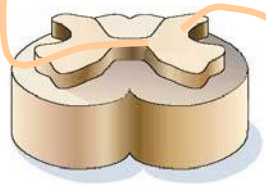
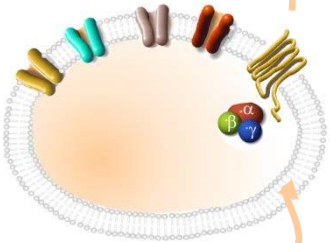
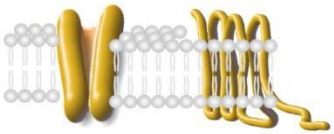
Major fields of research







NEUROPHYSIOLOGY	CARDIOVASCULAR PHYSIOLOGY	METABOLISM
System level		
- Circadian rhythms - Memory - Epilepsy - Alzheimer's disease - Pain	-Central and peripheral blood pressure control -Regulation of embryonic cardiac output -Pathophysiology of heart failure	-Neurohumoral control -Energy expenditure -Glucose homeostasis -Nutritional interventions -Metabolic syndrome -Biomarkers
Cellular level		
-Synaptic transmission -Neuromodulation -Nociception -Ionic channels: NMDA, TRP, nicotinic, purinergic -Metabotropic receptors: muscarinic, adrenergic -Secretion of pituitary hormones	-Calcium influx and calcium sensitization in contractility of resistance arteries -Calcium transients and ion channels -Gap junctional coupling -Isolated cardiac myocytes -Cell proliferation in cardiac growth and regeneration	-Signalling cascades -Mitochondrial (dys)function -Membrane biophysics
Molecular level		
-Opioid receptors -Gene and protein expression	-Adrenergic receptor number regulation -Mitochondrial membrane potential -RhoA/Rho kinase pathway in calcium sensitization	-Transport proteins -Reactive oxygen species -Structure of signalling proteins

INSTITUTE OF PHYSIOLOGY CAS

Department of Biochemistry of Membrane Receptors

Since 1989

MAIN RESEARCH ACTIVITIES

in 2010-2014:

(1a) Studies of desensitization and hyper-sensitization of opioid receptor signaling cascades and regulation of opioid receptor function by plasma membrane cholesterol level and monovalent cations.

(1b) Introduction of new methods of fluorescence spectroscopy and confocal fluorescence microscopy for analysis of the role of cholesterol in structural organization of G protein-coupled receptors, of hydrophobic matrix of plasma membrane and of interface between membrane and surrounding water molecules.

(1a) Introduction and social impact of our studies

The repeated exposure of human body to opioid receptor agonists morphine, codeine, heroine and other related pharmaceuticals results in state of addiction to these drugs.

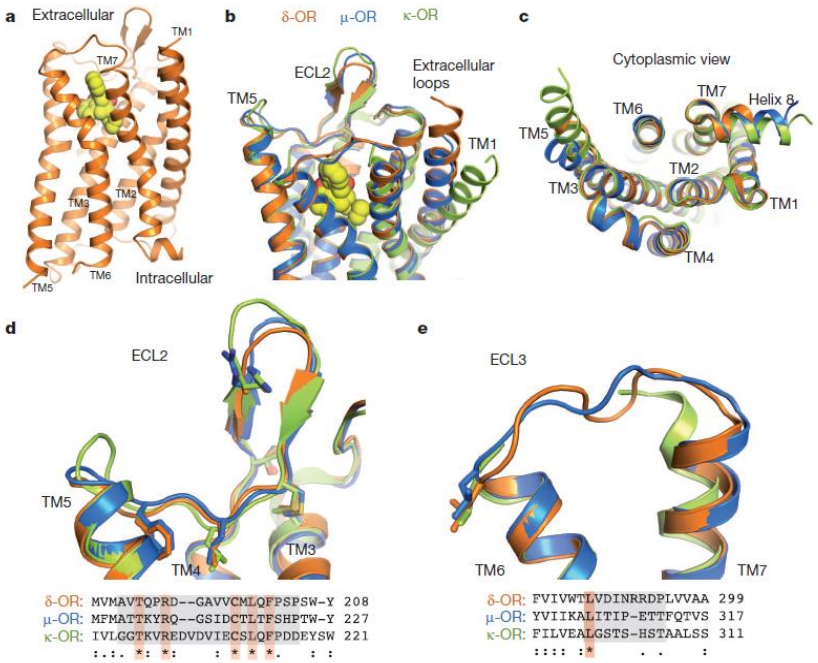
Drug addicts simply must have these drugs regardless of the price and negative consequences for their health and social live. They go step by step into the abyss of no return to the physiological norm.

Why? *They need higher and higher doses of these drugs because they develop the state of tolerance to these drugs.*

The consumption of opioid drugs represents one of the largest components of the *illicit* drug market worldwide.

Intravenous use of opioid drugs is a leading cause of death by overdose in Europe and North America and a major contributing factor to the worldwide AIDS epidemic.

