

CURRICULUM VITAE

Leonard Khiroug, Ph.D. (Passport spelling: Khirug)

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Date of Birth: January 16, 1971

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Academic Appointments:

Since April 2003 **Group leader** at the Neuroscience Center, University of Helsinki, Finland

Since August 2008 **Academy Research Fellow**, Academy of Finland (a 5 year post)

2007 - 2009 **Visiting Professor** at the Saint Petersburg State University, Russia

Since June 2005 **Docent of Neurobiology** at the University of Helsinki, Finland

Qualifications:

Ph.D. in Biophysics (July **1997**; International School for Advanced Studies, Trieste, Italy).

M.Sc. in Radiophysics and Electronics (**1993**; Kazan State University, Kazan, Russia).

Education and training:

2001–2003 Research Fellow at National Institute of Environmental Health Science, Durham, NC (NIEHS, part of National Institute of Health), Dr. **Jerrel Yakel's** group. Research project: Functional mapping and Ca²⁺ regulation of nicotinic and glutamate receptors in hippocampal interneurons using laser-based uncaging and confocal microscopy-based calcium imaging.

1998–2000 Postdoctoral fellow at the Department of Neurobiology, Duke University Medical Center (Durham, NC), laboratory of Prof. **George Augustine**. Studying mechanisms of memory expression on the single cell level, including: Spatial and temporal characteristics of intracellular calcium signaling in cerebellar long-term depression (LTD); Quantification of spread of LTD across the cell dendritic arbor, using caged compound photolysis

1993-1997 Graduate student at the International School for Advanced Studies (S.I.S.S.A.; Trieste, Italy), Ph.D. course in Biophysics under supervision of Prof. **Andrea Nistri**. Thesis entitled "Combined confocal microscopy and patch-clamp study of the effects of $[Ca^{2+}]_i$ and other modulators on ligand-gated channel desensitization". External examiner: Prof. Alan Marty.

1988-1993 Kazan State University (Kazan, Russia), Physics Department. Undergraduate course in Radiophysics and Electronics (Biophysics and Bionics). Thesis project under supervision of Prof. **Rashid Giniatullin** "Analysis of reliability factors of frog neuromuscular junction".

Research:

Current **research interests** include: synaptic function of the CNS neurons and astrocytes; morphological and functional plasticity of the "tripartite" synapse; protein targeting, trafficking and membrane insertion/removal mechanisms; intracellular Ca^{2+} dynamics; mitochondrial signaling and ROS-dependent modulation of synaptic function; glial-neuronal bidirectional signaling and roles of astrocytes in generation, maintenance and plasticity of synapses; KCC2-mediated intracellular Cl^- regulation in dendrites and axons.

The following **research methods** are used by the group: *in vivo* multiphoton imaging (thinned skull or cranial window); Investigation of vertebrate synaptic transmission using electrophysiological techniques (two-electrode voltage-clamp, whole cell patch-clamp) and computer modeling; single-cell electroporation; Confocal and multiphoton microscopy applied for 3-D analysis of cell structure and calcium imaging in living cells in combination with whole-cell patch-clamp recording and UV laser-based photolysis of caged compounds; Studying membrane-associated dynamics of tagged molecules using total-internal reflection fluorescence (TIRF) microscopy; Brain slice techniques (rat and mouse cerebellum and hippocampus); Cell-culture techniques (primary neurons and astrocytes; cell-lines).

Teaching experience:

a. Recent courses taught:

- 2008-10 Lecturer, Advanced Imaging Techniques course organized by HFIC, Helsinki
- 2007-10 Lecturer and instructor, the CIMO (Center for International Mobility) Winter School
- 2006-08 Lecturer and instructor, the EU (TEMPUS)-sponsored Master's Degree in Neuroscience program, University of Saint-Petersburg, Russia

