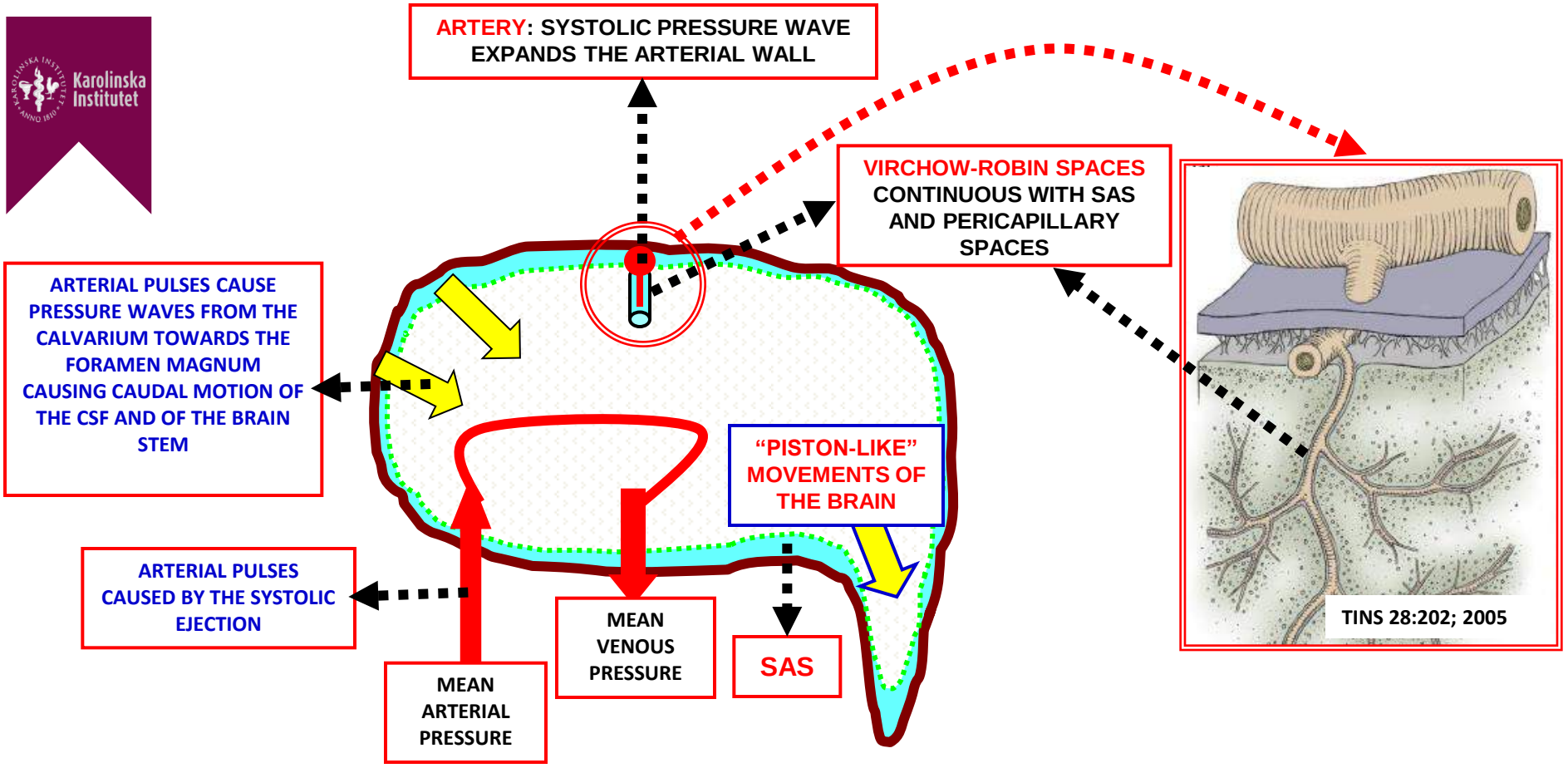




Modified from Nicholson & Sykova 1998 TINS

Höistad 2004

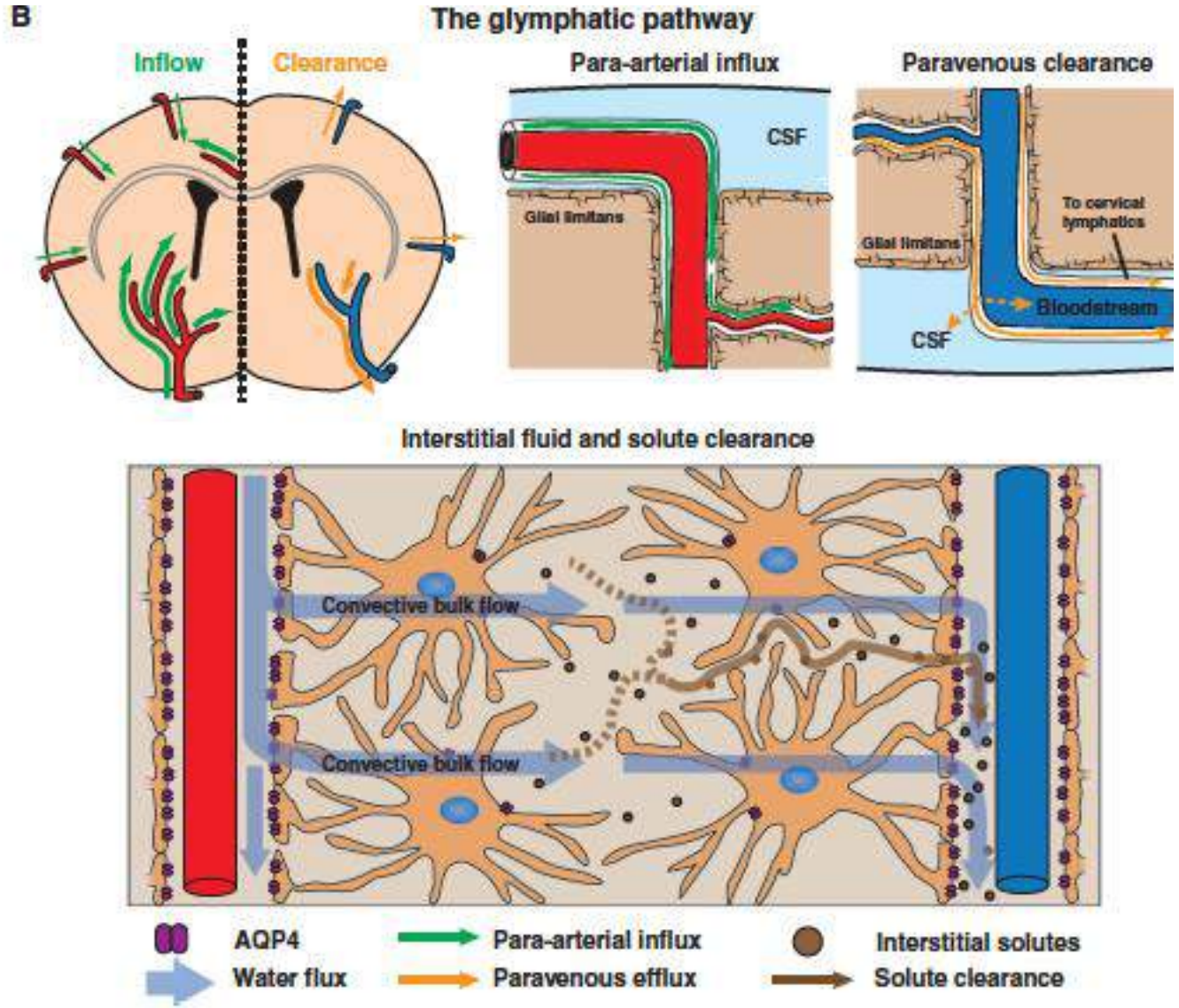
THE "TIDE HYPOTHESIS" OF VT-SIGNAL MIGRATION, CLEARANCE AND OF MODULATORY SIGNAL RELEASE



THE "TIDE HYPOTHESIS" OF VT-SIGNAL MIGRATION AND CLEARANCE (AGNATI, FUXE et al., 2004,2005)

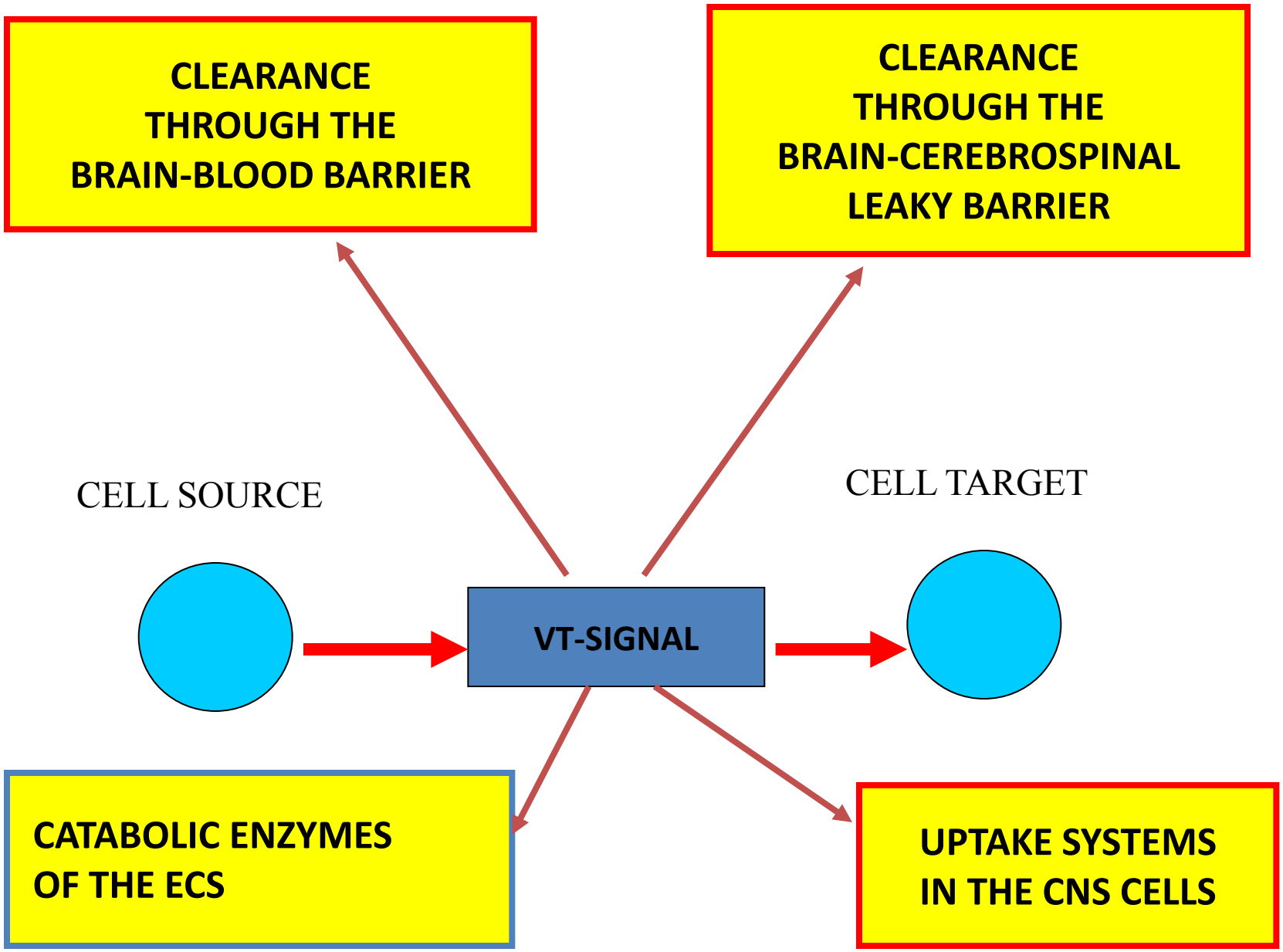
- THE ARTERIAL PULSE AS WELL AS THE "PISTON-LIKE" MOVEMENT OF THE BRAIN CAUSE CYCLIC PRESSURE OSCILLATIONS WITHIN THE SAS.
- THE CYCLIC PRESSURE OSCILLATIONS WITHIN THE SAS INDUCE "TIDE" MOVEMENTS OF THE FLUID IN THE VIRCHOW-ROBIN SPACES, HENCE IN THE PERICAPILLARY SPACES AND, EVENTUALLY, IN THE ECF-CHANNELS.
- THESE FLUID MOVEMENTS CONTRIBUTE TO THE VT-SIGNAL MIGRATION AS WELL AS TO THE HOMEOSTASIS OF THE INTERNAL MILIEU OF THE BRAIN (DIFFUSION OF NUTRIENTS AND CLEARANCE OF CATABOLITES e.g., Aß)

The glymphatic system supports interstitial solute and fluid clearance from the brain.



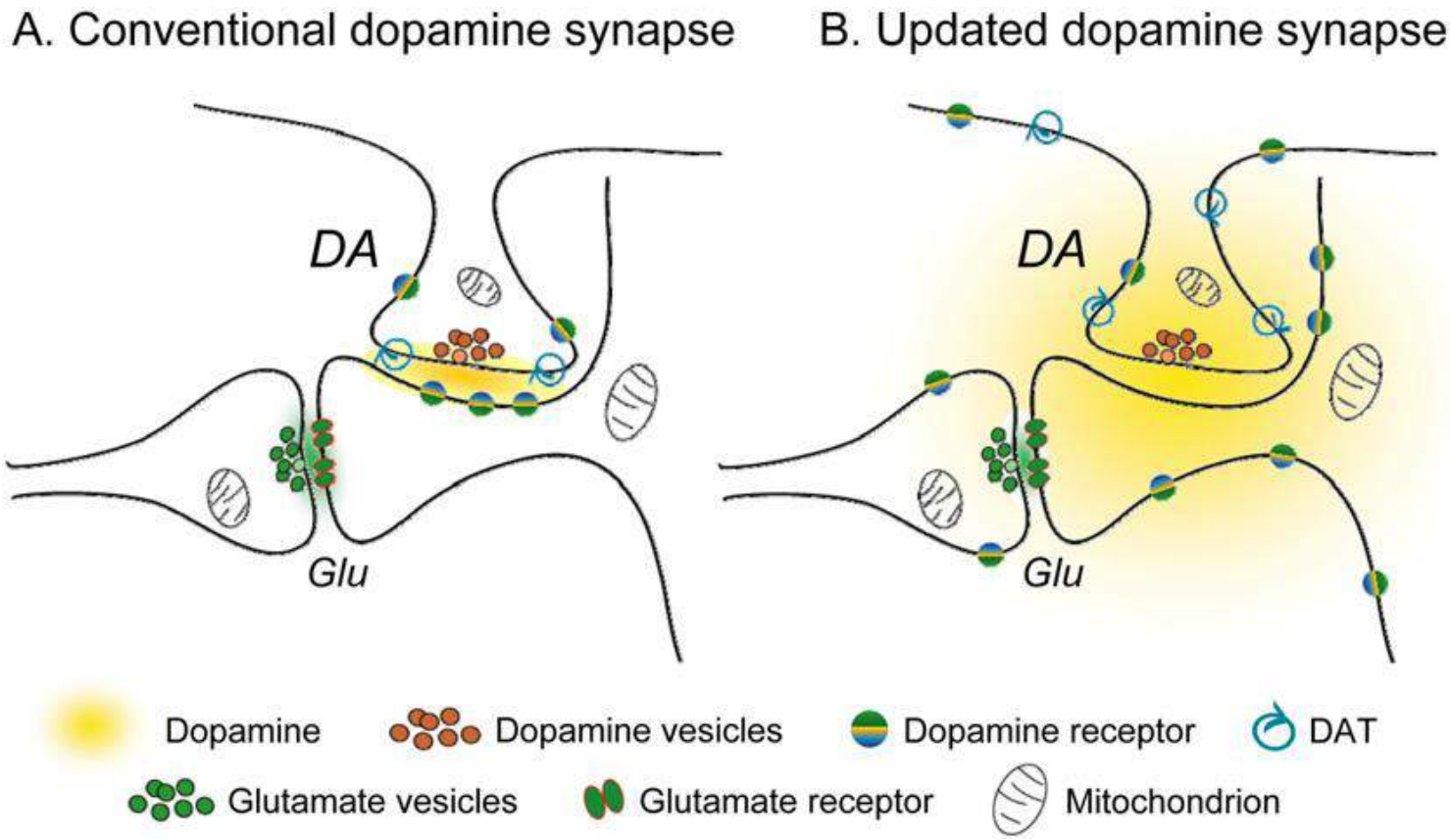
Schematic depiction of the glymphatic pathway. In this brain-wide pathway, CSF enters the brain along para-arterial routes, whereas ISF is cleared from the brain along paravenous routes.