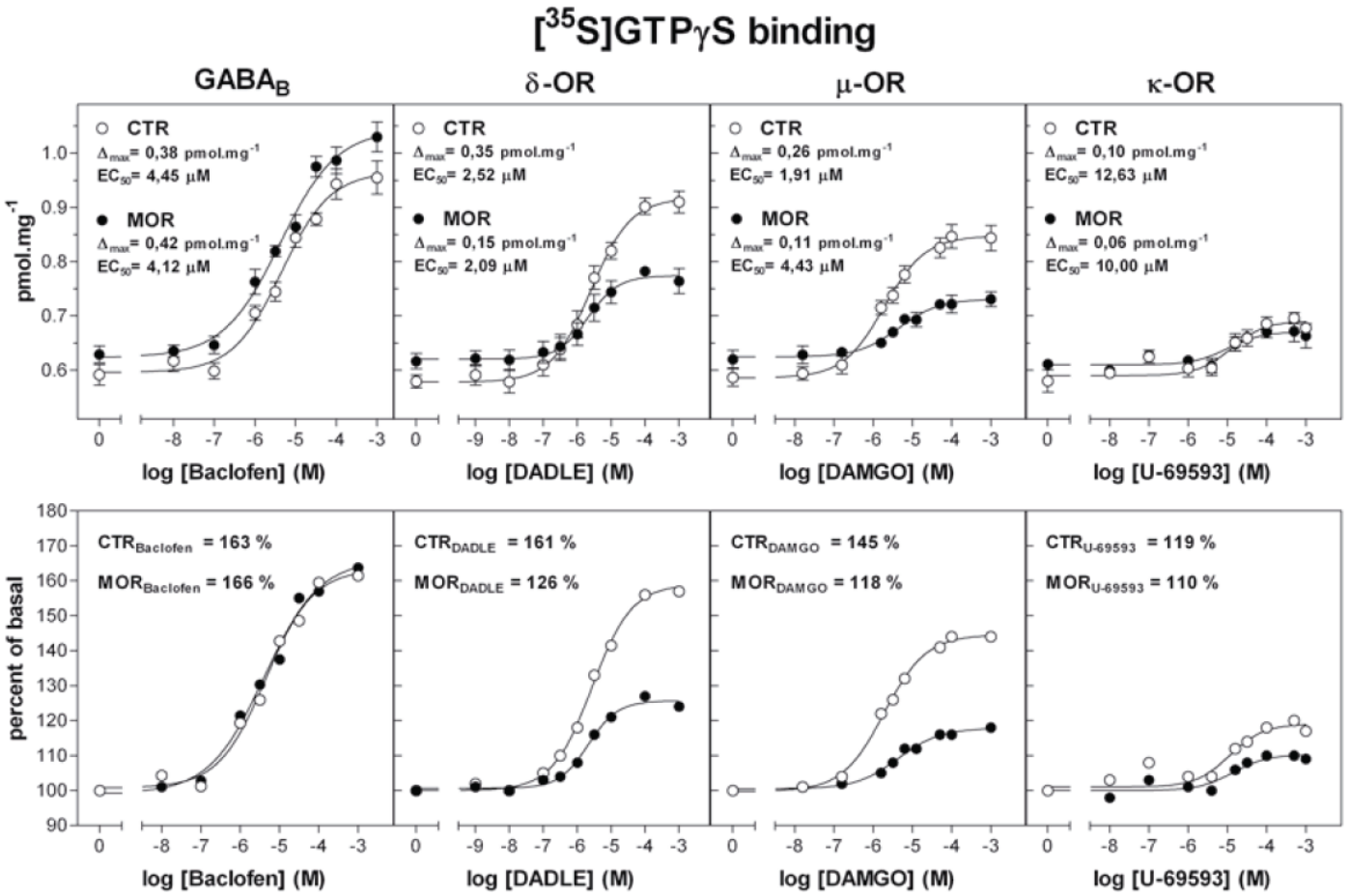
Morphine, codein and heroine bind to three different types of G-protein-coupled μ-, δ- and κ-opioid receptors which are present in the central nervous system as well as most other mammalian tissues.



The theoretical basis and molecular mechanism of **opioid addiction and tolerance** proceeding in CNS are not sufficiently known in spite of the fact that this research proceeds in ≈ 20 large world-class laboratories for about 40 years.

Main results / achievements

No. 1 Functional activity of G proteins in plasma membranes isolated from forebrain cortex of morphine-treated rats is decreased.



Our data support the view that the **primary mechanism of the long-term adaptation of brain to morphine is based on desensitization of G protein response to stimulation by μ-OR and δ-OR agonists.**

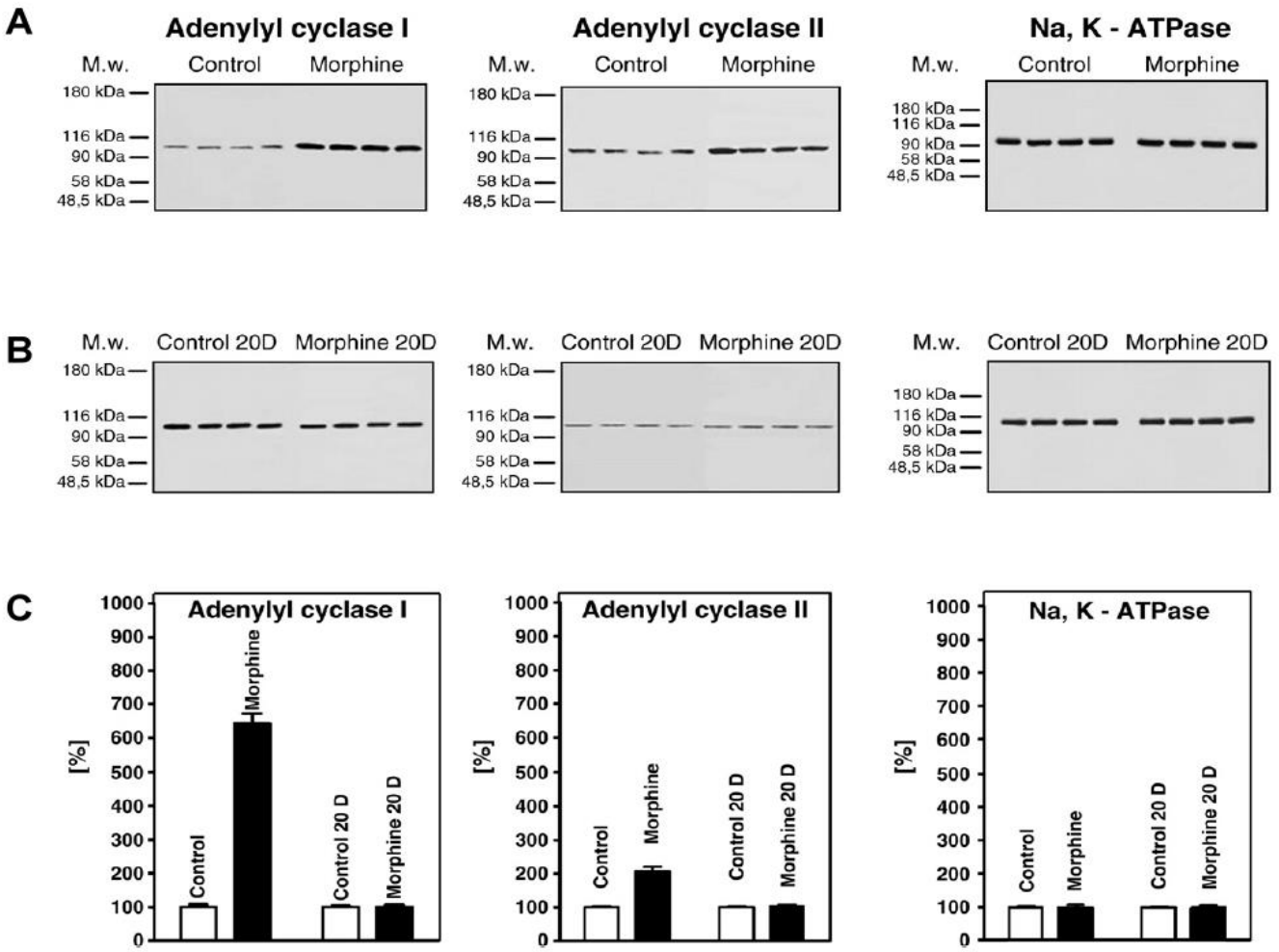
Bourova, L., Vosahlikova, M., Kagan, D., Dlouha, K., Novotny, J. and Svoboda, P. (2010) Long-term adaptation to high doses of morphine causes desensitization of μ-OR- and δ-OR-stimulated G protein response in fore brain cortex but not the decrease in the amount of G protein alpha subunits. Medical Science Monitor, 16(8), 260-270

