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Brain integration: From neural networks operating via synaptic and volume transmission to brain-body medicine

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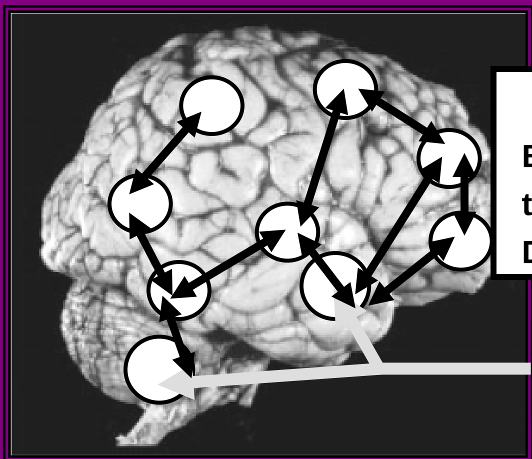
Consciousness as a virtual space and personage created by the existence of multiple interacting mirrors.

Cortical integration :Possible dynamic formation and disruption of mosaics of functional modules and reassembly into new mosaics of activated modules resulting in a novel function

NEUROBIOLOGICAL PHENOMENON



BINDING OF FUNCTIONAL MODULES especially thanks to the WIRING TRANSMISSION FORMING DYNAMIC CORTICAL MOSAICS

INPUTS FROM THE EXTERNAL ENVIRONMENT

EMERGENT PROPERTY

CREATION OF A VIRTUAL SPACE (THE INTERNAL THEATRE) AND OF A VIRTUAL PERSONAGE (THE SELF)

○ **FUNCTIONAL MODULES OF CELLUAR NETWORKS ORGANIZED AS RUSSIAN DOLLS.**

METAPHOR: THE INTERACTING FUNCTIONAL MODULES CAN BE REPRESENTED BY 'MIRRORS' THAT REFLECT THE IMAGES OF EACH OTHER BUT NOT IN A PASSIVE WAY

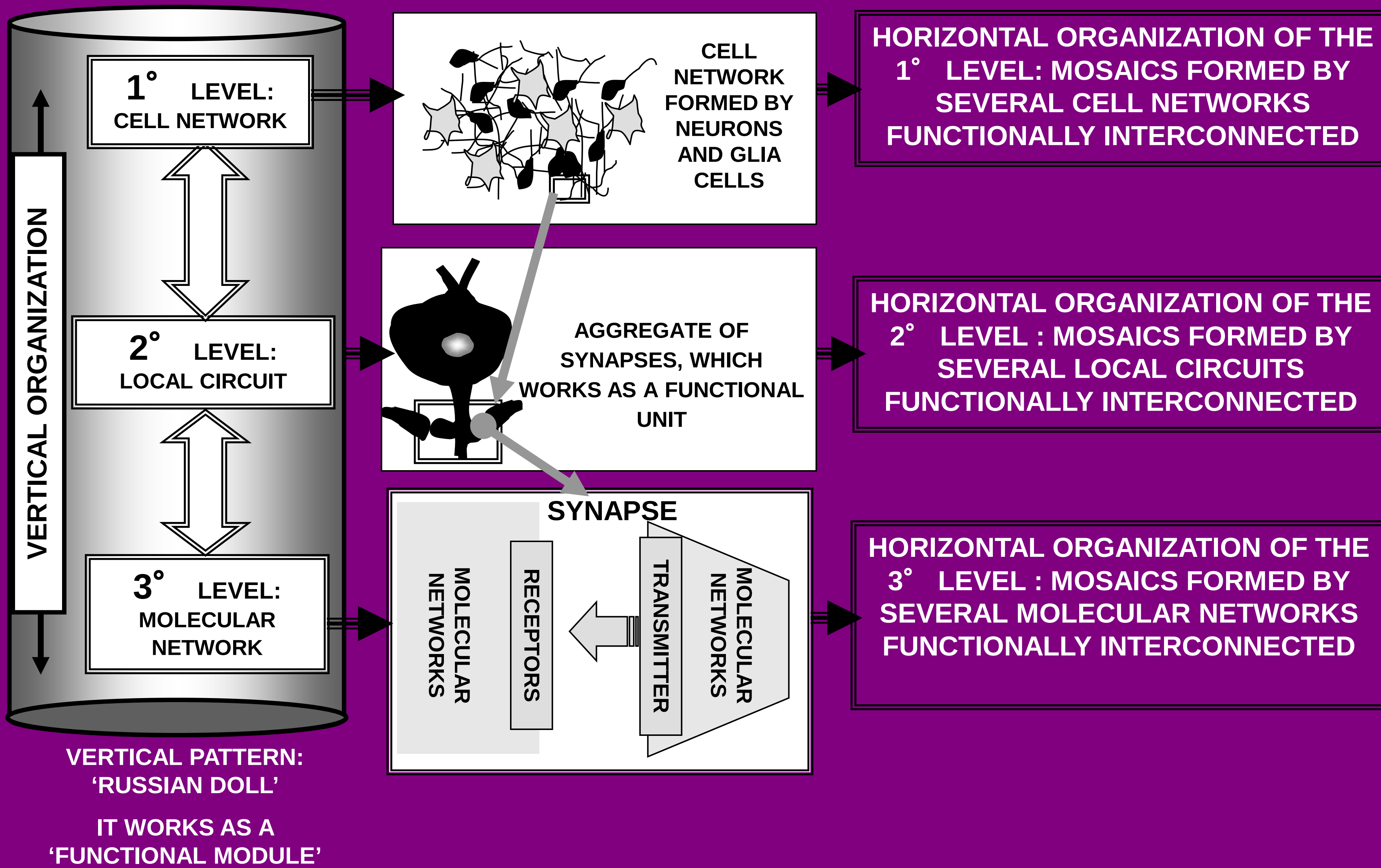
↔ **BINDING PHENOMENON CAPABLE OF INTERCONNECTING ACTIVE FUNCTIONAL MODULES.**

METAPHOR: PROJECTION OF IMAGES AMONG MIRRORS ACCORDING TO MULTIPLE RECURRENT PATTERNS

Neuronal correlates of consciousness as an epiphenomenon of multiple dynamic mosaics of cortical functional modules

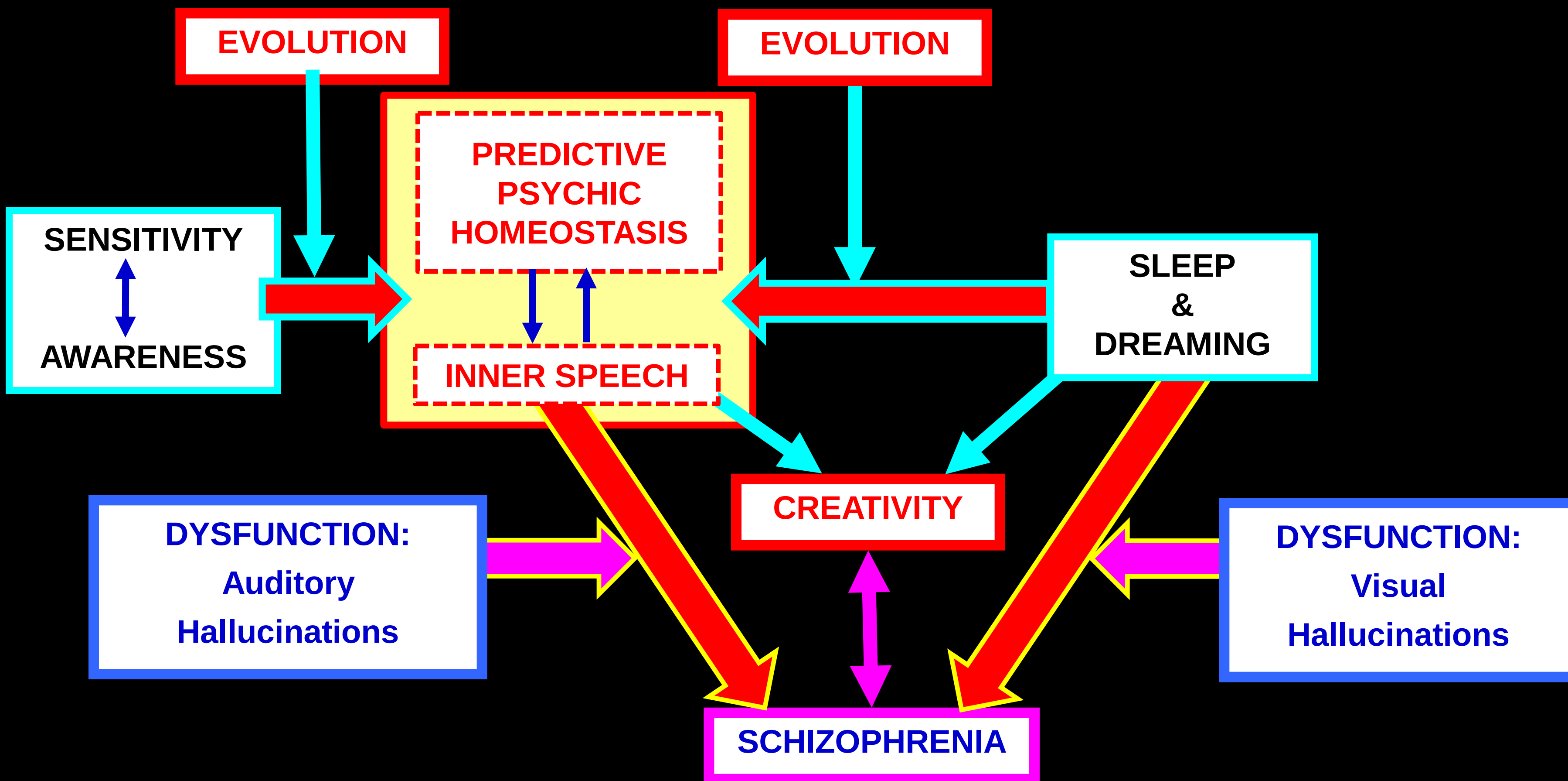
Agnati et al. 2012

Integration in functional modules



Agnati et al.2010,Fuxe et al.2010

The human dimension may develop out of inner speech, creativity and predictive psychic homeostasis

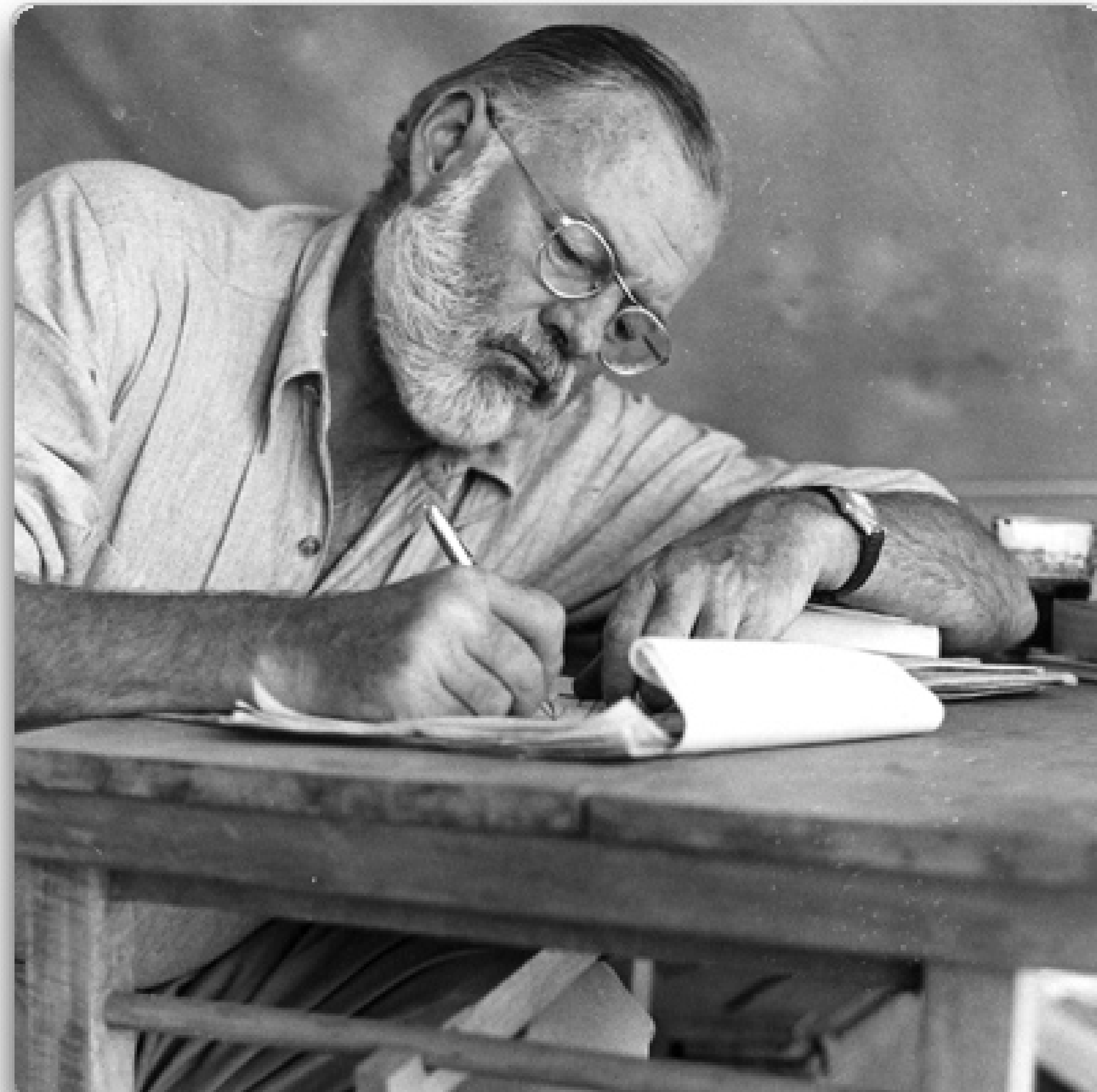


Evolution that has produced typical human capabilities (in red)