Jeffrey J. Iliff et al. 2012



Jansson et al.2000

Modeling extrasynaptic DA transmission



Schematic representation of an updated DA synapse that shows ready DA spillover, unconstrained by DATs. The cloud of released DA, diffusing in three dimensions, encounters predominantly extrasynaptic DA autoreceptors on DA axon terminals and heteroreceptors on neighboring dendrites, soma and terminals, including medium spiny neurons .

Rice and Cragg 2008

SYNAPSE 00:000–000 (2011)

Increased Affinity of Dopamine for D_2-Like Versus D_1-Like Receptors. Relevance for Volume Transmission in Interpreting PET Findings

DANIEL MARCELLINO,[1] JAN KEHR,[2] LUIGI F. AGNATI,[3] AND KJELL FUXE[1*]
[1]*Department of Neuroscience, Karolinska Institutet, SE-17177, Stockholm, Sweden*
[2]*Department of Physiology and Pharmacology, Karolinska Institutet, SE-17177 Stockholm, Sweden*
[3]*IRCCS San Camillo, Lido Venice, Italy*

-The IC50 values of DA at D2-like receptors in dorsal striatal membranes and CHO cell membranes were markedly and significantly reduced compared with the IC50 values of DA at D1-like receptors.

-The KiH value for DA at [3H] raclopride-labeled D2-like receptors in dorsal striatum was 12 nM, and this can help explain PET findings that amphetamine-induced increases in DA release can produce an up to 50% decrease of [11C] raclopride binding in the dorsal striatum in vivo.

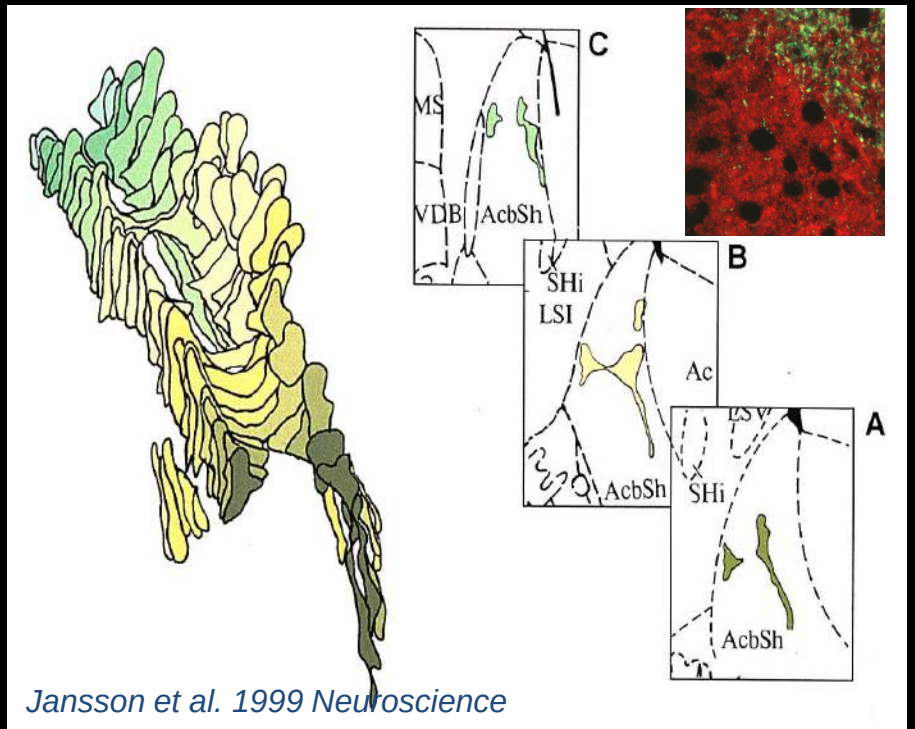
-These combined results give indications for the existence of striatal D2-like receptor-mediated DA VT at the local circuit level in vivo in the human striatum

D1 - TH mismatches in the Nucleus accumbens shell

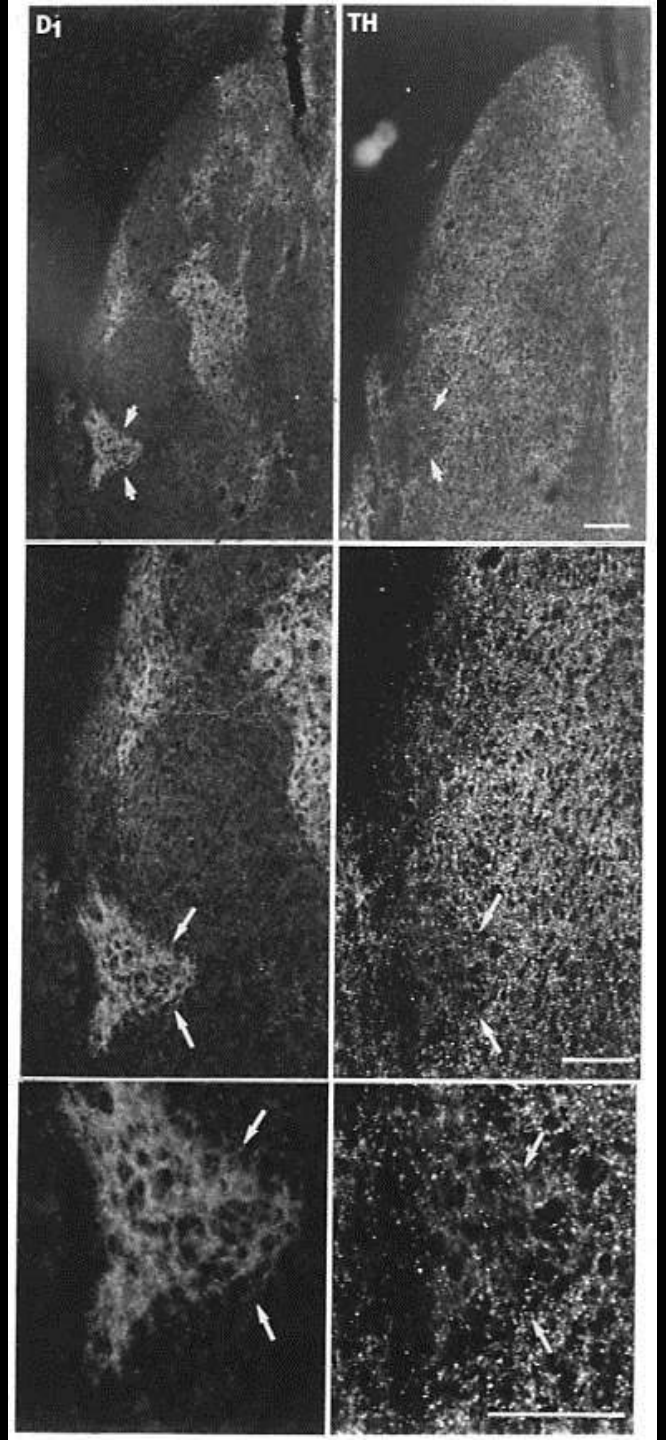
D1 rich islands surrounded by dense TH terminal networks

Function: temporal-difference learning based on VT ?

Due to different velocities of WT and VT , the mismatch VT signal is correlated to the synaptic DA signal at the source at an earlier time step. A prevision of the outcome at the next time step becomes possible. In the beginning DA neuronal activity is high at time of reward. At later trials it is high at the delivery of the sensory cue (Guidolin et al.2007)

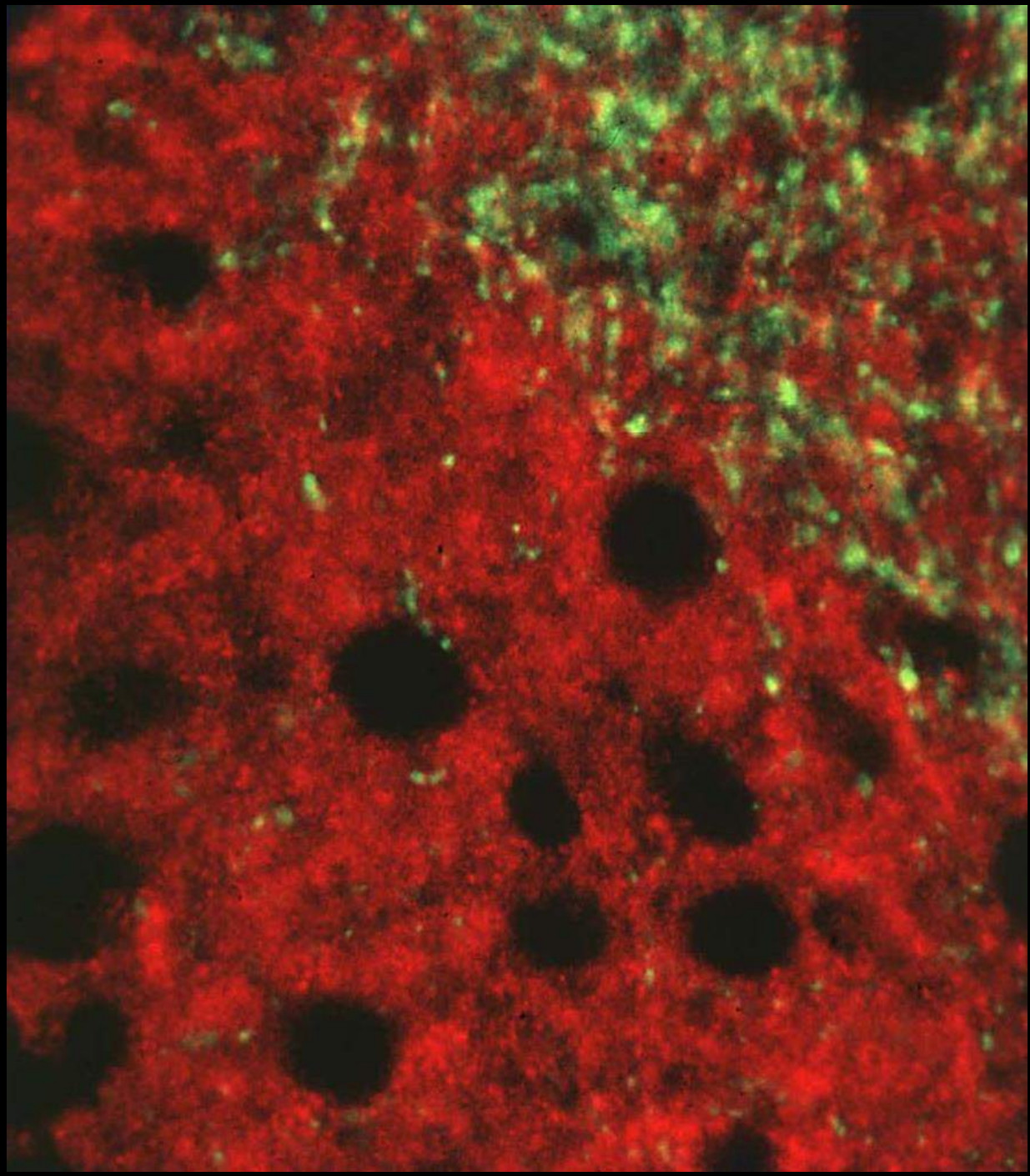


Jansson et al. 1999 Neuroscience



D1 - TH mismatch in nuc.accumbens shell

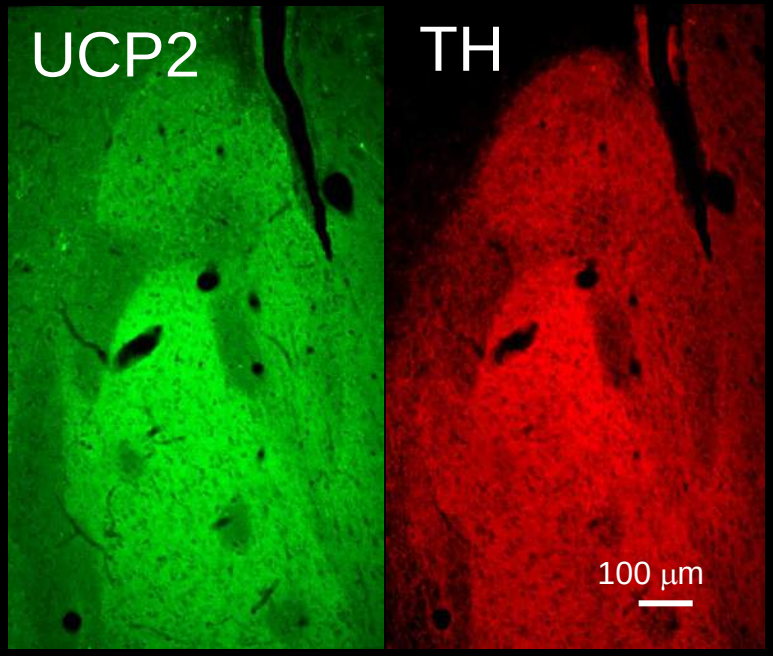
Confocal image



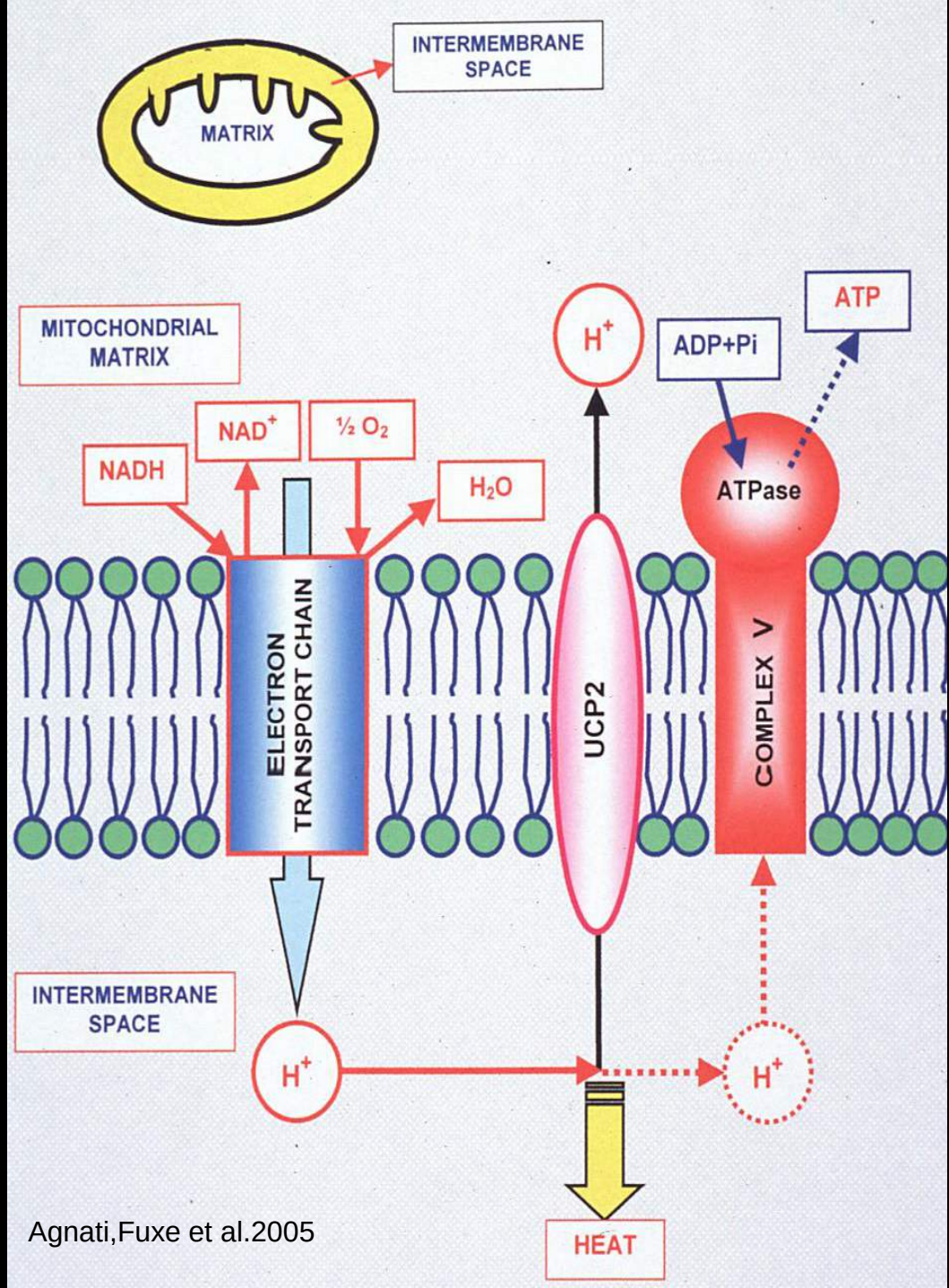
Jansson et al. 1999
Neuroscience

Brain Uncoupling Protein 2 (UCP2)