- 2006 Invited lecturer, the 3rd National Course on “Signal Transduction in Neurodegeneration and Neurophysiology” at AIVI in Kuopio, Finland.
- 2005-07 Invited teacher, lecture series covering the “Neural Signaling” section of the Neurobiology graduate course at Tallinn Technical University, Tallinn, Estonia.
- 2004-07 Coordinator and lecturer, Annual Course on “Advanced Light Microscopy”, Viikki Graduate School in Biosciences (co-organizer: Dr. Kimmo Tanhuanpää).
- 2005-07 Coordinator and lecturer, Annual Course on “Image Analysis”, Viikki Graduate School in Biosciences (co-organizer: Dr. Kimmo Tanhuanpää).
- 2005-10 Lecturer, graduate course on Molecular Neuroscience (the “Cell Signaling Microdomains in Neurons” section), Neuroscience Center, Helsinki.
- 2004 Lecturer, Course on “Imaging technologies of live cells and animals”, Helsinki Biomedical Graduate School, University of Helsinki.

b. Mentoring and thesis supervision:

Postdoctoral Fellows: Minna Nytytkoski (2009-present), Evgeny Pryazhnikov (2006-present), Olesya Okuneva (2009), Alexandra Shintyapina (2009), Andrej Golubtsov (2009), Elena Kremneva (2007-09), Sergey Saveliev (2007), Julia Kolikova (2004-08), Ramil Afzalov (2004-09)

Graduate Students: Dmitry Molotkov (2008-present), Alexey Yukin (2007-present), Ilya Kirilkin (2005-present), Mikhail Kislin (2010-present), Dmitry Toptunov (2010-present)

Undergrad. Students: Artyom Dugan (2008-present), Elizaveta Tilli (2008-09), Veronika Pylsy (2010), Elisabeth Rappou (2010)

Visiting Students: Vikram Sharma (2007), Ekaterina Mugantseva (2007, 2010), Yves Kellner (2007), Anastasia Griekhova (2008), Daniela Saderi (2009), Xiaoying Kang (2009-10)

Visiting Scientists: Alexander Surin (2005-09), Vladimir Motin (2005-06), Rasa Gabrenaite (2010)

Service to scientific community:

2009	Reviewer, doctoral thesis by Maxim Beshpalov
2009 – present	Member group leader, Viikki Research Group Organization in Mol. Biosci.
2009	Reviewer, doctoral thesis by Tommi Möykkynen
2008 – present	Grant Reviewer, Human Frontier Science Program Organization (HFSP)
2008 – present	Member group leader, Finnish Graduate School for Neuroscience (FGSN)
2008	Reviewer, doctoral thesis by Hanna-Kaisa Wigren
2008 – present	Co-organizer, lecturer, application reviewer: CIMO Winter School, Finland
2007	Evaluator, graduate student conference, Biocentrum Helsinki
2007	Grant Reviewer, Association Française contre les Myopathies
2006 - present	Reviewer for the international scientific journal “Glia”
2006	Reviewer of student applications for the 11 th CIMO Winter School, Finland
2006	Reviewer, doctoral thesis by Jukka S. Pakkanen
2006 - present	Expert, the Helsinki University Pool of Experts
2004 - present	Member of the Thesis Committee for six PhD projects: Evgeny Kuleskiy, Aino Vesikansa, Kathleen Gransalke, Riikka-Liisa Uronen, Marie-Estelle Hokkanen, Satu Leppänen (GSBM, FGSN)
2004 - present	Coordinator of the Neuroscience Seminar series, Neuroscience Center
2004 - present	Supervisor of Live Cell Imaging core facility, Neuroscience Center
2001 - 2002	Translator of the textbook “From Neuron to Brain” by Nicholls, Martin, Wallace and Fuchs; translation from English into Russian

Invited lectures, seminars and public appearances:

Dec 2009	Invited lecturer, Neurobiology course, University of Trieste, Trieste Italy
Jan 2009	Invited lecturer, “Pain, Degeneration and Development” course, Kuopio FI
Nov 2008	Workshop on Confocal and Multiphoton Microscopy, S-Petersburg, Russia

Oct 2008	Foreign Ministry of Finland, interview on the immigrant integration issues
Oct 2008	Nordic Imaging Network symposium, Turku, Finland
May 2008	Program for Molecular Neurology, Biomedicum, Helsinki FI
April 2008	Finnish State Radio YLE Mondo, interview on neurobiological research
March 2008	Russian State Television, interview on visualization of neuronal signaling
Nov 2006	Center for Biotechnology, Biocity, Turku Finland
Mar 2006	8 th International Neuroscience Winter Conference, Soelden, Austria
Oct 2005	Optical Molecular Imaging in Biomedical Science, Haapsalu Estonia
Jun 2005	NeuroImaging Workshop, RIKEN, Japan
Apr 2005	Time-Lapse Microscopy Short Course, Capri, Italy
Apr 2005	Section for Physiology, Umea University, Umea, Sweden
Jan 2005	Sechenov Institute of Evolutionary Physiology and Biochemistry, Russia
Dec 2004	Tallinn Technical University, Tallinn Estonia
Nov 2004	Institute of General Pathology and Pathophysiology, Moscow Russia
Nov 2004	Institute of Biochemistry and Biophysics, Kazan Russia