Dysfunction can produce typical human pathologies (in blue)

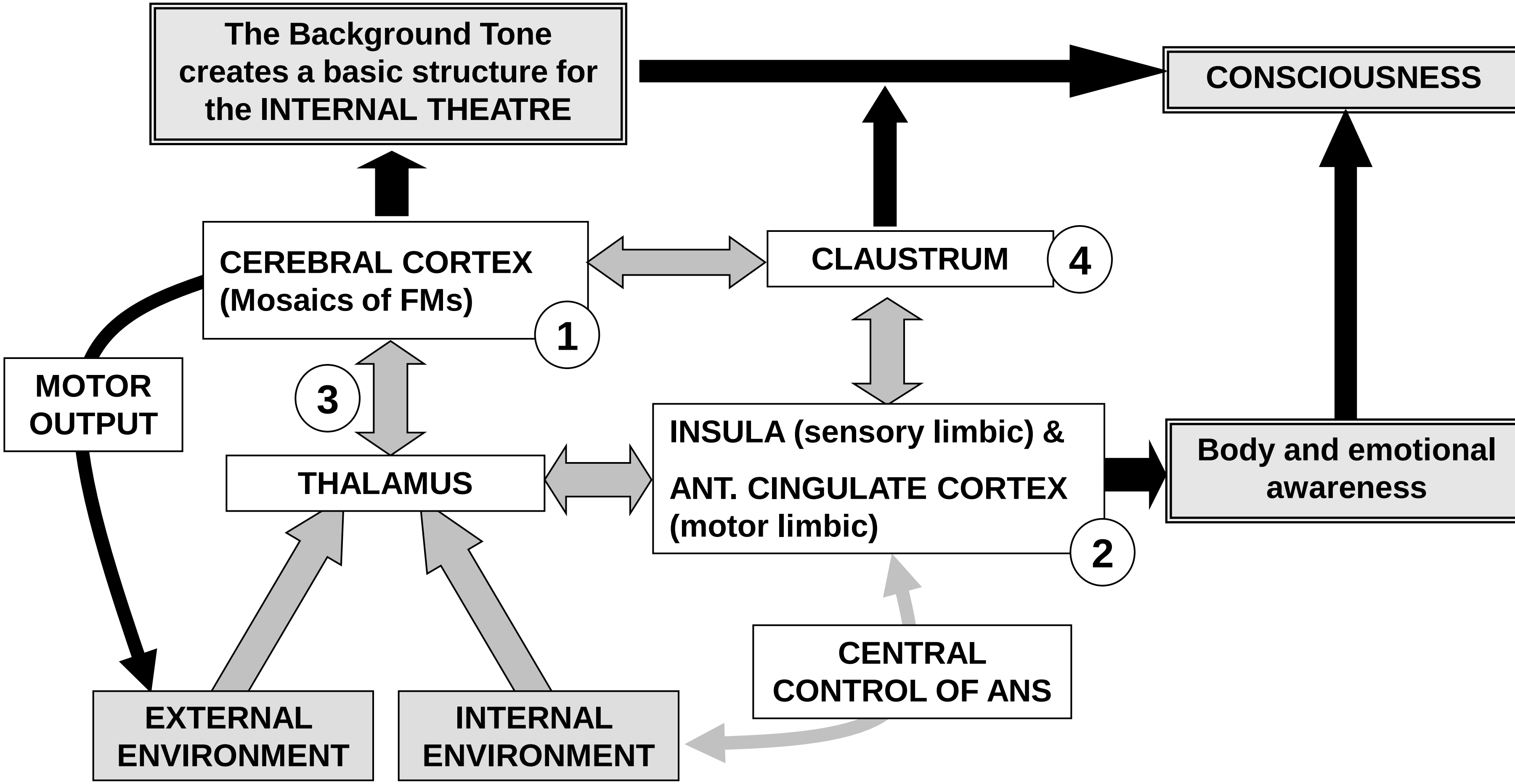
Agnati et al.2011, 2012

Ernest Hemingway (1899-1961)



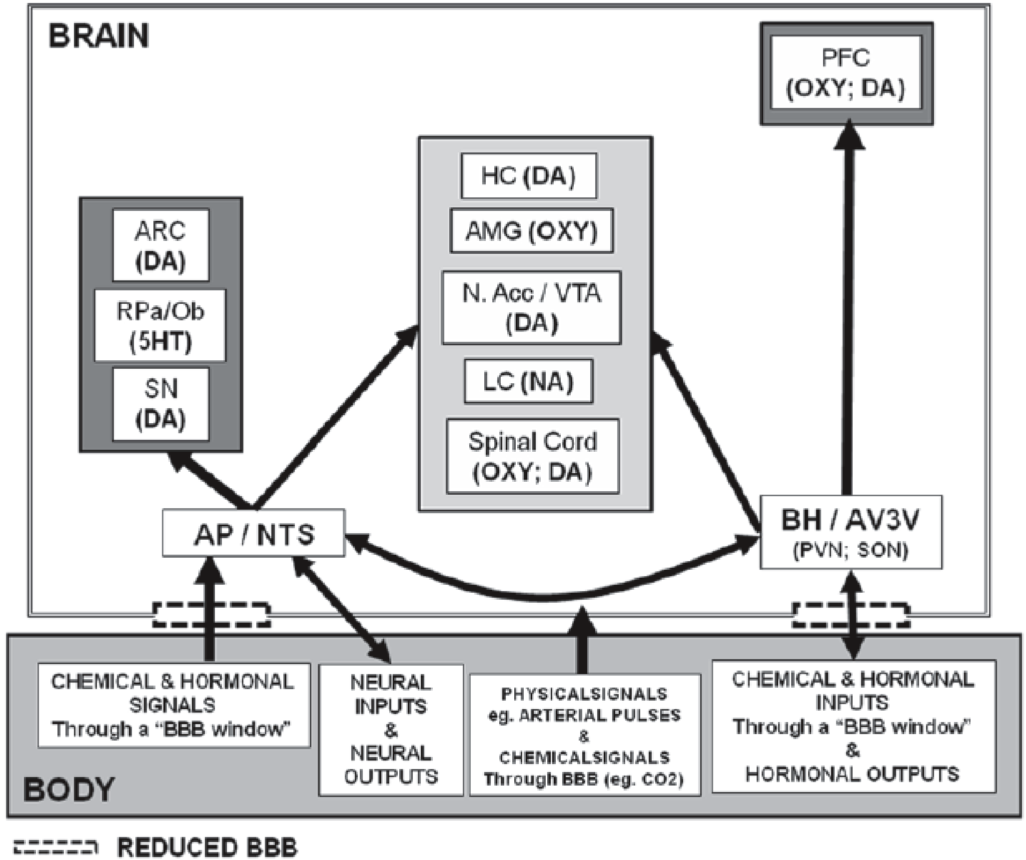
Ernest Hemingway Quote

His talent was as natural as the pattern that was made by the dust on a butterfly's wings. At one time he understood it no more than the butterfly did and he did not know when it was brushed or marred. Later he became conscious of his damaged wings and of their construction and he learned to think and could not fly any more because the love of flight was gone and he could only remember when it had been effortless



1.Coalitions of shifting assemblies of brain cells stabilized by re-entrant feed-backs (Edelman & Tononi, 2000) represent the NCCs
2. Special FMs could be found at the level of the insula and the anterior cingulate cortex (Craig, 2009) giving the body an emotional awareness.
3. As proposed by Llinas (Llinas et al., 1998) thalamo-cortical interconnections should represent the main system selecting and binding the FMs to form the NCCs
4. The claustrum could contribute to the process by acting as a ‘conductor’ giving the proper emphasis to each FM (Crick & Koch, 2003).

Agnati et al.2012



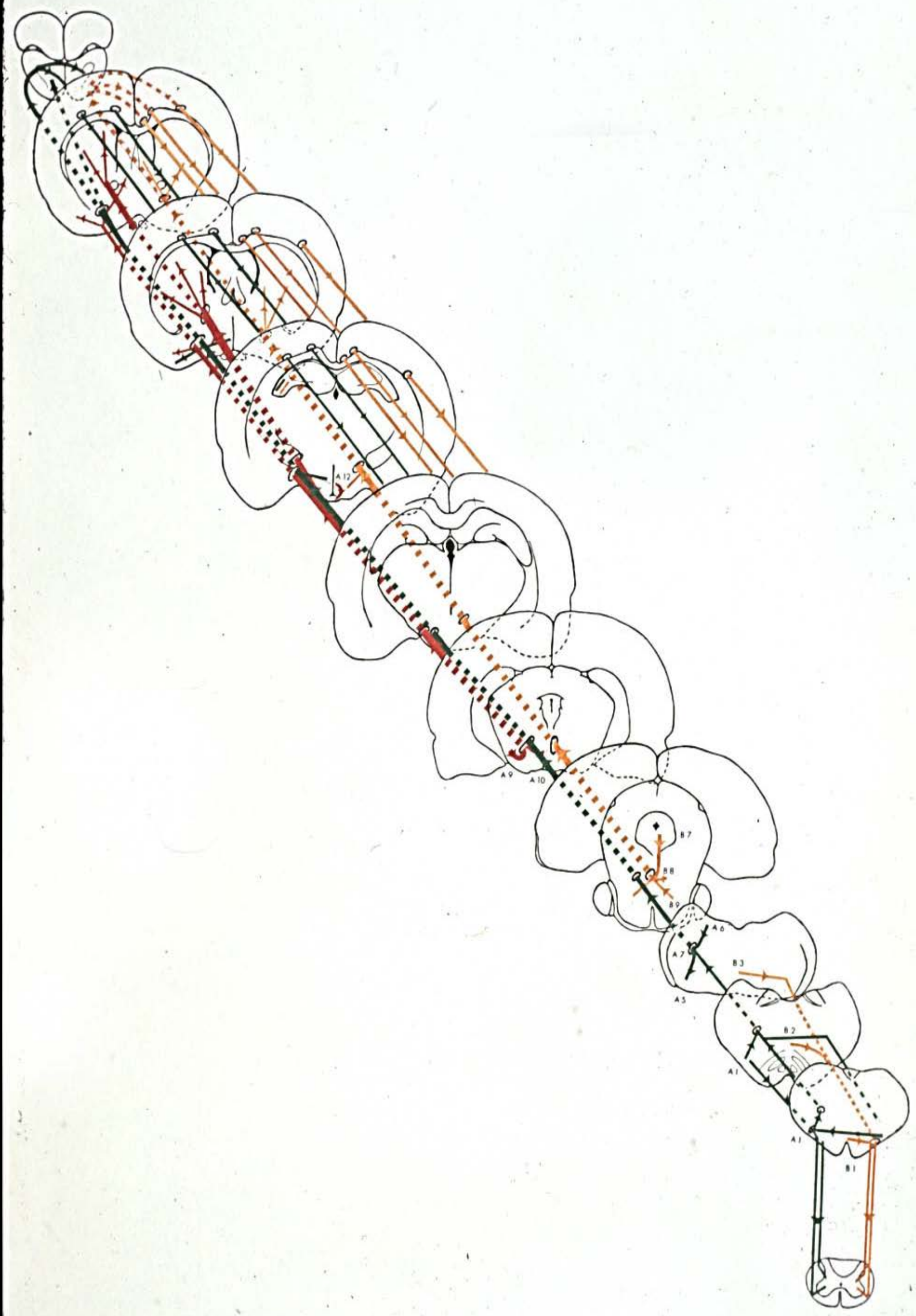
On the existence of a brain-body integrative structure formed by the area postrema/nucleus tractus solitarius (AP/NTS) and the anteroventral third ventricular region –basal hypothalamus with the median eminence. These interconnected regions represent interfaces between the body and remaining brain regions. They integrate brain-born and body born neural and humoral signals. The DA,NA and 5-HT brainstem pathways play a major role in mediating the body-born Signals to the cortical and subcortical structures