Uncoupled Neuronal Mitochondria Predict Thermal Synapses in Homeostatic Centers



Rivera et al.2005



Agnati,Fuxe et al.2005

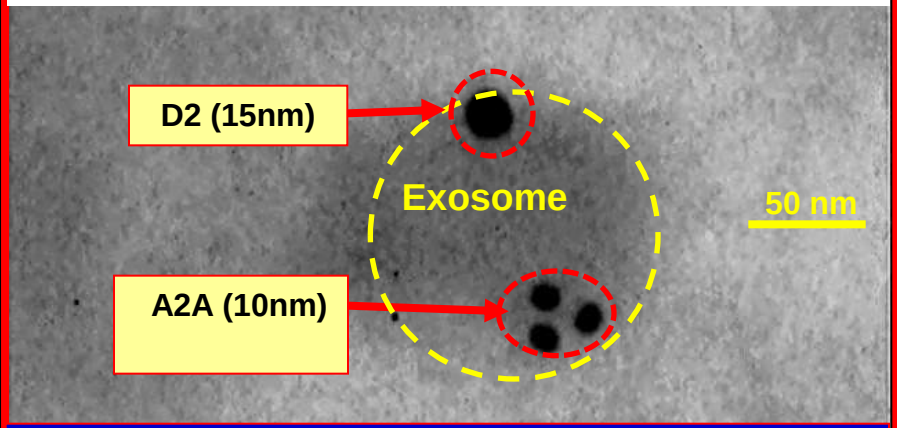
Classification of the extracellular vesicle (EV) mediated intercellular communication in the central nervous system into volume transmission

Intercellular communication mode	Channel	Cargo transfer	Mechanism of transport	Origin
EV type of VT (CNS: neurons, glial cells, brain endothelial cells) Simons and Raposo 2009; Schneider and Simons 2013	Extracellular space and CSF	**Exosomes** (40 – 100nm) containing **proteins Including receptors, lipids, mRNA, miRNA, mtDNA** **Shedding vesicles:** ***Microvesicles*** (100 – 1000nm) insufficiently known composition; cholesterol, tetraspanin ***Apoptotic bodies*** (1 – 4 um) Histones, DNA	Diffusion and flow along energy gradients in the extracellular space	Most cell types: **endosomes**, fuse with plasma membrane Most cell types: Plasma membrane All cell types: Plasma membrane Agnati and Fuxe 2014

NEW PERSPECTIVES OF THE COOPERATIVE INTERACTIONS BETWEEN COMMUNICATION MODES AND DECODING PROCESSING BASED ON EV TYPE OF VT AND ON REC HETEROMERS

GPCRs ARE PRESENT IN EXOSOMES

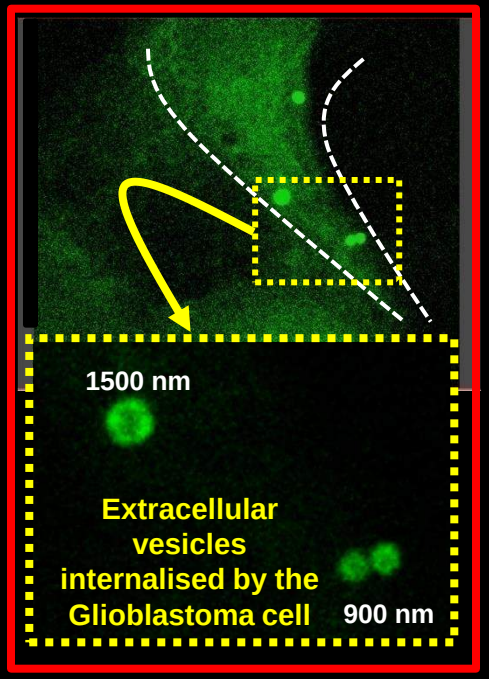
ELECTRON MICROSCOPE IMAGE OF EXOSOMES
D2 RECEPTORS were labelled by 15nm gold particles
A2A RECEPTORS were labelled by 10nm gold particles



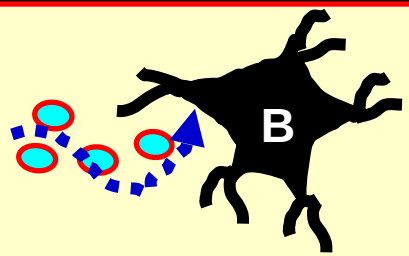
D2 – A2A IMMUNOGOLD STAINING SHOWS THE PRESENCE OF THE RECEPTORS IN THE EXOSOME

EXOSOMES ARE INTERNALISED BY CELLS

Glioblastoma cells were incubated with Exosomes prepared by CHO cells and containing A2A and D2 receptor



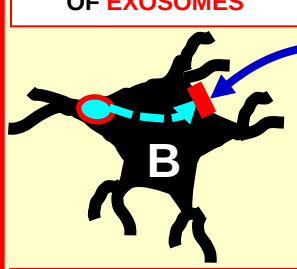
IMPLICATIONS OF THE CELL-TO-CELL TRANSFER VIA EXOSOMES OF GPCRs



CELL B IS A TARGET FOR EXOSOMES CONTAINING RECEPTORS THAT CELL B DOES NOT EXPRESS

Cell B, by internalising Exosomes can insert NEW TYPES OF GPCRs in the plasma membrane and hence decode previously undetected signals e.g., an undetected NEUROTRANSMITTER (NT) and/or can lead to formation of NEW RECEPTOR HETEROMERS

INTERNALISATION OF EXOSOMES



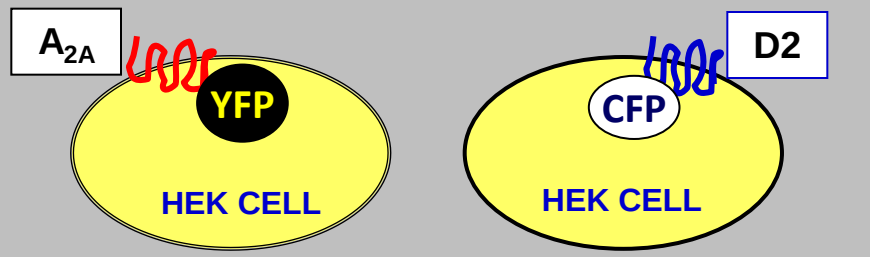
DECODING OF PREVIOUSLY UNDETECTED NEUROTRANSMITTER

INSERTION IN THE MEMBRANE OF NEW TYPES OF RECEPTORS

Guescini et al. 2012

FRET analysis indicates the existence of A2^D2 heteromers in HEK293 cells independently transfected with D2L-CFP and A2a-YFP

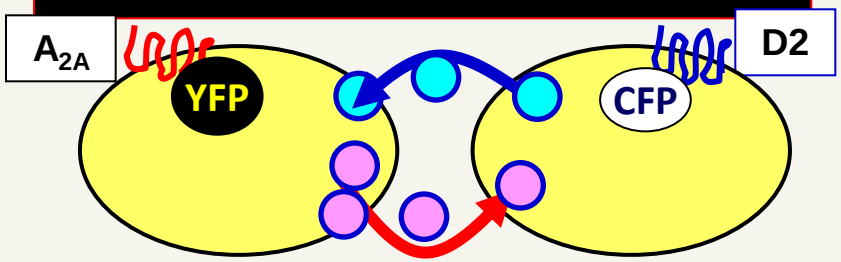
CO-CULTURE OF HEK CELLS TRANSFECTED WITH A_{2A}R-YFP AND D_2R-CFP



CFP (Donor: cyan); YFP (Acceptor: yellow)

WORKING HYPOTHESIS: POSITIVE FRET NEEDS LABELLED GPCR EXCHANGE

EXOSOME-MEDIATED ROAMER TYPE VT

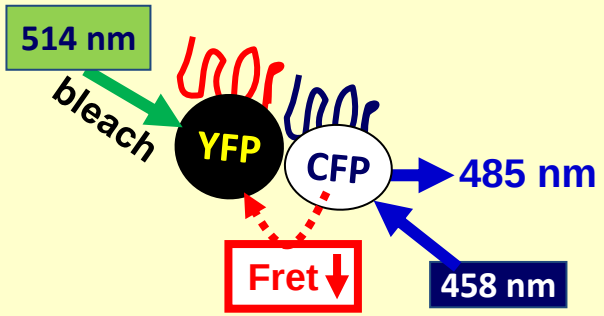


EXOSOMES THAT MAY CONTAIN D2-CFP

EXOSOMES THAT MAY CONTAIN A_{2A}-YFP

FRET ANALYSIS CAN DEMONSTRATE NOT ONLY INTERCELLULAR TRANSFER OF GPCRs BUT ALSO FORMATION OF A2a^D2 HETEROMERS

CO-CULTURED HEK CELLS: ACCEPTOR PHOTOBLEACHING



Fluorescence 485

Guescini et al.2012

Table 3 Characterization of the extracellular vesicle mediated volume transmission from the various cell types of the CNS

Cell type	Type of EVs°	Transfer compounds, acceptor cells, function
Neurons	Exosomes and microvesicles	Hsp, Flottilin, miRNA , GLuR2; nerve cells; ECV modulation of exocytosis and ECV internalization leads to neuronal plasticity
Astrocytes	Exosomes and microvesicles	Mitochondria, ATP,Hsp70, synapsin1, functional glutamate transporters, FGF-2,VEGF, matrix metallo-proteinases, microRNA-29; astrocytes and neurons; Potential role in repair,neuroinflammation and neurodegeneration

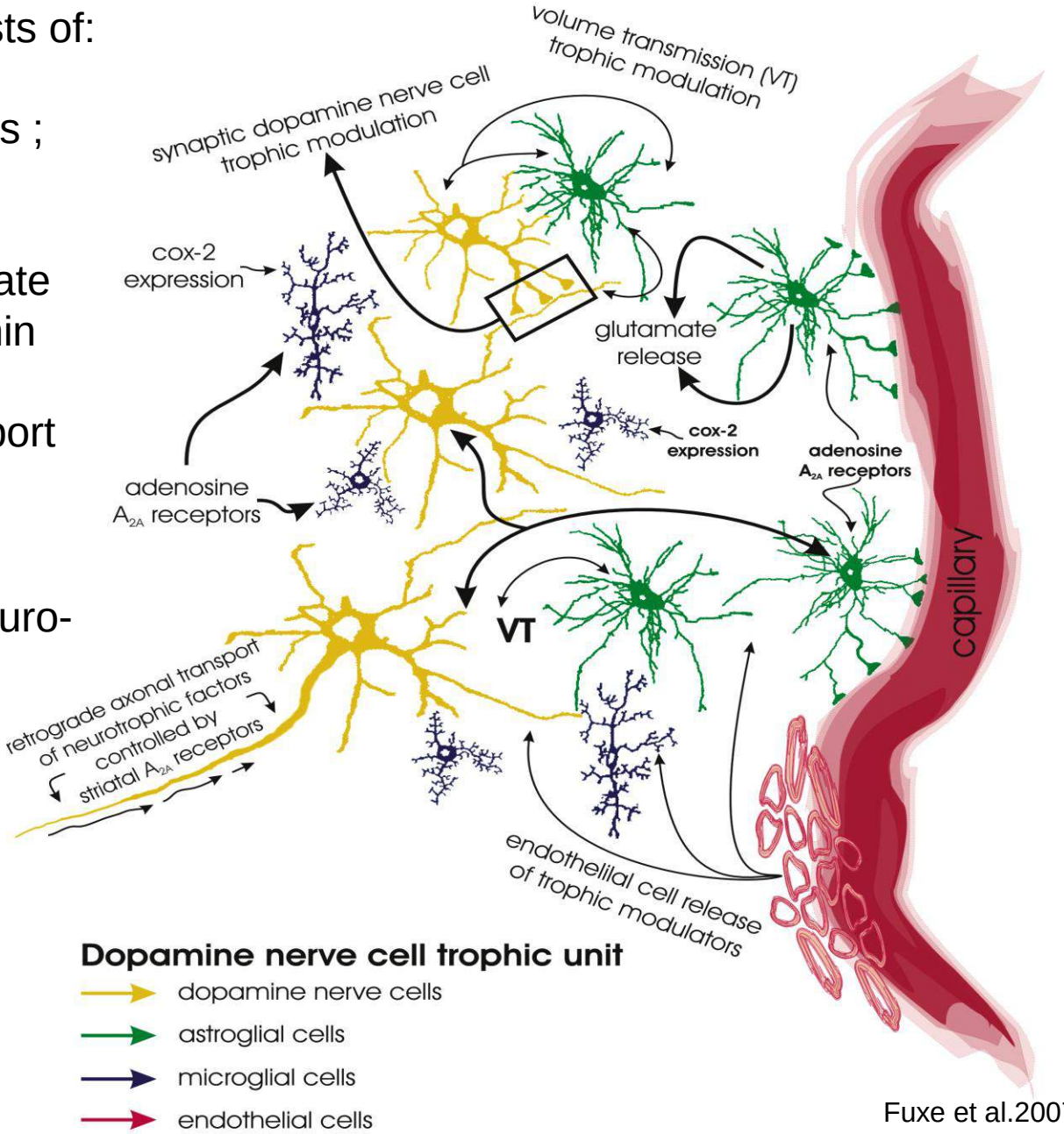
Agnati and Fuxe 2014

Substantia nigra, *pars compacta*

The CNS trophic unit consists of: neurons; glial cells; blood vessels with endothelial cells ; extracellular matrix

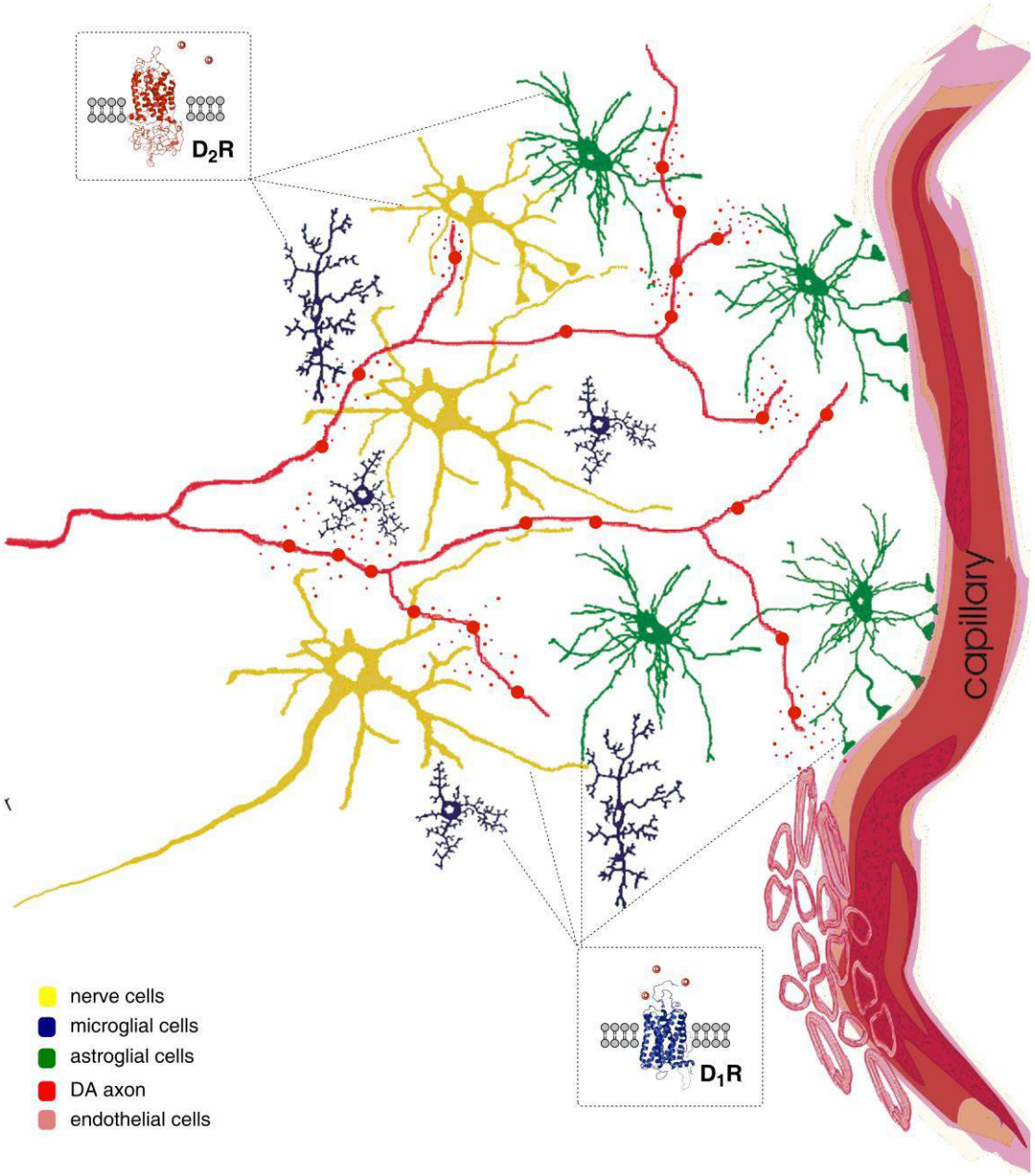
This term was used to indicate the smallest set of cells within the CNS which act in a complementary way to support the trophism of one another (Agnati,Cortelli, Pettersson,Fuxe 1995)