Professional membership:

2005-present	Societas Biochemica, Biophysica et Microbiologica Fenniae (Biobio)
2003-present	Federation of European Neuroscience Societies (FENS)
2003-present	Brain Research Society of Finland
1998-present	Society for Neuroscience (SfN), USA

External funding and grants:

2010	A one-year grant from the University of Helsinki for PhD Student Dmitry Molotkov
2009	A four-year grant from the Academy of Finland, Photonics program
2009	Two CIMO fellowships for Alexandra Shintyapina and Olesya Okuneva
2008	CIMO fellowship for Dmitry Molotkov, graduate student

- 2008 5-year funding for Academy Research Fellow post, **Academy** of Finland
- 2008 Grant from Finnish Cultural Foundation (**SKR**) for research on purinergic signaling
- 2008 An infrastructure grant from **Biocentrum** Helsinki for multiphoton microscopy
- 2007 Competitive renewal of the five-year position funded by the **Academy** of Finland as a group leader at Neuroscience Center, University of Helsinki, Finland
- 2007 Grant from the **Academy** of Finland for research on “Glial-neuronal signaling by reactive oxygen species at neuromuscular synapse”
- 2007 Four **CIMO** fellowships for Vikram Sharma, Ekaterina Mugantseva, Sergey Saveliev and Elena Kremneva, visiting students and postdoctoral fellows
- 2007 Grant from Finnish Cultural Foundation (**SKR**) for research on “Ca²⁺ homeostasis in a mouse model of Northern epilepsy” (collaboration with Prof. A-E. Lehesjoki)
- 2006 **CIMO** fellowship for Elena Vaschinkina, Visiting Student
- 2005 One-year funding from the **Academy** of Finland for research on “Extracellular ATP as a mediator of glial-neuronal communication”
- 2005 Postdoctoral fellowship from **CIMO** for Dr. Evgeny Pryazhnikov
- 2005 An infrastructure grant from **Biocentrum** Helsinki for multiphoton microscopy
- 2005 Two-year funding from the **Academy** of Finland for mitochondrial research
- 2005 One-year research grant from **RIKEN** Brain Science Institute, RIKEN, Japan
- 2004 Postdoctoral fellowship from **CIMO** for Dr. Ramil Afzalov

Publications by Leonard Khiroug

(Peer-reviewed Journals and Book Chapters)

1. Giniatullin, R.A., Kheeroug*, L.S. (1993). Comparative study of kinetics of pre- and postsynaptic processes in the neuromuscular synapse using original model of endplate current. *Neurofisiologia* 1(2), 126-132.
2. Giniatullin, R.A., Kheeroug*, L.S., Vyskocil, F. (1995). Modelling endplate current: dependence on quantum secretion probability and postsynaptic miniature current parameters. *Europ. Biophys. J.*, 23, 443-446.
3. Andjus, P.R., Khiroug, L., Yakel, J.L., Cherubini, E., Nistri, A. (1996). Changes in intracellular calcium induced by NMDA in cultured rat hippocampal neurones require exogenous glycine. *Neurosci. Letters*, 210, 25-28.
4. Andjus, P.R., Khiroug, L., Nistri, A., Cherubini, E. (1996). ALS IgGs suppress [Ca²⁺]_i rise through P/Q type calcium channels in central neurones in culture. *NeuroReport*, 7, 1914-1916.
5. Giniatullin, R., Khiroug, L., Talantova, M., Nistri, A. (1996). Role of calcium ions in neuronal cholinergic and purinergic receptors function. *Neurochemistry*, 13(3), 221.
6. Andjus, P.R., Khiroug, L., Nistri, A., Cherubini, E. (1996). Effects of amyotrophic lateral sclerosis IgGs on calcium homeostasis in neural cells. In: "Immunoregulation in Health and Disease", Lukic M.L., Colic M., Stojkovic M.M., and Cuperlovic K. (editors) *Academic Press*, London 1996.

