

International Society for Invertebrate Neurobiology



**12th SYMPOSIUM ON  
INVERTEBRATE NEUROBIOLOGY**

**PROGRAM and ABSTRACTS**

August 31–September 4, 2011  
Tihany, Hungary

**Supported**

**by**

**IBRO Central and East European Regional  
Committee**

**Balaton Limnological Research Institute,  
Hungarian Academy of Sciences**

# ISIN

## International Society for Invertebrate Neurobiology

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## 12th Symposium on Invertebrate Neurobiology

August 31–September 4, 2011, Tihany, Hungary

### Organizing Committee:

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## **Dates and Proceedings of the Former Symposia on Neurobiology of Invertebrates, Tihany**

1. September 1-4, 1967  
Neurobiology of Invertebrates. (Ed.: J. Salánki), Plenum Press and Akadémiai Kiadó, New York and Budapest, 1968
2. August 2-5, 1971  
Neurobiology of Invertebrates. Mechanisms of Rhythmic Regulation (Ed.: J. Salánki), Akadémiai Kiadó, Budapest.
3. September 8-12, 1975  
Neurobiology of Invertebrates. Gastropoda Brain (Ed.: J. Salánki), Akadémiai Kiadó, Budapest, 1976
4. July 10-13, 1980  
Neurotransmitters in Invertebrates. Advances of Physiological Sciences. Vol. 22 (Ed.: K. S.-Rózsa), Pergamon Press, Oxford and Akadémiai Kiadó, Budapest, 1981
5. July 20-24, 1980  
Mechanisms of Integration. Advances of Physiological Sciences. Vol. 23 (Ed.: J. Salánki), Pergamon Press, Oxford and Akadémiai Kiadó, Budapest, 1981
6. August 22- 26, 1987  
Neurobiology of Invertebrates. Transmitters, Modulators and Receptors., Symposia Biologica Hungarica, Vol. 36 (Eds.: J. Salánki, K. S.-Rózsa), Akadémiai Kiadó, Budapest, 1988
7. June 23- June 28, 1991  
Neurobiology of Invertebrates. Signal Molecules, Networks and Behaviour (Eds.: J. Salánki, K. S.-Rózsa, K. Elekes), Akadémiai Kiadó, Budapest, 1993; Acta Biologica Hungarica 43, 1992, and 44 (1), 1993
8. June 27- July 2, 1995  
Neurobiology of Invertebrates. Simple and Complex Regulatory Systems (Eds.: K. Elekes, J. Salánki), Akadémiai Kiadó, Budapest, 1995; Acta Biologica Hungarica 46 (2-4), 1995
9. July 1-5, 1999  
Neurobiology of Invertebrates. Membrane, Chemical Signalling and Systems Approach (Eds.: K. Elekes, J. Salánki), Akadémiai Kiadó, Budapest, 1995; Acta Biologica Hungarica 51 (2-4), 1995
10. July 5-9, 2003  
Acta Biologica Hungarica 55 (1-4), 2004
10. August 25-29, 2007  
Acta Biologica Hungarica 59 (Supplementum), 2008

# PROGRAM

## August 31, Wednesday

16:00-19.00 Registration

19:30- Get-together party

## September 1, Thursday

9.00-10.00 Registration

10.00-10-15 Opening of the symposium

10.15-12.00 Oral presentations  
Chairman: **J.-G. Hildebrand**

### *Ernst Florey Memory Lecture*

**Arthropod nervous systems: Their historical impact on ideas about the common origin of brains, and a modern rejection of convergent evolution.** N. J. Strausfeld (*Department of Neurosciences, University Arizona, Tucson, Arizona, USA*)

**The locust stomatogastric nervous system – recent updates.** A. Ayali (*Department of Zoology, Tel Aviv University, Tel Aviv*)

**Insect brain neurons labeled by actin isoforms.** F.-W. Schürmann (*University of Göttingen, Göttingen, Germany*)

12.00-13.30 Lunch

13.30-15.30 Oral presentations  
Chairman: **H.-J. Pflüger**

**Coding of essential ‘odor objects’ in the antennal lobe of the sphinx moth *Manduca sexta*.** J. G. Hildebrand (*Department of Neurosciences, University of Arizona, Tucson, USA*)

**The olfactory system of the red flower beetle, *Tribolium castaneum*: architecture and postmetamorphic plasticity.** J. Schachtner (*FB Biologie–Tierphysiologie, Philipps-Universität Marburg, Marburg, Germany*)

**Single-cell-based analysis of neuropeptide expression in *Periplaneta americana* antennal lobe neurons.** R. Predel (*Biocenter, Institute for Zoology, University of Köln, Köln, Germany*)

**The synaptology and 5-HT-immunoreactive innervation of the procerebrum cell body layer suggest a role of local circuits in olfactory integration.** K. Elekes,<sup>1</sup> I. Battonyai,<sup>1</sup> S. Kobayashi,<sup>2</sup> E. Ito<sup>2</sup> (<sup>1</sup>*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary;* <sup>2</sup>*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan*)

**15.30-15.45** Break  
**15.45-17.15** Oral presentations  
Chairman: **K. Elekes**

*Plenary lecture*

**Neuroanatomy for photoperiodism in insects.** S. Shiga (*Graduate School of Science, Osaka City University, Osaka, Japan*)

**Synaptic circuits in the *Drosophila* visual system: Serial EM yields progress but also some inconvenient truths.** I. A. Meinertzhagen,<sup>1,2</sup> S. Takemura,<sup>2</sup> Z. Lu,<sup>1,2</sup> L. Scheffer,<sup>2</sup> M. Chklovskii<sup>2</sup> (<sup>1</sup>*Life Sciences Centre, Dalhousie University, Halifax, Canada;* <sup>2</sup>*Janelia Farm Research Campus of HHMI, Ashburn, USA.*)

**Selective visual attention in *Drosophila*.** R. Wolf, P. Sareen, M. Heisenberg (*Rudolf-Virchow-Zentrum, DFG Research Center for Experimental Biomedicine University of Würzburg, Germany*)

**17.15-** Poster presentations

## September 2, Friday

**9.00-10.30** Oral presentations  
Chairman: **D. R. Nässel**

*Plenary Lecture*

**Neuropeptides and behavioural plasticity in *C. elegans*.** L. Holden-Dye, R. J Walker, V. O'Connor (*Institute for Life Sciences, Highfield Campus, University of Southampton, Southampton, UK.*)

**Serotonin inhibits dopamine-induced contraction of the snail salivary duct.** T. Kiss, L. Hiripi, L. Hernádi (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary*)

**High resolution spatial distribution of neuropeptides by MALDI imaging in the pond snail *Lymnaea stagnalis*.** L. Mark,<sup>1</sup> G. Maasz,<sup>1</sup> Z. Laszlo,<sup>2</sup> Zs. Pirger<sup>2</sup> (<sup>1</sup>*Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Pécs, Hungary;* <sup>2</sup>*School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom*)

**10.30-11.00** Break

**11.00-12.00** Oral presentations  
Chairman: **G. Kemenes**

*János Salánki Memorial Lecture*

**Genomic deciphering of memory mechanisms and multiple origins of neural circuits.** L. L. Moroz (*Department of Neuroscience, McKnight Brain Institute and Whitney Laboratory for Marine Bioscience University of Florida, Gainesville and St. Augustine, USA*)

**12.30-** EXCURSION (Visiting: Szigliget, fortress, XIII. century – Keszthely, castle, XVIII. century – Héviz, hot spring spa, romantic dinner) (Lunch on the board)

## September 3, Saturday

9.30-11.30 Oral presentations  
Chairman: **I. Meinertzhagen**

### *Plenary Lecture*

**Regulation of synaptic plasticity in simple nervous systems.** P. M. Balaban (*Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia*)

**Axonal spike processing and long-term associative memory in *Lymnaea*.** E. S. Nikitin,<sup>1</sup> T. Korshunova,<sup>1</sup> P. M. Balaban,<sup>1</sup> G. Kemenes<sup>2</sup> (<sup>1</sup>*Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia*; <sup>2</sup>*Sussex Centre for Neuroscience, School of Life Sciences, University of Sussex, Brighton, U.K.*)

**Insulin and memory in *Lymnaea*.** E. Ito,<sup>1</sup> Y. Sakamoto,<sup>1</sup> R. Okada,<sup>1</sup> K. Mita,<sup>1</sup> E. Otshuka,<sup>1</sup> A. Okuta,<sup>2</sup> H. Sunada,<sup>3</sup> M. Sakakibara<sup>3</sup> (<sup>1</sup>*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan*; <sup>2</sup>*Kyoto University, Kyoto, Japan*; <sup>3</sup>*Tokai University, Numazu, Japan*)

**Neurotransmitters regulating of insulin signaling in *Drosophila*.** D. R. Nässel, R. T. Birse, L. E. Enell, N. Kapan, J. Luo, J. A. E. Söderberg, Å. M. E. Winther (*Department of Zoology, Stockholm University, Stockholm, Sweden*)

12.00-13.30 Lunch

13.30-15.15 Oral presentations  
Chairman: **R. J. Walker**

**Adaptive control of cockroach locomotion.** A. Ayali (*Department of Zoology, Tel Aviv University, Tel Aviv*)

**In search for the neural basis of behavioral spontaneity in insects: Lessons from predatory wasps and zombie cockroaches.** F. Libersat (*Department of Life Sciences, Ben Gurion University, Beer Sheva, Israel*)

**Insect behaviour and the functional role of biogenic amines.** H.-J. Pflüger (*Institut für Biologie, Neurobiologie, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany*)

**Physiological, behavioral and life-span plasticity in *Drosophila*.** A. Ueda, H. Ruan, C.-F. Wu (*Department of Biology, University of Iowa, Iowa City, USA*)

### *Special Lecture*

Chairman: **Anonymous**

**The anti-intuitive visual system of the honey bee.** A. Horridge (*Australian National University, Canberra, Australia*)

15.15-15.30 Break

15.30-17.00 Poster presentations

17.00-19.00 Oral presentations

Chairman: **E. Ito**

**Mechanism of delayed action of serotonin overproduction in early molluscan development.** E. E. Voronezhskaya, M. Yu. Khabarova, E. G. Ivashkin (*Institute of Developmental Biology, RAS, Moscow, Russia*)

**Patterns of serotonin, FMRFamide and NO in the complex life cycle of *Digene*** O. O. Tolstakov,<sup>1</sup> N. B. Terenina,<sup>1</sup> M. K. S. Gustafsson<sup>2</sup> (<sup>1</sup>*Centre of Parasitology of A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia;* <sup>2</sup>*Department of Biology, Åbo Akademi University, Åbo, Finland*)

**NO-inducing signals are essential for the regeneration of the snail tentacular ganglion.** Z. Serfözö (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary*)

**The different strategy of muscle and neuronal systems development in larvae of closely related bivalve species.** V. Dyachuk<sup>1</sup>, N. Odintsova,<sup>1</sup> E. E. Voronezhskaya<sup>2</sup> (<sup>1</sup>*A. V. Zhirmunsky Institute of Marine Biology Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia;* <sup>2</sup>*Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia.*)

**DNA endoreplication in the brain neurons during body growth of an adult slug.** R. Matsuo (*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan*)

**Microglia recruitment during the CNS repair in the medicinal leech, *Hirudo medicinalis*.** A. Garçon-Bocquet, F. Croq, J. Vizioli, M. Tahtouh, C. Van Camp, P. Nagnan-Le Meillour, M. Salzet, P.-E. Sautière, C. Lefebvre (*Laboratoire de Spectrométrie de Masse Biologique Fondamentale et Appliqué, Université Lille1, Villeneuve d'Ascq France*)

## September 4, Sunday

9.00-12.00 Oral presentations

Chairman: **P. M. Balaban**

### *Plenary Lecture*

**Dynamic cellular and molecular mechanisms of memory: new insights from molluscan models** G. Kemenes (*Sussex Centre for Neuroscience, School of Life Sciences, University of Sussex, Falmer, Brighton, U.K.*)

**A systems level analysis of decision making: the interaction of feeding and withdrawal behaviors in *Lymnaea*.** I. Kemenes, Z. Pirger, Z. Laszlo, G. Kemenes, P. R. Benjamin, M. O'Shea (*Sussex Centre for Neuroscience, School of Life Sciences, University of Sussex, Falmer, Brighton, U. K.*)

**Analysis of feeding and withdrawal circuits reveals neural mechanisms of decision making.** Z. Pirger, Z. Laszlo, G. Kemenes, M. O'Shea, P. R. Benjamin, I. Kemenes (*Sussex Centre for Neuroscience, School of Life Sciences, University of Sussex, Falmer, Brighton, U.K.*)

**Repetitive noxious stimuli altered the shadow-induced withdrawal behavior in *Lymnaea*.** H. Sunada,<sup>1</sup> K. Lukowiak,<sup>2</sup> M. Sakakibara<sup>3</sup> (<sup>1</sup>*Graduate School of Bioscience, Tokai University, Numazu, Japan;* <sup>2</sup>*Hotchkiss Brain Institute, University of Calgary, Calgary, Canada;* <sup>3</sup>*School of High-Technology for Human Welfare, Tokai University, Numazu, Japan*)

**Neuroanatomical background of space positioning of olfactory organ in the snail, *Helix pomatia*.** L. Hernádi,<sup>1</sup> T. Teyke<sup>2</sup> (*Department of Experimental Zoology, Balaton Limnological Research Institute, Tihany, Hungary;* <sup>2</sup>*Zoological Institute, Johannes-Gutenberg University, Mainz, Germany*)

11.15-11.45 Break

11.45-13.30

**How and where should (and can) we publish?** (Discussion on the publication possibilities of invertebrate neurobiology – with the participation of Lindy Holden-Dye - *Invertebrate Neuroscience*, Ian Meinertzhagen – *Journal of Comparative Neurology*, John G. Hildebrand – *Journal of Comparative Physiology*)

**General assembly of ISIN**

**Closing remarks**

19.30- Farewell dinner

# POSTER SECTION

Posters Nos. 1-35, September 1-4 (Thursday-Sunday, continuous display)