Trophic unit linked to the neuro-vascular unit (BBB) with Endothelial cells, pericytes, and astrocytic perivascular endfeet



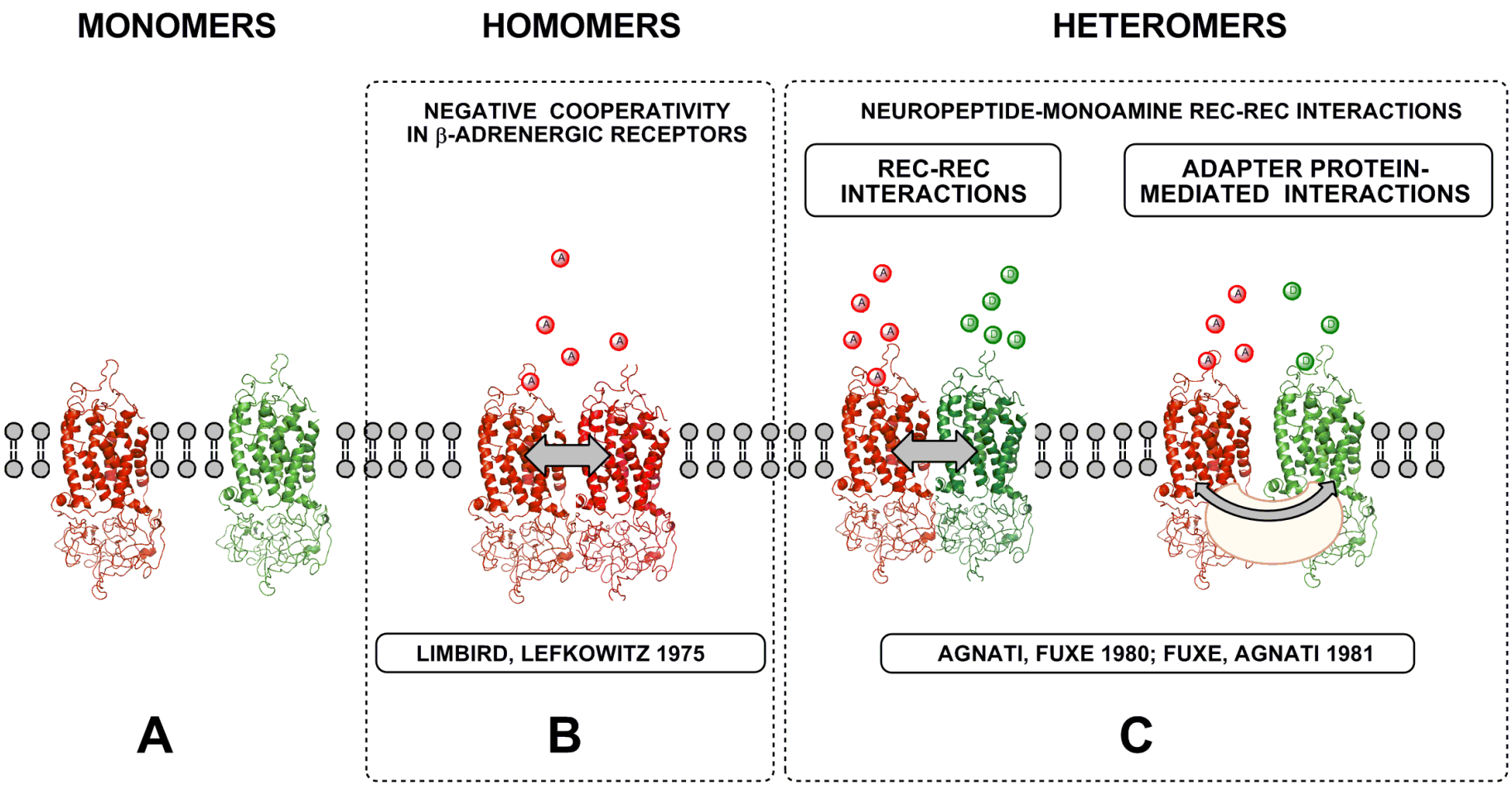
Fuxe et al.2007

DA VT from DA terminal networks in a trophic unit



Fuxe, Borroto-Escuela et al. 2015

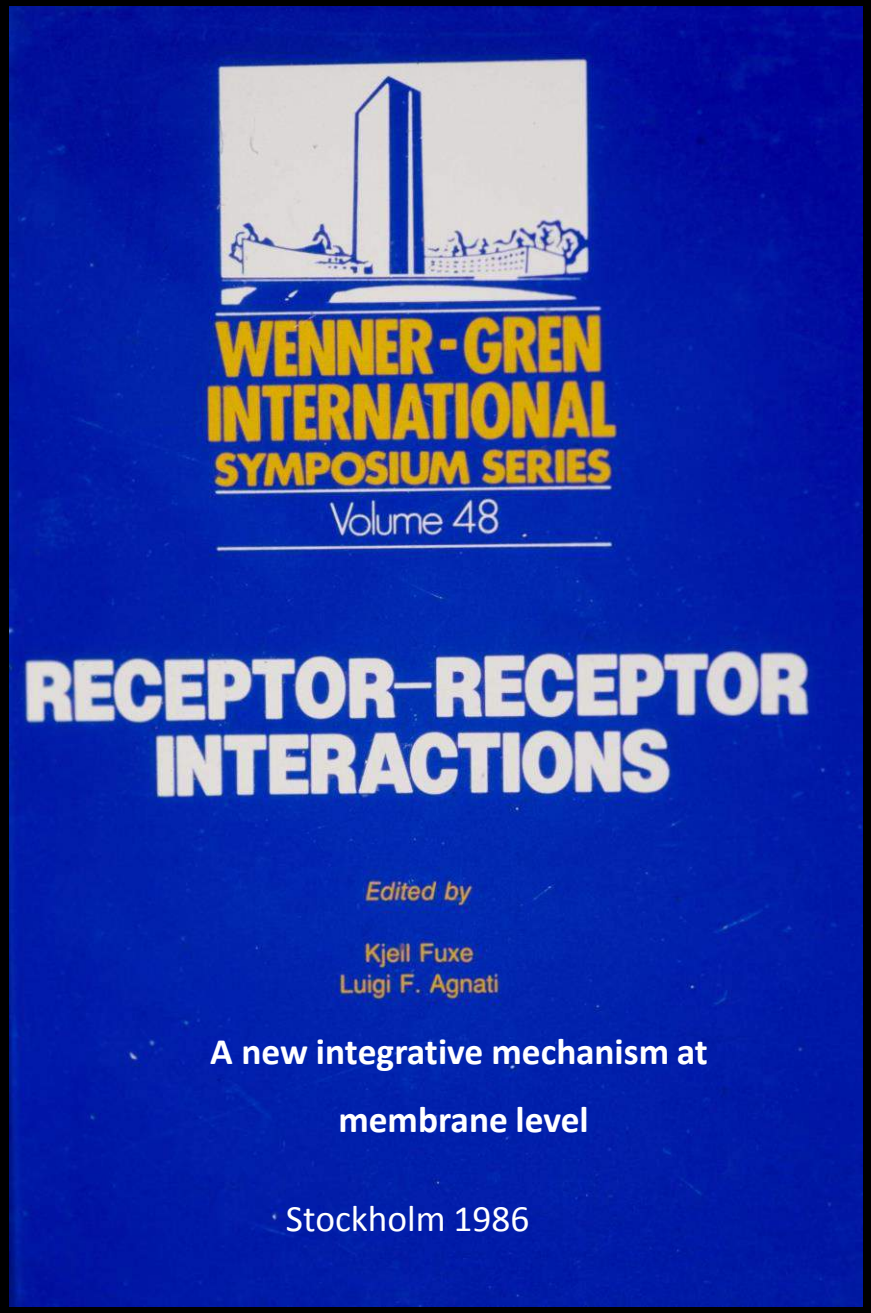
RECEPTOR-RECEPTOR INTERACTIONS: A NEW INTEGRATIVE MECHANISM AT MEMBRANE LEVEL



Zoli M, Agnati LF, Hedlund PB, Li XM, Ferré S, Fuxe K.
Mol Neurobiol. **1993** Fall-Winter;7(3-4):293-334. **The concept of receptor-receptor interactions in heteromers was introduced**

Intramembrane receptor-receptor interactions: **a novel principle in molecular medicine.**
Fuxe K et al J Neural Transm. 2007 114 :49-75.

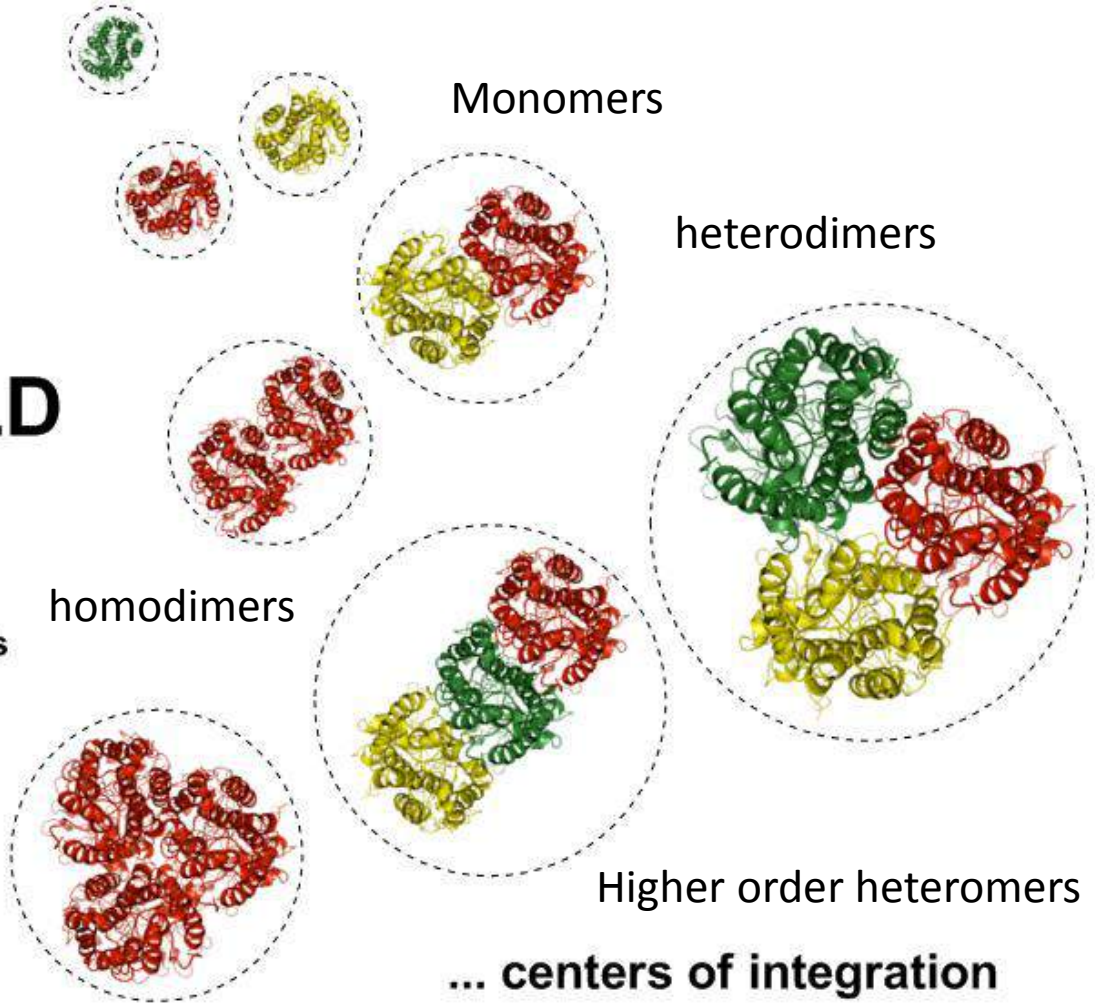
Fuxe et al.2010


WENNER-GREN
INTERNATIONAL
SYMPOSIUM SERIES
Volume 48
RECEPTOR–RECEPTOR
INTERACTIONS
Edited by
Kjell Fuxe
Luigi F. Agnati
A new integrative mechanism at
membrane level
Stockholm 1986