Agnati et al.2012

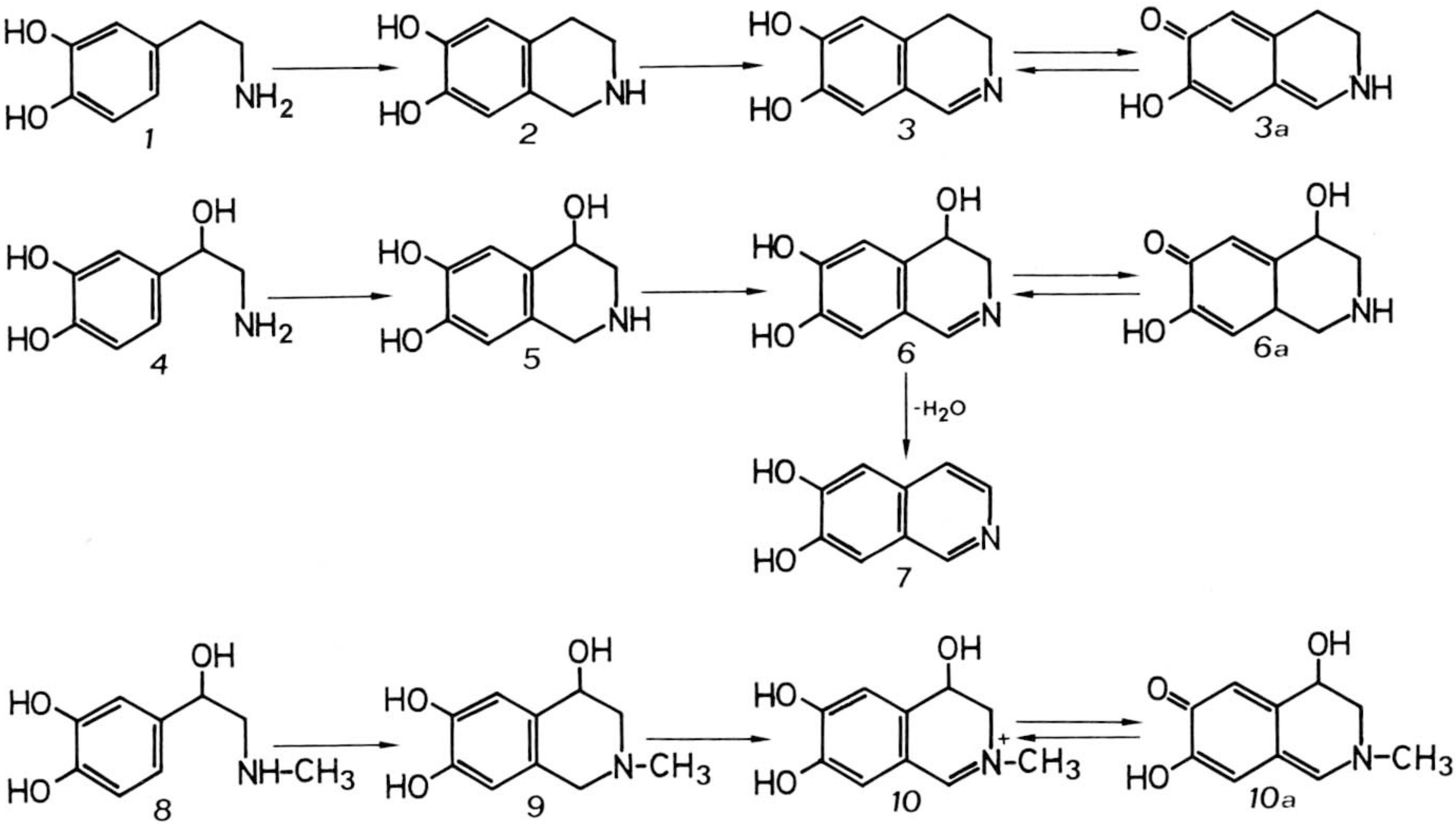
Schematic description of the DA, NA and 5-HT pathways from 1965

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

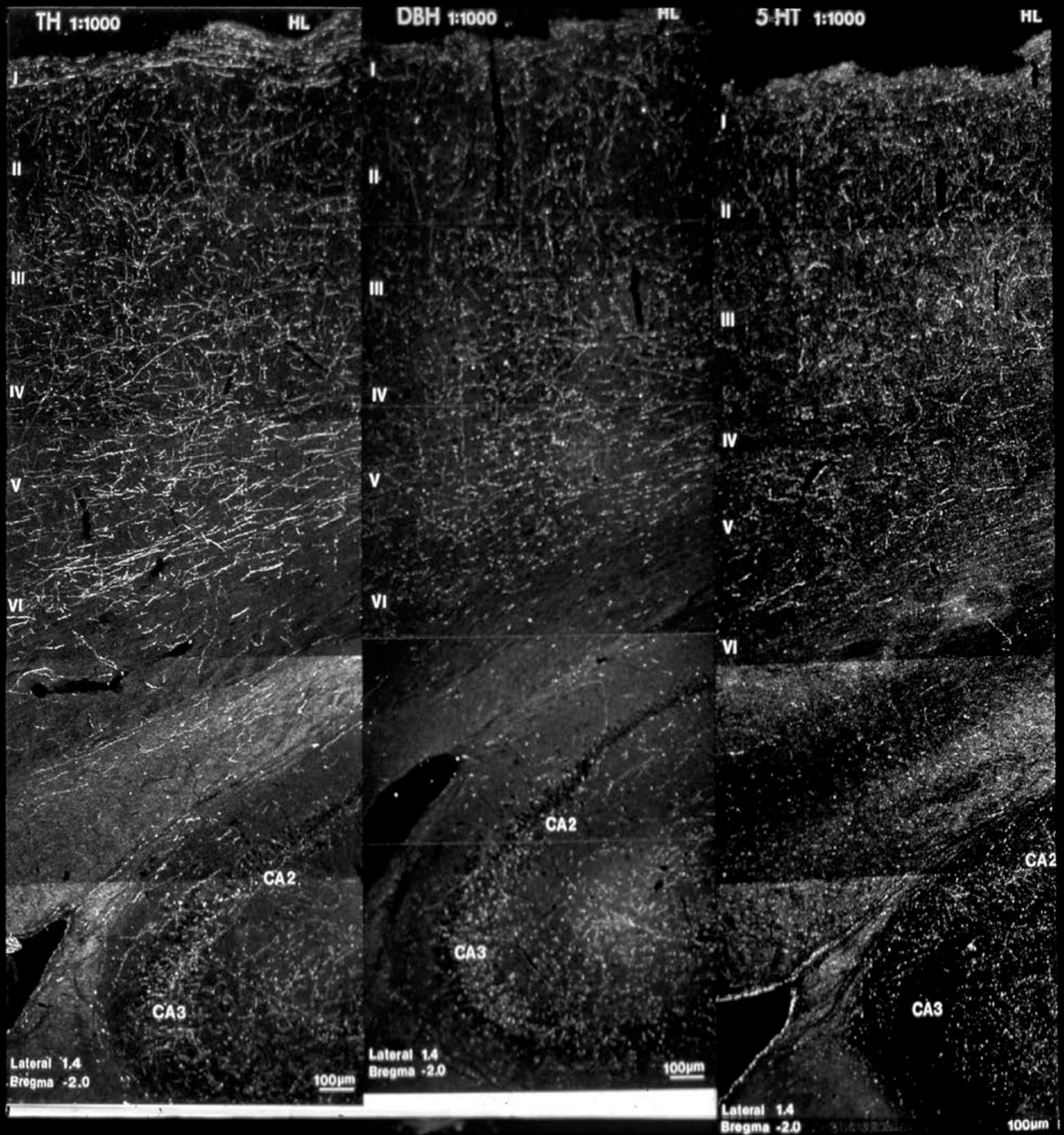


The chemistry of the CA and 5-HT histofluorescence method of Falck and Hillarp



The histochemical reaction between CA and formaldehyde.The amine condenses with formaldehyde forming a 1,2,3,4,-dihydroIsoquinoline subsequently dehydrogenated to the corresponding 3,4-dihydroisoquinoline in a protein promoted reaction.The latter Is in equilibrium with its quinoidal form which predominates and is responsible for the green fluorescence at 480 nm.

Fuxe et al.1970



Tinner,Fuxe Goldstein
unpublished

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).

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

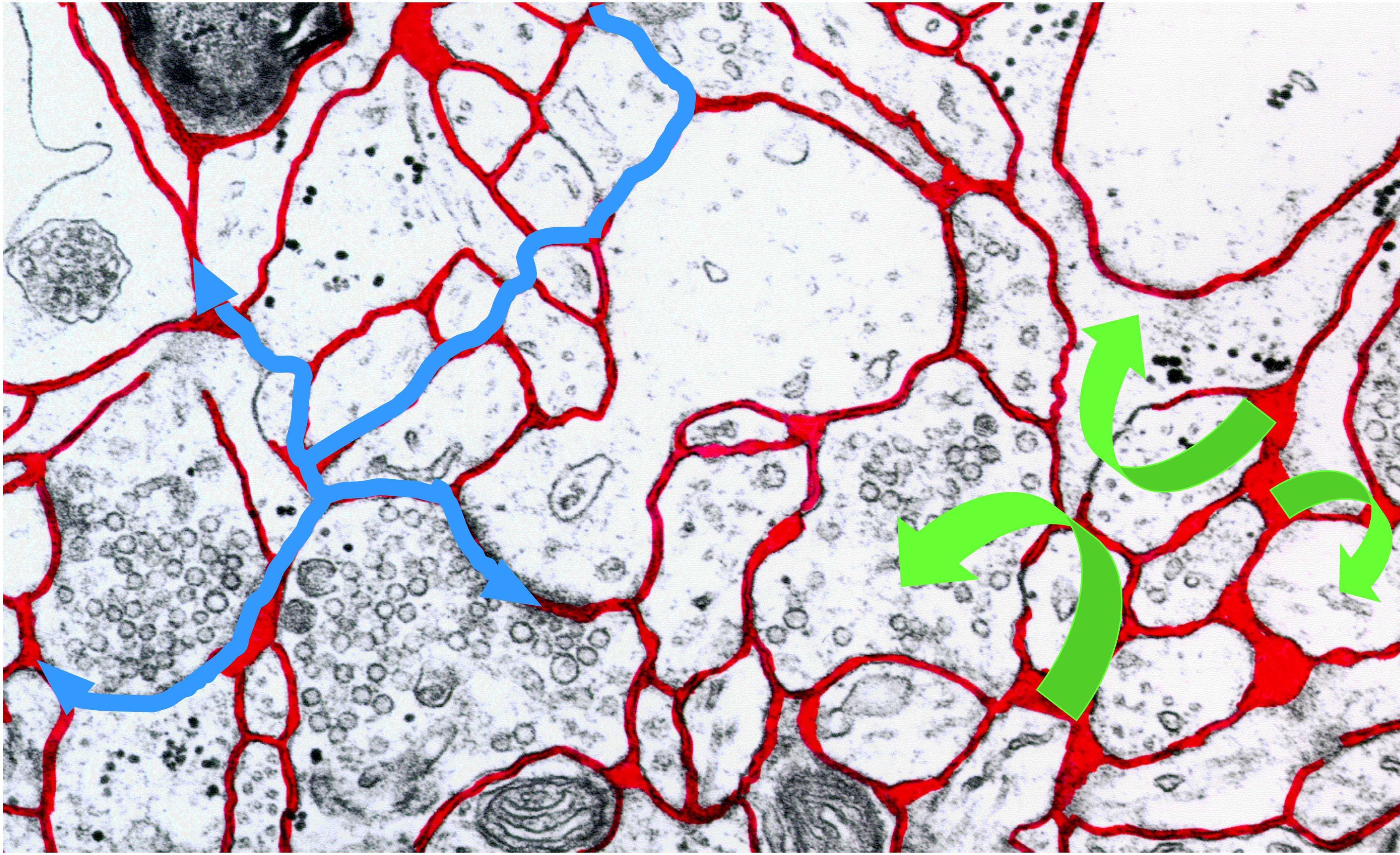
One subtype : gap junctions . privacy : broadcast