- 1. The 5-HT-immunoreactive innervation of the *Helix procerebrum*.** I. Battonyai, K. Elekes (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary*)
- 2. Potassium channels in the *Helix* central nervous system: preliminary immunohistochemical studies.** I. Battonyai, Z. Serfözö, K. Elekes (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary*)
- 3. Evidences for NO-signaling in the olfactory center of the snail, *Helix pomatia*. A biochemical and morphological study.** Nacsa, K., K. Elekes, Z. Serfözö (*Department of Experimental Zoology, Balaton Limnological Research Institut, HAS, Tihany, Hungary*)
- 4. GABAergic effects on the slow oscillatory neural activities in the procerebrum of *Limax valentianus*.** S. Kobayashi,<sup>1</sup> R. Matsuo,<sup>1</sup> H. Sadamoto,<sup>1</sup> S. Watanabe,<sup>2</sup> E. Ito<sup>1</sup> (<sup>1</sup>*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Shido, Sanuki, Kagawa, Japan;* <sup>2</sup>*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo Japan*)
- 5. Influence of serotonin and dopamine on functioning of neural network involved in olfaction and tentacle movements in *Helix* snail.** M. Roshchin, P. M. Balaban (*Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia*)
- 6. Immunohistochemical study on dermal photoreceptor in *Lymnaea*.** S. Takigami,<sup>1</sup> H. Sunada,<sup>2</sup> T. Horikoshi,<sup>3</sup> M. Sakakibara<sup>3</sup> (<sup>1</sup>*Graduate School of High-Technology for Human Welfare, Tokai University;* <sup>2</sup>*Graduate School of Bioscience;* <sup>3</sup>*Department of Biological Science and Technology, Tokai University, Numazu, Japan*)
- 7. In-vitro conditioning in *Lymnaea*.** H. Sunada,<sup>1</sup> K. Lukowiak,<sup>2</sup> M. Sakakibara<sup>3</sup> (<sup>1</sup>*Graduate School of Bioscience, Tokai University, Numazu, Japan;* <sup>2</sup>*Hotchkiss Brain Institute, University of Calgary, Calgary, Canada;* <sup>3</sup>*School of High-Technology for Human Welfare, Tokai University, Numazu, Japan*)
- 8. Pharmacological and anatomical background of twitching and quivering of the superior tentacles of *Helix*.** N. Krajcs,<sup>1</sup> L. Hernádi,<sup>1</sup> L. Márk,<sup>2</sup> K. Elekes,<sup>1</sup> T. Kiss<sup>1</sup> (<sup>1</sup>*Department of Experimental Biology, Balaton Limnological Research Institute HAS, Tihany, Hungary;* <sup>2</sup>*Department of Biochemistry and Medical Chemistry, University of Pécs, Pécs, Hungary*)
- 9. Nitric oxide is necessary for both erasure and consolidation of memory.** T. A. Korshunova, M. V. Roshchin, P. M. Balaban (*Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia*)
- 10. Coordination of feeding motor rhythms in semi-intact preparation of *Lymnaea stagnalis*.** I. A. Chistopolsky, V. E. Dyakonova (*Koltzov Institute of Developmental Biology, RAS, Moscow, Russia*)
- 11. Do the glutamatergic neurons of the *Lymnaea* CNS possess NMDA receptors?** L. Hernádi,<sup>1</sup> G. Kemenes<sup>2</sup> (<sup>1</sup>*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary;* <sup>2</sup>*School of Life Sciences, University of Sussex, Brighton, U.K.*)
- 12. Evidences for a possible cross-talk between serotonergic and dopaminergic neurons in the *Lymnaea* CNS.** L. Hernádi, Z. Serfözö, Á. Vehovszky (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary*)

13. **Control of locomotion in terrestrial snail by peptides.** I. S. Zakharov,<sup>1,2</sup> D. Boguslavsky,<sup>1</sup> A. V. Belyavsky,<sup>2</sup> P. M. Balaban<sup>2</sup> (<sup>1</sup>*Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia;* <sup>2</sup>*Institute for Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences, Moscow, Russia*)
14. **Genomic portrait of a neuron: identification and quantification of virtually all RNAs in single individually identified neurons** L. L. Moroz,<sup>1,2</sup> M. R. Citarella,<sup>1</sup> A. B. Kohn<sup>1</sup> (<sup>1</sup>*Univ. of Florida, Whitney Lab., St Augustine, FL 32080;* <sup>2</sup>*Dept. of Neuroscience, Univ. of Florida, Gainesville, USA*)
15. **Genome wide analysis of neurotransmitter signaling in the ctenophore, *Pleurobrachia bachei*.** M. Citarella,<sup>1</sup> D. O. Girardo,<sup>1</sup> J. J. Swore,<sup>1</sup> A. B. Kohn,<sup>1</sup> L. L. Moroz<sup>1,2</sup> (<sup>1</sup>*Whitney Lab for Marine Biosciences, USA;* <sup>2</sup>*Dept of Neuroscience, University of Florida, St. Augustine, Gainesville, USA*)
16. **Identification and expression analysis of the genes involved in the biogenic amine systems in the field cricket *Gryllus bimaculatus*.** T. Watanabe,<sup>1</sup> H. Sadamoto,<sup>2</sup> H. Aonuma<sup>1</sup> (<sup>1</sup>*Laboratory of Neuro-Cybernetics, Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan;* <sup>2</sup>*Laboratory of Functional Biology, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan*)
17. **Pumping off food (PoffF): A neuromodulatable adaptive response to chronic food withdrawal in *C. elegans*.** V. O'Connor, S. Luedtke, I. Casey, R. Hellmann, C. Murray, R. Walker, L. Holden-Dye (*Southampton Neuroscience Group (SoNG), University of Southampton, UK.*)
18. **Behavioral evidence for internal factors affecting duration of conglobation in pill bugs (*Armadillidium vulgare*).** T. Moriyama,<sup>1</sup> H. Matsuno<sup>2</sup> (<sup>1</sup>*Youn Researchers Empowerment Center, Shinshu University, Japan;* <sup>2</sup>*Department of Complex Systems Science, Future University Hakodate, Hakodate, Japan*)
19. **Fighting behavior of white-eye mutants in the cricket *Gryllus bimaculatus*.** M. Sakura,<sup>1,2</sup> T. Watanabe,<sup>2</sup> H. Aonuma<sup>2</sup> (<sup>1</sup>*Graduate School of Science, Kobe University, Kobe, Japan;* <sup>2</sup>*Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0812, Japan*)
20. **Evolutionary origin of the hooks in the buccal apparatus of *Clione limacina*: neurophysiological evidences.** A. Y. Malyshev,<sup>1</sup> A. V. Martynov,<sup>2</sup> P. M. Balaban<sup>1</sup> (<sup>1</sup>*Institute of Higher Nervous Activity and Neurophysiology of RAS, Moscow, Russia;* <sup>2</sup>*Zoological Museum of Moscow State University, Moscow, Russia*)
21. **Mathematical analysis of honeybee waggle dance.** R. Okada,<sup>1</sup> H. Ikeno,<sup>2</sup> T. Kimura,<sup>2</sup> M. Ohashi,<sup>2</sup> H. Aonuma,<sup>3</sup> E. Ito<sup>1</sup> (<sup>1</sup>*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Japan;* <sup>2</sup>*School of Human Science and Environment, University of Hyogo, Kobe, Japan;* <sup>3</sup>*Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan*)
22. **Octopaminergic control of task dependent aggression in the ant, *Formica japonica*.** H. Aonuma, T. Watanabe (*Laboratory of Neuro-Cybernetics, Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan*)
23. **Neuropeptides in the antennal lobe of *Tribolium castaneum*.** M. Binzer, C. M. Heuer, J. Schachtner (*Dept. Biology – Animal Physiology, Philipps-University Marburg, Marburg, Germany*)
24. **Neuropeptides in the mushroom bodies of *Tribolium castaneum*.** C. M. Heuer, M. Binzer, J. Schachtner (*Dept. Biology – Animal Physiology, Philipps-University Marburg, Marburg, Germany*)

25. **Some aspects of immunolocalization of FMRFamide in the nervous system of turbellarians *Girardia tigrina* and *Polycelis termis*.** N. D. Kreshchenko,<sup>1</sup> O. O. Tolstenkov<sup>2</sup> (<sup>1</sup>*Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, Russia;* <sup>2</sup>*Centre of Parasitology, Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia*)
26. **PACAP – a phylogenetically conserved neuropeptide.** Z. Pirger,<sup>1,2</sup> S. Naskar,<sup>2</sup> A. Pringle,<sup>2</sup> Z. Laszlo,<sup>1,2</sup> A. Vaczy,<sup>3</sup> G. Kemenes,<sup>2</sup> L. Mark,<sup>3</sup> T. Kiss,<sup>1</sup> D. Reglodi<sup>4</sup> (<sup>1</sup>*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary;* <sup>2</sup>*School of Life Sciences, University of Sussex, Falmer, Brighton, United Kingdom;* <sup>3</sup>*Departments of Biochemistry and Medical Chemistry, and* <sup>4</sup>*Anatomy, University of Pecs, Pecs, Hungary*)
27. **Expression of early genes in the CNS of snail *Helix*.** V. Ierusalimsky, P. M. Balaban (*Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences, Moscow, Russia*)
28. **Biochemical and physiological-pharmacological characterization of the 5-HTergic regulation of the peripheral feeding system (buccal mass) of *Lymnaea*.** G. Balog,<sup>1</sup> E. E. Voronezhskaya,<sup>2</sup> L. Hiripi,<sup>1</sup> K. Elekes<sup>1</sup> (*Department of Experimental Zoology, Balaton Limnological Research Institute, HAS, Tihany, Hungary;* <sup>2</sup>*Institute of Developmental Biology, RAS, Moscow, Russia*)
29. **Biogenic amines and FMRFamide in development of archiannelid *Dinophilus gyrociliatu*.** E. G. Fofanova, E. E. Voronezhskaya, L. P. Nezlin (*Institute of Developmental Biology, RAS, Moscow, Russia*)
30. **Transmembrane 5-HT transport during early development of molluscs.** E. G. Ivashkin, M. Yu. Khabarova, E. E. Voronezhskaya (*Institute of Developmental Biology, RAS, Moscow, Russia*)
31. **Neuronal modulation of larval growth in bay mussel *Mytilus trossilus* developed in various salinities.** E. Vekhova,<sup>2</sup> E. Ivashkin,<sup>1</sup> O. Yurchenko,<sup>2</sup> V. Dyachuk,<sup>2</sup> M. Khabarova<sup>1</sup>, E. Voronezhskaya<sup>1</sup> (<sup>1</sup>*Institute of Marine Biology Far Eastern Branch of RAS, Vladivostok, Russia ;* <sup>2</sup>*Institute of Developmental Biology, RAS, Moscow, Russia*)
32. **Origin of automatism and its regulation of the gut motility in gastropods, *Aplysia* and *Lymnaea*.** M. Kurokawa,<sup>1</sup> Y. Kasuya,<sup>1,2</sup> T. Okamoto<sup>1,3</sup> (<sup>1</sup>*Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan;* <sup>2</sup>*Meiji Milk Products Co. Ltd., Japan;* <sup>3</sup>*Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, USA*)
33. **Pharmacological analysis of ciliary rotation and heart contraction during *Helisoma* development.** M. Yu. Khabarova, E. E. Voronezhskaya (*Institute of Developmental Biology, RAS, Moscow, Russia*)
34. **Morphology of neuropeptide CNP2 modulation of heart activity in terrestrial snail.** N. Aseyev, I. S. Zakharov, P. M. Balaban (*Institute of Higher Nervous Activity and Neurophysiology of RAS, Moscow*)
35. **Pharmacological characterization of neurotoxic effects of cyanobacterial extracts in molluscan (CNS, heart) models.** Á. Vehovszky, A. W. Kovács, H. Szabó, J. Győri, A. Farkas (*Balaton Limnological Research Institute, Tihany, Hungary*)

# **ABSTRACTS**

# OCTOPAMINERGIC CONTROL OF TASK DEPENDENT AGGRESSION IN THE ANT, *FORMICA JAPONICA*

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Aggressive behavior is one of the common behaviors observed in all animals. In insects, offensive and defensive aggressive behaviors are observed as well as in vertebrates. In ants, offensive and defensive aggressions towards hetero-colonial intraspecific animals have been well studied. However, the neuronal mechanisms underlying the motivation of aggressive behavior are still lacking. Here, the role of biogenic amine octopamine (OA) in the brain was examined in the ants, *Formica japonica*. This ant is one of the most common species in Japan. The colonies of this species are largely polygynous and contain thousands of workers and brood. *Formica japonica* is a polyphagous and the foragers sometime hunt other small insects and take them into the nest as foods.

The worker ants in a colony have different tasks, for example, nursing, nest maintenance, guard and foraging. In this study, predatory aggression towards a cricket as a prey was examined in the nest-builder, forager and guard. Behavior experiments demonstrate that interspecific aggressiveness was significantly higher in foragers and guards than nest-builders. Foragers and guards show predatory aggressive behavior towards crickets but nest-builders showed escape behavior. Interestingly, this interspecific aggression was found to be maintained by intraspecific interactions. Social isolation decreased aggressiveness in foragers and guard, but increased in nest-builders. In order to investigate neuronal mechanisms underlying regulation of controlling motivation of predatory aggression, profile of biogenic amines in the brain in workers was examined using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). Octopamine (OA) level in the brain was found to be significantly higher in foragers and in guards than in nest builders. Social isolation decreased OA level in the brain of foragers and guards but increased in the brain of nest-builders. Pharmacological experiments were then performed to examine if OA increases aggressiveness of the workers. These experiments strongly suggested that OA in the brain regulates task-dependent aggressive motivation in the ants.

## **MORPHOLOGY OF NEUROPEPTIDE CNP2 MODULATION OF HEART ACTIVITY IN TERRESTRIAL SNAIL**

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A family of neuropeptides called Command Neuron Peptides (CNPs) was described ten years ago as the protein products of the gene HCS2, specifically expressed in the identified interneurons of the nervous system of terrestrial snail (*Helix lucorum* L. and *H. pomatia* L.). Recently, the CNP-like peptides have been detected by immunochemistry and immunoblotting in nervous systems of representatives of different invertebrate phyla (Mollusca, Annelida and Insecta). Still, the function of these peptides remains largely unknown. In *Helix* it is shown that CNPs: modulate the electrical activity of unidentified central neurons, modulate the pneumostome motoneurons, stimulate neural cones growth in neural cultures. Here, we describe for the first time the CNPs-immunoreactive neural fibers in walls of both auricle and ventricle of the snail heart. We show that application of the synthetic neuropeptide CNP2 (DYPRLamide) in perfusion saline affects heart rate and magnitude of beats in isolated snail heart. The results suggest that in *Helix* the command neuron peptides could participate in neural modulation of cardiovascular system.