THE CHANGING WORLD OF GPCRs

The Royal Swedish Academy of Sciences

Stockholm, May 18, 2010

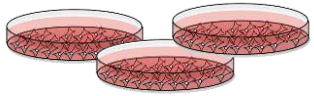


In tissues

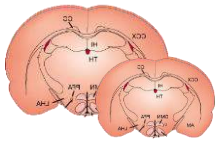
heteroreceptor complexes

Organizer. Fuxe

WORKFLOW



Cellular Models



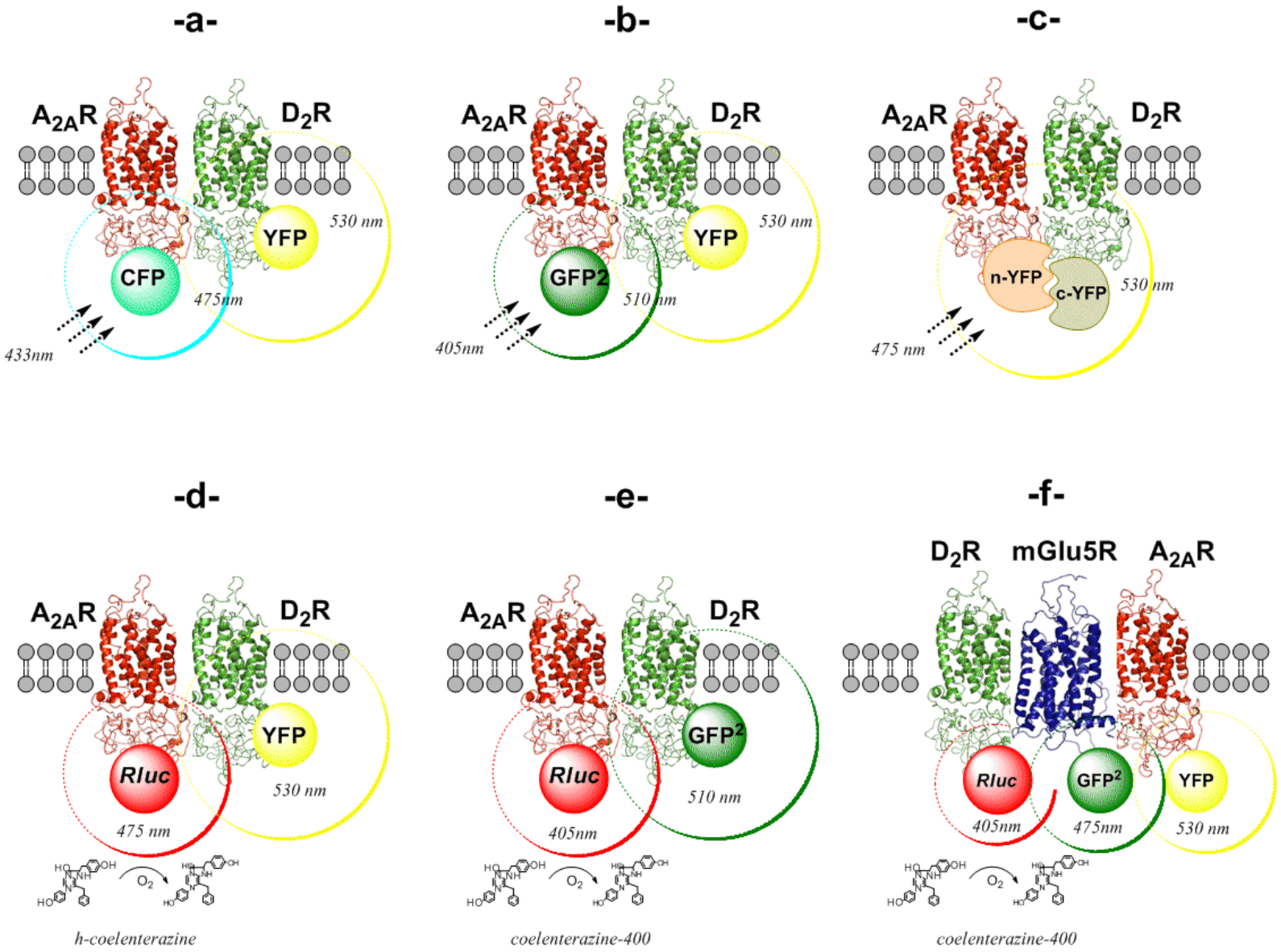
Brain tissue



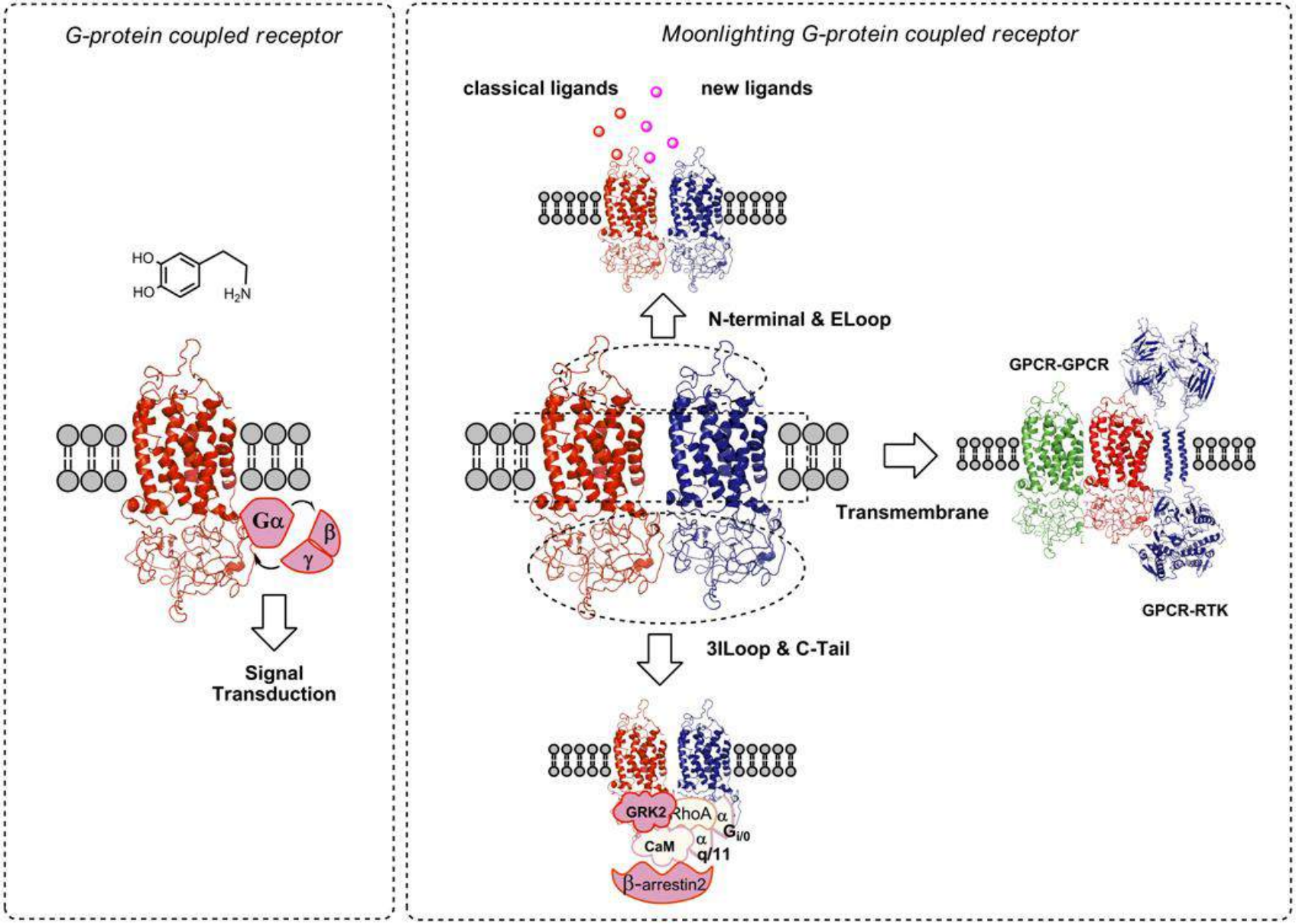
In vivo

	Cellular Models	Brain tissue	*In vivo*
Heteromer Structure	FRET, RT-FRET, BRET, BiFC, FCCS, *In situ* PLA, CoIP	*In situ* PLA, CoIP	
Receptor interface characterization	Small interface interfering peptides (SIIP) Site-direct mutagenesis	Small interface interfering peptides (SIIP) Site-direct mutagenesis	Small interface interfering peptides (SIIP)
Allosteric receptor-receptor interactions	Binding assay, GTPγS, Gene reporter assay, B-arresting interaction phosphoproteomics	Binding assay, GTPγS, Rec autoradiography, ERK1/2, CREB & c-Fos	
Transgenic rats		A2A knock-in rat line A2A OE rat line	A2A knock-in rat line A2A OE rat line
The neuronal level		Neurophysiological studies	
Brain circuit level			Microdialysis, MRI, fMRI, *in vivo* binding PET
Global network level			Behavioral pharmacology

Illustration of the FRET and BRET methods: $A_{2A}R$-D_2R



Fuxe et al.2012



Jeffrey (1999): Moonlighting protein is a multifunctional protein where different functions are found in single strands of amino acids not linked to splicing etc

GPCR protomers can moonlight through allosteric receptor-receptor interactions

Borroto-Escuela et al.2011

Heteroreceptor complexes formed between DA receptors and NMDA receptors

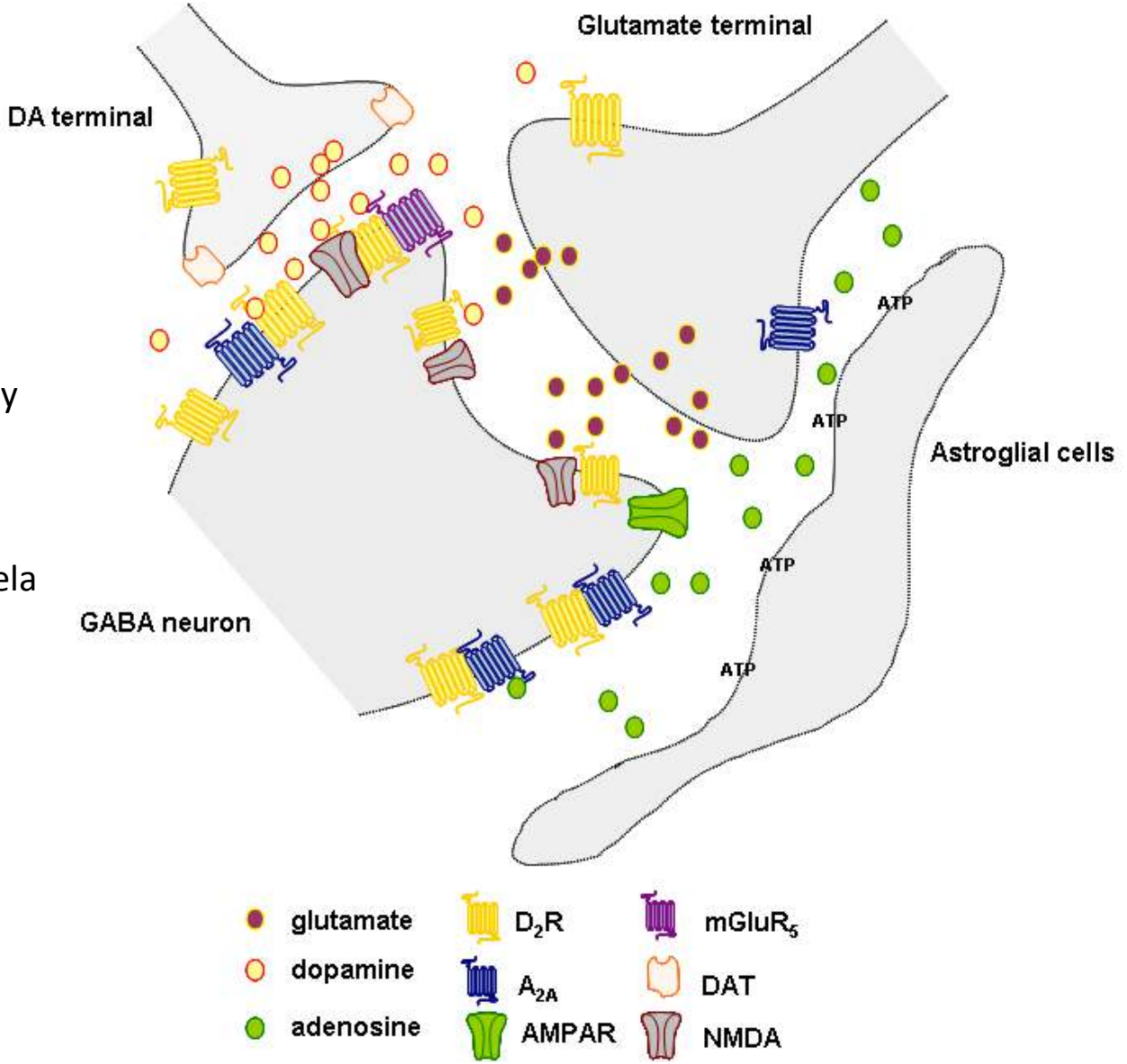
Heteroreceptor complex and location	Signaling	Potential relevance PD
D1R-NMDAR (D1-NR1) Hippocampus, striatum (synapse);*Lee et al.2002, Fiorentini et al.2003,Woods et al.2005*	NMDAR increased D1R signaling. **D1R activation** upregulates NMDA dependent LTP and **promotes working memory and NR1-CaMKII coupling**	Cognitive dysfunction after uncoupling of the receptor complex ;*Nai et al. 2010* **Can contribute to improvement of cognitive and motor functions (direct pathway) with L-DO**PA.
D2R-NMDAR (D2-NR2B) ; striatal glutamate synapses *Liu et al.2006*	This complex **blocks CaMKII-NR2B interaction** with **reduction of NR2B phosphorylation and NMDAR currents** Disruption of the D2R-NR2B receptor complex reduced cocaine induced locomotion. The complex is increased by cocaine	**Contributes to increasing motor function by reducing NMDAR drive in the Indirect pathway**

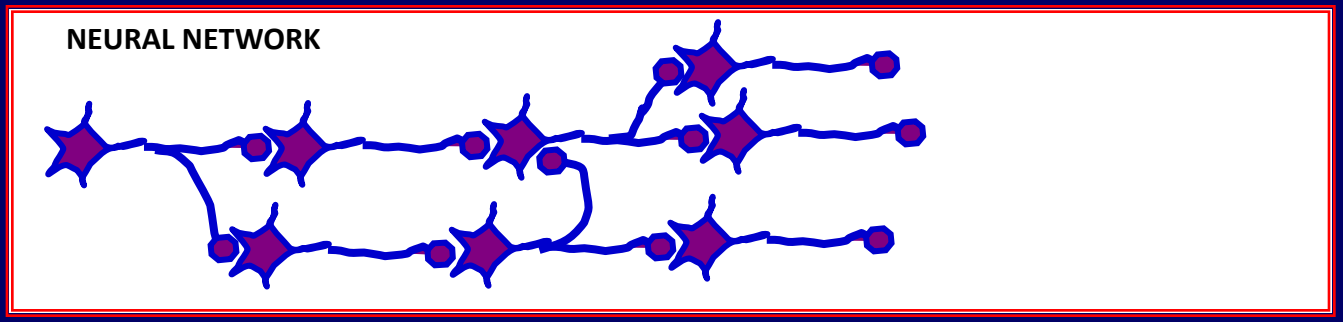
DA VT via D2R modulates glutamate WT via antagonistic receptor-receptor interactions with NMDA receptors in heteroreceptor complexes in the indirect pathway.

✓ **D2-NMDA** (NR2B) ;indirect pathway (Liu et al.2006).

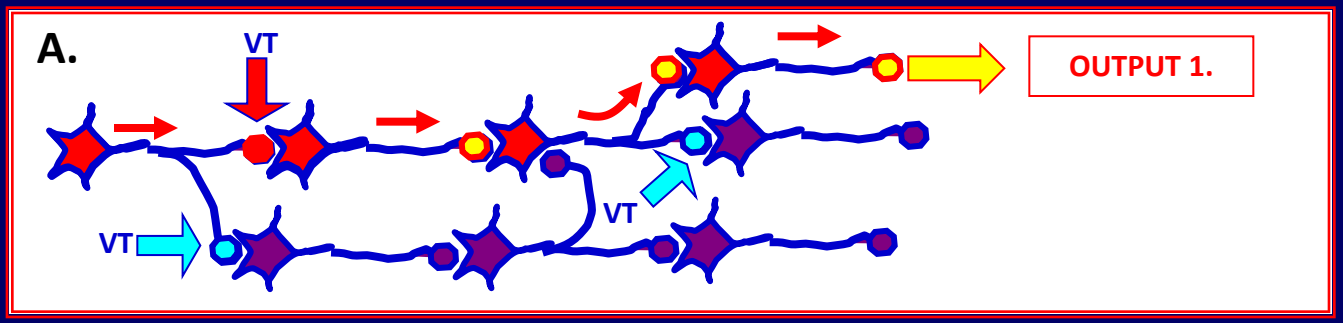
✓ **D1-NMDA** (NR1) ; direct pathway (Lee et al.2002)

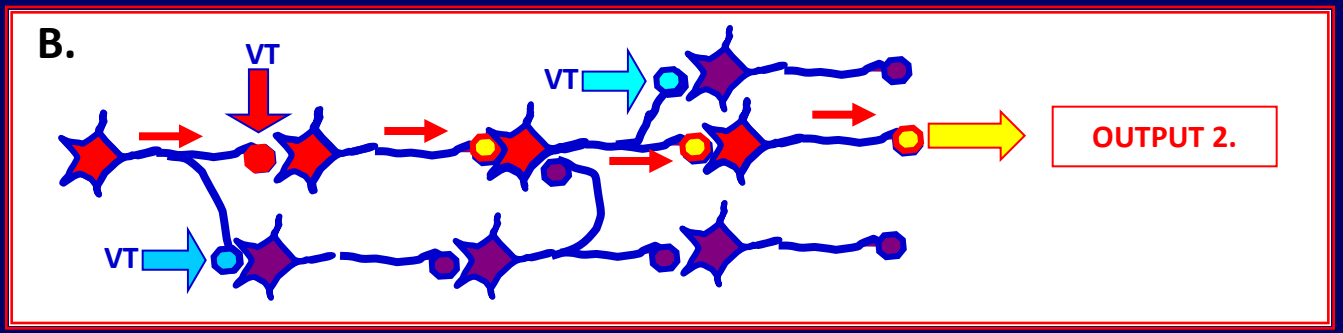
✓**D2-5-HT2A**; dorsal striatum and nuc accumbens (Borroto-Escuela et al. 2010, 2014 ; Lukasiewicz et al.2010)

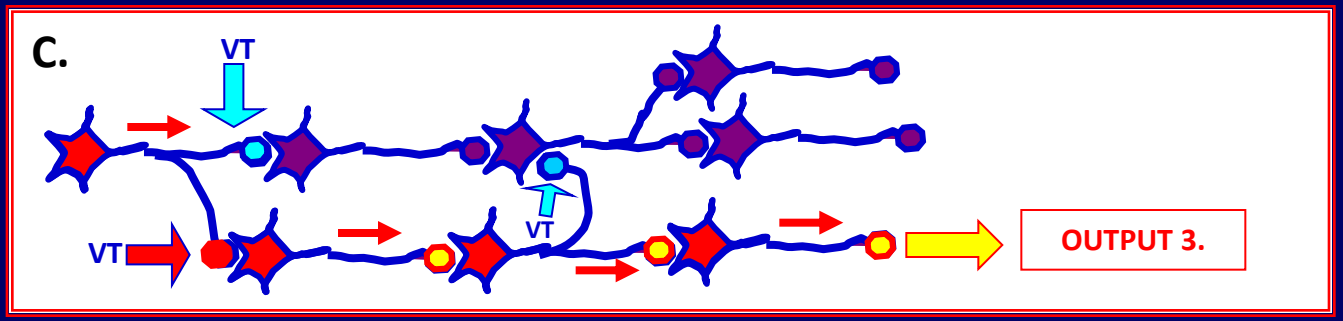




UPREGULATING VT SIGNAL
DOWNREGULATING VT SIGNAL
UPREGULATED SYNAPSE
DOWNREGULATED SYNAPSE
ACTIVE SYNAPSE
INACTIVE SYNAPSE
INFORMATION FLOW







Karolinska Institutet

THREE DIFFERENT OUTPUTS

1. 2. 3.