7. Giniatullin, R., Khiroug, L., Talantova, M., Nistri, A. (1996). Fading and rebound of currents induced by ATP on rat PC12 cells. *Br. J. Pharmacol.*, 119, 1045-1053.
8. Khiroug, L., Giniatullin, R., Talantova, M., Nistri, A. (1997). Role of intracellular calcium in fast and slow desensitization of purinoceptors in PC12 cells. *Br. J. Pharmacol.*, 120, 1552-1560.
9. Khiroug, L., Giniatullin, R., Talantova, M., and Nistri, A. (1997). The effect of the neuropeptide Substance P on desensitization of ATP receptors of PC12 cells. *Br. J. Pharmacol.*, 121, 497-502.
10. Khiroug, L., Giniatullin, R., Sokolova, E., Talantova, M., and Nistri, A. (1997). Imaging of intracellular calcium during desensitization of nicotinic acetylcholine receptors of rat chromaffin cells. *Br. J. Pharmacol.*, 122, 1323-1332.
11. Khiroug, L., Sokolova, E., Giniatullin, R., Afzalov, R., and Nistri, A. (1998). Recovery from desensitization of neuronal nicotinic acetylcholine receptors of rat chromaffin cells is modulated by intracellular calcium through distinct second messengers. *J. Neurosci.* 18, 2458-2466.
12. Giniatullin R, Di Angelantonio S, Marchetti C, Sokolova E, Khiroug L, Nistri A. (1999). Calcitonin gene-related peptide rapidly downregulates nicotinic receptor function and slowly raises intracellular Ca^{2+} in rat chromaffin cells in vitro. *J Neurosci.* 19, 2945-2953.
13. Wang S.S.-H., Khiroug L. and Augustine G.J. (2000). Quantification of spread of cerebellar long-term depression using chemical two-photon uncaging of glutamate. *Proc Natl Acad Sci USA* . 97(15), 8635-8640.
14. Miyata M., Finch E.A., Khiroug L., Hashimoto K., Emdad L., Siddiq A., Hayasaka S., Dekker-Ohno K., Inouye M., Takagishi Y., Augustine G.J., Kano M. (2000). Local IP3-mediated Signaling Required in Purkinje Cell Dendritic Spines for Long-term Synaptic Depression. *Neuron* 28(1), 233-44.
15. Ribar T.J., Rodriguiz R.M., Khiroug L., Wetsel W.C., Augustine G.J. and Means A.R. (2000) Ca^{2+} /calmodulin kinase null mice exhibit profound cerebellar deficits due to stalled Purkinje cell maturation. *J Neurosci* 20(22), RC107.
16. Khiroug S.S., Harkness P.C., Lamb P.W., Sudweeks S.N., Khiroug L., Millar N.S. and Yakel J.L. (2002). Nicotinic acetylcholine receptor $\alpha 7$ and $\beta 2$ subunits co-assemble to form functional heteromeric nicotinic receptor channels. *J Physiol* 540(2), 425-34.
17. Khiroug L., Giniatullin R, Klein RC, Fayuk D, and Yakel J.L. (2003) Functional mapping and Ca^{2+} regulation of nicotinic acetylcholine receptor channels in rat hippocampal CA1 neurons. *J Neurosci.* 23:9024-31
18. Khiroug, S.S., Khiroug L. and Yakel J.L. (2004) Rat nicotinic ACh receptor $\alpha 2\beta 2$ channels: Comparison of functional properties with $\alpha 4\beta 2$ channels in *Xenopus* oocytes. *Neuroscience* 124(4):817-22.
19. Khiroug, S., Huttu, K., Ludwig, A., Smirnov, S., Voipio, J., Rivera, C., Kaila, K. and Khiroug, L. (2005) Distinct properties of functional KCC2 expression in immature mouse hippocampal neurons in culture and in acute slices. *Eur J Neurosci* 21:899-904.