## REGULATION OF SYNAPTIC PLASTICITY IN SIMPLE NERVOUS SYSTEMS

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Cellular mechanisms of learning and memory have been highly conserved during evolution. In many models it was shown that induction of synaptic plasticity takes place in postsynapse, short and intermediate changes involve presynaptic terminals, and long-term changes involve pre- and postsynapse. Persistent phosphorylation by the atypical protein kinase C isoform PKM $\zeta$  was shown to be required for maintaining long-term sensitization in *Aplysia* (Cai et al., 2011). This molecule controls trafficking of AMPA receptors in postsynapse and it is suggested that long-term memory in *Aplysia* is maintained via a positive-feedback loop involving PKM Apl III-dependent protein phosphorylation.

Using a simple experimental model consisting of 3 identifiable interconnected neurons we investigated mechanisms of associative plasticity in *Helix*. Monosynaptic connection between the mechanosensory glutamatergic cell and the withdrawal behavior interneuron was shown to be modulated by a single "reinforcing" serotonergic neuron in a pairing-specific way.

We investigated involvement of cannabinoids in regulation of this identified synaptic contact. Obtained results demonstrate that (i) cannabinoid receptors homologous to mammalian CB1 receptors exist in the nervous system of terrestrial snail, and that density of receptors is different in different parts of the nervous system. (ii) Application of synthetic cannabinoid anandamide selectively changed effectivity of some synaptic contacts while no changes were observed in other. (iii) Application of selective antagonist of CB1 receptors AM251 was effective only in situations with preliminary intracellular activation of a postsynaptic neuron suggesting involvement of endocannabinoids in plasticity phenomena induced by postsynaptic activation.

Nitric oxide (NO) is one of the most common molecules involved in regulation of many brain processes. One of the ways of NO influence on neuronal activity and synaptic plasticity is S-nitrosylation, the covalent attachment of NO group to the thiol side chain of cysteine, changes function of existing proteins, inhibiting their normal role in physiological functions, including memory. Influence of NO via guanylyl cyclase (GC) activates intracellular signaling cascades and triggers increased synthesis of proteins.

In our experiments in terrestrial snail *Helix* we tested the idea that NO is involved in erasure of memory during relearning. Three groups of snails were trained to remember the context in which they were shocked. Group #3 was each day injected with NO-synthase inhibitor N-omega-nitro-L-arginin (L-NNA), and showed no learning. Groups #1 and 2 were sham-injected and showed excellent memory for the context in which they were shocked. Then Group # 2 was injected (before each session) with L-NNA, and both groups (#1 and 2) were learned for three days to percept the context in which they were shocked as a positive one by feeding them only in this context. All groups demonstrated normal feeding in previously noxious context. Testing of context memory revealed that the control group (#1) relearned perfectly well, while the group #2 (L-NNA injected) still demonstrated selective aversive context memory. Results suggest that inhibition of NO synthesis during relearning may inhibit erasure of old memory and prevent new memory formation.

In the next series of experiments we investigated influence of the protein synthesis blocker anisomycin (AN) on contextual memory in the snail. Prior to the training session, the behavioral responses in two contexts were similar. One day after a session of electric shocks in one context only, the context conditioning was observed as the significant difference of behavioral response amplitudes in two contexts. On the day following testing of context learning, a session of "reminding" was performed, before which the snails were injected with L-NNA, L-NNA+AN or AN only. Next day testing of long-term context memory has shown that AN and L-NNA injections impaired the context conditioning, while under injection of L-NNA+anisomycin no impairment of the long-term context memory was observed. It suggests that without NO the memory is not erased.

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**BIOCHEMICAL AND PHYSIOLOGICAL-PHARMACOLOGICAL  
CHARACTERIZATION OF THE 5-HT<sub>6</sub> RECEPTOR REGULATION OF THE PERIPHERAL  
FEEDING SYSTEM (BUCCAL MASS) OF *LYMNAEA***

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The 5-HT<sub>6</sub> control of the buccal musculature which is responsible for the execution of feeding (radula protraction, rasping and retraction) was investigated from the embryonic appearance of this behavior throughout the maturation until adulthood in the pond snail, *Lymnaea stagnalis*, by biochemical and physiological-pharmacological assays. HPLC assay showed a gradual increase of the 5-HT level in the buccal mass during development. A single component high affinity 5-HT uptake system, coupled with a Na<sup>+</sup>-Ca<sup>+</sup> dependent release was demonstrated; the latter could be evoked by high (100 mM) K<sup>+</sup> concentration and blocked in Ca<sup>2+</sup> free medium. The uptake system was also characterized by a gradual increase from late embryonic development to adulthood. The kinetic analysis of membrane preparations revealed a single high affinity 5-HT receptor binding site that was positively coupled to cAMP. Pharmacological manipulation of the adenylyl cyclase activity also indicated the presence of a 5-HT<sub>6</sub>-like receptor type in the buccal mass. *In vivo* physiological-pharmacological assays confirmed, on the one hand, the role of a 5-HT<sub>6</sub>-like receptor type in the muscle activity, and on the other, they proved the involvement of two additional, the inhibitory 5-HT<sub>1</sub>- and the stimulatory 5-HT<sub>7</sub>-like receptors in the regulation of radula protrusion. Enhancement of the 5-HT level by adding 5-HTP in higher (100 μM) concentration evoked an increased frequency of rasping, meanwhile pCPA treatment, that is blocking 5-HT synthesis, decreased it. However, when pCPA treatment was combined with 5-HTP administration the radula activity increased again, showing that the effect of pCPA can be rescued by inducing 5-HT synthesis. Our results suggest a complex role of 5-HT in the peripheral regulation of feeding behavior.

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# THE 5-HT IMMUNOREACTIVE INNERVATION OF THE *HELIX* PROCEREBRUM

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The procerebrum (PC), which is the center of olfactory learning and memory formation in terrestrial pulmonate snails, receives extensive projections from both the olfactory nerve and other parts of the CNS. Although the cellular organization of the PC has been known for a while, there are a number of details to be revealed such as the cellular interactions involved in the integration of odor information and olfaction based behaviors. A high number of signal molecules have been demonstrated to participate in olfactory processes of terrestrial pulmonate snails, including a widely distributed biogenic amine, serotonin (5-HT). In the PC, 5-HT is a key factor for generation of synchronous oscillatory activity, up-regulation of oscillation frequency, and to influence odor learning capability. Although 5-HT was visualized by fluorescence and peroxidase immunohistochemistry in the PC, yet there is little information on the precise distribution and intercellular contacts of 5-HT-containing elements innervating the olfactory lobe. Therefore, we have studied the characteristics of the 5-HT-immunoreactive (5-HT-IR) innervation at distinct anatomical levels of the PC of *Helix pomatia*, applying correlative light- and electron microscopic immunocytochemistry. It was demonstrated that the PC received a dense innervation of 5-HT-IR fibers of extrinsic origin, entering from other regions of the cerebral ganglion and displaying different organization patterns in the different anatomical subdivisions of the PC. Accordingly, a perisomatic basket-like innervation was characteristic for the cell body layer, meanwhile varicose fiber systems of different density occurred in the terminal and internal neuropil. By camera lucida tracing, further details of the 5-HT-IR innervation were resolved. Corresponding to previous studies, we failed to find immunoreactive somata in the PC. At ultrastructural level, 5-HT-IR varicosities were found to contact procerebral (globuli) cell bodies, contacting sometimes more than one cell, and they also formed contacts with different axon profiles in the local neuropils of the cell body layer as well as in the main, terminal and internal neuropil areas. Most of the labeled structures established close, but unspecialized membrane contacts with different postsynaptic profiles. Our findings are the first anatomical evidence for the involvement of 5-HT in intercellular contacts in the PC, underlying its modulatory role in cellular activities at different levels of olfactory processing and learning. The overall dense distribution of 5-HT-IR innervation suggests a role for 5-HT in the coordination of local field potential oscillation events.

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## POTASSIUM CHANNELS IN THE *HELIX* CENTRAL NERVOUS SYSTEM: PRELIMINARY IMMUNOHISTOCHEMICAL STUDIES

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Voltage gated ion channels, including potassium (K) channels play an important role in neuronal communication. Among voltage gated ion channels K-channels are exceptionally significant because they possess widely different molecular structures, and so play a key role in many elementary neural functions, for example generation of action potentials and setting the resting membrane potential. Meanwhile there is an increasing amount of information on channel distribution patterns in vertebrates, especially in mammals, little information exist on this matter in gastropods. Therefore we have started a study to visualize and identify K-channels in the *Helix* CNS, in order also to possibly match their distribution with known and/or novel functional characteristics of the nerve cells/neural networks. As a first attempt fluorescence immunohistochemistry and Western blot analysis were applied to map the distribution of four different K-channels:  $K_v1.2$ , generating sustained, and  $K_v1.4$ ,  $K_v4.2$ ,  $K_v4.3$ , generating transient currents. Clustered cells displaying immunoreactivity for the subunits were observed in the CNS, however, in strongly variable number and intensity. So far, the most remarkable and unequivocal labeling was obtained by the antibody raised against  $K_v4.3$  subunit. The buccal ganglion, the meta(post)cerebral and procerebral lobes and the pedal ganglia contained the most labeled neurons in the CNS, whereas in the SOG complex the pleural and parietal but not the visceral ganglion displayed labeled cells. Yet no previously functionally identified neuron could be found immunolabeled. All neuropils were innervated by  $K_v4.3$  immunopositive fibers, and a network of brightly stained varicose fibers was seen in the thick connective tissue sheath. Western blot analysis for  $K_v4.3$  channel performed on total CNS, or separately on desheathed CNS, procerebrum and connective tissue sheath revealed a labeled band indicative of a 73 Kd weight protein. The pattern of distribution of  $K_v4.3$  subunit suggests both diverse and specific roles in cell signaling pathway(s) in the *Helix* CNS. Our preliminary results provide a steady basis for ultrastructural experiments aiming at the resolution of the membrane compartmentalization of  $K_v4.3$ , as well as the characterization of the entire K-channel subunit family.

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## NEUROPEPTIDES IN THE ANTENNAL LOBES OF *TRIBOLIUM CASTANEUM*

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From an evolutionary perspective, olfaction represents the most ancient of the senses and it is one of the most important ways of interaction with the environment for the majority of metazoan animals. In insects, the antennal lobes (ALs) act as primary olfactory centers that receive direct input from olfactory receptor neurons. While these paired deutocerebral neuropils are also intimately linked to higher integrative centers such as the mushroom bodies, early processing and modification of olfactory signals already occurs at the level of the olfactory glomeruli that reside within the ALs.

Beyond classical neurotransmitters like acetylcholine and  $\gamma$ -aminobutyric acid, studies in different insect species have revealed AL neurons to express a variety of different neuropeptides. Representing the largest and most diverse group of neuroactive substances, neuropeptides are often considered to act as cotransmitters that are released in concert with a principal transmitter, thereby modulating the activity pattern of a neuronal circuit.

To illuminate the potential role of neuropeptides in the AL, we investigate the neuropeptidergic repertoire of these neuropils in the emerging model organism *T. castaneum*. To assess the full range of the AL neuropeptidome, isolated AL tissue samples are subjected to MALDI-TOF mass spectrometry by direct peptide profiling. Immunohistochemical stainings are used to confirm the presence of neuropeptide transmitters and to localize sites of expression in the ALs of *T. castaneum*.

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# COORDINATION OF FEEDING MOTOR RHYTHMS IN SEMI-INTACT PREPARATION OF *LYMNAEA STAGNALIS*

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The feeding behavior of *Lymnaea stagnalis* is a popular model system for studying the mechanisms of motor program generation. Located in the buccal ganglia, the central pattern generator (CPG) is known to produce the three-phase rhythm, responsible for radula movements and food swallowing (Benjamin and Rose, 1979). Besides this well-characterised standard rhythm, a number of other fictive rhythms can be observed in this system in preparations of isolated CNS. The recent investigation has also revealed several possible types of coordination between the buccal CPG and the B2 neurons, controlling gut contractions (Dyakonova and Dyakonova, 2010). It remains however unclear whether this variety of fictive rhythms in the buccal system corresponds to real behavioral repertoire.

Here, we studied the radula movements and gut contractions in semi-intact preparations of *L. stagnalis* using video registration and an event recorder. Snails were anaesthetised with an injection of 0.1 mM MgCl<sub>2</sub> and pinned down to Sylgard in a chamber. The cut was made at the dorsal side, the skin was pinned down to reveal the CNS, the buccal mass and the gut. The motor activity of the buccal mass and gut contractions normally occurred within a few minutes after MgCl<sub>2</sub> replacement with Ringer. A one-character code was assigned to each of the different components of feeding behavior, and the respective key on a computer keyboard was pressed to indicate each start and end of a behavioral sequence. To study the possible influence of the distal gut area on the buccal rhythm, the distal part of the intact esophagus was placed in a small plastic container of 2 mm in diameter. Then, the container was hermetically sealed by mixture of vaseline, parafilm and wax. The selected area of esophagus was stimulated either by adding 0.1M KCl in the container or by a touch with a glass rod.

Analysis of the buccal mass activity in 16 animals has revealed various modifications of the standard three phase rhythm. The foregut contractions could occur in the absence of the buccal rhythm. During the buccal rhythm, they were coordinated with the feeding cycles. In ten preparations demonstrating 3-5 feeding cycles per minute, strong contraction of the foregut occurred after the third phase (swallowing). In two preparations demonstrating fast feeding (12 cycles per minute), foregut contracted weakly only during the second phase of the buccal rhythm (radula retraction). The transection of dorsal buccal nerves connecting the buccal ganglia with salivary glands and gut resulted in the loss of coordination between the buccal mass movements and gut contractions.

When the buccal mass was at rest, stimulation of the distal esophagus caused rhythmic movements of the buccal mass. Physical stimulation of the distal esophagus initiated one or two cycles of the standard three-phase rhythm of the buccal mass. Stimulation by 0.1 M KCl under similar conditions caused a series of about 10 cycles of standard rhythmic movements. Earlier the role of signals from the rostral part of the gut in termination of the feeding rhythm has been demonstrated (Elliott and Benjamin, 1989).

We propose this experimental model as suitable for studying central-peripheral interactions and coordination of motor rhythms within the feeding system of *Lymnaea*. Our data suggest that the motor repertoire of the feeding system is wider than it was thought earlier. It is likely that the buccal system of *Lymnaea* is a multifunctional neuronal ensemble, which rhythmic activity depends upon various factors including signals from rostral and distal parts of the gut.