Agnati and Fuxe, 1986, 2000,Fuxe and Agnati 1988, 1991,2009

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

α **volume fraction** λ **tortuosity** κ **clearance**

Increase in path length compared to a straight course

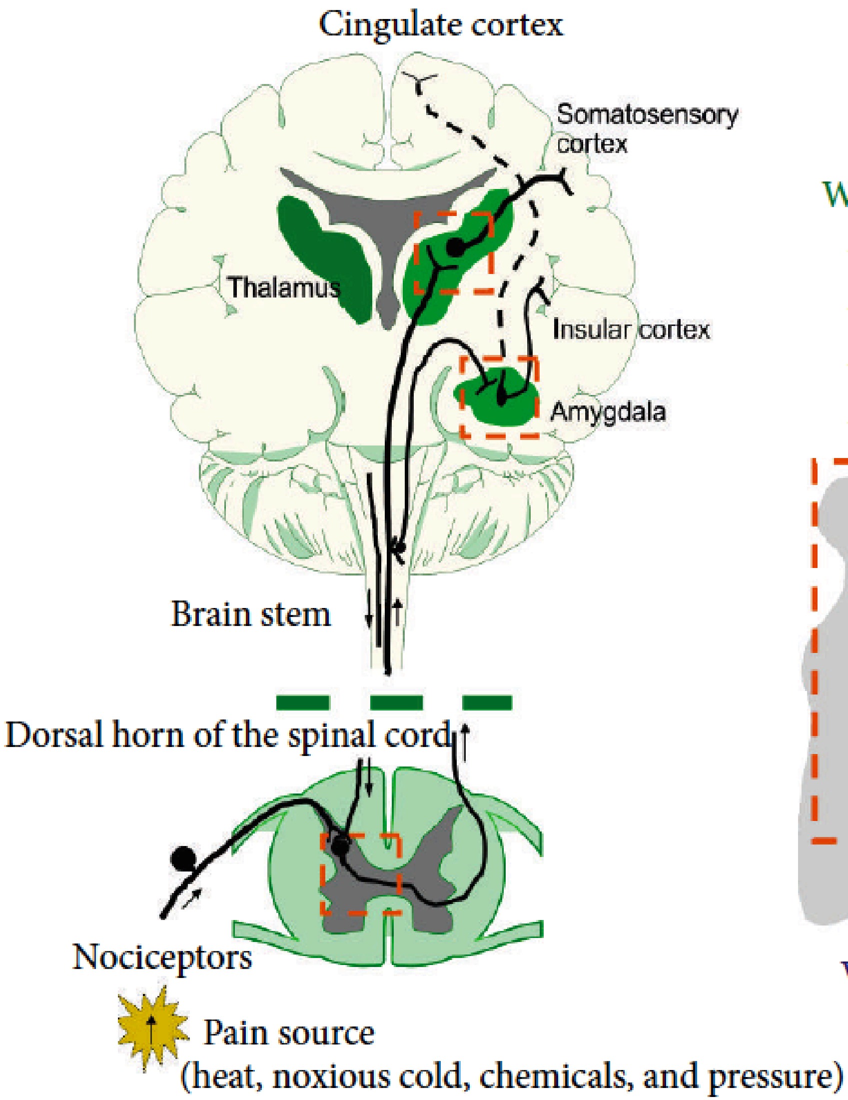


Modified from Nicholson & Sykova 1998 TINS

Porous fluid filled space with a foam-like structure

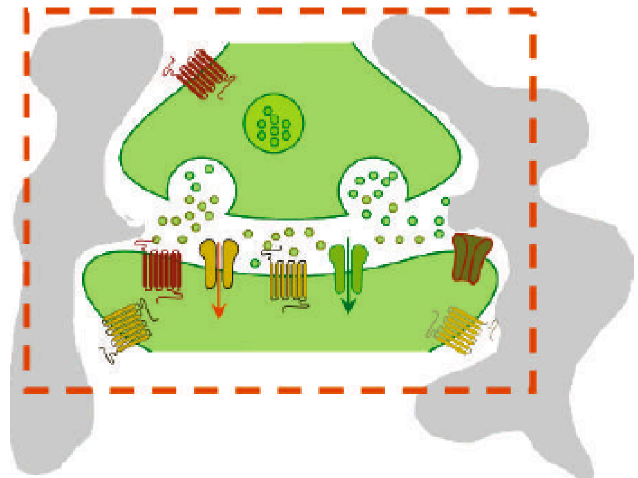
Höistad 2004

Pain pathways and their regulation through WT and VT signals integrated through receptor-receptor interactions in heteroreceptor complexes



Wiring transmission

- Ions and neurotransmitters
- Usually a high chemical concentration at receiver
- Usually a low receiver affinity for chemical signal
- A low transmission delay



Volume transmission (VT)

- Ions, neurotransmitters, neuropeptides, gas
- Usually a low chemical signal concentration at receiver
- Usually a high receiver affinity for chemical signal
- A high transmission delay

Borroto-Escuela et al.2013

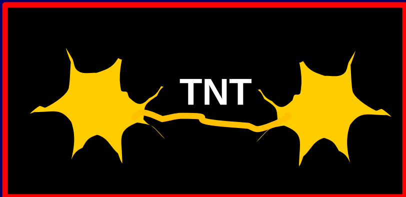
Roamer type of volume transmission

-Release of extracellular vesicles (microvesicles ,MVs; exosomes and shedding vesicles) containing e.g. proteins and RNAs from cellular networks in the CNS into the extracellular flluid and CSF represents a special form of volume transmission,the Roamer subtype of VT.
The MVs are like the roamer's bag filled with important materials (see Agnati et al.2010, Guescini et al.2012,Fuxe et al.2012,2013).

- GPCRs may also be transferred from cell to cell via MVs. The transferred mRNAs coding for A2A receptor and/or the corresponding protein are shown to be functionally competent.Target cells can thus acquire the ability to transiently recognize and decode signals via receptors they do not normally express and also form receptor heteromers resulting in novel integrated signaling.

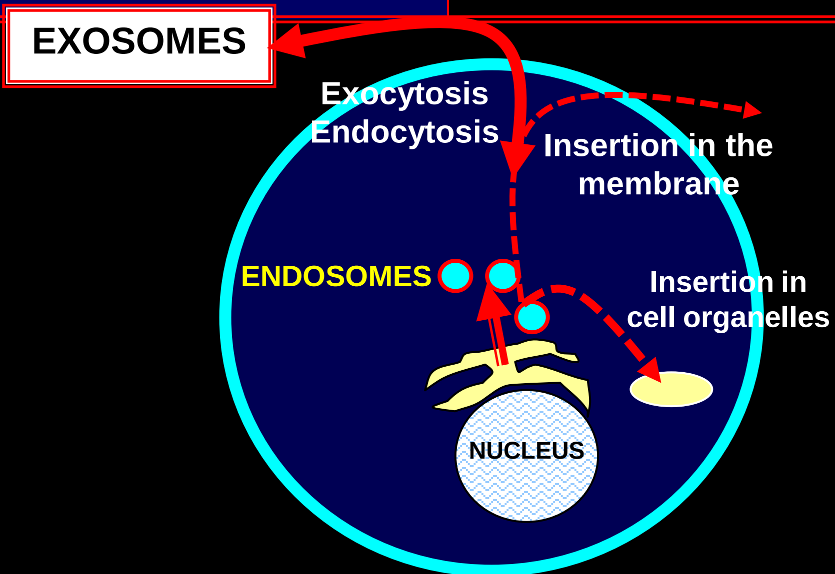
-MV release may represent a novel mechanism for neuronal plasticity and plasticity in glia-neuronal communication involving also receptor transfer at the protein and mRNA level which can form novel receptor heteromers in the target cells. It may also lead to the spread of pathological proteins in the CNS (see Brundin and colleagues,University of Lund)

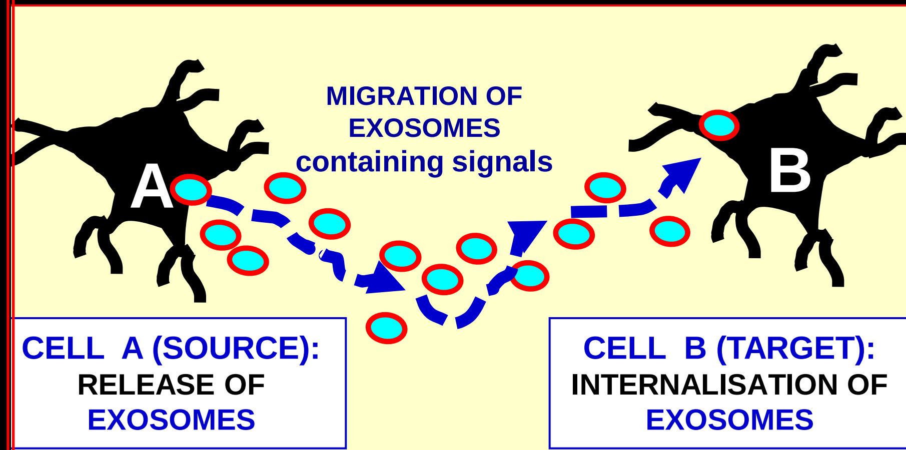
NEW MODES FOR INTERCELLULAR COMMUNICATION: NANOTUBES (TNTs) and ROAMER TYPE OF Volume Transmission



Main features of Tunneling Nano tubes (TNTs):

radius of 25–100nm; lengths of up to several cell diameters straight, transient, connections between





EXOSOMES as 'safe containers' of signals released by the cell or impinging on the cell

Exosomes contain: mtDNA; ncRNAs, Proteins

If internalised, they can markedly modify the biochemical machinery of the recipient cells

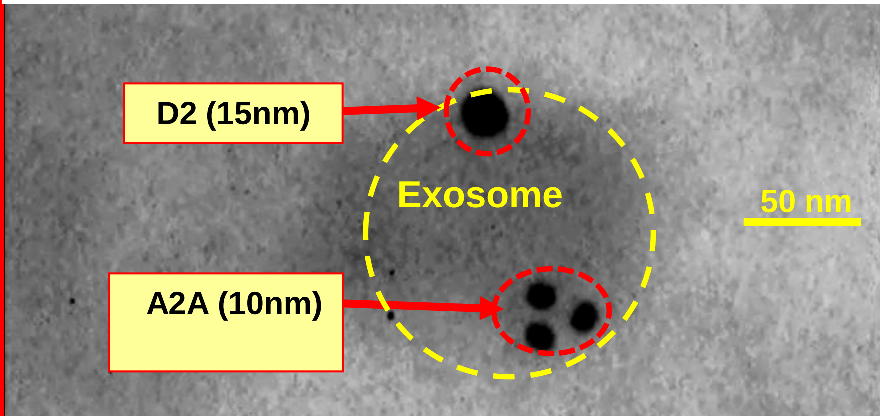
- Are also receptors (e.g., GPCRs) transferred from cell to cell by means of Nanotubes and/or Exosomes. Can these receptors form new Receptor Mosaics?

- Can be surmised new aspects of brain plasticity and possible new modes for spread of neurodegeneration based on intercellular transfer of pathogenic molecules and organelles?

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

GPCRs ARE PRESENT IN EXOSOMES

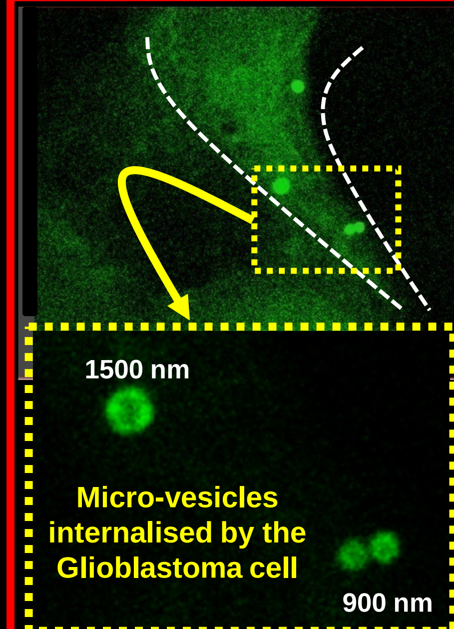
ELECTRON MICROSCOPE IMAGE OF EXOSOMES
D2 RECEPTORS were labelled by 15nm gold particles
A2A RECEPTORS were labelled by 10nm gold particles