Than we tried to understand what happens if the morphine-induced inhibition of G proteins proceeds for the long period of time (10 days).

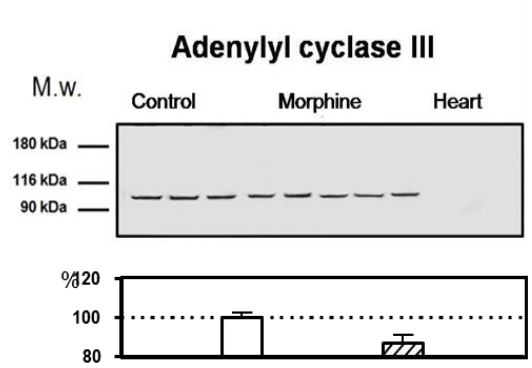
Result **No. 2**

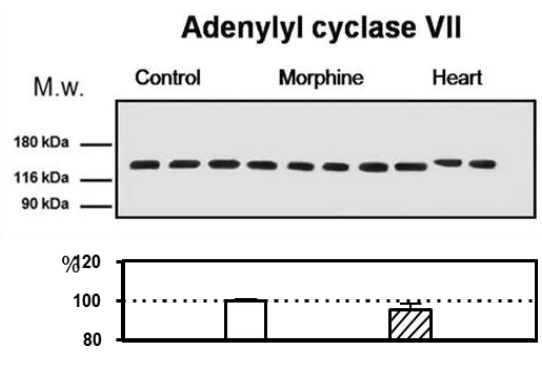
Adenylylcylase I and II, the enzymes producing 3´,5´-cyclic AMP, were up-regulated.

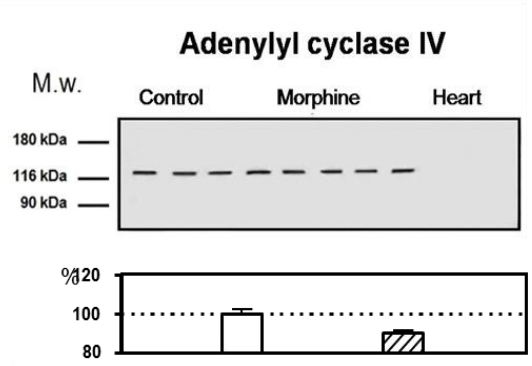
Immunoblot analysis of plasma membranes isolated from forebrain cortex of rats exposed to morphine for 10 days (**A, upper columns)** and rats exposed to morphine for 10 days and subsequently nurtured for 20 days in the absence of this drug **(B, lower columns).**

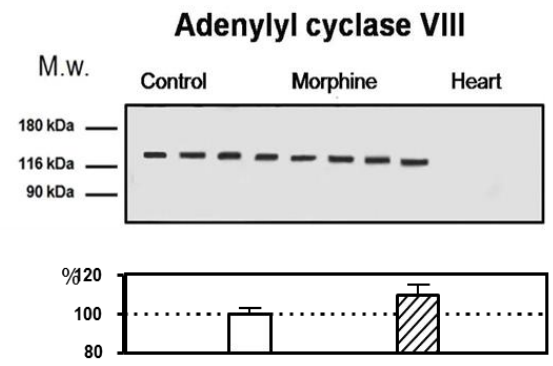


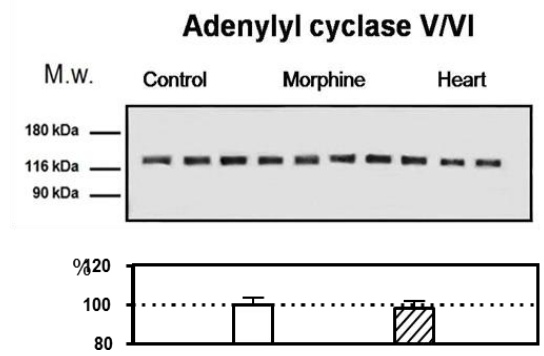
The other AC isoforms (III-X) were unchanged.