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# GENOME WIDE ANALYSIS OF NEUROTRANSMITTER SIGNALING IN THE CTENOPHORE, *PLEUROBRACHIA BACHEI*

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Ctenophores, like poriferans, cnidarians and placozoans, are among the earliest branching lineages at the very base of the animal tree. These are the animals that might preserve the earliest designs of neuro-muscular organization. Ctenophores are even more basal than cnidarians (e.g. polyps or jellyfishes). The unique characteristics of the ctenophore body plan such as their biradial symmetry, definitive muscle cells, characteristic design of nerve nets and unique neural cords make them useful for understanding animal evolution and development.

To explore the molecular bases underlying the origin of neuronal organization, the Moroz lab, in collaboration with Dr. E. Rogaev's group (UMass), have now sequenced the genome of the ctenophore, *Pleurobrachia bachei*. This work was followed by extensive analysis of the transcriptomes from all major tissues in ctenophores. To our surprise, many components of 'classical' neurotransmitter machinery were not detected in *Pleurobrachia*. However there is initial evidence for the presence of peptide-type signaling, suggesting that peptides (rather than classical neurotransmitters) might be the earliest inter-neuronal messengers and integrators of behaviors. Here, we identified more than 50 novel peptide prohormones likely representing the largest complement of intercellular neuronal signaling in Ctenophores.

Glutamate and ATP might be two likely low molecular weight transmitter candidates in Ctenophores. Indeed, multiple receptors for these molecules are present in both genomic and transcriptome datasets. To validate the hypothesis for the presence of glutamergic and purinergic neurotransmission, we first cloned and localized more than 20 ligand-gated ion channels with remarkable cell specific expression. Interestingly, many other bilaterian-specific components controlling neuronal type specification (e.g. Hox genes) either are not found in the *Pleurobrachia* genome or, if present, show non-neuronal expression further suggesting that ctenophores have one of the most unique nervous systems among animals. Possibly Ctenophores preserved one of the earliest designs of nervous systems.

Combined, the apparent absence/underrepresentation of classical neurotransmitter and other neurogenic systems suggest that neurons might have evolved independently in different animal lineages (see also Moroz, 2009). Thus, further studies of ctenophores are absolutely critical to understand the origins and fundamental principles underlying the organization of neural circuits and behavior.

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## THE DIFFERENT STRATEGY OF MUSCLE AND NEURONAL SYSTEMS DEVELOPMENT IN LARVAE OF CLOSELY RELATED BIVALVE SPECIES

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In a variety of trochophore animals two sets of neurons are the earliest to differentiate: apical cells and pioneer neurons (Croll, 2009; Voronezhskaya and Ivashkin, 2010). The apical cells have been considered to be homologous according to their origin, location within the larval body and transmitter content, while the pioneer cells express high diversity of all mentioned features. Is this diversity reflects taxonomic relations or differences in larval life style? We investigated in detail neuro- and muscle development of two bivalve species: the mussel *Mytilus trossulus* and the oyster *Crassostrea gigas*. Larvae of both species pass through the trochophore, the veliger and the pediveliger stages which have similar external morphology and occupy the same ecological niche. Staining with phalloidin, specific antibodies (AB) against the thick muscle proteins as well as AB against peptide FMRFa (FMRFa) and serotonin (5-HT) has been combined with LCSM and 3D reconstruction. In both species neurogenesis starts at the apical extreme of trochophore with the appearance of 5-HT-ergic and FMRFa-ergic cells. In *Mytilus* this larval neuronal structure (apical organ) is surrounded by distinct muscle ring which is not detected in *Crassostrea*. During the veliger stage additional FMRFa-ergic cells (pioneer neurons) appear. In *Mytilus* they are located in the pretrochal ventral region, while in *Crassostrea* they are occurred postrochal dorsally and within the developing foot. The processes of pioneer cells run underneath the apical organ then turn and terminate within the foot. Retractor and adductor muscles are developed in both species. By the pediveliger stage cerebral, pedal and visceral ganglia appear along the pathway established by pioneer fibers at the ventral side of the larvae. Velum and foot retractors as well as anterior adductor have similar pattern in both species.

Thus, while pioneer neurons and pioneer myocytes differ in number and location between the species, they form similar final pattern of muscle and neuronal structures in the course of development. Our finding suggests that the morphology of early muscle and nervous elements more likely reflects their morphogenic and physiological function than phylogenetic relationships or larval life style.

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# THE SYNAPTOLOGY AND 5-HT IMMUNOREACTIVE INNERVATION OF THE PROCEREBRUM CELL BODY LAYER SUGGEST A ROLE OF LOCAL CIRCUITS IN OLFACTORY INTEGRATION

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The synaptology and 5-HT-immunoreactive innervation of the cell body layer of the olfactory center, procerebrum (PC) was investigated in two prominent terrestrial pulmonate gastropod species, *Helix pomatia* and *Limax valentianus*, with special attention to the local neuropils in the cell body layer. These local neuropils were of different size, consisted of a variable number of profiles, up to the fine arborization of varicose fibers which formed both synaptic and non-synaptic connections in both species. PC (globuli) cell perikarya were richly innervated by varicosities meanwhile these axon profiles also established contacts with each other. Different forms of synaptic configurations occurred. The unspecialized but close axo-somatic and axo-axonic membrane contacts regularly displayed membrane configurations of exocytosis, which refers to an extensive modulatory influence of the procerebral cell body layer activity. In *Helix*, the cell mass layer was shown to receive a rich 5-HT-immunoreactive innervation, forming a dense network around and among the cell bodies. At ultrastructural level, 5-HT-immunoreactive varicosities contacted both cell bodies and different unlabeled axon profiles. In contrast, only a weakly stained 5-HT-immunoreactive network in the main neuropil regions could be demonstrated in the *Limax* PC, whereas marked fibers were only rarely seen in the cell body layer. Local application of 5-HT evoked increased bursting activity of PC cells of *Limax*. Our results suggest that i) local circuits are anatomically distinguished functional units which play a decisive role in the regulation of integrative processes, including oscillation, in the PC, and ii) 5-HT is a major candidate of extrinsic modulatory substances, capable of maintaining under permanent influence the entire cell body layer.

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**BIOGENIC AMINES AND FMRFAMIDE IN DEVELOPMENT OF ARCHIANNELID  
*DINOPHIUS GYROCILIATUS*.**

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Archiannelid group Dinophilidae includes small interstitial marine worms whose morphological archetype and phylogenetic position are still under discussion. To shed light on this question, we transferred a widespread species *Dinophilus gyrociliatus* to the laboratory culture and studied its morphology using fluorescence histo- and immunochemical technique and laser scanning confocal microscopy.

We used monoclonal antibodies against acetylated alpha-tubulin to stain neurotubules and cilia, polyclonal antibodies against neurotransmitter serotonin (5-HT) and RF-peptide family (FMRFa), glyoxilic reaction to stain catecholamine elements (CA) and phalloidin to mark muscles in adult and juvenile worms. In consistence with earlier studies on other archiannelid species, segmented pattern was attributed to the surface cilia (circular ciliary bands) and protonephridia. No signs of segmentation were found in the distribution of nervous and muscular elements. Positively stained cell bodies was visualized along the ventral nerve cords for 5-HT, around cerebral ganglia for FMRFa and in the periphery for CA.

Thus, the inner morphology of adult *D. gyrociliatus* differs from the Annelid archetype in many respects. Modern high-sensitive and specific method added detailed information about archiannelid morphology. However further investigation of early developmental stages is necessary to clarify the phylogenetic position of archiannelid among Lophotrochozoa and possible evolutionary events within this group.

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## MICROGLIA RECRUITMENT DURING THE CNS REPAIR IN THE MEDICINAL LEECH, *HIRUDO MEDICINALIS*.

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The leech, *Hirudo medicinalis*, has the capacity to regenerate its nervous system following injury. After damage, the microglial cells migrate and accumulate at the lesion site, and this phenomenon is essential for the CNS repair. The first experiments carried out in our laboratory showed that the leech CNS-conditioned medium (leech CM), medium in which crushed nerve cords dissected out were incubated, has chemoattractant factors, *HmIL-16* and *HmC1q*, two molecules which are homologous to mammalian IL-16 and C1q.

The *HmIL-16* characterization showed that *HmIL-16* presents different forms having different molecular weight. Its C-terminal end possesses significant homology to the human IL-16 active form, a 12kDa cytokine. The human form is initially produced as large precursor proteins which are cleaved by caspase 3 to produce a N-terminal prodomain and a C-terminal active form (IL-16). The active IL-16 can recruit CD4<sup>+</sup> T cells and macrophages via CD4 receptor and its functional activity is optimal when the cytokine is homo-tetramerized. In order to correlate the different aggregation states with the activity, respective forms of *HmIL-16* were isolated by RP-HPLC from leech CM, characterized by mass spectrometry and, finally tested on microglial cells using chemotaxis assay. Moreover, the results of chemotaxis assays performed with (i) *HmIL-16* on human CD4<sup>+</sup>T cells and (ii) with the human IL-16 on leech microglial cells suggest the conservation of functional IL-16/CD4 interaction in leech. To confirm these hypotheses, a characterization strategy of *HmIL-16* receptor was defined from leech microglia. Because the receptor for IL-16 is still unknown on human nerve cells, such a study is also primed from human microglia.

In a second part, *HmC1q*, a chemotactic factor homologous to human C1q/TNF family, was identified in the leech CNS. In mammals, the collagen tail of C1q appeared to mediate chemotaxis on human eosinophils and neutrophils by the recognition of the gC1qR (also called C1qBP for C1q Binding Protein). To investigate the functional properties of *HmC1q* and characterize its specific receptor on leech microglial cells, the recombinant form (r*HmC1q*) was produced. The leech microglia migration was driven by r*HmC1q* in a dose dependent manner and the incubation of cells with anti-human gC1qR antibodies prior to chemotactic assays highly reduced *HmC1q*-mediated leech microglia recruitment. It suggests that *HmC1q* could recruit a leech microglia subset via a leech gC1qR. Strategies of purification are carried out to definitely demonstrate the natural interaction between *HmC1q* and *HmC1qBP*.

Taken together, these results contribute to a better understanding of microglia activation leading to the CNS repair in leech.

# NEUROANATOMICAL BACKGROUND OF SPACE POSITIONING OF OLFACTORY ORGAN IN THE SNAIL, *HELIX POMATIA*

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The posterior tentacles of the terrestrial stylommatophoran snails possess olfactory organs on their tips. The space position of the olfactory organ changes during olfactory orientation related to different behaviors. For the space positioning the tentacles require a complex motor program to execute the observed movements (lateral and downward bending, twitching, quivering and graded withdrawal). Since flexor muscles have not yet been identified which could execute bending of tentacles therefore it is difficult to explain how the tentacles realize these complicated movements.

In this study we describe the gross anatomy of three distinct novel muscles with flexor function as well as their innervation pattern by cerebral neurons. Applying retrograde NB tracing via the olfactory nerve (ON) and the peritentacular nerves (PTN) which innervate the tentacles, we identified the cerebral neurons that supplying these muscles, meanwhile applying anterograde neurobiotin (NB) tracing via these nerves we revealed the innervation pattern of these neurons in the flexor muscles as well as the tegumental musculature. Furthermore, by the combination of retrograde tracing via these nerves and the 5-HT immunocytochemistry we identified the serotonergic neurons that send axons to the flexor and the tegumental muscles.

The gross anatomical survey revealed three fine string-like muscles originating from the base of the olfactory sensory pad. Two of them (thin muscles) originated from the digits of the tentacular ganglion whereas the third (thick) one originated from the tip of the withdrawal musculature. The thick muscle was anchored to the base of the tentacle at the external side. One of the thin muscles was anchored near to the thick one whereas the other one to the internal side of the tentacle. Anterograde NB tracing via the PTNs showed that they densely innervated the external and internal thin flexor muscles as well as the tegumental musculature from the base to the tip of the tentacles. Contrary, NB tracing via the ON revealed that it densely innervated all the three flexor muscles as well as the tegumental musculature from the tip to the base of the tentacle. Retrograde NB tracing demonstrated that neurons sending axonal processes via the ON and PTNs were located in a large group in the ventral metacerebrum. The combination of retrograde NB tracing and 5-HT immunocytochemistry visualized the 5HT-IR neurons innervating the flexor and tegumental muscles.

The present results show that the three novel flexor muscles co-operating with the tegumental and the withdrawal musculature enable the tentacles to execute bending or lateral movements orienting the olfactory organ toward any point of the space.

## DO THE GLUTAMATERGIC NEURONS OF THE *LYMNAEA* CNS POSSESS NMDA RECEPTORS?

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Glutamate has been shown to be present as an excitatory transmitter in the nervous system of snails. Glutamate may exert its effects via different glutamate receptors including NMDA type receptors that have been shown to function in a variety of different molluscan neurons. In *Lymnaea*, the presence of type 1 NMDA receptor (R1) has been shown by *in situ* hybridization, which however outlines reliably only the cell bodies of neurons but not their neurites. In this study we applied commercial antibodies raised against different NMDA type receptors (R1, R2A, R2B) to give a detailed description of the distribution of NMDA containing neurons in the CNS of *Lymnaea*. Furthermore, using antibodies against vesicular glutamate transporters (VGT), which are specific markers of glutamatergic neurons, we applied double immunostaining on NMDA receptor labeled cryostat sections to establish possible connections between NMDA R1 and VGT or glutamate immunolabeled neurons in the CNS of *Lymnaea*.

The NMDA immunostaining showed that NMDA R1 immunoreactivity was present in both small and large neurons in each ganglion of the CNS. NMDA R2A immunoreactivity was seen predominantly in two large clusters of the cerebral ganglia. The location of NMDA R2B immunostained neurons nearly overlaps the location of NMDA R1 immunostained neurons. VGT immunocytochemistry demonstrated numerous immunostained neurons with similar distribution to that of NMDA labeled neurons. The double immunostaining experiments showed that NMDA immunostained neurons do not receive VGT immunolabeled fibers but each NMDA R1 immunostained neuron contains VGT immunoreactivity, a specific marker of the glutamatergic neurons.

These unexpected observations demonstrate that only glutamatergic neurons contain NMDA type receptors, which suggests that glutamatergic neurons have an excitatory autaptic feedback route via their own NMDA type glutamate receptors. This positive feedback mechanism may play a role in the increase of glutamatergic tone in the CNS of *Lymnaea* or some other self-regulatory process. It is also possible however that each glutamatergic neuron synapses only onto non-glutamatergic neurons that only contain non-NMDA type glutamate receptors (e.g., GluA1 type AMPA receptors). Only a combination of electrophysiological and neuropharmacological methods targeting the glutamatergic and NMDA receptor containing neurons detected immunohistochemically in the present work can elucidate the functional connections of these cells.