FROM THE SAME NEURONAL NETWORK THANKS TO THE MODULATORY ACTIONS OF VT SIGNALS

Agnati and Fuxe 2000,
Fuxe et al. 2013

Direct modulation glutamate excitotoxicity through Cytokine receptor-ionotropic glutamate receptor Interactions in heteroreceptor complexes

Cytokine	Receptor heteromer	Source
IFNgamma	IFNGR/GluR1	Mizuno et al. (2008)
TNFalpha	?	Pickering et al. (2005)
IL-1beta	?	Viviani et al. (2003)
IL-6	?	Qiu et al. (1998)

IFNgamma directly induces neuronal damage in mouse cortical neurons through a unique neuron-specific receptor complex (receptor heteromer) containing the IFNgamma receptor (IFNGR) and AMPA GluR1 receptor.

IFNgamma synergistically enhances glutamate neurotoxicity mediated by AMPA receptors but not NMDA receptors.

Novel mechanisms of neuronal excitotoxicity based on direct and specific cytokine–glutamate receptor interactions, which may occur in MS.

A new road to neuroinflammation and neurodegeneration in Parkinson's disease (Fuxe et al.2008)

Abnormal allosteric receptor-receptor interactions may also contribute to a microglia-mediated neuroinflammatory response (Block et al., 2007), found in the SN of individuals with PD.

Tarakanov et al.2009

Summary

- The DA, NA and 5-HT neurons served a model neurons for the introduction of the theory of volume transmission (extrasynaptic , long distance and CSF VT)

- At the nerve terminal level soluble VT signals dominate, mainly released from synaptic and other vesicles by exocytosis

- At the soma-dendritic level not only extrasynaptic exocytosis of soluble VT signals exists but also extracellular vesicle mediated VT (exosomes and shedding vesicles) containing e.g. proteins incl. **receptors**, lipids and mRNA ; transport in ECF

- The major target for integration of synaptic and VT signals is the trophic unit including the BBB and nerve, glial and endothelial cells (the smallest set of cells to support the trophism of one another)

- Extrasynaptic DA,NA and 5-HT VT operates by targeting DA, NA and 5-HT receptor subtypes on the nerve ,astroglial, microglial cells and cells of the BBB of the trophic units. Assemblies of trophic units build up the neural-glial networks of the CNS.

- The molecular targets for the integration of synaptic and volume transmission are the heteroreceptor complexes in the pre-and postjunctional membranes through their allosteric receptor-receptor interactions (Fuxe et al.2013)

Karolinska Institutet

Acta Physiol Scand 1986, 128, 201–207

A correlation analysis of the regional distribution of central enkephalin and β-endorphin immunoreactive terminals and of opiate receptors in adult and old male rats. Evidence for the existence of two main types of communication in the central nervous system: the volume transmission and the wiring transmission

L. F. AGNATI, K. FUXE,* M. ZOLI, I. OZINI, G. TOFFANO and F. FERRAGUTI
Department of Human Physiology, University of Modena and *Department of Histology, Karolinska Institute, Stockholm, Sweden

Karolinska Institutet

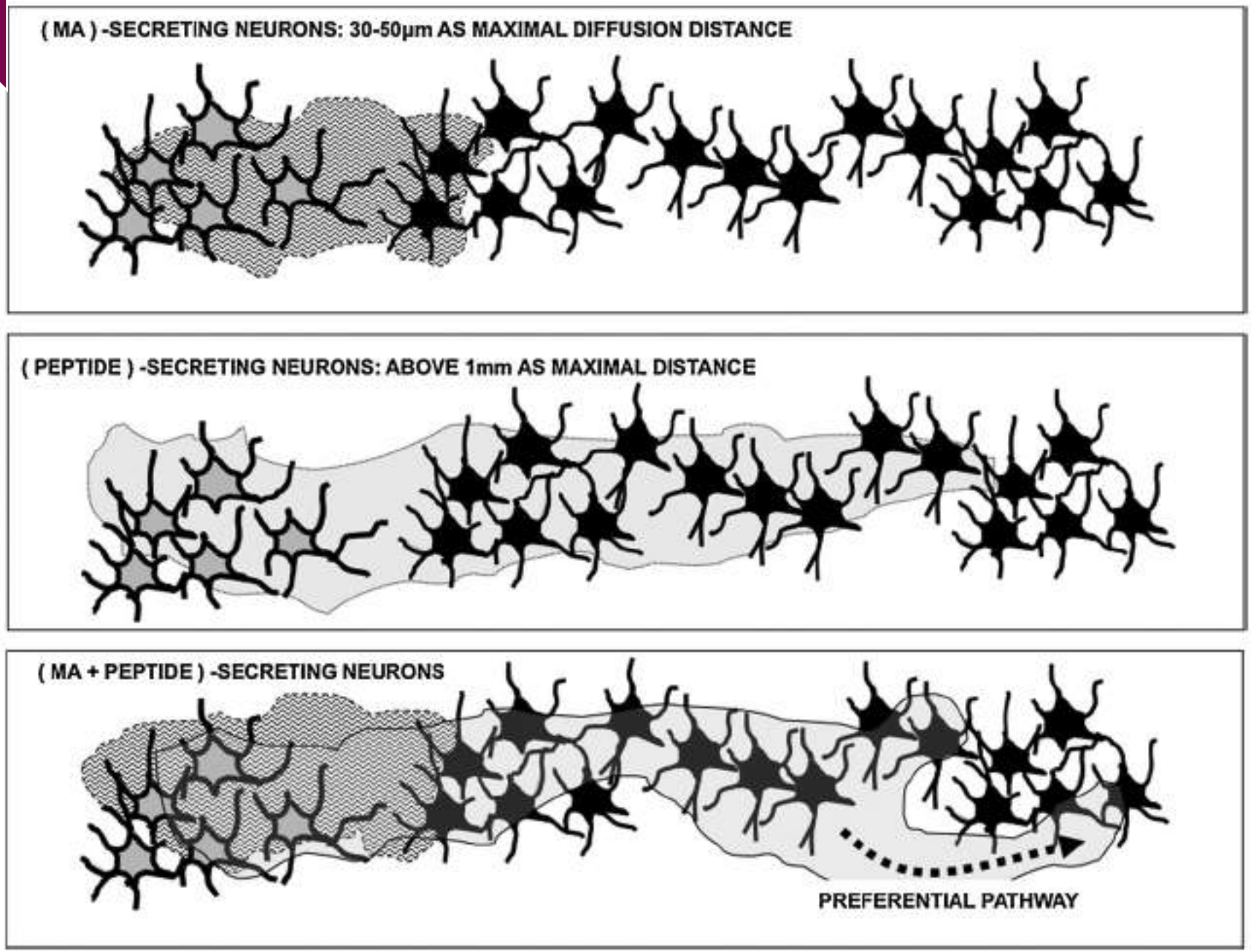
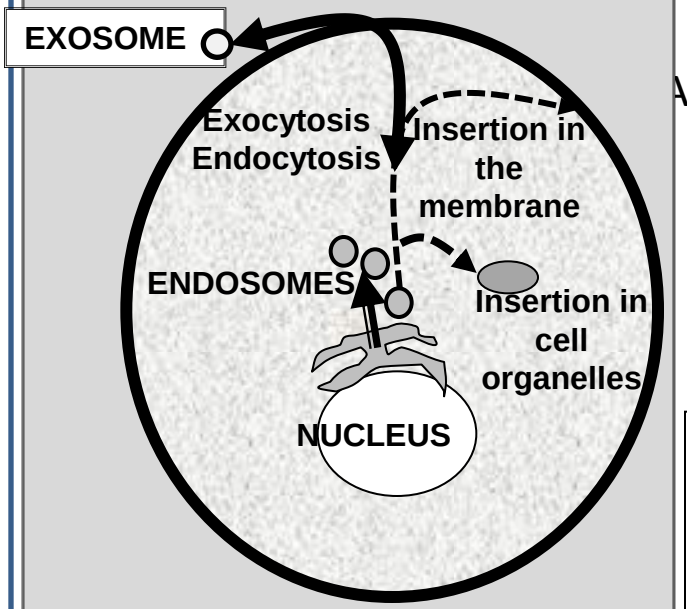


Table 2
Classification of the tunneling nanotube (TNT) and extracellular vesicle (EV) mediated intercellular communication in the central nervous system into wiring transmission and volume transmission, respectively

Intercellular communication mode	Channel	Cargo transfer	Mechanism of transport	Origin
TNT type of WT (cultures of neuronal and glial cells) Rustom et al.2004 Gerdes et al.2007	TNT (15-60 µm long; 50-200nm diameter) transient, membrane continuity between cells	Organelles (especially vesicles), but also mtDNA, proteins, … inside or along the TNT plasma membrane surface	Actin driven	No in vivo data in adult CNS
EV type of VT (CNS: neurons, glial cells, brain endothelial cells) Simons and Raposo 2009; Schneider and Simons 2013	Extracellular space and CSF	**Exosomes** (40 – 100nm) containing proteins, lipids, mRNA, miRNA, mtDNA **Shedding vesicles:** ***Microvesicles*** (100 – 1000nm) insufficiently known composition ***Apoptotic bodies*** (1 – 4 um) Histones, DNA	Diffusion and flow along energy gradients in the extracellular space	Most cell types: endosomes, fuse with plasma membrane Most cell types: Plasma membrane All cell types: Plasma membrane Agnati and Fuxe 2014

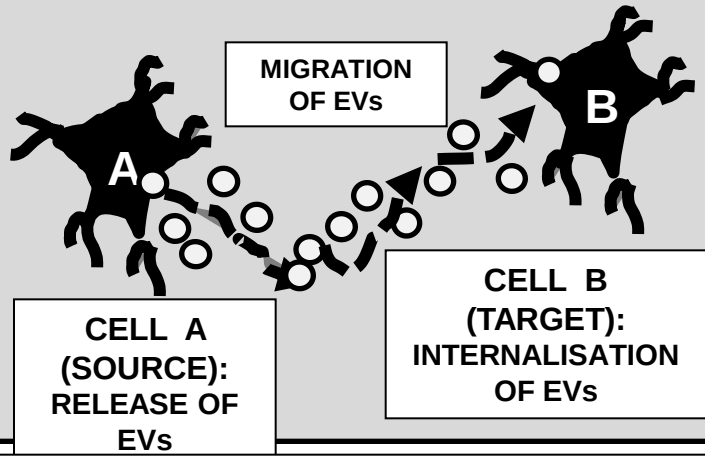
THE VOLUME TRANSMISSION CAN BE MEDIATED BY EXTRACELLULAR VESICLES (ECVs)

EXOSOMES (40-100nm) are 'SAFE CONTAINERS' of signals (mtDNA, mRNA, miRNA, Proteins, Lipids, …) released by cells



THE ECV MEDIATED VT

EVs (such as exosomes) allow the transfer of molecules from a source cell to a target cell



- **EVs (e.g., exosomes) can be internalized by Cell B that transiently modifies its biochemical machinery (i.e., the cell neurochemical phenotype)**

Agnati and Fuxe 2014: Borroto-Escuela et al 2015

Table 2

Classification of the tunneling nanotube (TNT) and extracellular vesicle (EV) mediated intercellular communication in the central nervous system into wiring transmission and volume transmission, respectively

Intercellular communication mode	Channel	Cargo transfer	Mechanism of transport	Origin
TNT type of WT (cultures of neuronal and glial cells) Rustom et al.2004 Gerdes et al.2007	TNT (15-60 µm long; 50-200nm diameter) transient, membrane continuity between cells	Organelles (especially vesicles), but also mtDNA, proteins, … inside or along the TNT plasma membrane surface	Actin driven	No in vivo data in adult CNS
EV type of VT (CNS: neurons, glial cells, brain endothelial cells) Simons and Raposo 2009; Schneider and Simons 2013	Extracellular space and CSF	**Exosomes** (40 – 100nm) containing proteins, lipids, mRNA, miRNA, mtDNA **Shedding vesicles:** ***Microvesicles*** (100 – 1000nm) insufficiently known composition ***Apoptotic bodies*** (1 – 4 um) Histones, DNA	Diffusion and flow along energy gradients in the extracellular space	Most cell types: endosomes, fuse with plasma membrane Most cell types: Plasma membrane All cell types: Plasma membrane Agnati and Fuxe 2014

Table 3 Characterization of the extracellular vesicle mediated volume transmission from the various cell types of the CNS

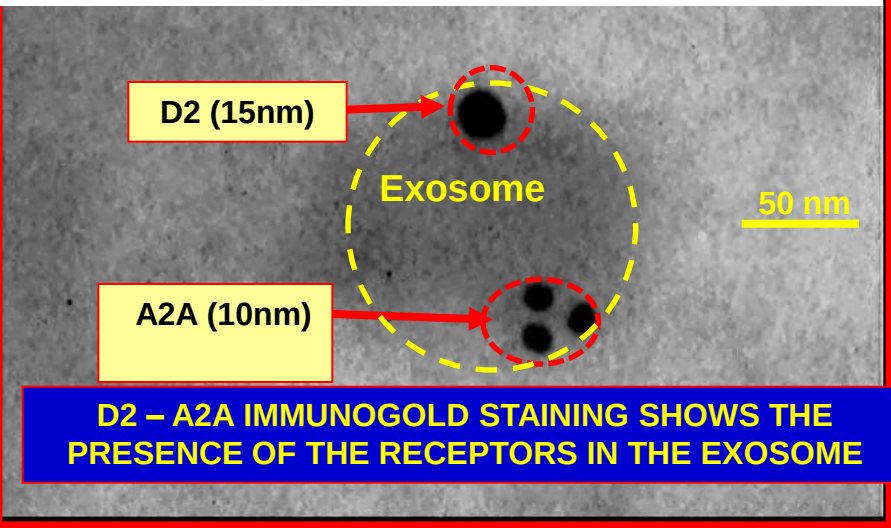
Cell type	Type of EVs°	Transfer compounds, acceptor cells, function
Neurons	Exosomes and microvesicles	Hsp, Flottilin, miRNA , GLuR2; nerve cells; synaptic and extrasynaptic exocytosis leading to neuronal plasticity
Astrocytes	Exosomes and microvesicles	Mitochondria, ATP,Hsp70, synapsin1, functional glutamate transporters, FGF-2,VEGF, matrix metallo-proteinases, microRNA-29; astrocytes and neurons; Potential role in repair,neuroinflammation and neurodegeneration

Agnati and Fuxe 2014

NEW PERSPECTIVES ON ROAMER TYPE OF VT

GPCRs ARE PRESENT IN EXOSOMES

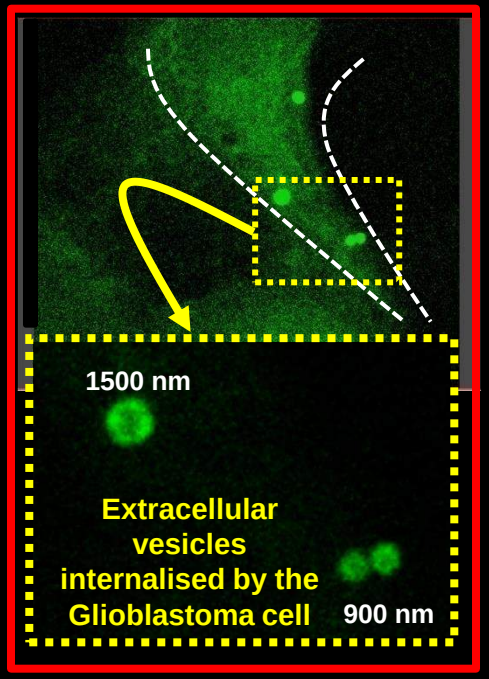
ELECTRON MICROSCOPE IMAGE OF EXOSOMES
D2 RECEPTORS were labelled by 15nm gold particles
A2A RECEPTORS were labelled by 10nm gold particles