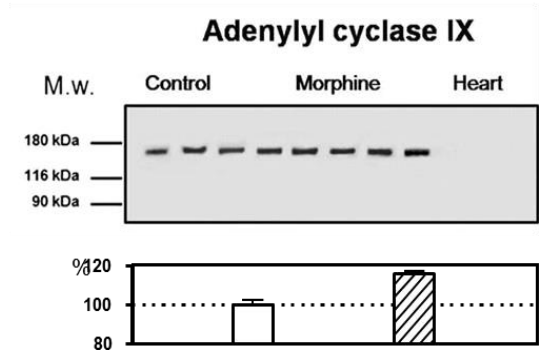










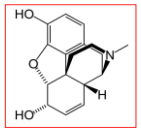


Decrease of ACI and ACII back to control level after 20 days of morphine withdrawal represents a **“good message“** for drug addicts, because the dramatic morphine-induced change of the crucial component of opioid receptor cascade, represented by up-regulation of ACI and ACII, was fully reversible after withdrawal of the drug.

Ujcikova, H., Dlouha, K., Bourova, L., Vosahlikova, M., Kagan, D., Svoboda, P. (2011) Up-regulation of adenylylcyclase I and II induced by long-term adaptation of rats to morphine fades away 20 days after morphine withdrawal. BBA General Subjects, 1810, 1220-1229

Proteomic analysis of forebrain cortex of rats exposed to morphine for 10 days (+M10) and rats exposed to morphine and subsequently nurtured for 20 days without the drug (+M10/-M20)

Opioid alkaloids: **morphine treatment** of male Wistar rats **+** 20 days of **morphine withdrawal**

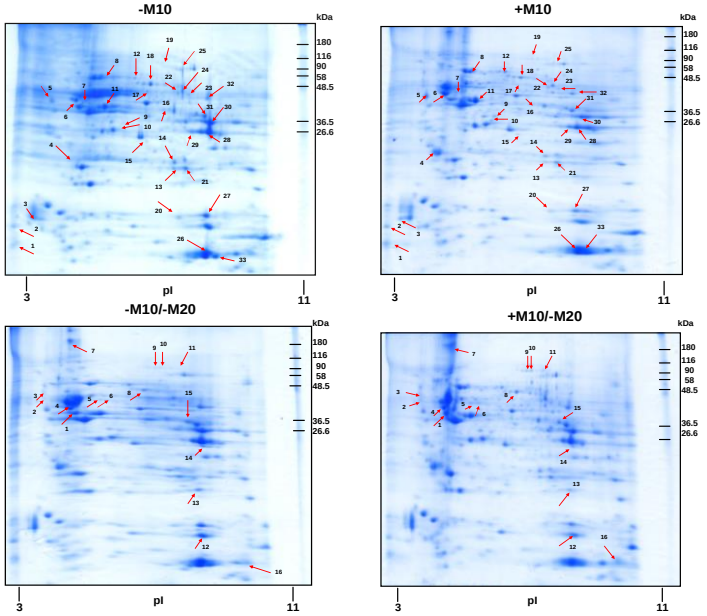


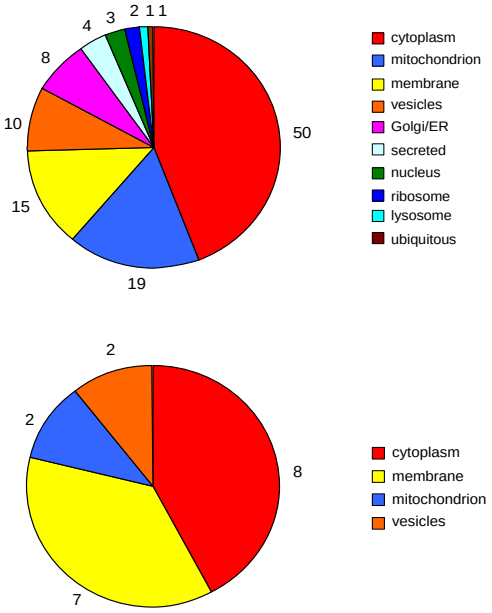
10 mg/kg (day 1 and 2)
15 mg/kg (day 3 and 4)
20 mg/kg (day 5 and 6)
30 mg/kg (day 7 and 8)
40 mg/kg (day 9)
50 mg/kg (day 10)

forebrain cortex → **PNS** → **2D ELFO**

+M10 **-M10** **+M10/-M20** **-M10/-M20**

CBB staining → **MALDI-TOF MS/MS** **+** **label-free quantification (MaxLFQ)**





Result **No. 3**

Exposure of rat forebrain cortex to morphine for 10 days resulted in alteration of 28 proteins. Good message is that the number of altered proteins was decreased to 16 after 20 days of the drug withdrawal.

The impact of the drug on the brain cell metabolism is very strong and 20 days without the drug are not sufficient to repair all damages. Longer time is needed for complete recovery.

Hana Ujcikova, Adam Eckhardt, Dmytro Kagan, Lenka Roubalova and Petr Svoboda (2014) Proteomic analysis of post-nuclear supernatant fraction and Percoll-purified membranes prepared from brain cortex of rats exposed to increasing doses of morphine. Proteom Science 12 : 11

Result **No. 4**

Perturbation / alteration of optimum plasma membrane composition by cholesterol depletion deteriorated the functional coupling of δ-OR to G proteins. The agonist binding site of the receptor molecule was unchanged.

The biophysical state of hydrophobic plasma membrane interior has to be regarded as one of regulatory factors of δ-OR-signaling cascade

Brejchová, J., Sýkora, J, Dlouhá, K., Roubalová, L., Ostašov, P., Vošahlíková, M., Hof, M., Svoboda, P. (2011) Fluorescence spectroscopy studies of HEK293 cells expressing DOR- Gi1α fusion protein; *the effect of cholesterol depletion.* BBA Biomembranes, 1808, 2819-2829

Research area (1b) Methods of fluorescence spectroscopy and confocal fluorescence microscopy were used for analysis of the role of cholesterol in

- mobility of receptor molecules at the surface of living cells by Fluorescence Lifetime Imaging (FLIM), Fluorescence Recovery Ater Photobleaching (FRAP) and Raster Image Correlation Spectroscopy (RICS)