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## EVIDENCES FOR A POSSIBLE CROSS-TALK BETWEEN SEROTONERGIC AND DOPAMINERGIC NEURONS IN THE *LYMNAEA* CNS

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It has been shown earlier that during the external stimuli induced increased behavioral arousal serotonergic (5-HTergic) neurons are activated both in the central and peripheral nervous system, and liberate 5-HT. In *Lymnaea*, the cerebral 5-HTergic feeding modulator neuron the CGC innervates and excites the neuronal elements of the feeding central pattern generator (CPG), hence may induce feeding. It was also demonstrated that both external DA and the activation of intrinsic dopaminergic (DAergic) neurons of the feeding CPG are able to initiate feeding movements in snails. During feeding arousal intracellular level of both 5-HT and DA was found to be increased, whereas by satiation it significantly decreased, suggesting that the 5HTergic and DAergic systems have a cross-talk in the regulation of feeding behavior.

In the present study the possible neuroanatomical connection between the 5-HTergic and DAergic neurons was investigated in the CNS applying double immunolabeling with anti-5-HT, anti-tyrosine-hydroxylase (TH, putative DA indicator) as well as anti-5-HT<sub>3</sub> and anti-D1A receptor antibodies. In addition, the action potential generation of the CGC and the DAergic RPeD1 neurons were tested in electrophysiological experiments after extracellular application of relevant concentrations of DA and 5-HT.

In morphological preparations DAergic neurons were found to receive 5HTergic (5HT-ir) inputs whereas 5HTergic neurons were found to receive DAergic (TH-ir) inputs. Further double immunolabeling experiments revealed that 5-HT-ir neurons contain D1A receptor immunoreactivity, and vice-versa, TH-ir neurons contain 5-HT<sub>3</sub> receptor immunoreactivity, which suggests a mutual correspondence between 5-HTergic and DAergic neurons. Western blot analysis confirmed that both receptor antibodies used specifically labeled protein bands that molecular weights (D1A: nearly at 50 and 75 kDa; 5-HT<sub>3</sub> nearly at 60 kDa) well correspond to the mammalian homologues, respectively, which were the source of the antibody epitopes. Intracellular recordings showed that low concentrations of DA could slightly increase the spike generation and firing frequency of the CGC. Similarly, the RPeD1 was excited by low concentrations of 5-HT.

All these observations indicate a possible excitatory cross-talk between the 5-HTergic and DAergic system during the increase of feeding arousal in *Lymnaea*.

## NEUROPEPTIDES IN THE MUSHROOM BODIES OF *TRIBOLIUM CASTANEUM*

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Neuropeptides are a highly diverse group of signaling molecules that affect a broad range of biological processes in insects, including development, metabolism, behavior, and reproduction. In the central nervous system, neuropeptides are usually considered to act as neuromodulators and co-transmitters that modify the effect of ‘classical’ transmitters at the synapse, thus shaping activity and output patterns of neuronal circuits. While neuropeptides have been shown to play key roles in regulating endocrine events and coordinating behavioral actions, they are also thought to be involved in processes related to neuronal plasticity, the substrate for learning and memory.

Mushroom bodies (MBs) are prominent neuropils of the insect brain that are often regarded as principal sites of learning and memory. Probably of ancient evolutionary origin, these integrative brain centers are intimately connected with primary olfactory neuropils but may also receive information from other sensory modalities. In the red flour beetle *T. castaneum*, the MBs comprise a single knob-shaped calyx, a stalk-like peduncle, and a medial and vertical lobe, each consisting of distinct subdivisions. The whole structure is formed by dendritic and axonal processes of intrinsic MB neurons, the Kenyon cells.

Studies in a variety of insects have demonstrated the presence of different neuropeptides in either intrinsic or extrinsic MB neurons, suggesting this group of signaling molecules to play an important role in information processing in the MBs. To further elucidate these functions, we investigate the neuropeptide repertoire of the MBs in the coleopteran model organism *T. castaneum*. Neuropeptide distribution is examined in immunohistological preparations using confocal laser scanning microscopy. To assess the full range of the MB neuro-peptidome, isolated MB tissue is analyzed by means of MALDI-TOF mass spectrometry. The findings are compared to other insect species, including holometabolous and hemimetabolous taxa, as well as ancestral representatives.

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# CODING OF ESSENTIAL ‘ODOR OBJECTS’ IN THE ANTENNAL LOBE OF THE SPHINX MOTH *MANDUCA SEXTA*

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A principal goal of our work is to understand neurobiological mechanisms through which information about behaviorally significant olfactory stimuli is encoded, processed, and integrated with inputs of other modalities in the insect brain and specifically how olfactory information ultimately initiates and controls the characteristic, natural behavioral responses of the giant sphinx moth *Manduca sexta*.

Our studies have focused mainly on the antennal lobes (ALs), the primary olfactory centers in the moth's brain. As in most vertebrates and invertebrates, the ALs are characterized by glomeruli – condensed-neuropil structures in which primary-sensory and central neural elements interact synaptically. We seek to understand how primary-sensory inputs from olfactory receptor cells are processed in glomeruli and represented in their outputs.

We focus on olfaction-dependent behaviors that are crucial for the survival of the moths – mate-seeking and interactions with host plants for feeding and oviposition. In some of those studies, olfactory neurobiology has led us to recognize naturally occurring volatiles that function in the chemical mediation of such interactions.

Insights from the sex-pheromonal communication system led to recent analysis of olfaction-dependent interactions with host plants. A multidisciplinary approach combining chemical characterization of natural volatiles, behavioral experiments in a laboratory wind tunnel, and electrophysiology has enabled us to determine how mixtures of volatiles, at natural concentrations, control flight behavior and are encoded in the ALs. Mounting evidence points to coincident firing of output neurons of glomeruli as a mechanism for neural coding of the context or significance of an odor. Gas chromatography coupled with multi-channel CNS recording has enabled identification, in complex floral mixtures, of key odorants to which olfactory-lobe neurons are particularly responsive. Mixtures containing only a few of those floral odorants are as effective as the complete, natural floral blend in modulating flight.

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## NEUROPEPTIDES AND BEHAVIOURAL PLASTICITY IN *C. ELEGANS*

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We have been establishing behavioural paradigms in *C. elegans* in which we can interrogate the neural substrates of behavioural plasticity from the molecular genetic level, through to neural circuits and the intact behaving animal. In particular we have been interested to address the question as to what extent, and how, neuropeptides are involved in remodelling neural networks as an animal undergoes an adaptive behavioural response. Recently we have developed two models in which animals are exposed to an environmental challenge namely, acute food deprivation or conditioning in ethanol. We have monitored the behaviour of the animals over extended time-courses and provided a precise description of time-dependent changes in behaviour. Intriguingly, in both of these paradigms we find that animals that are deficient in neuropeptides (a proconvertase mutant *egl.-3*) are devoid of the adaptive behavioural responses. Currently we are analysing neuropeptide and receptor mutants in order to provide further insight into the neural mechanisms that underpin the adaptive behaviour.

# THE ANTI-INTUITIVE VISUAL SYSTEM OF THE HONEY BEE

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Because insects fly around, visit flowers and chase mates, we conclude intuitively that they see things as we do. When their vision turns out to be quite different, we say it is anti-intuitive. Some examples are described.

Detailed tests of trained bees have demonstrated small feature detectors for modulation of the blue receptors and separately of the green receptors, for edge orientation and for area of black or colour. They are small, about  $3^\circ$  across, independent, and not re-assembled to make lines, shapes or textures. Instead, the responses of each type of feature detector are summed quantitatively to form cues in each local region irrespective of the pattern. Trained bees remember the modulation, positions of the average edge orientation, of areas of black or colour, and positions of hubs of radial and circular shapes in each local region, but not the original feature detector responses, so all pattern detail is dissolved.

When they discriminate between vertical and horizontal gratings, the preferred cue is the modulation difference, not the orientation. When trained to a single bar versus a plain white target, bees learn its position, not the orientation. When they discriminate between bars at  $45^\circ$  and  $135^\circ$ , located at the same place on the targets, they learn only the edge orientation because all the other cues are the same on the two targets. When bees discriminate a ring, spokes, a square, or a square cross, they remember none of these shapes, but the modulation and the positions of the hubs are remembered.

When presented with a colour on a background, the preferred cue is not a measure of the photon flux at each wavelength, but is the sum of the modulation detected at edges by the green and by the blue receptors separately in local regions.

## EXPRESSION OF EARLY GENES IN THE CNS OF SNAIL *HELIX*

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Basic physiological mechanisms of CNS reaction to the changing environmental stimuli are to be common within vertebrates and invertebrates. One of the most important systems of regulation is the expression of early genes as an answer to the external stimuli. Nevertheless, the data on early genes expression in mollusks are poor and random. In our work we made an attempt to detect early genes *cfos* and pCREB by means of immunocytochemistry and mRNA hybridization. Amongst several tested commercial antibodies to the pCREB, the better results were obtained with the sc7978. We detected the specific staining restricted to the cell nuclei. Nevertheless, the pattern of staining was stable, and did not depend on the state of animal. This dependence is typical for the early genes detection in vertebrates. Moreover, curiously, only part of the sections was stained within the whole set on the slide, thus reflecting some methodical problem.

The search of the conservative region in the structure of *cfos*-encoding RNA's of different species resulted in the recognition of potentially meaningful sequence. The first attempt of preparing the mRNA probe for detection of the *cfos* in *Helix* CNS resulted in specific, stable from preparation to preparation pattern of staining. Unfortunately, the pattern of staining was the same as previously detected by us using of FMRFamide probe, thus reflecting that the probe produced "false" staining. Now we are preparing three different mRNA probes to *cfos*, varying slightly the used area of synthesis.

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## INSULIN AND MEMORY IN *LYMNAEA*

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The pond snail *Lymnaea stagnalis* is capable of learning conditioned taste aversion (CTA) and consolidating this CTA into long-term memory (LTM). Previous studies have shown the following three results to help us understand the cellular and molecular mechanism underlying the CTA-LTM in *Lymnaea*. (1) The molluscan insulin-related peptide II (MIP II) was up-regulated in snails exhibiting CTA-LTM. (2) MIP II was only observed in the cerebral ganglia, but the MIP receptor was distributed throughout the entire CNS including the buccal ganglia. (3) The application of exogenous mammalian insulin or the secretions from *Lymnaea* MIP II-containing cells evoked long-term potentiation (LTP) at the synapses between the cerebral giant cell (a key interneuron for CTA) and the B1 motoneuron (a buccal motoneuron). We thus hypothesized that MIP II and MIP receptors play an important role at the synapses underlying the LTM consolidation process. To further examine this hypothesis, we applied the antibody, which recognizes the binding site of mammalian insulin receptors, to the *Lymnaea* CNS. The application of the antibody for insulin receptors to the isolated CNS blocked LTP, and the injection of the antibody into the *Lymnaea* abdominal cavity inhibited the LTM consolidation, but not the CTA formation. Moreover, snails exhibited CTA-LTM by training after 1-day starvation, but they failed it after 5-day starvation. Because the hemolymph level of glucose was changed according to the starvation period, this result may be explained by the hemolymph level of MIP II in *Lymnaea*. All our results suggest that insulin in the CNS is involved in LTP and CTA-LTM in *Lymnaea*.

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## TRANSMEMBRANE 5-HT TRANSPORT DURING EARLY DEVELOPMENT OF MOLLUSCS

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Serotonin (5-HT) is expressed at very early developmental stages in a wide variety of animals (Buznikov, 1990). All 5-HT receptors known to date are expressed at the cell membrane and react to the extracellular 5-HT. In mammals (mouse), systems of 5-HT synthesis and specific transmembrane transport (SERT) are expressed and functionally active from the oocyte stage up to the blastocyst (Amireault and Dube, 2005a; b). However mammals are viviparous animals and their embryonic development take place within the mother reproductive system, which is the source extracellular 5-HT. How does the 5-HT system functioning in aquatic invertebrates which have external fertilization and subsequent development? For our recent investigation of transmembrane 5-HT transport during early development we used freshwater pulmonate snail *Lymnaea stagnalis*. Immunocytochemical technique combined with LCS microscopy and ELISA were used to analyze internal and external 5-HT level at various developmental stages from zygote to blastula.

Incubation in 5-HT itself did not change intracellular 5-HT content at zygote-morula stages. Note, that incubation at blastula stage resulted in dramatic increase in intracellular 5-HT level. To the contrary, at all stages investigated incubation in biochemical 5-HT precursors tryptophan and 5-hydroxytryptophan (5-HTP) resulted in significant increase of intracellular 5-HT level. ELISA revealed the presence of low amount of 5-HT in incubation medium (filtered water) which increased in two-three folds following pre-incubation in 5-HTP. Simultaneous incubation in 5-HTP and serotonin specific reuptake inhibitor citalopram resulted in decrease in released 5-HT.

Our data demonstrated that systems of both 5-HT synthesis and reuptake are physiologically active at the early cleavage and blastula stages in *Lymnaea* embryo. Contrary to the mammalian embryo, the direction of transmembrane transport in molluscan embryo is altered during development. At early cleavage stages no 5-HT uptake is detected, while active release of 5-HT is obvious. The situation inverted at blastula stage and the uptake became predominant feature.

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## DYNAMIC CELLULAR AND MOLECULAR MECHANISMS OF MEMORY: NEW INSIGHTS FROM MOLLUSCAN MODELS

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An important discovery of research into the neurobiology of learning and memory in the past 30 years or so was that memory has several distinct phases, from short-term through intermediate to long-term. These phases can be distinguished by both temporal and biochemical/molecular criteria. The standard view has been that short-term memory is only dependent on co-valent changes in protein structure whereas longer-lasting forms of memory require the synthesis of new proteins around the time of training. In addition, stable long-term memory depends on the transcription of new mRNA molecules, which in turn are translated into regulatory and structural proteins required for the synaptic plasticity underpinning memory. Although this type of textbook categorisation of memory phases is still valid, it is based exclusively on the different requirements for macromolecular synthesis for different phases of memory and ignores the very dynamic nature of cellular and molecular changes mediated by the activation and inactivation of a large number of signalling molecules involved in early and later phases of the consolidation as well as the reconsolidation of memory. Recent research on molluscan model systems, particularly *Aplysia* and *Lymnaea*, have made a key contribution to understanding the dynamic role of a number of evolutionarily conserved molecules in shaping both behavioural memory and its underlying cellular mechanisms. These molecules include cAMP and protein kinase A (PKA), mitogen activated protein kinase (MAPK), nitric oxide (NO), calcium-calmodulin dependent kinase II (CaMKII), NMDA and AMPA type glutamate receptors, the protein kinases C and M (PKC and PKM) as well as the peptides sensorin and the vertebrate-like pituitary adenylate cyclase activating polypeptide (PACAP). Different molecules or even the same molecule can play very different roles in the consolidation, maintenance and reconsolidation of intermediate versus long-term memory, or even in different phases (early or late) of the consolidation of the same type of memory. Here I will review results emerging from recent research in *Aplysia* and *Lymnaea* that cast a new light on the highly dynamic and complex nature of cellular and molecular changes underlying even relatively simple forms of behavioural plasticity, such as sensitisation and classical conditioning.