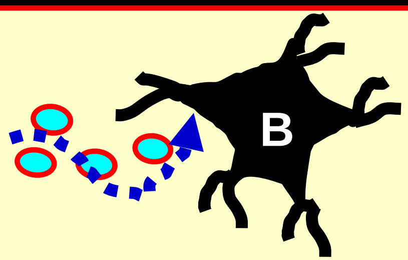
D2 – A2A IMMUNOGOLD STAINING SHOWS THE PRESENCE OF THE RECEPTORS IN THE EXOSOME

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

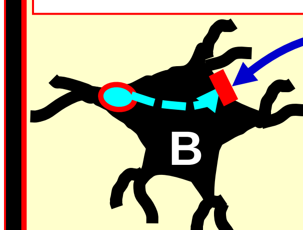


CELL B IS A TARGET FOR EXOSOMES CONTAINING RECEPTORS THAT CELL B DOES NOT EXPRESS

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

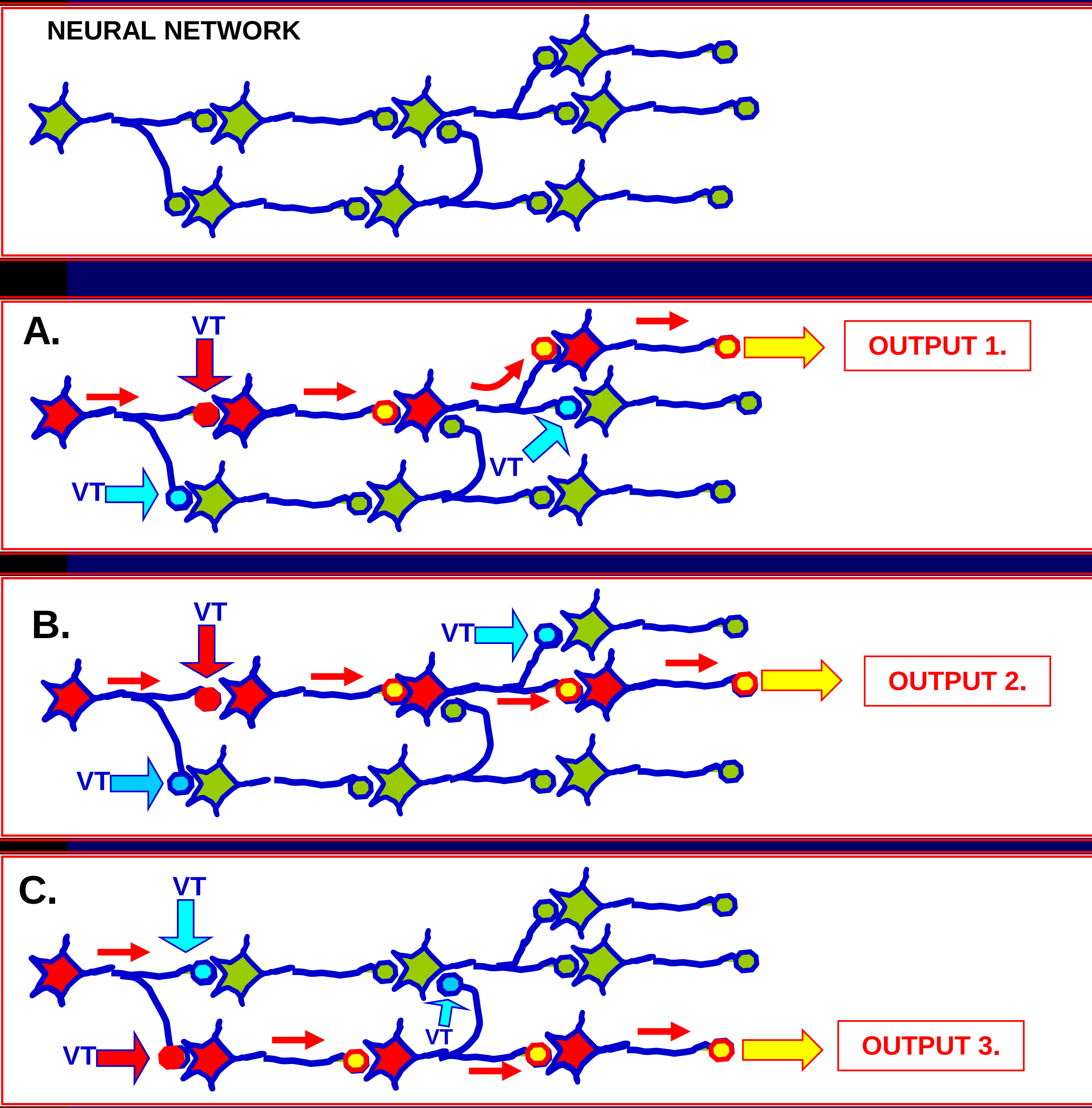
NT

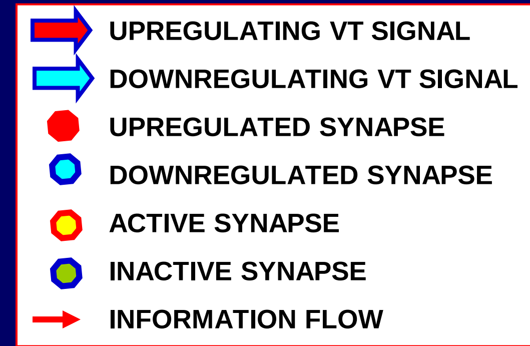


DECODING OF PREVIOUSLY UNDETECTED NEUROTRANSMITTER

INSERTION IN THE MEMBRANE OF NEW TYPES OF RECEPTORS

Guescini et al. 2012





Changes in the balance of VT and WT have a fundamental role in brain circuit control

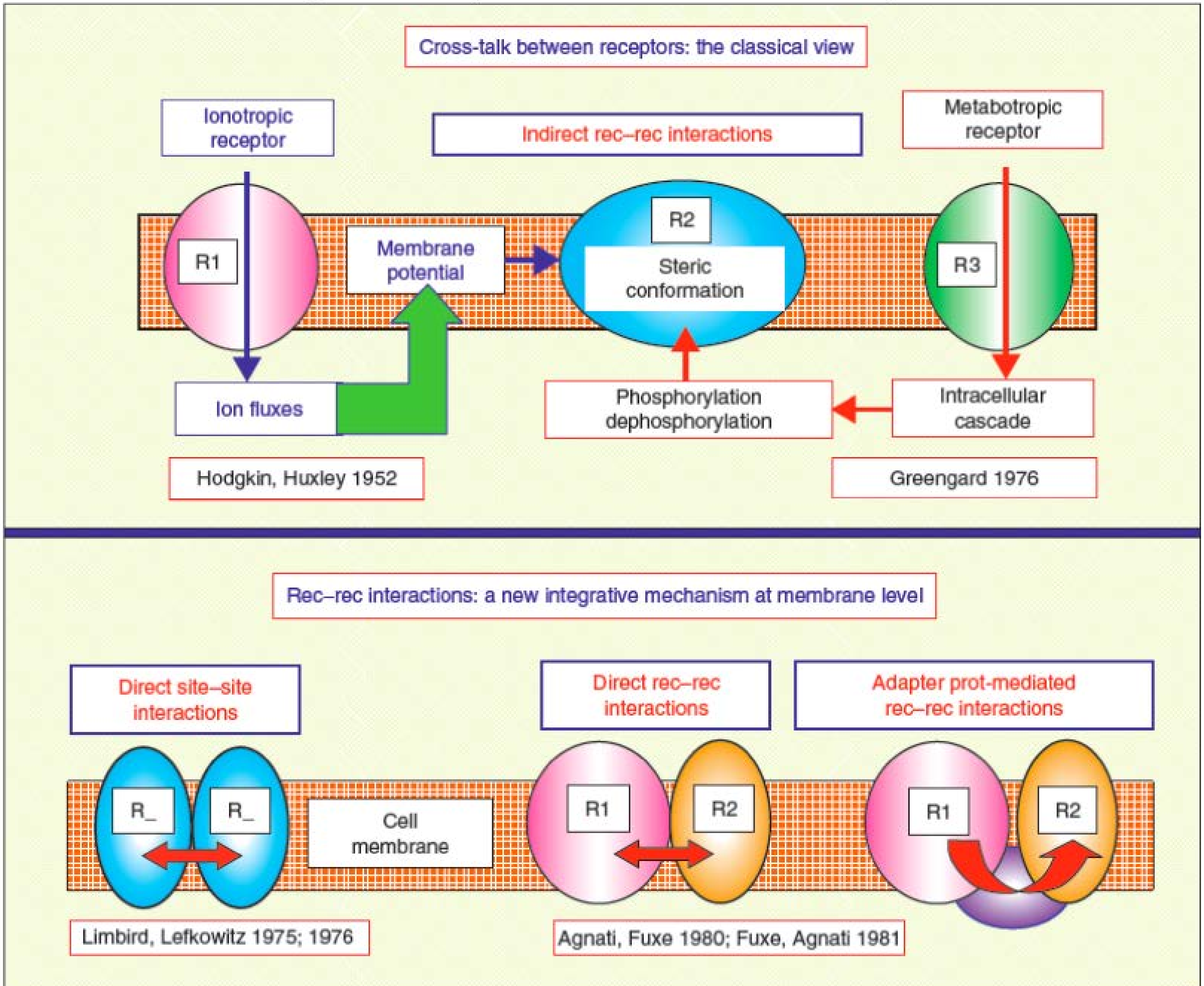
THREE DIFFERENT OUTPUTS

1. 2. 3.