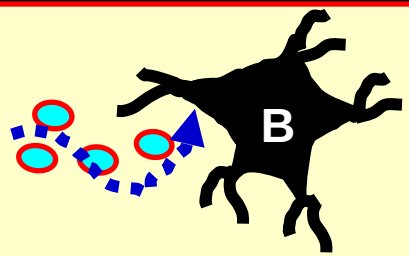
D2 – A2A IMMUNOGOLD STAINING SHOWS THE PRESENCE OF THE RECEPTORS IN THE EXOSOME

EXOSOMES ARE INTERNALISED BY CELLS

Glioblastoma cells were incubated with Exosomes prepared by CHO cells and containing A2A and D2 receptor



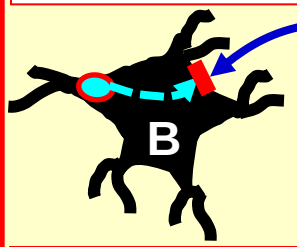
IMPLICATIONS OF THE CELL-TO-CELL TRANSFER VIA EXOSOMES OF GPCRs



CELL B IS A TARGET FOR EXOSOMES CONTAINING RECEPTORS THAT CELL B DOES NOT EXPRESS

Cell B, by internalising Exosomes can insert NEW TYPES OF GPCRs in the plasma membrane and hence decode previously undetected signals e.g., an undetected NEUROTRANSMITTER (NT) and/or can lead to formation of NEW RECEPTOR MOSAICS

INTERNALISATION OF EXOSOMES



NT

DECODING OF PREVIOUSLY UNDETECTED NEUROTRANSMITTER

INSERTION IN THE MEMBRANE OF NEW TYPES OF RECEPTORS

Guescini et al. 2012

FEATURES OF VOLUME TRANSMISSION

- **TYPE OF SIGNALS**

  Chemical : neurotransmitters, neuromodulators,

  growth factors, ions, gases (NO, CO, CO_2)

- **PATHWAYS**

  ECS preferential pathways

  CSF

- **POTENTIAL ENERGY GRADIENTS FOR SIGNAL MIGRATION**

  Concentration gradients

  Temperature gradients

  Pressure gradients

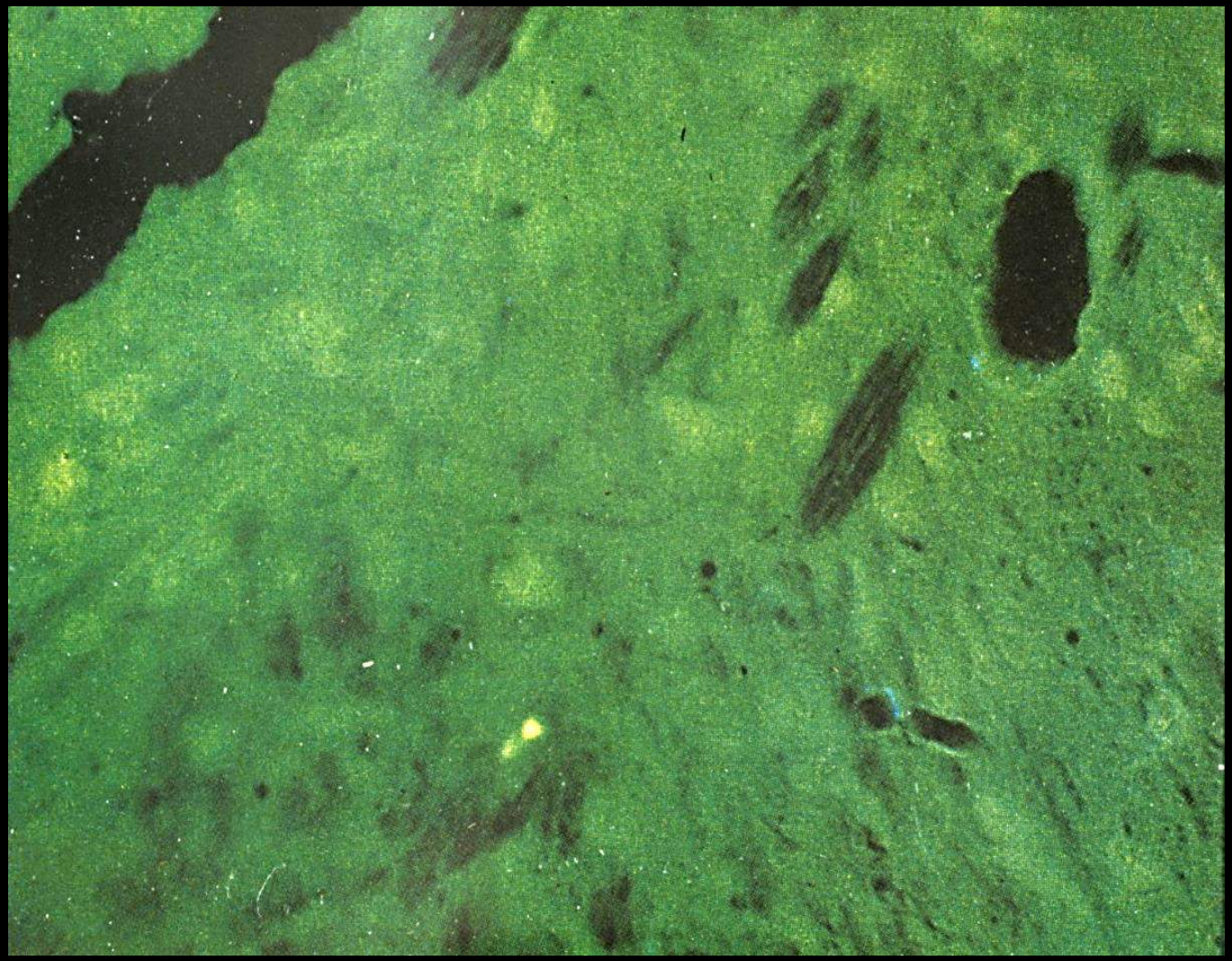
Convective forces

Convective forces

DECODING SYSTEMS Receptors, enzymes, ion channels

Appearance of extraneuronal CA fluorescence in the A9-A10 after amphetamine treatment in reserpine-nialamide-L-dopa treated rats

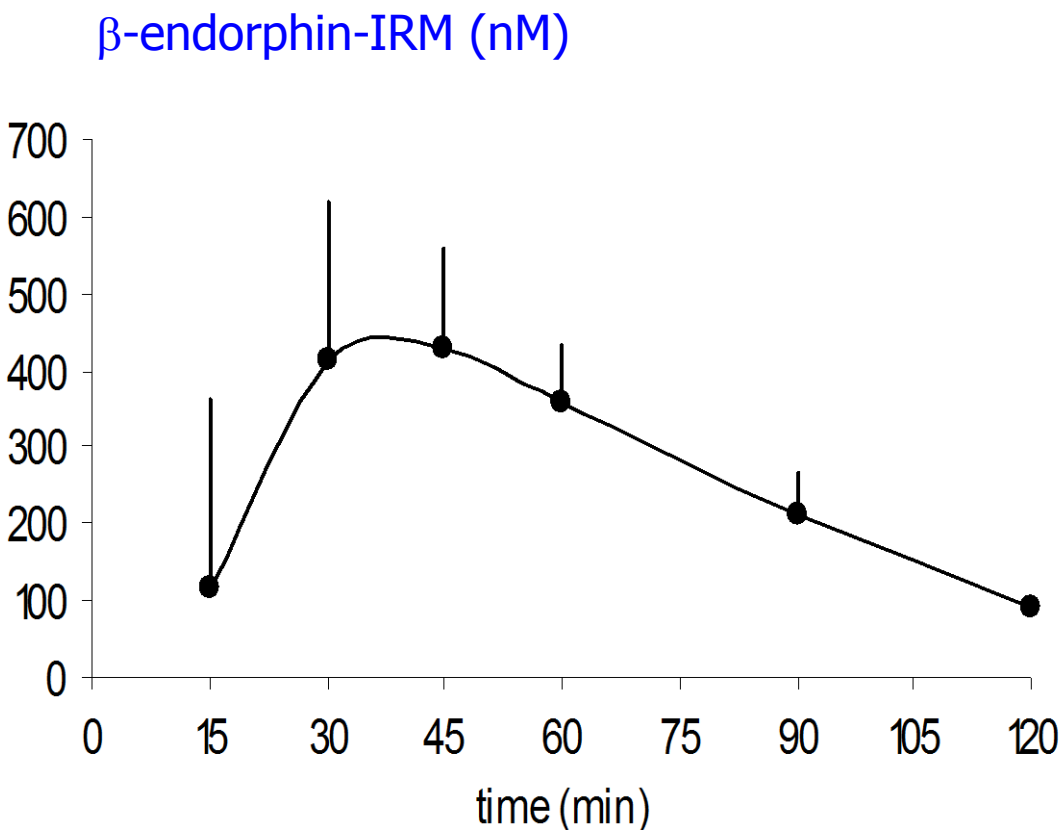
*Fuxe and Ungerstedt **1970** in Amphetamines and Related Compounds*



DA Microinjected into the striatum can diffuse

Ungerstedt, Butcher,Butcher Anden and Fuxe 1969

Radio-immunoassay of β-endorphin in the CSF



Intrastriatally administered beta-endorphin can migrate within the brain parenchyma and reach the CSF compartment as an intact peptide.

Beta-endorphin may employ the CSF as an complementary communication pathway, to also reach and activate distant opioid receptors.

The activation of opioid receptors was suggested from the fact that intrastriatally injected beta-endorphin was found on the cell surface of mu-opioid receptor-IR nerve cells on remote cells.

Hoistad et al. 2005

Summary II

- The molecular targets for the integration of synaptic and volume transmission are the heteroreceptor complexes in the pre-and postjunctional membranes through allosteric receptor-receptor interactions (Fuxe et al.2013)

- The receptor interface in GPCRs involves helix-helix interactions, electrostatic interactions in the intracellular loops and C-terminal tail and protriplet amino acid homologies representing adhesive guides .
  The triplet puzzle theory is based on a bioinformatic approach (Tarakanov Fuxe 2010)

- 5-HT1A autoreceptor-FGFR1 heteroreceptor complexes on serotonin cell bodies located extrasynaptically are a novel target for antidepressants. Synergistic allosteric receptor-receptor interactions upon coagonist treatment recruit 5-HT1A autoreceptors into 5-HT1A-FGFR1 heteroreceptor complexes (Borroto-Escuela et al.2012)

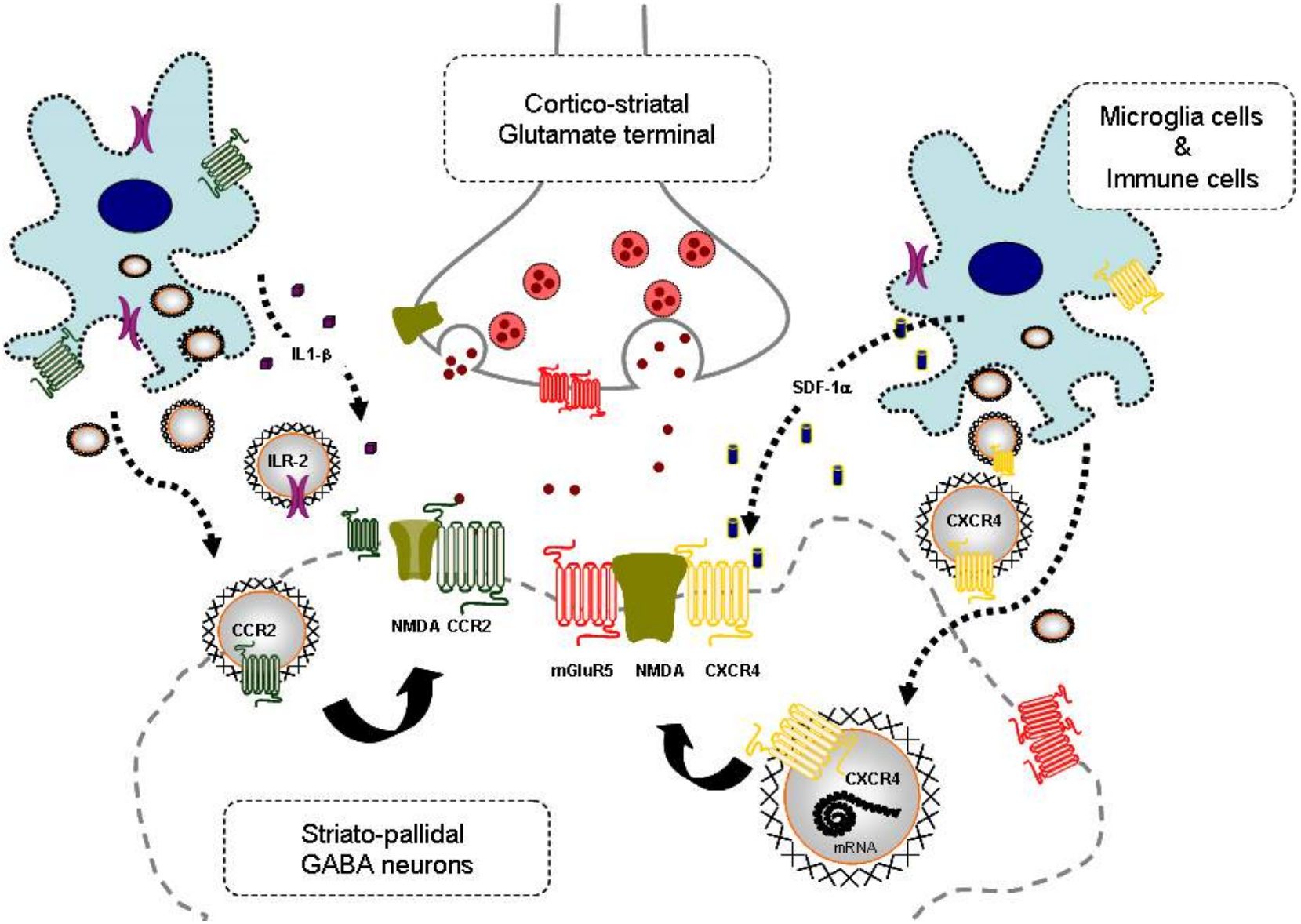
- One proposed role of ECV mediated glial VT in schizophrenia: Based on the triplet puzzle, CXCR4,CCR2 and ILR2 can in mild neuroinflammation if internalized into neurons interact with NMDAR and GABAA/BR via distinct triplet homologies.Pathological allosteric receptor-receptor interactions can develop contributing to schizophrenia symptoms (Fuxe et al.2013)

Summary II

- The molecular targets for the integration of synaptic and volume transmission are the heteroreceptor complexes in the pre-and postjunctional membranes through allosteric receptor-receptor interactions (Fuxe et al.2013)