# A SYSTEMS LEVEL ANALYSIS OF DECISION MAKING: THE INTERACTION OF FEEDING AND WITHDRAWAL BEHAVIOURS

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We aim to understand how networks interact to produce alternative behaviours such as feeding or withdrawal. Pond snails (*Lymnaea stagnalis*) respond to food stimuli by producing a three-phase feeding motor program. Feeding movements consist of cyclic protraction, rasp and swallow phases, corresponding to the movements of the buccal mass, the feeding organ of the snail. Aversive chemical stimuli, such as quinine or lemon grass, inhibit feeding and activate the withdrawal system, whose main effector organ is the columellar muscle that withdraws the body into the shell. Withdrawal can also be elicited by strong tactile stimulation. The key neurons generating feeding and withdrawal are well characterised but how the two systems interact is unknown. Here we used a semi-intact preparation containing the lip chemo- and mechano-sensory areas, the buccal mass and the columellar muscle as well as the CNS to study the effects of contrasting appetitive and aversive stimulation. Two force transducers were used, one to measure the contractions of the buccal mass (feeding) and another to monitor the columellar muscle (withdrawal) simultaneously. First we established the relative intensities of aversive and appetitive stimuli leading to the generation of either feeding or withdrawal. Then we measured the intensity of chemical and tactile stimuli needed to switch from feeding to withdrawal. This enables us to investigate changes at the cellular level which mediate decision making. Backfilling of the lip nerves and columellar nerve highlighted the cells potentially participating in the interaction between the two opposing behaviors. Putative motoneurons and interneurons were then impaled with microelectrodes and stimulated by injected current while muscle contractions involved in feeding and withdrawal were recorded. The effects of sensory stimulation with aversive and appetitive stimuli were also investigated on the same neurons. Nerve stimulation was also applied to further map the connections between the feeding and withdrawal system. As a result of these experiments we have now identified areas of the brain and individual neurons that are crucial for the generation of feeding and withdrawal and are potential sites of decision making.

## PHARMACOLOGICAL ANALYSIS OF CILIARY ROTATION AND HEART CONTRACTION DURING *HELISOMA* DEVELOPMENT

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We demonstrated earlier that serotonin (5-HT) emitted from the apical sensory neurons modulate development of molluscan larvae (Voronezhskaya et al., 2004). The effect of apical cells activation was found to be bidirectional in freshwater pulmonate snail *Lymnaea* and *Helisoma*, and both developmental tempo and embryonic behavior programs (locomotion, buccal rhythm and heart contraction) were affected. While at larval stages before metamorphosis all listed above events were diminished, at postmetamorphic stages the same events were enhanced (Voronezhskaya et al., 2008).

At the present work we investigated ciliary rotation (early stages), gliding locomotion (late stages) and heart contraction of *Helisoma* embryo during normal development and under modulation with 5-HT agonists. Ciliary rotation gradually decreased from the trochophore to metamorphic stage. Gliding locomotion slightly gradually increased after metamorphosis. Heart contraction started at the beginning of metamorphosis, the frequency of the rhythm increased in two folds by the end of metamorphosis and then it gradually decreased up to initial level. Agonist of 5-HT<sub>4</sub> receptor RS 67333 2,5-1  $\mu$ M dramatically (in 2-3 folds) retarded both ciliary rotation and gliding locomotion and significantly decreased heart rhythm at premetamorphic stages. Note that 1  $\mu$ M RS 67333 has no effect on heart contraction after metamorphosis and 0,1  $\mu$ M RS 67333 even enhance rhythm frequency at all stages. Agonist of 5-HT<sub>1</sub> and 5-HT<sub>7</sub> receptors 8-OH-DPAT 5  $\mu$ M has no significant effect on both ciliary and gliding locomotion but slightly retard heart contraction at premetamorphic stages. Agonist of 5-HT<sub>1</sub>, 5-HT<sub>5</sub> and 5-HT<sub>7</sub> receptors 5-CT significantly (up to 2 folds) increased ciliary rotation but had no effect on gliding locomotion after metamorphosis.

Our pharmacological analysis demonstrated that several types of 5-HT receptors are involved in regulation of locomotion and heart contraction in *Helisoma* embryo. Affinity or expression of each particular receptor is not identical at different developmental stage. We suggested that such alterations can underlay the well-coordinated character of serotonin-dependent behavior throughout embryonic development of freshwater molluscs.

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## SEROTONIN INHIBITS DOPAMINE-INDUCED CONTRACTION OF THE SNAIL SALIVARY DUCT

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Dopamine (DA) is an important neurotransmitter and modulator substance both in vertebrate and invertebrate species. Along with serotonin (5-HT) DA is widely distributed throughout the nervous system and peripheral organs of mollusks. It has been found that the balance between DA and 5-HT concentrations modulates the central arousal in snails. DA has also been shown to play a fundamental role in the generation of the feeding motor program and resultant feeding behavior in several molluscan species. In terrestrial gastropods the saliva is forwarded by cilia in the small diameter ducts and by cilia and muscular peristalsis along the main ducts. Previously we have shown that peristaltic movements of the salivary duct (SD) muscles are regulated and modulated by an interaction of 5-HT and different neuropeptides co-released or co-localized with the 5-HT. The relaxing effect is mediated by 5-HT<sub>2</sub>-like receptors, whereas the contraction is mediated by 5-HT<sub>3</sub> and D<sub>1</sub>-like receptors. Recently, we have also observed that 5-HT effectively attenuated DA-elicited contractions. In the present study, using a battery of receptor ligands it is shown that 5-HT receptors play a prominent role in the control of DA-mediated functions in SD muscles. Tyrosine hydroxylase and DA immunoreactivity was observed in the nerves innervating the SD. Further on, applying immunocytochemistry, it has been found that both 5-HT and DA containing axons display overlapping innervation pattern in the muscle. In addition the simultaneous occurrence of D<sub>1</sub>- and 5-HT<sub>3</sub>-receptors could be demonstrated in the innervating axons. Our data aiming to reveal the presence of 5-HT and DA receptors on the muscle remained so far ambiguous. Based on our pharmacological data obtained on salivary duct muscle the cross-regulation of several specific pathways, including the cross-talk between different receptors and signaling pathways, is suggested. We shall discuss our results in the light of a possible 5-HT-DA receptor interaction.

# GABAERGIC EFFECTS ON THE SLOW OSCILLATORY NEURAL ACTIVITIES IN THE PROCEREBRUM OF *LIMAX VALENTIANUS*

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Network oscillations are generated by gamma-aminobutyric acid (GABA) neurons in the central nervous systems (CNSs) of various animals. In the terrestrial mollusk *Limax valentianus*, oscillatory activity encoding the olfactory information is recorded in the procerebrum (PC), the secondary olfactory center. However, GABAergic actions in the PC have not yet been clarified. Previous studies showed that there are GABA-like immunoreactive neurons in the *Limax* CNS, but there has been no detailed analysis of such neurons in the PC. In the present study, we examined the modulation mechanism of the frequency of ‘slow’ oscillation (0.5 – 1.0 Hz) in the PC by GABA and its analogues. First, application of GABA-receptor agonists to the isolated CNS increased the frequency of a periodic local field potential in the PC, whereas application of its metabotropic receptor antagonists decreased it. Second, the GABA-like immunoreactivities were found in the neuropil layers and the cell body layer of the PC. To our knowledge, our present findings are the first evidence showing that GABA modulates the frequency of ‘slow’ oscillation via the metabotropic receptors in the CNS.

## NITRIC OXIDE IS NECESSARY FOR BOTH ERASURE AND CONSOLIDATION OF MEMORY

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In our experiments in terrestrial snail *Helix lucorum* we tested the idea that NO is involved in erasure/suppression of memory during relearning. Three groups of snails were trained to remember the context in which they were shocked. Group 3 was each day injected with the NO-synthase inhibitor N-omega-nitro-L-arginin (L-NNA), and showed no learning. Groups 1 and 2 were sham-injected and showed excellent memory for the context in which they were shocked. Then Group 2 was injected with L-NNA, and all groups were relearned for three days to percept the context in which they were shocked as a positive one by feeding hungry snails only in this context. During relearning session all groups demonstrated normal feeding in previously noxious context. Testing of context memory revealed that the control group 1 relearned perfectly well, while the group 2 (L-NNA injected) still demonstrated selective aversive context memory. Results suggest that inhibition of NO synthesis during relearning may inhibit erasure/suppression of old memory and prevent new memory formation.

In the next series of experiments we investigated influence of the protein synthesis blocker anisomycin (AN) on contextual memory in the snail. Prior to the training session, the behavioral responses in two contexts were similar in all snails. One day after a 10-day session of electric shocks in one context only, the context conditioning was observed as the significant difference of behavioral response amplitudes in two contexts. On the day following testing of context learning, a session of “reminding” was performed, before which the snails were injected with AN, L-NNA+AN or L-NNA only. Testing of long-term context memory has shown that the reminder under AN injections impaired the context conditioning, while the reminder under combined injection of L-NNA+AN showed no impairment of the long-term context memory. It can be interpreted as participation on NO in erasure/suppression of the existing memory, and that NO is necessary for development of new memory.

## PHARMACOLOGICAL AND ANATOMICAL BACKGROUND OF TWITCHING AND QUIVERING OF THE SUPERIOR TENTACLES OF *HELIX*

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Bending, twitching and quivering are types of tentacle movements which can be observed during the snail's olfactory orientation. These movements cannot be explained by the tentacular withdrawal mechanism. We assume that three special flexor muscles (see Hernádi and Teyke in this program booklet), spanning the length of superior tentacles from the tip to the base, are responsible for the execution of these movements. To execute complex flexor movements these muscles have to perform both extreme relaxation during tentacle protraction and different rate of contraction during twitching and quivering. In this study we have investigated transmitter and modulator candidates responsible for the contraction or relaxation of the muscles. In addition the localization of the effective signal molecules and the ultrastructure of the muscles were studied, the latter with special attention to whether the organization of the contractile elements fulfill the criteria of the extreme relaxation and graded contraction requirements.

For the functional characterization of these muscles, quantitative isometric tension measurements were made by calculating the integral of the responses evoked by pharmacological agents. It was observed that contractions elicited by high (40 mM) K<sup>+</sup> were highly dependent on the extracellular Ca<sup>2+</sup> concentration. In pharmacological experiments acetylcholine (Ach, 10<sup>-5</sup>-10<sup>-4</sup> M) and dopamine (DA, 10<sup>-9</sup>-10<sup>-4</sup> M, KD<sub>50</sub>=10<sup>-6</sup> M) proved to be effective in eliciting contraction. Serotonin (5-HT), on the other hand, evoked contraction at low (10<sup>-7</sup>-10<sup>-5</sup> M) and relaxation at high (10<sup>-4</sup> M) concentrations. Immunocytochemical experiments revealed that D<sub>1A</sub> receptor was located exclusively in the axonal fibers suggesting that DA modulate the liberation of excitatory transmitter eliciting contraction of the muscle. Pharmacological assay with DA receptor antagonists, however, revealed that D<sub>2</sub>-like receptor subtypes might be present in the muscle. 5-HT and tyrosine hydroxylase immunoreactivity was observed in nerve fibers, supporting the transmitter and/or modulatory role of 5-HT and DA in these muscles. Ultrastructural study revealed that the muscles are composed of a small number of smooth muscle fibers that are located relatively far from each other never forming bundles. The muscle fibers contain densely arranged, parallel contractile elements, centrally located clusters of mitochondria. The neighboring muscle fibers are connected with each other by shorter or longer sarcoplasmic processes.

According to our observations these flexor muscles are regulated by the interaction of different signal molecules, modifying a complex contraction and relaxation pattern.

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## SOME ASPECTS OF IMMUNOLocalIZATION OF FMRFAMIDE IN THE NERVOUS SYSTEM OF TURBELLARIANS *GIRARDIA TIGRINA* AND *POLYCELIS TENUIS*.

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Details of morphology of the nervous system has been investigated in two planarian (Platyhelminthes, Turbellaria) species *Girardia tigrina* and *Polycelis tenuis* using immunostaining to neuropeptide FMRFamide. Abundant FMRF-immunoreactivity (IR) has been revealed in central and peripheral nervous systems of both species.

Cells and fibres immunoreactive (IR) to FMRFamide have been observed in the ventral nerve cords, the transverse connecting commissures and the paired cephalic ganglions of *P. tenuis* (bilobbed, butterfly-like) and *G. tigrina* (horseshoe-shaped). Intensive staining has been found in the cells and fibres of the peripheral nerve plexus. Muscular pharynx is supplied with FMRF-IR nerves, and flexible and mobile tail body region is highly innervated by FMRF-IR nervous elements. Net of numerous FMRF-IR fibres has been detected in the genital structures of the sexually reproduced *P. tenuis*. Intensive FMRF-IR has been observed on the ventral side of the body in cells and fibres surrounding a mouth opening in *G. tigrina*. On anterior end of the body the triangular auricles of *G. tigrina* were deeply innervated by FMRF-IR fibres, and some thin FMRF-IR fibres were seen bordering the photoreceptors in both planarian species. Observations on specific immunostaining to FMRFamide in the neurons of the body regions, deeply supplied with receptor's structures (mouth, eyes, body edges, auricles), may indicate the possible involvement of FMRF-like neuropeptides in realization of receptor's functions in flatworms.

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## ORIGIN OF AUTOMATISM AND ITS REGULATION OF THE GUT MOTILITY IN GASTROPODS, *APLYSIA* AND *LYMNAEA*

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The enteric nervous system (ENS) consists of a network of neurons that are intrinsic to the gastrointestinal tract in a wide variety of animals. We have shown the existence of neurogenic automatism in addition to myogenic one in the gastrointestinal motility of *Aplysia* and *Lymnaea*. In this study we examined whether ENS neurons are competent to control ordered autonomous peristalsis, which is apparent in the crop of *Aplysia* and in the esophagus of *Lymnaea*. Autonomous bursting activities of ENS neurons were shown to be responsible for the rhythmic automaticity of peristalsis. Interestingly, the "pacemaker region", which regulates the rhythm of peristaltic movements, was found in the gizzard in *Aplysia* and in the crop in *Lymnaea*, which are both located distal to the regions with peristalsis. Thus, the bursting activities of ENS first occurred in the gizzard of *Aplysia* or in the crop of *Lymnaea* and then were propagated in an ascending direction (i.e., in the opposite direction of peristalsis) along the crop or esophagus, respectively. The conduction velocity of the peristaltic movement was faster in the esophagus of *Lymnaea* than in the crop of *Aplysia*. Different mechanisms were supposed to be involved in producing gastrointestinal peristalsis between the two species.