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

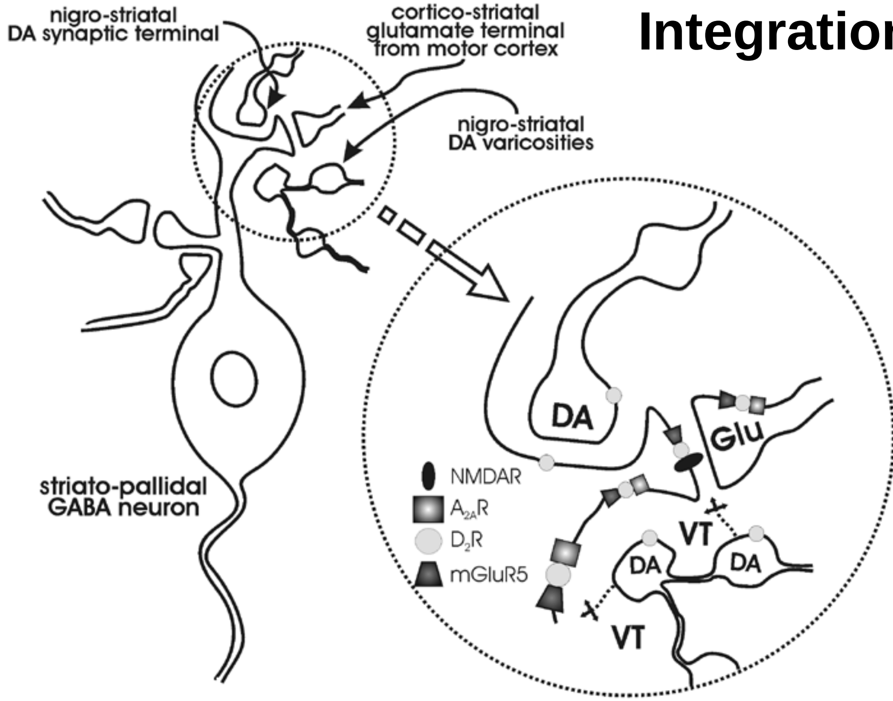
Agnati and Fuxe 2000

Allosteric receptor-receptor interactions vs receptor crosstalk



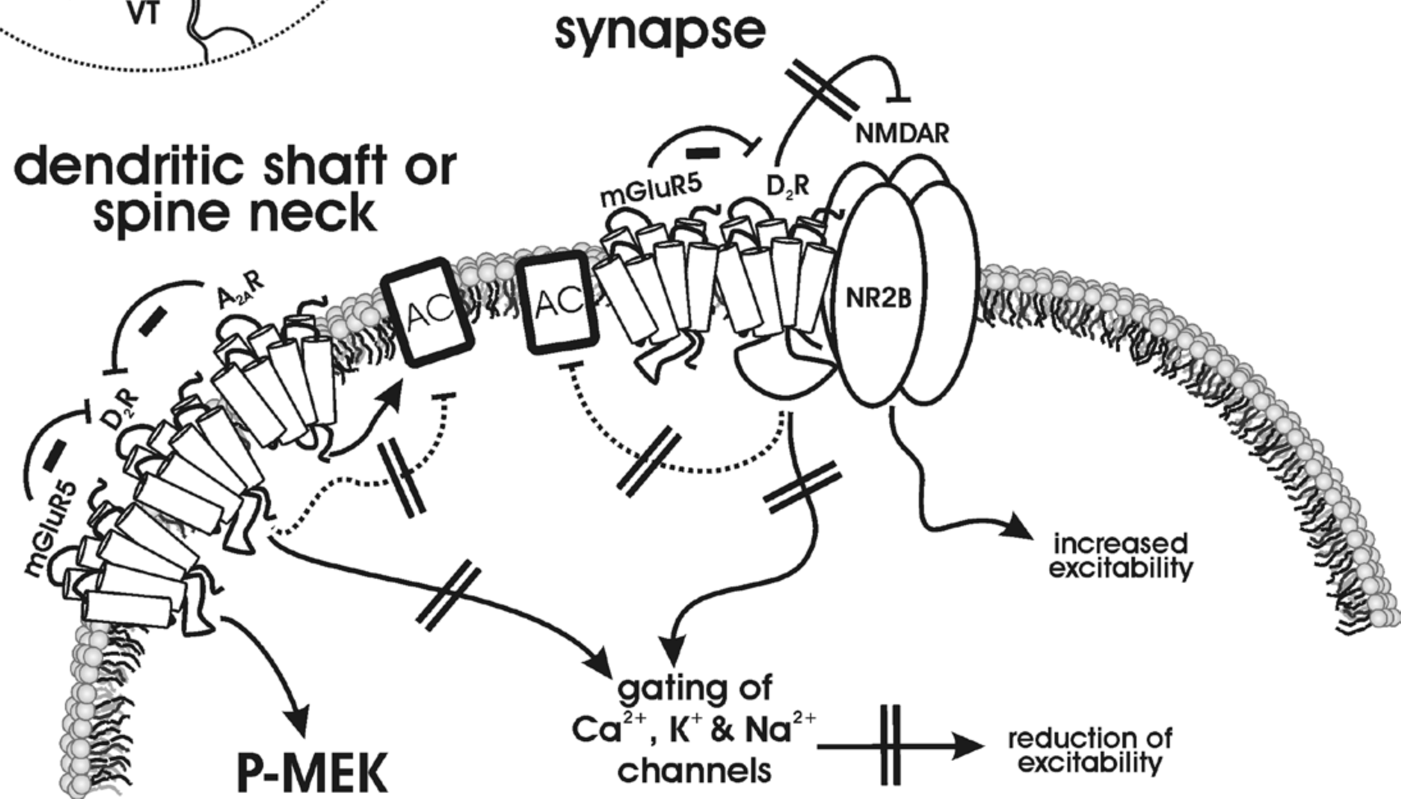
Fuxe and Agnati 2009

Integration and balancing of WT and VT signals in the synapse



DA VT modulates glutamate WT via receptor-receptor interactions with NMDA receptors in heteroreceptor complexes.

1. D2-NMDA(NR2B);indirect pathway (Liu et al.2006)
2. D1-NMDA (NR2A and NR1.1) ; direct pathway (Lee et al.2002)

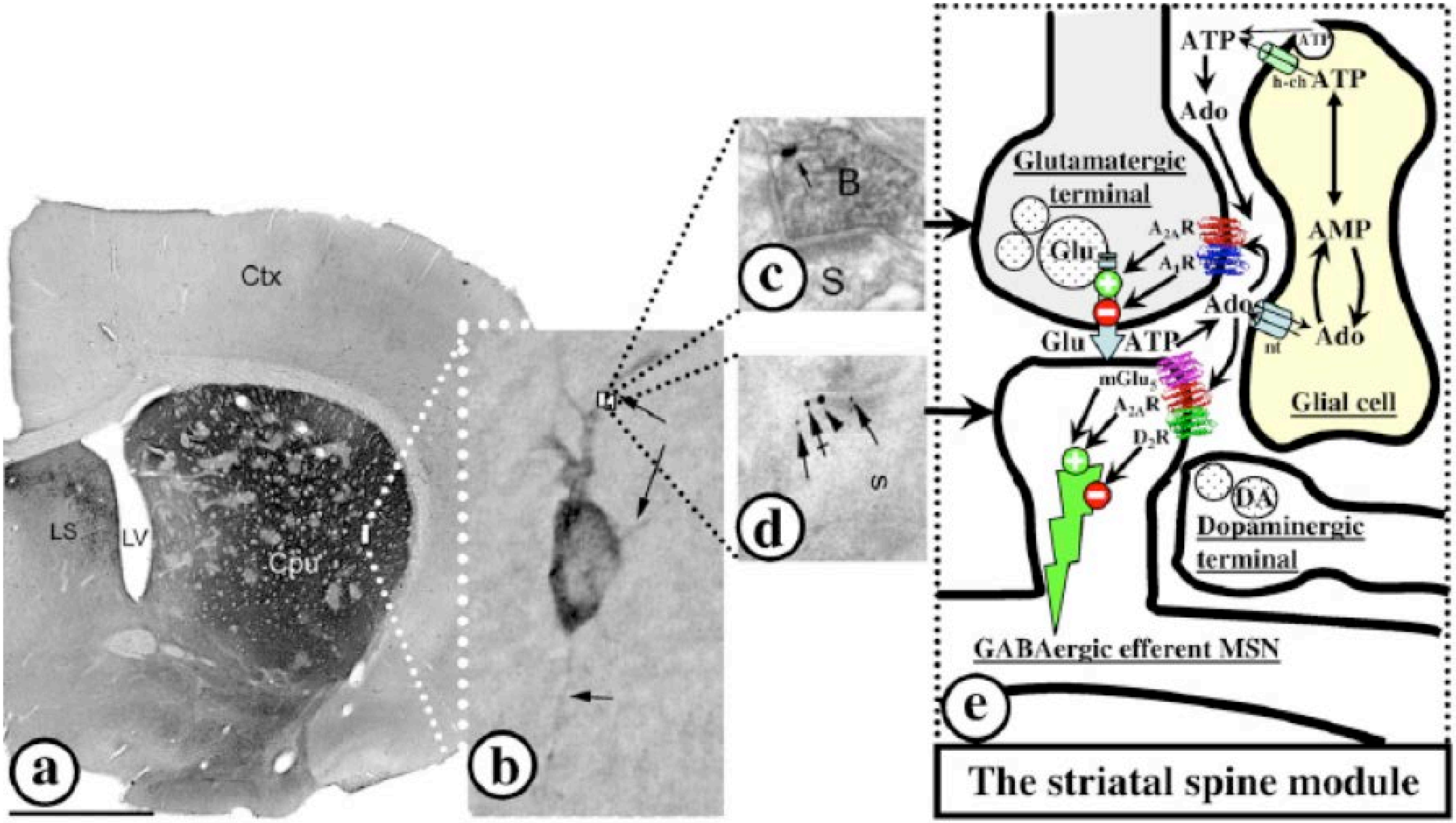


Fuxe et al.2008

Integration of neuronal and glial signaling via heteroreceptor complexes

The striatal spine module (SSM) within the striatal spiny neuron (MSN).

(a) Immunoreactivity of NECAB2, an A2AR interacting protein, in the rat striatum.



Ciruela et al.2011

Psychoneuroendocrinology (2005) 30, 1050-1058

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www.elsevier.com/locate/psyneuen

Psychosomatics and psychopathology: looking up and down from the brain

Julian F. Thayer[a,*], Jos F. Brosschot[b]

[a]*NIA/GRC/LPC, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA*
[b]*University of Leiden, Leiden, The Netherlands*

Prefrontal cortex ‘offline’: parasympathetic inhibitory action is withdrawn and a relative sympathetic dominance takes place associated with disinhibited defensive circuits
This state is indicated by low heart rate variability (HRV), a marker for low parasympathetic activation and prefrontal hypoactivity.
Low HRV predicts hypervigilance and inefficient allocation of attentional and cognitive resources.

This autonomic imbalance is likely the major mechanism linking psychosomatics to psychopathology

NeuroImage 47 (2009) 1135–1140

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Editorial

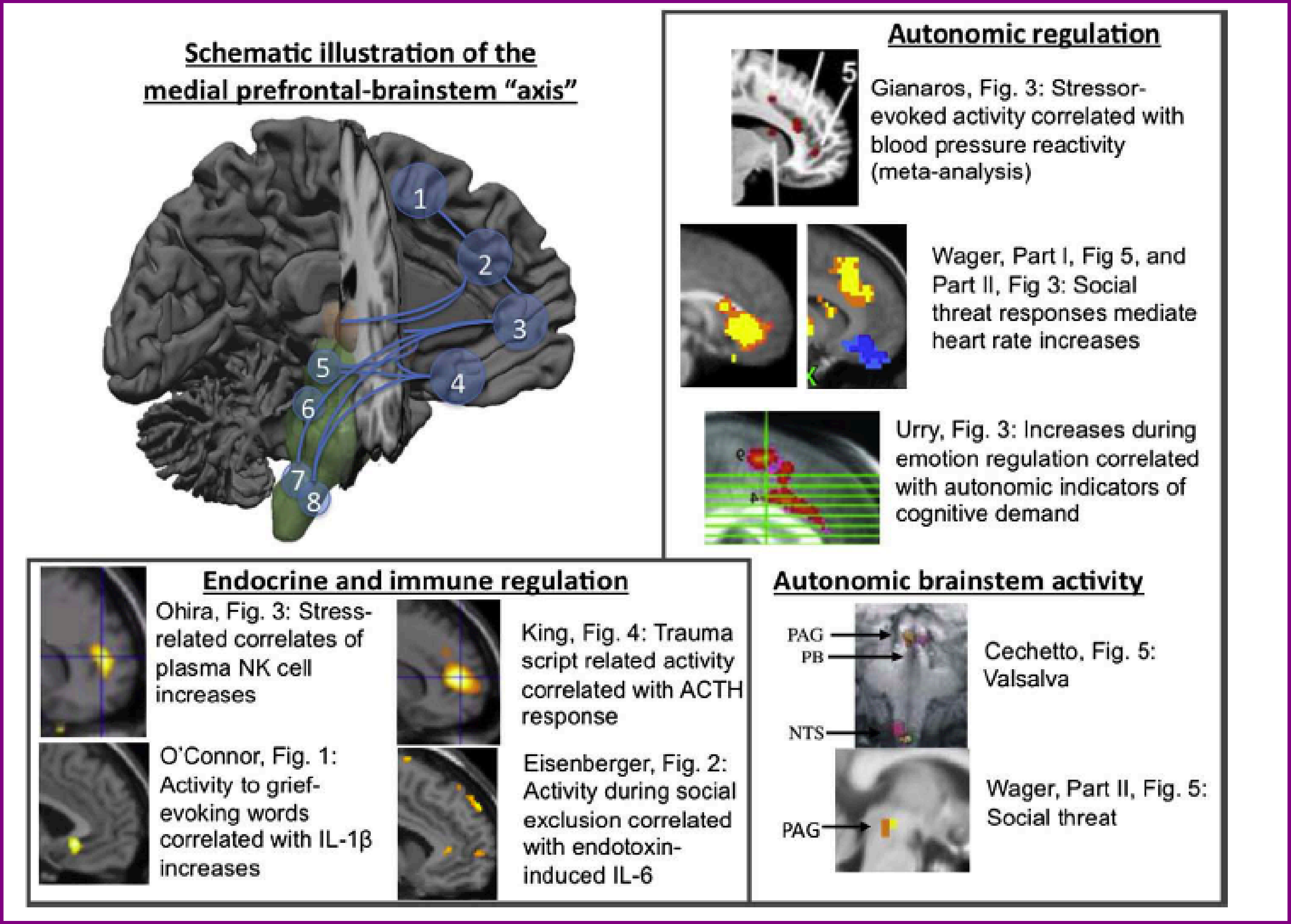
Richard D. Lane

The new field of Brain–Body Medicine: What have we learned and where are we headed?

A unifying framework that links many of the neuroimaging papers in this Special Issue is that brainstem mechanisms directly regulate vital bodily functions, and that cortical–subcortical interactions modulate the function of these brainstem mechanisms (Lane et al.2009) Lane et al., 2009a).