20. Safiulina, V.F., Afzalov, R., Khiroug, L., Cherubini, E. and Giniatullin, R. (2006) Reactive oxygen species mediate the potentiating effect of ATP on GABAergic synaptic transmission in the immature hippocampus. *J Biol Chem* 281: 23464-23470.
21. Blaesse, P., Schweizer, M., Schindler, J., Doncheva, E., Delpire, E., Khiroug, L., Friauf, E. and Nothwang, H.G. (2006) Inactive and active K⁺-Cl⁻-cotransporter 2 localize to the plasma membrane of developing rat superior olivary complex neurons. *J Neurosci* 26(41):10407-19.
22. Sokka, A.-E., Putkonen, N., Mudo, G., Pryazhnikov, E., Reijonen, S., Khiroug, L., Belluardo, N., Lindholm, D. and Korhonen, L. (2006) Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. *J Neurosci*, 27(4):901-8.
23. Kolikova, J., Afzalov, R., Giniatullina, A., Surin, A., Giniatullin, R., and Khiroug, L. (2006) Calcium-dependent trapping of mitochondria near plasma membrane in stimulated astrocytes. *Brain Cell Biology*, 35:75-86.
24. Tanaka, K., Khiroug, L., Santamaria, F., Doi T., Ogasawara, H., Ellis-Davies, G, Kawato, M, and Augustine, G.J. (2007) Ca²⁺ requirements for cerebellar long-term synaptic depression: role for a postsynaptic leaky integrator. *Neuron*, 54(5):787-800.
25. Pangrsic, T., Potokar, M., Stenovec, M., Kreft, M., Fabbretti, E., Nistri, A., Pryazhnikov, E., Khiroug, L., Giniatullin, R., and Zorec, R. (2007) Exocytotic release of ATP from cultured astrocytes. *J Biol Chem*, 282(39):28749-58.
26. Ahtiainen, L., Kolikova, J., Mutka, A.-L., Luiro, K., Gentile, M., Ikonen, E., Khiroug, L., Jalanko, A., Kopra, O. (2007) Palmitoyl protein thioesterase 1 (Ppt1) deficient mouse neurons show alterations in cholesterol metabolism and calcium homeostasis prior to synaptic dysfunction. *Neurobiol Disease*, 28(1):52-64.
27. Li, H., Khirug, S., Cai, C., Ludwig, A., Kolikova, J., Afzalov, R., Coleman, S., Lauri, S., Airaksinen, M., Keinänen, K., Khiroug, L., Saarma, M., Kaila, K. and Rivera, C. (2007) KCC2 Interacts with the Dendritic Cytoskeleton to Promote Spine Development. *Neuron*, 56(6):1019-33.
28. Pryazhnikov, E. and Khiroug, L. (2008) Sub-micromolar increase in [Ca²⁺]_i triggers delayed exocytosis of ATP in cultured astrocytes. *Glia*, 56(1):38-49.
29. Khirug, S., Yamada, J., Afzalov, R., Voipio, J., Khiroug, L. and Kaila, K. (2008) GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J Neurosci*, 28(18):4635-4639.
30. Van Ryssen, M.P., Avlonitis, N., Giniatullin, R., McDougall, C., Carr, J.L., Stanton-Humphreys, M.N., Borgström, E.L.A., Brown, C.T.A., Fayuk, D., Surin, A., Niittykoski, M., Khiroug, L. and Conway, S.J. (2009) Synthesis, photolysis studies and *in vitro* photorelease of caged TRPV1 agonists and antagonists. *Organic and Biomol. Chem.*, 7:4695–4707.
31. Khirug, S., Pryazhnikov, E., Coleman, S.K., Jeromin, A., Keiänen, K. and Khiroug, L. (2009) Dynamic visualization of membrane-inserted fraction of pHluorin- tagged channels using repetitive acidification technique. *BMC Neurosci* 10:141.
32. Moise, L., Liu, J., Pryazhnikov, E., Khiroug, L., Jeromin, A. and Hawrot, E. (2009) Kv4.2 channels tagged in the S1-S2 loop for alpha-bungarotoxin binding provide a new tool for studies of channel expression and localization. *Channels* 4(2).

33. Molotkov, D., Yukin, A., Afzalov, R. and Khiroug, L. (2010) Gene Delivery To Postnatal Rat Brain By Non-ventricular Plasmid Injection and Electroporation. ***Journal of Visualized Experiments*** JoVE <http://www.jove.com/index/details.stp?id=2244>, doi: 10.3791/2244.