# IN SEARCH FOR THE NEURAL BASIS OF BEHAVIORAL SPONTANEITY IN INSECTS: LESSONS FROM PREDATORY WASPS AND ZOMBIE COCKROACHES

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If we assume that humans possess “free will” in that it reflects our ability to make our own spontaneous choices, then this ability must be embedded in our brains. A crucial question then comes to mind: is “free will” unique to humans or is it a trait shared by other organisms as well, and, if so, can we track and identify precursor forms of choice and spontaneity in non-human organisms.

‘Lower’ organisms such as invertebrates, are often seen merely as complex ‘automatons’ that respond stereotypically to environmental cues. However, much like humans, invertebrates may choose to initiate behavior based on their "internal state" rather than as a response to external stimuli alone. The neuronal underpinnings responsible for generating this ‘internal state’, however, remain elusive and the next logical step is to localize and understand the brain circuits responsible for such spontaneous behaviors. In our lab, we work on a unique and naturally-occurring phenomenon in which one insect uses neurotoxins and inject these into the brain of another to apparently ‘hijack its free will’. This phenomenon, a result of millions of years of co-evolution between a cockroach and its wasp parasitoid, offers a unique opportunity to study the roots and mechanisms of spontaneous behavior in nonhuman organisms. To demonstrate that the wasp venom injection into the head ganglia selectively depresses the motivation to move, we have adjusted behavioral paradigms traditionally employed to test mammalian models for motivational states. Stung cockroaches demonstrated the following behavioral deficits: Decreased spontaneous and evoked walking, analgesia, an increased threshold for walking initiation in response to escapable foot shocks or repetitive stimuli, and decreased swimming duration in the Forced Swimming Test with no change in the swimming motor pattern. Furthermore, our recent data indicates that the behavioral manipulation of cockroaches is achieved by, at least, venom-induced inhibition of neuronal activity in the sub-esophageal ganglion (SEG). We found that spontaneous and stimulus-evoked neuronal activity in the SEG is decreased in stung cockroaches, and that experimental injections of venom or local anesthetics into the SEG perturb walking initiation in un-stung cockroaches. Thus, our investigations point to one possible neuronal substrate involved in the regulation of spontaneous behavior in insects.

## EVOLUTIONARY ORIGIN OF THE HOOKS IN THE BUCCAL APPARATUS OF *CLIONE LIMACINA*: NEUROPHYSIOLOGICAL EVIDENCES

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*Clione limacina* and other mollusks from the order Gymnosomata have a unique feeding structure reflecting their high food specialization - chitinous hooks. The functional role of the hooks is to grab the soft tissue of the prey (another Pteropod mollusk, named *Limacina*) and to pull it out of the shell into the buccal cavity during the feeding. However the question of evolutionary origin of the hooks is absolutely unclear now. The buccal apparatus in many gastropod mollusks consists of the radula and the jaws. As we believe there are two possible evolutionary scenarios of the appearance of the hooks in Gymnosomata: 1) hooks evolved from the jaws 2) hooks evolved from the radula. Here we try to address this question by analyzing the neural network controlling hooks and radula movements in *Clione* in comparison with described in the literature neural network controlling jaws and radula movements in *Aplysia*, which could be considered as an ancestral form for Gymnosomata. Such an analysis brought us to conclusion that hooks in *Clione* most likely evolved from the radula while hook controlling neural network originated from the appropriate network of the radula in the hypothetical ancestor. Most important, that comparative-morphological study of the hooks in *Clione* and the jaws in *Aplysia* also strongly supported this idea. Thus, analyzing the neural network controlling the structure, we were able to make a conclusion on the evolutionary origin of this structure.

## HIGH RESOLUTION SPATIAL DISTRIBUTION OF NEUROPEPTIDES BY MALDI MS IMAGING IN THE POND SNAIL, *LYMNAEA STAGNALIS*

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Mass spectrometry based imaging technologies are suitable for label-free, high-throughput discovery of multiple classes of biomolecules directly from a tissue section surface, and can be combined with other routine imaging and proteomic methods. Wide broad biological and medical application of imaging mass spectrometry (IMS) has been published. IMS was used from discovery of neurodegenerative diseases related lipids, peptides and proteins to detection of protein biomarkers on tumor xenograft and human formalin-fixed paraffin-embedded breast cancer. The localization and chemical modifications of pharmaceuticals and their metabolites can be followed in animal whole-body sections, as well as in sections of specific organs. The most widespread IMS scanning technique is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS). High sensitivity and mass accuracy, broad mass range detection, good mass resolving power, high speed, MS/MS capabilities and good spatial resolution make it excellent tool for tissue-based imaging purposes.

In this study MALDI TOF/TOF and MALDI LTQ Orbitrap Imaging were used for direct molecular imaging of nervous tissue samples at micrometer spatial resolution. Cryosections of the cerebral ganglia and whole embryos of the pond snail *Lymnaea stagnalis* were placed onto ITO-coated conductive glass slides. Novel, highly reproducible matrix deposition and fixation techniques were used for the investigations. High-resolution local alterations of several well-known neuropeptides (eg. PACAP-38, PACAP-27, FMRFa, PMSMLRLa) were directly mapped by IMS for the first time. MALDI Imaging allows localization and direct identification of the peptides and proteins as well as their post-translational modifications from tissue sections.

# DNA ENDOREPLICATION IN THE BRAIN NEURONS DURING BODY GROWTH OF AN ADULT SLUG

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It has been hypothesized that the size of neurons of gastropods becomes larger in parallel with an increase in body size. DNA endoreplication (DNA synthesis without cell division) is thought to be involved in this process to meet the increasing demand for macromolecules in neurons. There is, however, no experimental evidence for this hypothesis to date. Here we investigated quantitatively: (1) the size of the brain and each ganglion, (2) the size of identified neurons, (3) the total number of neurons undergoing DNA endoreplication, (4) the total number of the neurons containing a cardioexcitatory peptide, and (5) the gene expression level per neuron, using adult terrestrial slugs whose body growth was regulated through the amount of food supplied. The body growth was accompanied by increases in the sizes of both neurons and ganglia, and triggered more frequent DNA endoreplication events in each ganglion of the growth-promoted slugs, without increasing the total number of neurons. Increase in the neuronal size also involved the increase in the amount of transcripts expressed in a single neuron. This is the first quantitative evidence showing that the DNA endoreplication, neuronal size, and gene expression are increased concomitantly with body growth in adult slugs. In this symposium, I also would like to present the recent data on the experiments to distinguish whether the increase in the amount of genomic DNA reflects the whole genome replication (polyploidy) or the local DNA amplification (polyteny).

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## SYNAPTIC CIRCUITS IN THE *DROSOPHILA* VISUAL SYSTEM: SERIAL-EM YIELDS PROGRESS BUT ALSO SOME INCONVENIENT TRUTHS

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Nervous systems are frequently depicted as circuit diagrams. Such schemata represent varying degrees of abstraction, however, being in the most extreme cases simply models or mnemonics to interpret electrophysiological network interactions. Text-book accounts usually portray neural circuits as relay networks incorporating neurons with terminals that are clubbed and exclusively presynaptic, and dendrites that are fine, branched and exclusively postsynaptic. Terminals are then presumed to contact dendrites according to Cajal's law of dynamic polarization. Such contact is often claimed from light microscopy, at co-arborising sites in the same neuropile stratum or glomerulus, and synaptic transmission is then said to occur at individual sites between a single postsynaptic element and a single presynaptic release site. Although the vertebrate retina is accepted to diverge from some of these assumptions, the insect brain disregards all of them, as shown by our serial-section EM studies on the second optic neuropile or medulla in *Drosophila melanogaster*, the largest neuropile of the fly's brain. Our findings derive from a series of 1700 40-nm sections imaged over  $\sim 100\mu\text{m} \times 100\mu\text{m}$  and begin to reveal synaptic relay pathways formed by the neurons of a single column, but simultaneously the extraordinary richness of local microcircuits and of interconnections between these pathways. All synapses are divergent polyads often with four postsynaptic elements abutting a T-bar synaptic ribbon at each release site. The column. Each medulla column receives 10 input terminals: photoreceptors R7 and R8, lamina cells L1-L5, centrifugal cells C2 and C3, and an enigmatic medulla cell T1. A single column has been reconstructed within a  $\sim 12 \times 12\mu\text{m} \times 50 \mu\text{m}$  volume. This contains  $\sim 10,000$  presynaptic T-bar ribbons, with  $\sim 40,000$  postsynaptic dendrites. Of the 10,000 synapses only  $\sim 30\%$  ( $\sim 3,000$ ) belong to a single column, but column borders are ill-defined, a major difference between the medulla column and lamina cartridge. The column contains a total of 35 medulla columnar neurons. These represent only some of the  $>60$  reported medulla columnar cell types; at least some of the 60 must not therefore exist in all columns.

Synaptic features. The 45 input terminals and medulla cells themselves bear  $\sim 1800$  synapses, so maybe 1200 synapses are from elements that enter or leave the column volume. Presynaptic sites vary  $\sim 10$ -fold in number for different cells, between 27 (for R7) and 229 (for Mi1). Synapses, roughly 1.5 per  $\mu\text{m}^3$  of neuropile, are packed unevenly, most densely in stratum 9 of the proximal medulla. Pathway strength between synaptic partners varies widely, with fewer than 6 to 144 synapses. Many neuron pairs are reciprocally connected; importantly, L1 and L2 are both reciprocally connected to L5.

Pathways. R7 and R8 provide inputs to spectral pathways. L1 and L2 provide 'off' and 'on' responses to moving stripes (Joesch *et al.*, *Nature*, 2010) and their pathways can respond differentially to motion in opposite directions (Rister *et al.*, *Neuron*, 2007), in the posteroanterior and anteroposterior directions, respectively. These cells' medulla targets are not symmetrical however. L2 provides input to Tm1 and Tm2, which are paired at many synapses, while L1 provides input to Mi1, which receives input along with L5, C2, C3 and Tm3 cells and terminates in the proximal medulla. There, it provides inputs to dendrites of T4 cells that provide motion-sensitive input in turn to directionally selective lobula plate giant tangential neurons. The two centrifugal cells C2 and C3 provide an inhibitory feedback loop to the lamina, receiving input in the medulla predominantly from L1, which is probably glutamatergic, and GABAergic output in the lamina predominantly to L2 and L2 (C2). The functional significance of all these circuits remains to be determined.

# BEHAVIORAL EVIDENCE FOR INTERNAL FACTORS AFFECTING DURATION OF CONGLOBATION IN PILL BUGS (*ARMADILLIDIUM VULGARE*)

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Pill bugs, *Armadillidium vulgare*, can effectively conglobate (roll up into a ball), drawing its antennae inside to form an uninterrupted sphere. This behavior can be triggered by strong vibrations or pressure, and offers protection against predators such as ants and spiders. Observations suggest that pill bugs recover from conglobation without external stimuli. In this study, we performed behavioral experiments to investigate possible internal factors affecting duration of conglobation. Eighty individuals were individually placed in an arena (2 cm in diameter) connected to a linear pathway (15 cm in length). Each individual walked the path to reach another arena (2 cm in diameter). Then, it was moved to a petri dish (6 cm in diameter) on its back. In the first experiment, the ventral surface of individuals was stimulated by a puff of air, and 69 individuals conglobated. When they began to recover from conglobation, they initially moved their antennae or legs. In the second experiment, immediately after they stretched their body after conglobation a puff of air was directed toward their ventral surface, and 60 individuals conglobated. Locomotion time along the straight pathway ( $t_0$ ) and duration of conglobation ( $t_1$  and  $t_2$  respectively) were measured in each experiment. In both the first and second experiment, 20 individuals moved their antennae and another 20 moved their legs when recovering from conglobation (Antenna-Antenna group; AA and Leg-Leg group; LL). In the remaining 20 that conglobated, when recovering from conglobation, 15 moved their antennae in the first experiment and legs in the second one (AL), and the last 5 moved their legs in the first experiment and antennae in the second one (LA). In the AA and LL groups, there was significant positive correlation between  $t_1$  and  $t_2$  (AA:  $r_s = 0.657$ ,  $p < 0.01$ ; LL:  $r_s = 0.476$ ,  $p < 0.05$ ). However, in the LA group, there were significant positive correlations between  $t_0$  and both  $t_1$  and  $t_2$  ( $t_0 - t_1$ :  $r_s = 0.563$ ,  $p < 0.05$ ;  $t_0 - t_2$ :  $r_s = 0.725$ ,  $p < 0.01$ ), while there was no correlation between  $t_1$  and  $t_2$ . These results suggest that there are a variety of internal factors, which may include CNS, affecting duration of conglobation in *A. vulgare*.

# GENOMIC DECIPHERING OF MEMORY MECHANISMS AND MULTIPLE ORIGINS OF NEURAL CIRCUITS

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Advantages of massive parallel sequencing technologies revolutionized virtually all fields of biomedicine and neuroscience in particular. Here, I will summarize the use of molecular and comparative approaches (i) to probe genomic organization of memory-forming circuits at the single cell resolution; and (ii) to reconstruct the origins of neurons and complex brains in major animal lineages. Specifically, our team developed an unbiased assay of genome-wide DNA methylation events complemented by nearly complete transcriptome analysis from the same sample or a single cell. As a result, this methodology allowed us to identify >4,000 genes and their regulatory regions in the *Aplysia* neuronal genomes that changed their DNA methylation status and expression during plasticity tests with a remarkable cell specificity. However, we do identified a core of evolutionary conserved molecular modules shared across different neuronal subtypes suggesting that learning and persistent memory are in fact true epigenetic processes involving large scale chromatin remodeling and changes in DNA methylation within a neuron's genome. We discovered that dynamic interaction between 5-mC and 5-hmC (5<sup>th</sup> and 6<sup>th</sup> bases in DNA) underlie and integrate transcriptional outputs from neurons at different functional states including transcription-dependent regulation of excitability and synaptic efficacy in memory circuits. Consequently, we and other found that memory mechanisms share a lot of similarities with long-term modifications during neuronal injury, and hypothesized that memory of injury can be a predecessor of all memory forms (Walters, Moroz, 2009). The origins of neurons can also be linked to adaptations to stress/injury factors in a form of integrated regeneration-type cellular response (Moroz, 2009). To further reconstruct the parallel evolution of nervous systems, we implemented genomic approaches to probe enigmatic neurons of basal metazoans, selected lophotrochozoans and deuterostomes. Both cladistic and genomic analyses suggest that neurons evolved multiple times from polarized secretory cells both as a response to injury and to coordinate ciliated locomotion in early animals. Surprisingly, our phylogenomic analysis also suggests that complex brains can be evolved at least 5-9 times independently within different animal lineages; only within the phylum Mollusca cephalization might occur at least 4 times. At the genomic level such transitions might be achieved by changes in expression just a few transcriptional factors – not surprisingly such events might happen multiple times over 600 million years of animal evolution.