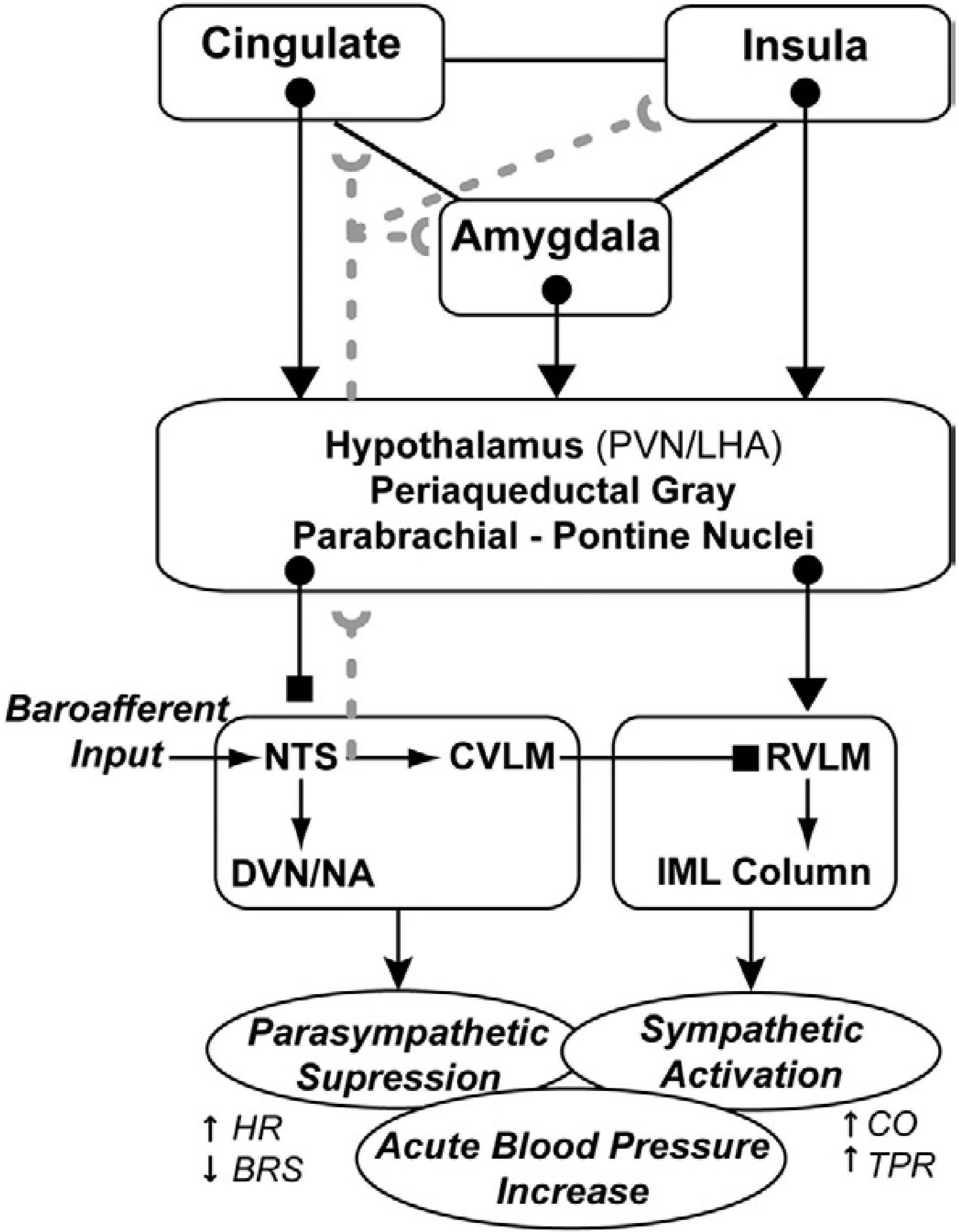
Integration of human and animal work
Integration of neuroimaging and genetics
Highlights new findings with fMRI and PET

Activity measures in the medial prefrontal-brainstem "axis" related to autonomic, endocrine and immune functions



1)Pre-supplementary motor area (SMA)/mid-cingulate cortex (MCC); 2) anterior MCC/rostral dorsal cingulate; 3) anterior cingulate/ pregenual cingulate; 4) subgenual cingulate/ventromedial prefrontal cortex/ medial orbitofrontal cortex; Prefrontal cortical networks related to visceral function and mood (Price 1999)

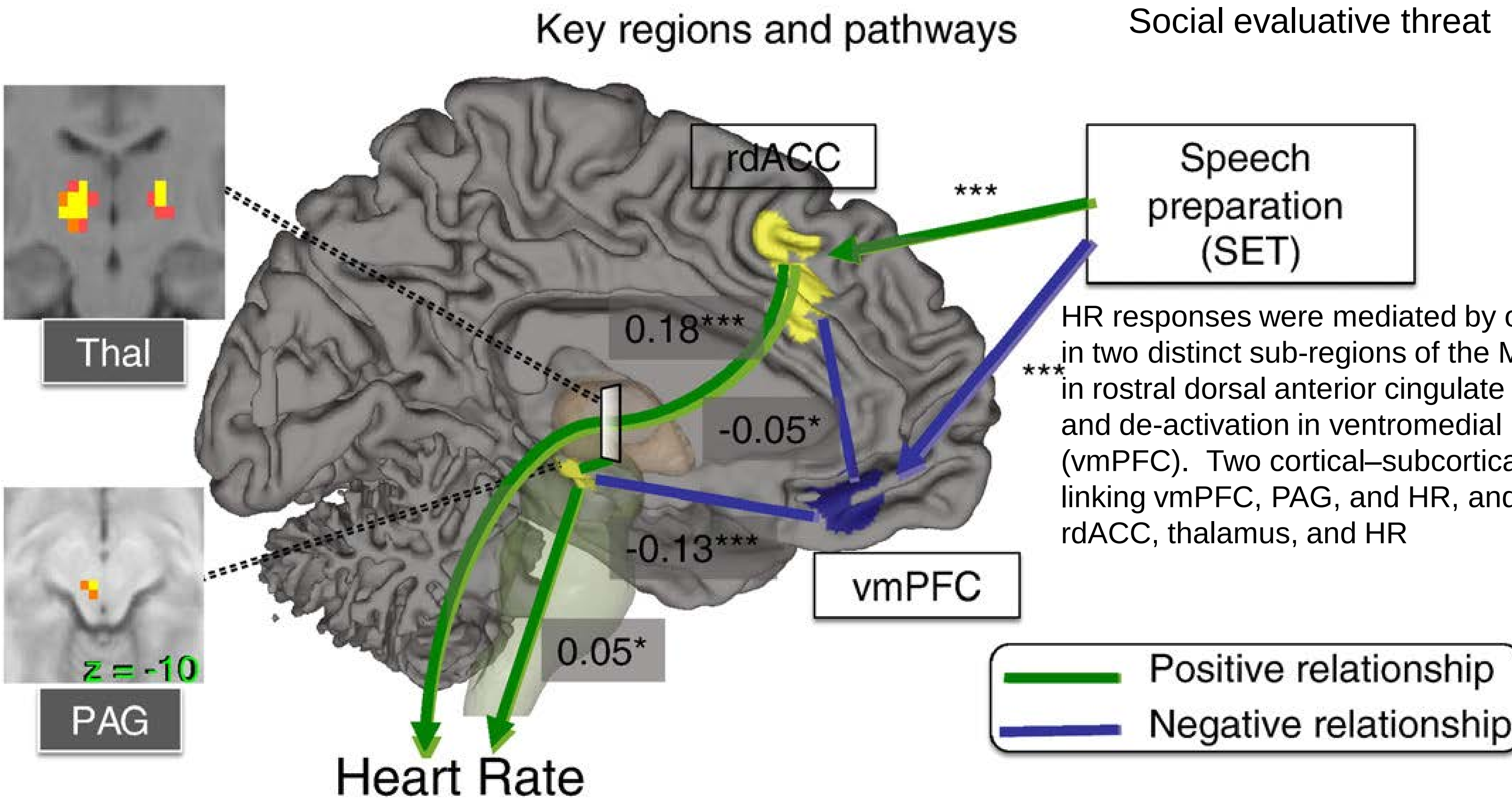
Lane et al.2009



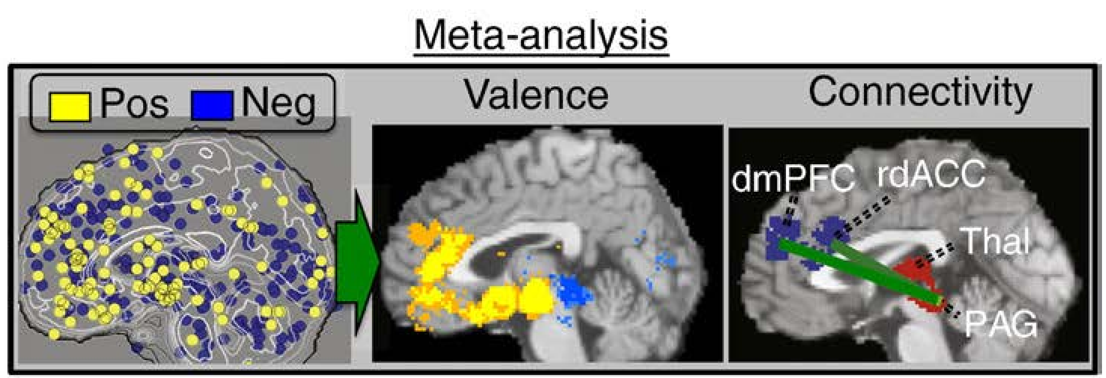
Brain systems involved in the expression of stressor-evoked blood pressure reactivity

Gianaros and Sheu 2009

Cortical-brainstem and cortical-heart rate connections
Brain mediators of cardiovascular responses to social threat

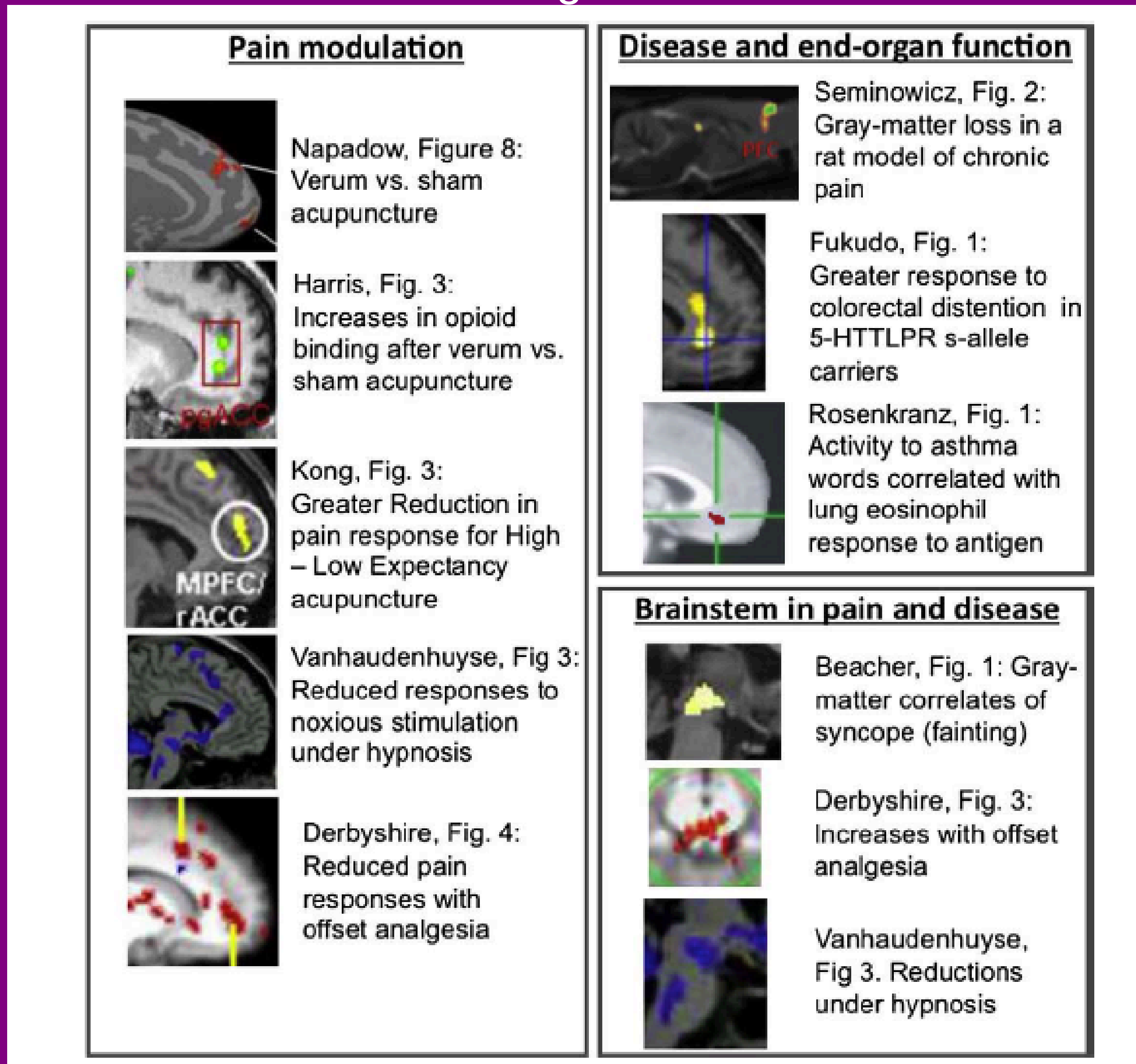


HR responses were mediated by opposing signals in two distinct sub-regions of the MPFC—increases in rostral dorsal anterior cingulate cortex (rdACC) and de-activation in ventromedial prefrontal cortex (vmPFC). Two cortical–subcortical pathways: one linking vmPFC, PAG, and HR, and another linking rdACC, thalamus, and HR



Wager et al.2009

Brain activity measures related to pain,other disease processes and end-organ function



Lane et al.2009

Brain encoding of acupuncture sensation--coupling on-line rating with fMRI.
Napadow V, Dhond RP, Kim J, LaCount L, Vangel M, Harris RE, Kettner N, Park K. Neuroimage. 2009 Sep;47(3):1055-65.
" increased cognitive load and dmPFC activity may be a salient component of acupuncture analgesia — sensations focus attention and accentuate bodily awareness, contributing to enhanced top-down modulation of any nociceptive afference and central pain networks. Acupuncture may function as a somatosensory-guided mind–body therapy".

Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on mu-opioid receptors (MORs). Harris RE, Zubieta JK, Scott DJ, Napadow V, Gracely RH, Clauw DJ. Neuroimage. 2009 Sep;47(3):1077-85.
"Acupuncture therapy evoked short- and long-term increases in MOR binding potential, in multiple pain and sensory processing regions including the cingulate (dorsal and subgenual) and insula,. These effects were absent in the sham group where small reductions were observed, an effect more consistent with previous placebo PET studies. Divergent MOR processes may mediate clinically relevant analgesic effects for acupuncture and sham acupuncture."

An fMRI study on the interaction and dissociation between expectation of pain relief and acupuncture treatment. Kong J, Kaptchuk TJ, Polich G, Kirsch I, Vangel M, Zyloney C, Rosen B, Gollub RL. Neuroimage. 2009 Sep;47(3):1066-76

"We hypothesize that as a peripheral-central modulation, acupuncture needle stimulation may inhibit incoming noxious stimuli; while as a top-down modulation, expectancy (placebo) may work through the emotional circuit.It also induces analgesia exclusively in regions of the body on which expectation is focused"

ISOPROTERENOL INDUCED INCREASE IN PLASMA CONTENT OF EXTRACELLULAR VESICLES AND ITS POSSIBLE LINK TO ANXIETY

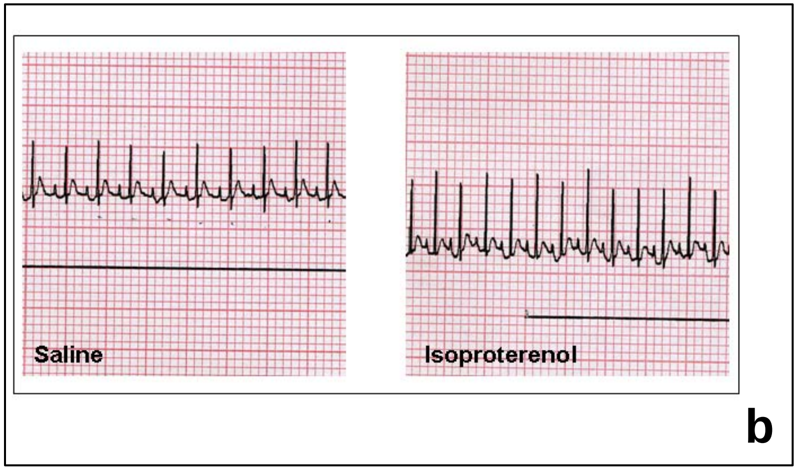
Leo G. , Guescini M, Genedani S., Stocchi V. , Carone C., Sisti D. , Guidolin D. , Fuxe K., Agnati LF.

β-Adrenergic receptor function seems to be altered in patients with anxiety disorders and decreased β-adrenergic receptor responsiveness has been reported in patients with panic disorder. There is a link between heart rate, anxiety and panic disorders.

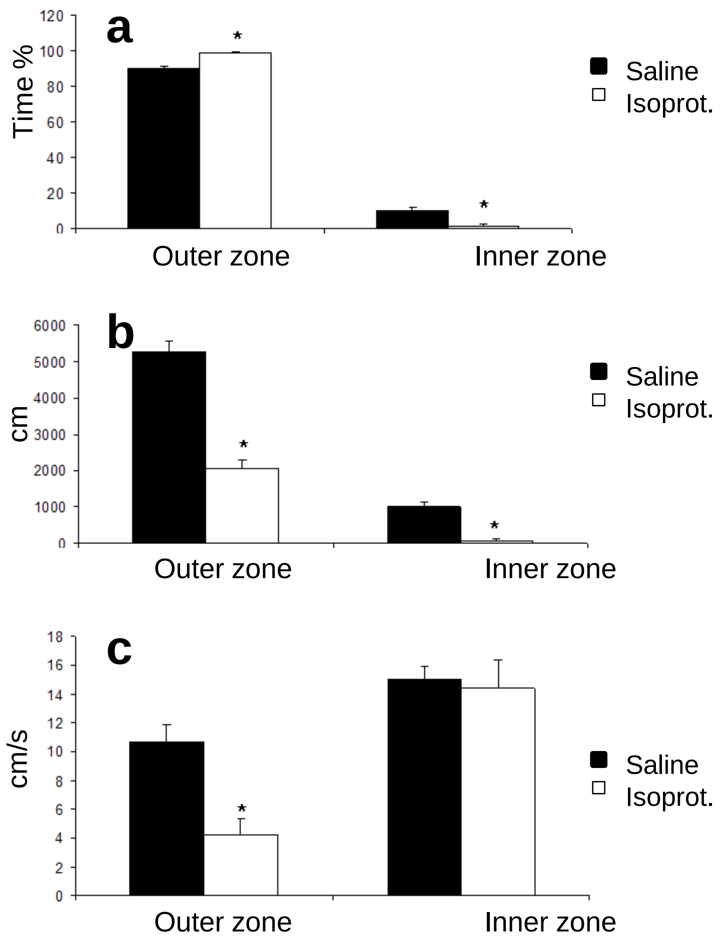
The effect of the β-adrenergic receptor agonist isoproterenol, at a dose inducing heart rate increase, has now been evaluated on anxious behaviour of normal rats by means of the open field test focusing on possible mechanisms involved

-James and Lange theory of emotion: Emotion is the feeling of bodily changes, which follow the perception of an exciting event. Bodily and behavioural responses in emotion are prior events (see Lang 1994)

-Cannon critique (1987): Artificial visceral stimulation does not produce strong emotions Visceral changes when presented with an external stimulus are slower than the emotional reaction

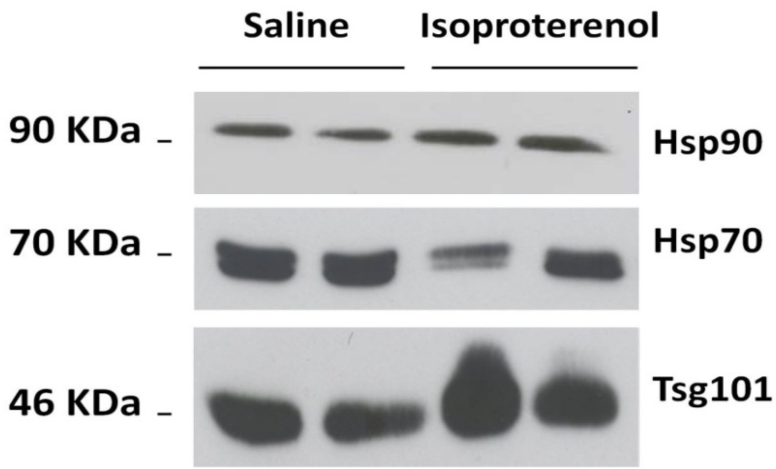


a) Heart rate obtained by Ecg recorded for 5 min in rats 45 min after the intraperitoneal injection of saline or isoproterenol 5 mg/kg. Data are expressed as mean±SEM of 10 animals/group. b) Examples of Ecg tracing in saline and isoproterenol treated animals showing no modification of the rhythm (constancy of the R-R interval).
*p< 0.01 vs. saline (t Student's test).



Effect of isoproterenol intraperitoneally injected at the dose of 5 mg/kg on behaviour of rats in the open-field test. Isoproterenol or saline were injected 45 min before the test. a) Percentage of time spent in the outer and inner zone; b) total distance (in cm) traveled in the outer and inner zone respectively; c) speed (cm/s) in the outer and inner zone. Data are reported as mean±SEM of 10 animals/group. *p< 0.01 vs. saline (t Student's test).

A

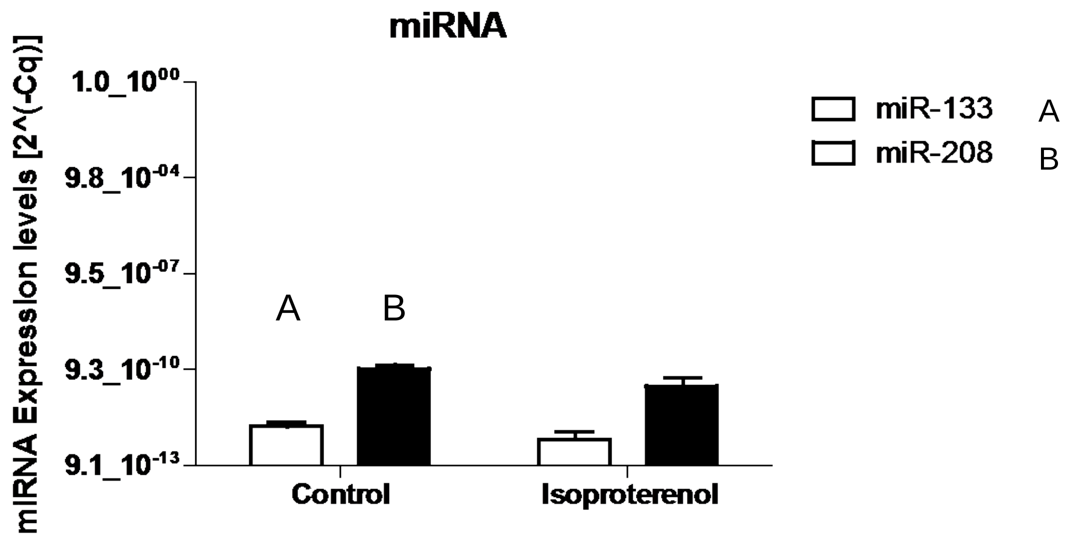


B

Treatment	Hsp70 (O.D.)	Hsp90 (O.D.)	Tsg101 (O.D.)
Saline	50.35 ± 5.136	18.35 ± 6.12	63.31 ± 10.49
Isoproterenol	34.83 ± 4.072*	26.20 ± 5.80	98.89 ± 11.08 **

A) Representative western blots of Tsg101, Hsp70 and Hsp90 performed on extracellular vesicles (EVs) isolated from plasma of two animals treated with isoproterenol 5 mg/kg/i.p. or saline. B) Table show the values of Tsg101, Hsp70 and Hsp90 reported as optical density (O.D.). Data are expressed as mean±SEM of 10 animals/group. **$p<0.01$; *$p<0.05$ vs. saline (t Student's test).

Leo et al.



Analysis of the expression levels of miR-133 (A) and miR-208 (B) from plasma EVs. EVs were isolated from control and treated rats using the reported serial ultra-centrifugation protocol starting from equal plasma amount (2 ml) for each rats. Subsequently, the EV miRNA content was evaluated by Real-time RT-PCR, the reported data represents means + S.E.M.

Leo et al.

Conclusions

Hypothesis: The increase of HR induced by isoproterenol can stimulate the release of EVs, probably from endothelial cells, carrying a modified Hsp content which may contribute to induction of an anxious behaviour by interacting with limbic system structures, especially amygdala. EVs could provide a slow but long lasting action.

See paper by Lakhal and Wood 2011 in Bioessays on Exosome nanotechnology. An emerging paradigm shift in drug delivery.

The increase in visceral sensory input to the Nucleus Tractus Solitarius by the increase in heart rate may also play an important role by giving a fast input to the limbic structures, especially amygdala via the NTS and parabrachial regions.

Understanding Brain-Body medicine

Cognition requires the Environment-Body-Brain system

1. External and internal sensory signals, including neuronal and glial transmitters/modulaters, and hormones are decoded by brain and spinal cord adapters.
   Extracellular vesicles in blood may be a novel peripheral signal modulating psychic homeostasis.

-The adapters are functional neuro-glial modules operating mainly via synaptic transmission and volume transmission and their integration through allosteric receptor-receptor interactions in synaptic and extrasynaptic heteroreceptor complexes.

-The search is for Free Energy Minimization in the brain circuits to increase self and species survival.

- Of special relevance are the functional brainstem modules of the hypothalamus with the median eminence and the nucleus solitarius region with the area postrema.

Understanding Brain-Body medicine (cont.)

2. The brain adapters in the brain stem and subcortical structures after decoding then send the alphabet to the functional modules of the cerebral cortex , especially the prefrontal cortex, via ascending projections.

   The dynamic formation and disappearance of mosaics of functional cortical modules builds the internal world (Consciousness). Do they produce the mind? in turn

3. The functional cortical modules especially of the medial prefrontal cortex can top-down regulate the autonomic and neuroendocrine mechanisms of the brainstem and the immune responses. Hypofrontality leads to sympathetic dominance over a low parasympathetic tone indicated by a low heart rate variability. Psychopathology can develop.