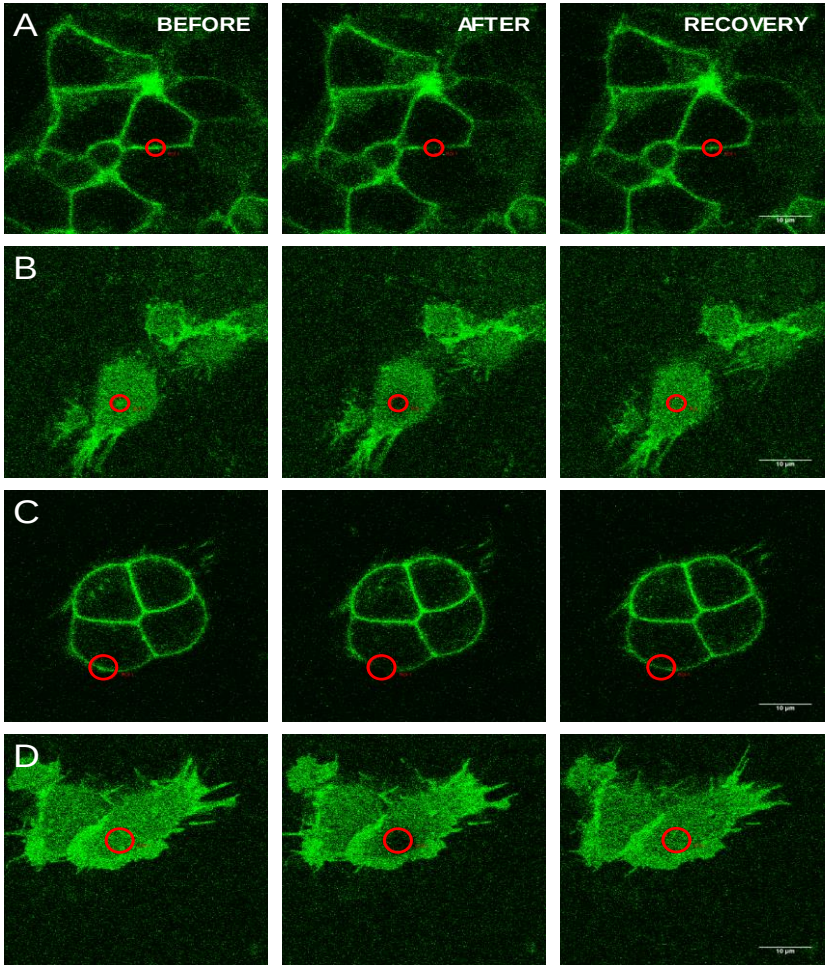
- and in organization of hydrophobic matrix and membrane-water interface of plasma membrane (steady-state and time-resolved anisotropy of fluorescence of membrane probes DPH, TMA-DPH and Laurdan.

Pavel Ostasov, Jan Sykora, Jana Brejchova, Agneszka Olzynska, Martin Hof and Petr Svoboda (2013) FLIM studies of 22- and 25-NBD-cholesterol in living HEK293 cells; plasma membrane change induced by cholesterol depletion. Chemistry and Physics of Lipids, 167-168, 62-69

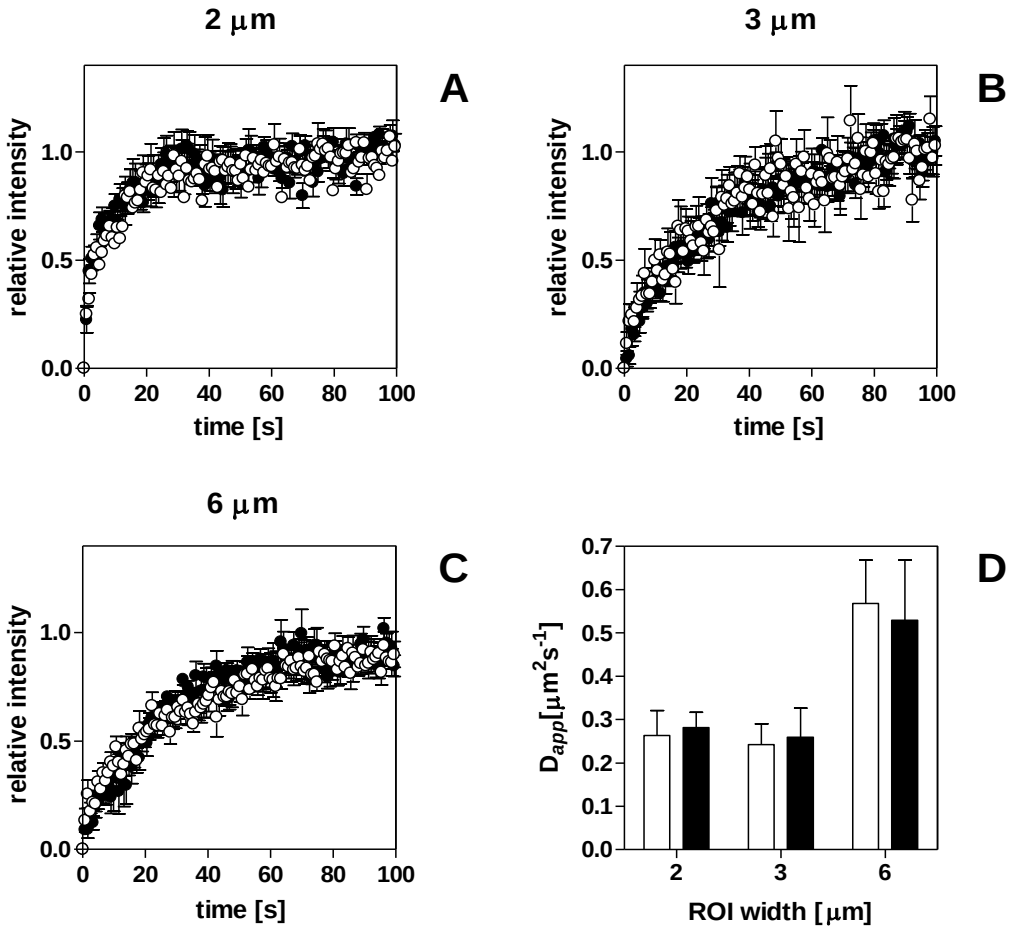
Jana Brejchová, Jan Sýkora, Pavel Ostašov, Ladislav Merta, Lenka Roubalová, Jiří Janáček, Martin Hof and Petr Svoboda(2015) TRH-receptor mobility and function in control and cholesterol-depleted plasma membrane of HEK293 cells stably expressing TRH-R-eGFP. BBA-Biomembranes, 1848, 781-796