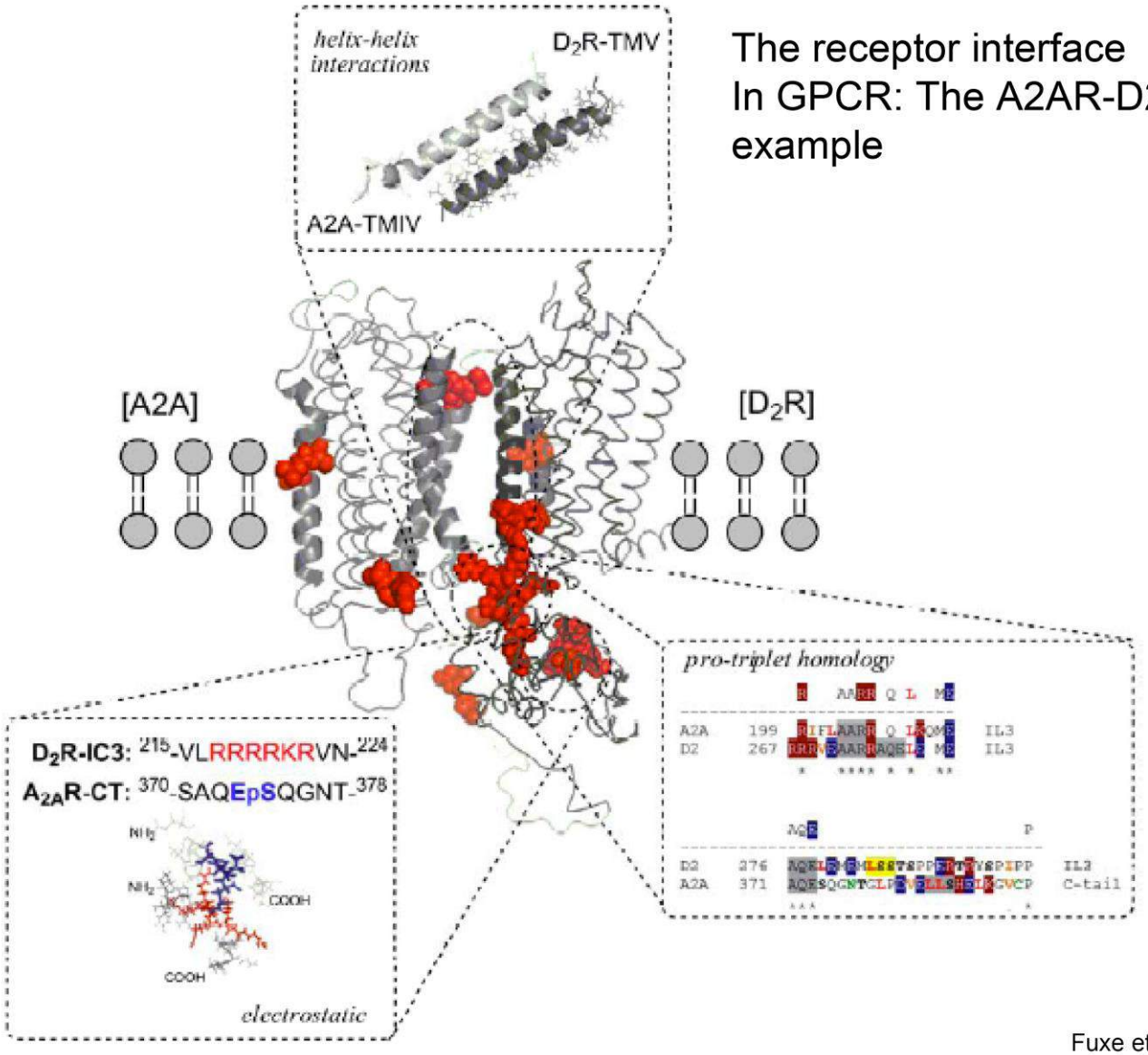
- One proposed role of ECV mediated glial VT in schizophrenia: Based on the triplet puzzle, CXCR4,CCR2 and ILR2 can in mild neuroinflammation if internalized into neurons interact with NMDAR and GABAA/BR via distinct triplet homologies.Pathological allosteric receptor-receptor interactions can develop contributing to schizophrenia symptoms (Fuxe et al.2013; Bechter 2013)

Illustration of potential chemokine & cytokine receptor transfer via EV-VT from immune and glial cells to neurons in inflammation and formation of putative novel NMDA heteroreceptor complexes



Fuxe et al.2015

The receptor interface In GPCR: The A2AR-D2R example



Fuxe et al.2012

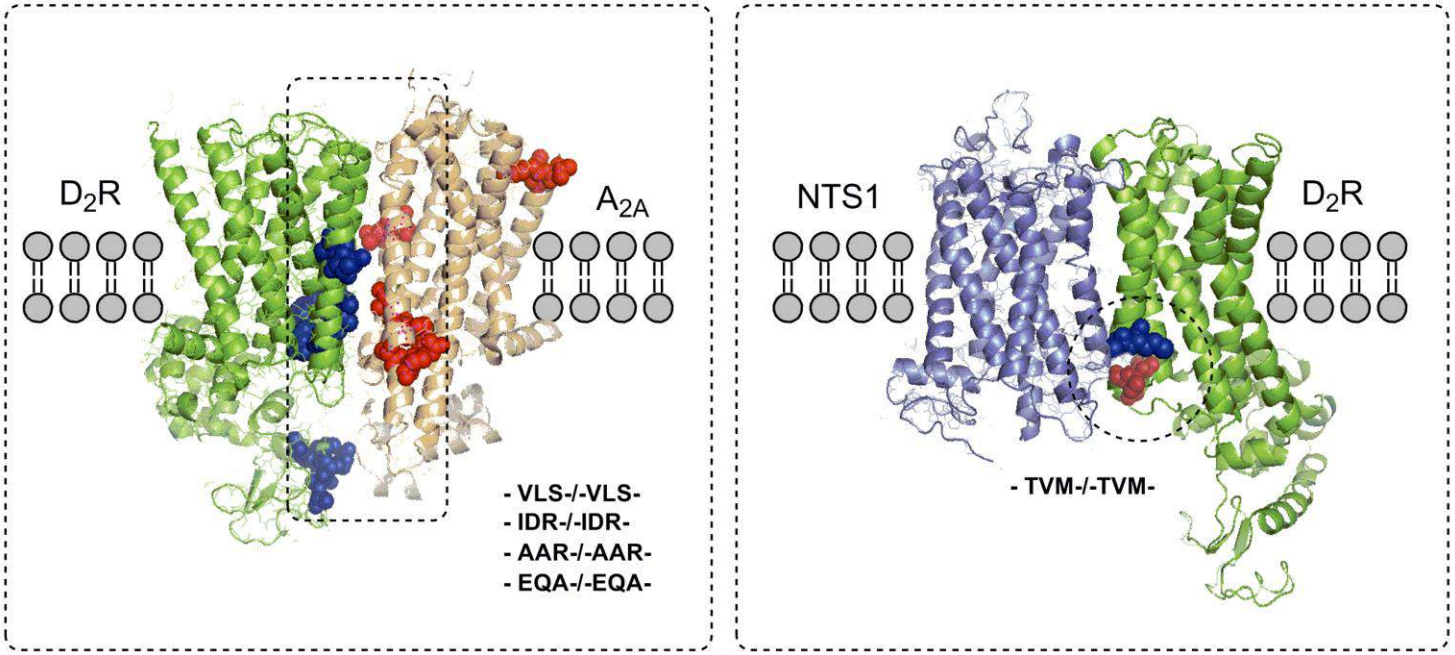
The triplet Puzzle: Homologies of amino acid protriplets in Receptor Heteromers

Rank	Triplets
40 pro-triplets (for heterodimers)	
8	LNL LVI LPF AIV IDR
7	LLT VLS ILS TSL LIL ILI APL LYS AAR
6	LLI LVA TLV LSL SSL ASI IIY TAV ITL PFF AAV
5	LLP LLE LLG LVL KSL NSL IIL VIL ATL FNI FGN SVS VAI VFV AIS
4 contra-triplets (against heterodimers)	
2	IMG IIN VTN GNT

Through a mathematical approach, we have deduced, based on 48 pairs of receptors that form or not form heteromers, sets of triplet amino acid homologies that may participate in receptor interface.

They give a kind of code that help determine which receptors should or should not form heterodimers .

Illustration of homology protriplets in A2A-D_{2L}R and D_{2L}R-NTS1 heteromers



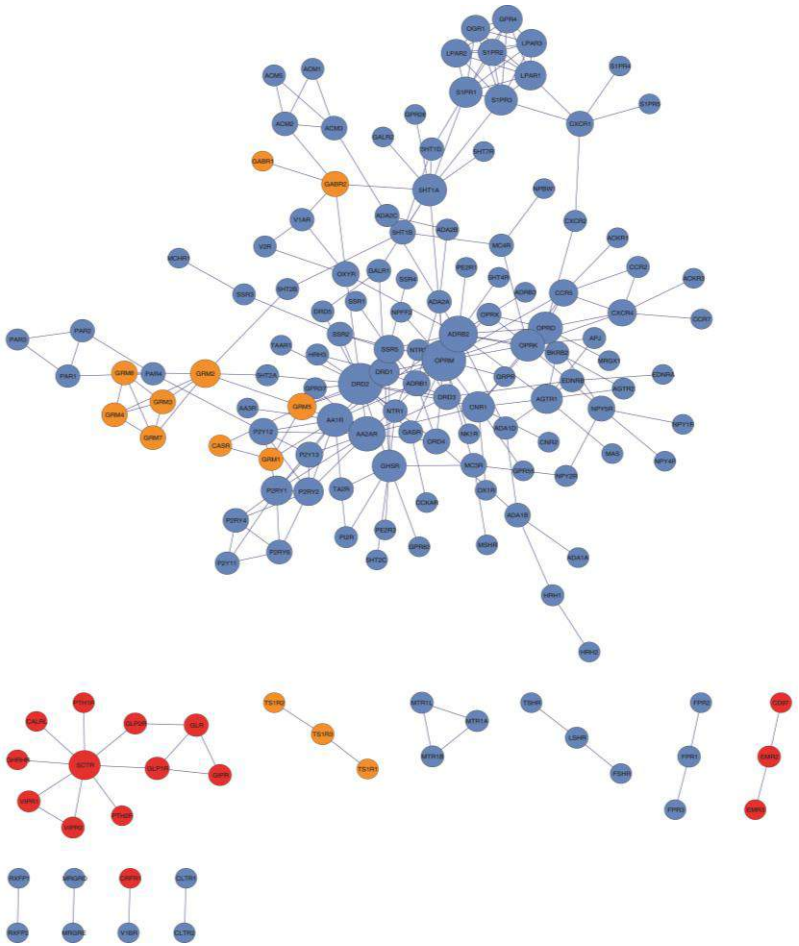
We propose a 'guide-and clasp' manner for receptor–receptor interactions where 'adhesive guides' may be the triplet homologies.

Tarakanov and Fuxe 2010

GPCR heterodimer network

Class A or rhodopsin-like: **blue**;
Class B or Secretin: **red**;
Class C or Metabotropic: **orange**.

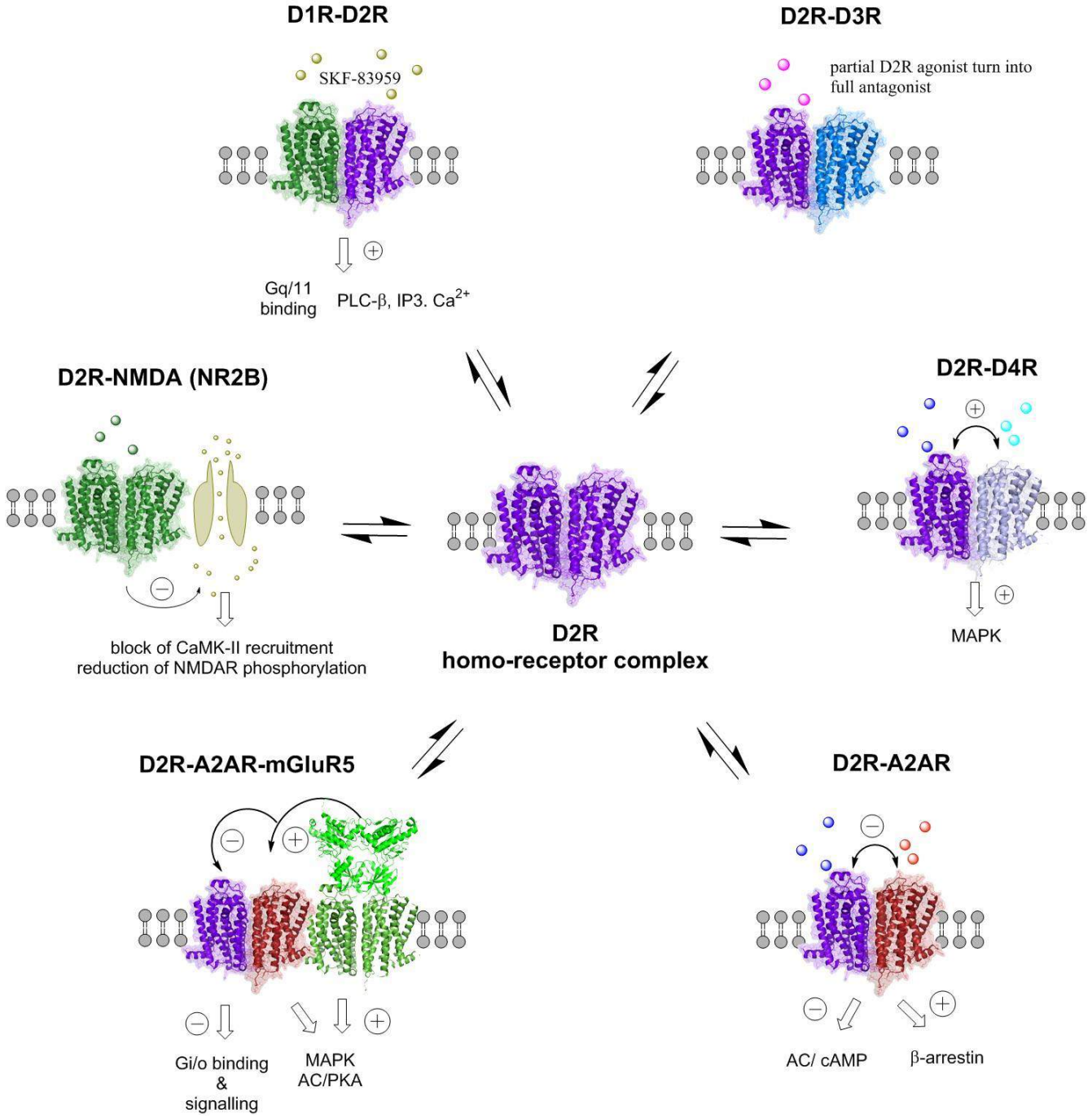
Experimental data from the String and Scopus databases and using Cytoscape software
Implemented in Java



156 protomers /nodes
260 interactions /edges
Scale- free topology:
Most nodes have few interactions
Nine nodes have ten or more inter actions : e.g. D2, 5-HT1A, A2A, A1

http://www.iiia.csic.es/~ismel/GPCR-Nets/index.html

Borroto-Escuela et al. 2014


D1R-D2R
SKF-83959
Gq/11
binding
PLC-β, IP3. Ca2+
D2R-D3R
partial D2R agonist turn into
full antagonist
D2R-NMDA (NR2B)
block of CaMK-II recruitment
reduction of NMDAR phosphorylation
D2R
homo-receptor complex
D2R-D4R
MAPK
D2R-A2AR-mGluR5
Gi/o binding
&
signalling
MAPK
AC/PKA
D2R-A2AR
AC/ cAMP
β-arrestin

Heteroreceptor complexes among DA receptor subtypes

Heteroreceptor complex and location	Signaling	Potential relevance PD
D1R-D2R ; subsets of MSNs in accumbens (DYN,ENK,GABA/glutamate) *Lee et al.2004,*	**Gq**, Ca release via IP3, recruitment of **BDNF** via CaMKII and MeCP2, blocked by D1R and D2R antag.;inactivates GSK-3β SKF 83959: **D1-D2 heteromer agonist**	Hyperdopaminergia increases function of the D1R-D2R heteromer; **mental side-effects** with L-DOPA and D2 agonist including addiction *Perrault et al. 2010-2013; O'Dowd et al.2012*
D1R-D3R; certain striato-nigral GABA neurons *Bordet et al.1997;Ridray et al.1998; Marcellino et al.2008 Fiorentini et al.2008*	D3R enhancement of D1R affinity and postsynaptic signaling	**D3R enhances D1R induced locomotion**. *Marcellino et al.2008, Fiorentini et al.2008*
D2R-D3R; ventral striatum *Scarcelli et al. 2001; Pou et al.2012*	**Partial D2R agonists turn into D2R antagonists at the D2-D3 heteromer**	Partial D2R agonists in PD treatment have **reduced mental side-effects** *Maggio and Millan 2010*

Heteroreceptor complexes among DA receptor subtypes

Heteroreceptor complex and location	Signaling	Potential relevance PD
D2R-D4R: **D2LR–D4.2R , D2LR–D4.4R** but not D2LR-D4.7R heteromers; short form of D2R also form hetromers with D4.2 and D4.4; colocalization in striatum *Borroto-Escuela et al.2011, Gonzalez et al.2012*	Combined D2 and D4 agonist treatment resulted **in potentiating effects on ERK1/2 phosphorylation** for D4.2R, D4.4R but not for D4.7R containing heteromers	The synergistic effects on ERK signaling may **increase plasticity** responses to L-DOPA treatment.The D4.7 variant may be linked to ADHD. *Borroto-Escuela et al.2011, Gonzalez et al.2012*
D2R-D5R: colocalization cortex and ventral pallidum *So et al.2009, O'Dowd et al 2013*	**Gq-PLC signaling upon combined agonist** treatment leads to small intracellular calcium release and then to large extracellular influx of calcium	**Possible activation of CaMKII , linked to involvement in drug addiction**. Possible side-effect of L-DOPA treatment in PD. *So et al.2009, O'Dowd et al 2013*