FRAP

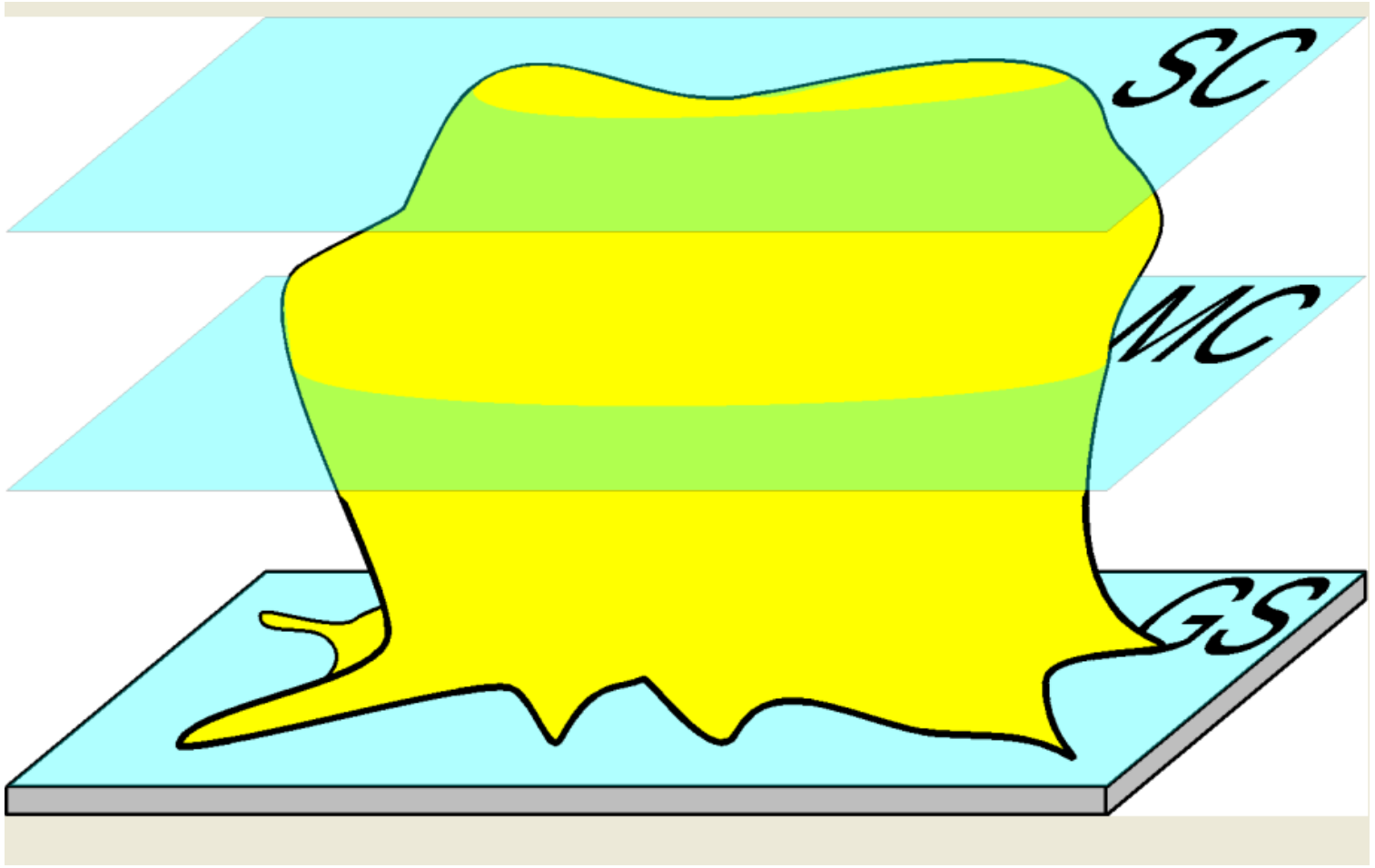
Step. 1 Small area of surface of the cell containing the fluorescent version of receptor is bleached.



Step. 2. The time-course of recovery of fluorescence is measured in different bleach spots and corresponding control spots, data collected and mathematically analyzed.



Analysis of primary FRAP data is difficult because the cells move in and out of confocal plain and the scatter of collected data is large and different in spots of different sizes.



TRH-R-eGFP diffusion coefficient in control and cholesterol depleted HEK293 cells

Diffusion coefficient ($\mu m^2.s^{-1}$)

ROI µm	Control cells	Cholesterol-depleted cells	
MC mode1st (*circle*-based analysis)			
2 µm	0.26 ± 0.06 (N=14)	0.28 ± 0.04 (N=12)	p > 0.05, NS
3 µm	0.24 ± 0.05 (N=10)	0.26 ± 0.07 (N=10)	p > 0.05, NS
6 µm	0.57 ± 0.10 (N=9)	0.53 ± 0.14 (N=9)	p > 0.05, NS
MC mode2nd (*rectangle*-based analysis)			
3 µm	0.124 ± 0.009 (N=15)	0.138 ± 0.031 (N=19)	p > 0.05, NS
5 µm	**0.271 ± 0.022 (N=19)**	**0.168 ± 0.012 (N=17)**	**p < 0.01**
GS mode (*circle*-based analysis)			
3 µm	0.115 ± 0.011 (N=19)	0.102 ± 0.006 (N=17)	p > 0.05, NS
5 µm	**0.191 ± 0.009 (N=16)**	**0.146 ± 0.012 (N=16)**	**p < 0.01**

Result **No. 5**

Diffusion coefficient of fluorescent version of TRH-R receptor molecule in living cells was decreased by cholesterol depletion.

Our results support the „Corral model“ of plasma membrane organization

Result **No. 6**

The numerical value of inhibition constant of high-affinity sodium binding sites in **δ-OR** determined by Vosahlikova et al. (*Naunyn Schmiedebergs Arch Pharmacol 387: 487- 502, 2014)*

K_i = 7.9 mM

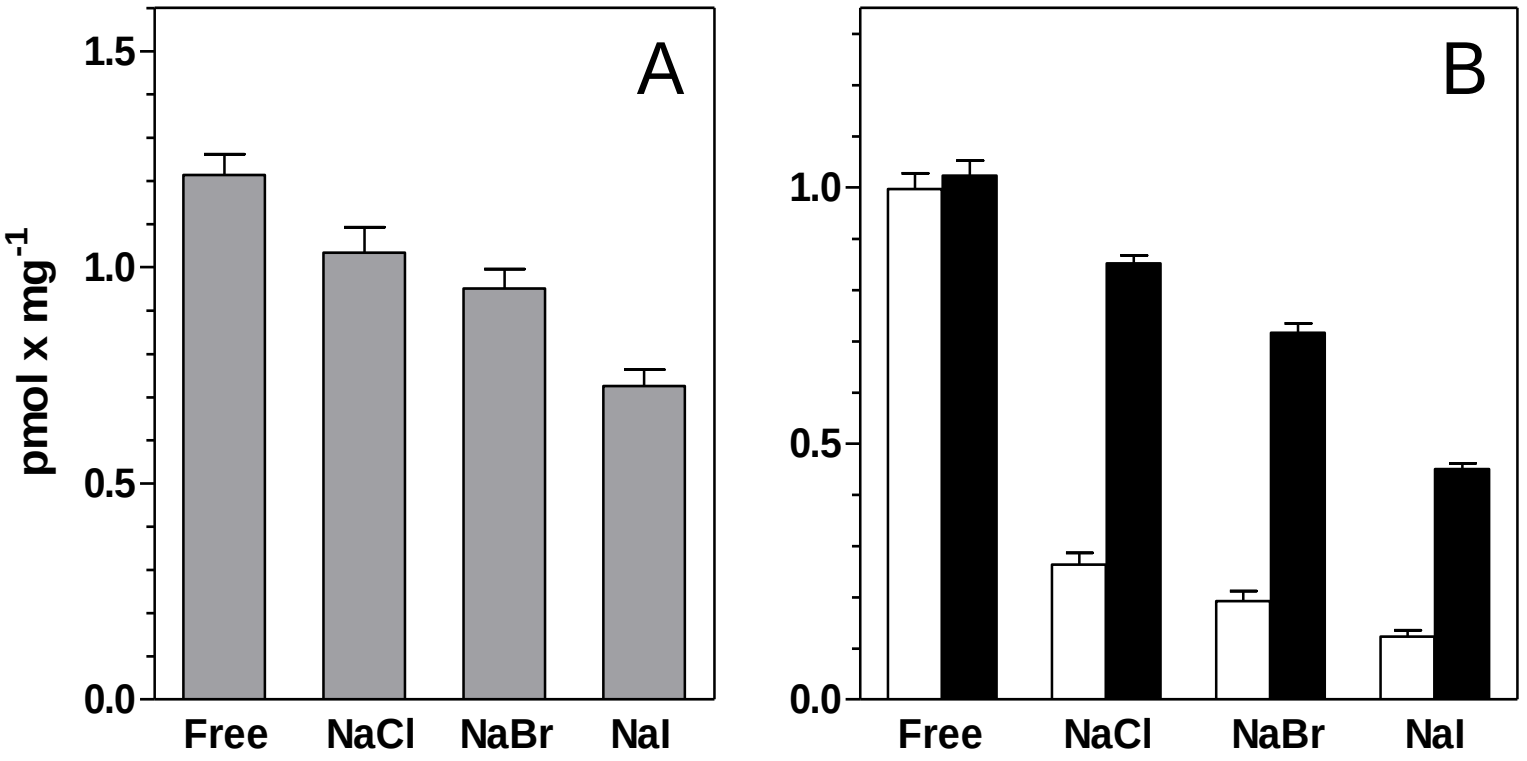
was very close to the value of allosteric constant of sodium binding to **δ-OR** determined by *Fenalti et al., (Nature, 506, 191-6, 2014)*

K_B = 13.3 mM.

The order of potency of monovalent cations when inhibiting agonist binding to δ-OR was: Na+ >> Li+ > K+ > (+)NMDG.

Our results further indicated that inhibition of opioid binding to δ-OR (A) and opioid-stimulated G protein activity (B) was increased by increase of the hydrophobicity of the counter ion:

chloride < bromide < iodine.



This result was fully in agreement with that what is known in psychiatry for more than 100 years but not included in the present theories of molecular bases of lithium therapy:

"I have used the bromide of lithium in cases of acute mania and have more reason to be satisfied with it than with any other medicine calculated to diminish the amount of blood in the cerebral vessels, and to calm any nervous excitement that may be present.

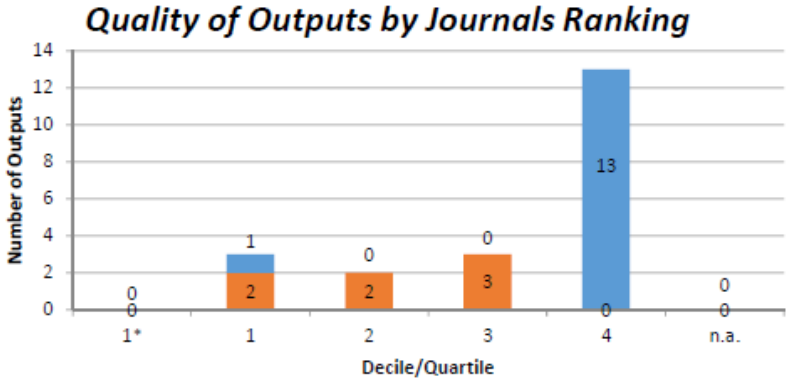
Hammond W. A. in „A treatise on diseases of the nervous system (p. 371), D. Appleton and Company (Edit.), New York, 1871.

Quality of publications and our contribution

22 papers in impacted journals 2010-2014

71% corresponding authors
on the papers selected for evaluation

59% corresponding authors
on all the papers published



Societal impact of our research

Educational activities

Lectures and practical courses:

Molecular Pharmacology I (**2** / **3** hours per week, winter semester, Department of Physiology, Biology, Faculty of Science, Charles University in Prague); L. Roubalova, J. Brejchova, M. Vosahlikova, D. Kagan, P. Svoboda

Molecular Pharmacology II (2 hours per week, winter semester, Department of Biochemistry, Chemistry, Faculty of Science, Charles University in Prague)
P. Svoboda

Members of Boards for doctoral studies :

Animal Physiology (Faculty of Science, Charles University in Prague)
Pharmacology and Toxicology (Medical Faculty, Charles University in Prague)
P. Svoboda

Popularization
1x CT24, opioid addiction

Involvement of students in research and supervision of students

BS Degree (3)

Ladislav Merta (**2012**), Adéla Provazníková (**2014**), Kristina Cechová (**2014**)

MS Degree (6)

Alexandra Rejhová (**2011**), Lenka Ulrychová (12 months at Glasgow University, **2011**), Barbora Volfová (12 months at Glasgow University, **2011),** Kateřina Višněvská (**2013**), Karolina Kettnerová (**2014**), Ladislav Merta (**2014**)

At present – Kristina Cechová

PhD Degree (5)

Pavel Ostašov (**2012**), Hana Ujčíková (**2014**), Miroslava Vošahlíková (**2014**), Jana Brejchová (6 months at Milano University, **2014**), Dmytro Kagan (**2015**)

At present: Kateřina Stolařová, Michaela Czerneková, MartinaHloušková

63% of publications with PhD students

All grant projects involve PhD students

Position in international and national contexts

Intramural collaboration

Department of Biomathematics (J. Janáček, Z. Burdíková, L. Kubínová)
Department of Protein Structures (T. Obšil, V. Obšilová and J. Teisinger)

Major domestic collaboration

Department of Biophysics, Heyrovsky Institute of Physical Chemistry, ASCR,
M. Hof, J. Sýkora, P. Jurkiewitz
Department of Physiology, Faculty of Science, Charles University in Prague
J. Novotný, L. Hejnová, Z. Drastichová, I. Svandova

International collaborations

M. Parenti, Department of Pharmacology, University of Milano-Biccoca, Italy
V. Hruby, Department of Chemistry and Biochemistry, University of Arizona, Tucson, USA
Tae-Weon Lee, Amgen, Palo Alto, USA
M. Buhneman, Department of Pharmacology and Clinical Pharmacy, Marburg, Germany
A. Faussner, Institute for Cardiovascular Prevention, Munich, Germany
G. Milligan, Glasgow University, Scotland

Foreign scientists and students

4 scientists giving a lecture
2 students spending > 1 month

Financial resources – grants and projects

2006-2010, AS CR IAA500110606, Delta-opioid receptor targeting to membrane domains: the role of palmitoylation.

2005-2011, Centrum of Neurosciences (project of MEYS LC554), L. Vyklicky, project leader.

2006-2011, Centrum of Fluorescence spectroscopy (project of MEYS LC06063), M. Hof, project leader.

2011-2015, GA CR P303/11/0298, Biochemical and morphological aspects of early postnatal development of rat heart and brain. The role of free radicals.

2012-2016, GA CR P207/12/0919, The role of hydrophobic plasma membrane interior in regulation of functional activity of δ-opioid receptors.

2012-2018, Project of Excellence in Neurosciences, L. Vyklicky, project leader (GA CR P304/12/G069)

100% funding from domestic grants and projects

Team members (December 2014)

Senior Scientists

P. Svoboda, Assoc. Prof. , DSc (team leader); H-index 22
- graduated at Faculty of Science, Charles Univ., Prague in 1977 – 3 years abroad (USA, Sweden, Scotland)

L. Roubalová, PhD; H-index 10
- graduated at Faculty of Science, Charles Univ., Prague in 2003 – 1 year abroad (Sweden, Canada)

J. Wilhelm, Prof., PhD; H-index 18
- graduated at Medical Faculty, Charles Univ., Prague in 2005 – 2 years abroad (USA)

Junior Scientists

H. Ujčíková, PhD
- graduated at Faculty of Science, Charles Univ., Prague in May 2014

M. Vošahlíková, PhD; graduated at 1st Medical Faculty, Charles Univ., Prague in June 2014

- **J. Brejchová,** PhD; graduated at Faculty of Science, Charles Univ., Prague in November 2014, 6 months abroad (Italy)

-K. Stolařová, on maternity leave since April 2015.

Department of Biochemistry of Membrane Receptors

1989-2014

The existence of Department was terminated on December 31, 2014 by director of Institute of Physiology, Dr. Lucie Kubínová, CSc. This decision was based on conclusions of the co-called „*Search and evaluation committee*“ which was looking for a new and better leader of this department.

Since 2015, we are organized as Laboratory of Membrane Receptors within Department of Mathematics under leadership of Dr. J. Janáček. In 2006-2011, we have participated in „Centrum of Fluorescence spectroscopy“ (project MSMT IC06063, Prof. M. Hof), Dr. Kubínová and Dr. J. Janáček.

Strategy and aims for the future

We shall try to understand how opioids and related peptides interact with OR present in selected parts of brain and in lymphocytes. Formulation of „biased signaling mechanism“ of GPCR action by R.J. Lefkowitz and B. Kobilka presents a new approach how to understand the difference in action of different agonists using the same receptor in the same cell and specificity of action of the same receptor in different cell types.

It also gives a guiding light in effort to understand more clearly the time-course of the route of mammalian organism to hell of drug addiction in molecular terms.