# GENOMIC PORTRAIT OF A NEURON: IDENTIFICATION AND QUANTIFICATION OF VIRTUALLY ALL RNAs IN SINGLE INDIVIDUALLY IDENTIFIED NEURONS

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It might not be an overstatement to say that virtually every neuron in our brain is unique in its position, wiring, morphological and functional properties. However, the molecular and genomic bases of this remarkable neuronal specificity are largely unknown. We simply do not know how many genes are expressed in any given neuron, how different one neuron is from another, or the scale of transcriptional changes in a neuron following physiological or pathological changes. Here we completed deep transcriptome analysis of four single identifiable neurons using three complementary sequencing platforms (Illumina, SOLiD, and 454; with an order of 0.5-1 million reads per given neuronal type). Specifically, we selected: (i) a serotonergic interneuron (MCC), (ii) a cholinergic motor neuron R2, as well as (iii) glutamergic mechanoreceptor neurons and (iv) peptidergic L7 motoneurons known as pre- and postsynaptic components of a simple memory-forming circuit in the sea hare *Aplysia californica*. Our protocol insures that each sequencing read corresponds to exactly one copy of the transcript within the cell. We also preserved the directionality of transcripts, providing accurate levels of both sense and anti-sense expression. A comparative neurogenomics database was developed to support these massive genomic datasets. As a result of initial mapping to the *Aplysia* genome, we estimated that >70% of the genome is expressed in a given neuronal type, generating on the order of 100,000 unique transcripts (including a very large fraction of ncRNAs). Finally, to find epigenetic markers and associate them to differential gene expression profiles, we have complemented RNA-seq by direct single DNA methylation analysis from the same identified neurons and hosted it on a genome browser. We proved that each neuron has its own distinct methylome supporting the maintenance of a unique expression blueprint. Combined, our data reveal an unprecedented genomic complexity of neurons, providing the first insight into the complete genomic portrait of not one, but four identifiable neurons directly involved in regulation of feeding and defensive behaviors. We argue that implementation of unbiased single-cell genome-wide approaches is a vital, challenging, but currently achievable landmark to understand both the full scale of the molecular bases of integrative activity of a neuron and to decipher learning and memory mechanisms.

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# EVIDENCES FOR NO-SIGNALING IN THE OLFACTORY CENTER OF THE SNAIL, *HELIX POMATIA*. A BIOCHEMICAL AND MORPHOLOGICAL STUDY

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The olfactory center (the procerebral lobe) of stylommatophoran terrestrial snails is known to produce nitric oxide (NO)-modulated local field potential oscillatory waves when the animal receives odor stimulus. This electrical wave propagation forms the basis of olfactory information processing and learning. Up to now only few literature data are available on the precise distribution, activity and signaling pathway of NO in this system. Therefore, our aim was to visualize NO synthase (NOS) in protein homogenates and histological preparations, and evaluate the activity of NOS in the procerebrum.

Performing *ex vivo* NOS assay on procerebral homogenate the basal NOS activity was found  $455 \pm 24$  nmol nitrite/mg protein, determined as NO metabolites (NO<sub>x</sub>) by an NO gas analyzer. Western blot of protein homogenate probed by an anti-NOS antibody showed a 150 kDa mw band. Applying NADPH-diaphorase histochemistry as well as NOS immunohistochemistry on serial cryosections, identical structures were labeled by both techniques in the procerebrum. NADPH-diaphorase reactive and NOS-immunoreactive globuli cells and their projections with variable intensity of labeling could be visualized in the cell body layer. Intensive NOS signal was detected in the internal and lateral neuropil. The internal neuropil received NOS containing intrinsic fibers from the globuli cells and extrinsic fibers from the upper tentacular nerve. Extirpation of the tentacles showed that the lateral neuropil was innervated by NADPH-diaphorase reactive fibers originating both from the upper and the lower tentacles as well as from the sensory epithelium of the mouth region. At ultrastructural level NADPH-d cytochemistry revealed that NOS was bound to the nuclear envelope and endoplasmic reticulum elements in the globuli cells, whereas it was attached to the membrane of agranular synaptic vesicles and medium electron dense granular vesicles in the neuropil. It suggests the involvement of NO both in the process of transmitter release and intercellular signaling in the neuropil. Our observations provide a clear evidence for the cellular presence and activity of NOS in the procerebrum. Based on the present results the search for molecular downstream targets of NO in the procerebrum will be in the focus of our future research activity.

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## NEUROTRANSMITTERS REGULATING INSULIN PRODUCING CELLS IN *DROSOPHILA*

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*Drosophila* insulin-like peptides (DILPs), like their counterparts in worms and mammals, play important hormonal roles in regulation of metabolic carbohydrate and lipids, but also in reproduction, growth, stress resistance and ageing. In spite of intense studies of insulin signaling in *Drosophila* the regulation of DILP production and release is poorly understood. The insulin producing cells (IPCs) of the *Drosophila* brain are located in the median neurosecretory cell group. These IPCs produce three of the seven insulin-like peptides, DILP2, 3 and 5, and are presumed to release these into the circulation from axon terminations in the neurohemal organ corpora cardiaca. Our recent studies, and those of others, have suggested neuronal control of the activity of brain IPCs in *Drosophila*, and a few neurotransmitters have been suggested as regulators in these neuronal systems: short neuropeptide F (sNPF), *Drosophila* tachykinin (DTK) octopamine (OA), GABA, and serotonin (5HT). Of these, sNPF and OA seem to stimulate IPCs and insulin signaling, whereas GABA, DTK and 5HT are inhibitory. The receptors expressed on IPCs for these neurotransmitters have been identified and targeted for genetic manipulations to study effects on insulin signaling and fly physiology. Our data suggest that the IPCs are under tight neuronal control by multiple neurotransmitters, probably released from distinct neuronal systems, and that insulin signaling is not only depending on circulating nutrient signals.

## **SINGLE-CELL-BASED ANALYSIS OF NEUROPEPTIDE EXPRESSION IN PERIPLANETA AMERICANA ANTENNAL LOBE NEURONS.**

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An important prerequisite to understanding the role of neuropeptides in olfactory processing is to match the functionally different cell types in the antennal lobe with their peptide profiles by using electrophysiological recordings combined with immunocytochemical studies and/ or single cell mass spectrometry. The olfactory system of *Periplaneta americana* is particularly well suited to accomplish this goal, because several physiologically distinct neuron types can be unequivocally identified. In our study, we systematically analyzed different parts of the antennal lobe by MALDI-TOF mass spectrometry. Altogether, 50 ion signals could be assigned to products of 10 neuropeptide genes. We also demonstrated the feasibility of MALDI-TOF mass spectrometric profiling of single antennal lobe neurons, which is an important precondition for combining electrophysiology with peptide profiling at the single cell level.

## AXONAL SPIKE PROCESSING AND LONG-TERM ASSOCIATIVE MEMORY IN *LYMNAEA*

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Although synaptic plasticity is generally regarded as the primary mechanism of memory, non-synaptic plasticity, such as persistent somal depolarization also makes contributions to the storage of long-term memory in both vertebrates and invertebrates. There is however little information on how other neuronal compartments are affected by learning-induced maintained soma level changes of membrane potential. Specific mechanisms of non-synaptic plasticity may contribute to the storage and retrieval of memory in a manner strongly dependent on the functional morphology of individual neurons. Here we show that somal depolarization of the CGCs (Cerebral Giant Cells) of the pond snail *Lymnaea stagnalis* spreads to the distal end of an axonal side branch to enhance synaptic outputs of the cell. In intact cerebral ganglia we recorded optically with calcium-sensitive and voltage-sensitive probes the activity of one of the axonal side branches of the CGC as in this type of preparation its fine neurites are inaccessible for electrophysiological techniques. At recorded somal membrane potential levels, the amplitudes of optically recorded action potentials (APs) and calcium transients displayed a significant decay along the side branch towards its distal end where a high density pool of active synapses was clustered. Depolarization of the CGC soma by current injection resulted in an increase in AP area and amplitude, which was more pronounced in the proximal segments of the side branch. Bath application of 4AP increased the amplitude of APs and calcium transients at the distal end of the side branch but not in the proximal locations, suggesting that an A-type potassium current regulates the AP propagation along the side branch. Confocal microscopy with FM4-64 dye revealed positive synaptic-like spots of 0.5-2  $\mu\text{m}$  size co-localized with the distal ramifications of the side branch. Our subsequent experiments with single-trial classical food-reward conditioning demonstrated that the amplitude of AP-evoked calcium transients at the distal end of the side branch correlated with the learning-induced persistent depolarization and was increased in the trained group. Thus the suppression of attenuation of calcium transients traveling towards the synapses of the side branch was concomitant with and can extensively contribute to the emergence of long-term associative memory. Our findings provide new insights into how learning-induced membrane level changes are translated into a morphologically relevant form of long-lasting non-synaptic plasticity specific to particular neuronal compartments.

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## **PUMPING OFF FOOD (POFFF): A NEUROMODULATABLE ADAPTIVE RESPONSE TO CHRONIC FOOD WITHDRAWAL IN *C.ELEGANS*.**

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Presentation or withdrawal of food provides a powerful environmental cue that modulates many aspects of *C.elegan's* behaviour. Food removal is known to have progressive effect on the locomotory behaviour depending on the time the organism is in the absence of food. Such adaptive behaviours are suggestive of a complex integrative response driven from external and potential internal cues. We have defined an adaptive response in pharyngeal pumping (feeding behaviour) in response to chronic food withdrawal. Well fed worms (L4+1 day) placed on a no food arena for 5 hours show a complex change in their pattern of pharyngeal pumping. This is defined by three phases encompassing a i) progressive increase in pumping reaching ii) steady state prior to iii) an erratic phase in which the pharynx fluctuates between very high pump rate and no pumping. Overall the pumping during PoffF rises to about 30 % of the rate seen in animals placed on food. This appears driven by distinct pathways to those that increase pharyngeal pumping on food. PoffF is not affected by the *tph-1* mutants which otherwise show a severe inhibition in the increased pumping seen in worms on food. In a similar way many transmitters that embedded in the pharyngeal microcircuit do not have a marked effect on this behaviour. In contrast cutting the cuticle to isolate the pharynx from overriding extra-pharyngeal nervous system sees a loss in the PoffF behaviour. This suggests that the primary drive for PoffF lies within the extrapharyngeal circuit. Surprisingly, the PoffF is markedly different in mutants lacking glutamate (*eat-4*) and these animals show an instant elevation in pumping that is sustained at a much higher level than in the wild-type animals off food. In view of the pivotal role of extrapharyngeal glutamate pathways in mediating chemosensory withdrawal suggests that activation such pathways may act to inhibit the pharyngeal pumping during food withdrawal. Interestingly *unc-31* which is thought to play a pivotal role in peptidergic signalling exhibits a PoffF that phenocopies the *eat-4* mutants which might suggest an important interaction between glutamate and peptidergic signalling. Surprisingly PoffF is completely absent in *egl-3* mutants deficient in neuropeptide which raises important issues about the relationship between *unc-31* and peptide transmission. However, the observed converse relationship between glutamate (*eat-4*) and neuropeptide signalling with respect to PoffF suggests an integrative interaction in which an upstream glutamate pathways acts to inhibit an excitatory peptide pathway or visa versa. Overall these data reinforce how the pharyngeal circuit can be used to reveal important principles of neuroadaptive responses that follow changes in environment.

## MATHEMATICAL ANALYSIS OF HONEYBEE WAGGLE DANCE

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A honeybee informs her nestmates of the location of a flower by a waggle dance. To reveal the effects of the waggle dance in terms of the colony's benefit, we have created mathematical models and performed computer simulations.

To obtain biological parameters for improvement of our mathematical description, we observed bee behavior in the field. Video analysis showed that the bee does not dance in a single, random place in the hive but waggled several times in one place and several times in another. It also showed that the information of the waggle dance is not so precise. Angle and duration of waggle runs varied from run to run with the range of  $\pm 15^\circ$  and  $\pm 15\%$ , respectively, even in a series of the waggle dance of an individual. We also found that a follower that listens to the waggle dance attended to multiple dancers before her flight and that most dance followers turned away from the dancer after one or two sessions of listening.

We, then, created a Markov model of bee foraging-related behavior inside and outside of the hive and performed simulation experiments by incorporating those biological parameters. The simulations showed that information transfer by the dance was important for the effective food collection. A colony in which honeybees danced and correctly transferred information made 2.15 times more successful visits to food sources compared to a colony with non-accurate information transfer. Furthermore, our simulations suggested that accuracy of orientation information must increase along the increase of the distance between the food sources and the hive.

## INSECT BEHAVIOUR AND THE FUNCTIONAL ROLES OF BIOGENIC AMINES, IN PARTICULAR TYRAMINE AND OCTOPAMINE.

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Biogenic amines act as important neuromodulators and neurohormones and affect many different behaviours such as motivation, learning, memory formation, locomotion, aggression, gregarisation, reproduction and many others. In insects such as the locust, an important amine is octopamine which is synthesized from tyramine. In addition, tyramine can act as an independent transmitter. Immunohistochemical studies showed many tyraminergetic and less tyraminergetic/octopaminergetic neurones in the brain and only a few tyraminergetic but many tyraminergetic/octopaminergetic neurones in the thoracic ganglia. When animals were treated differently, for example were kept undisturbed or were stressed prior to immunohistochemistry, some of the previously tyraminergetic neurones were now producing octopamine. This way, a new descending class of descending brain interneurons was discovered that extends until the fourth abdominal ganglion.

In order to test differential effects of octopamine and tyramine, fictive flight was elicited in an isolated moth (*Manduca sexta*) ventral cord by the application of chlordimeform (an octopamine-receptor agonist). The additional application of tyramine led to an increased recruitment of neurones of the depressor system. The application of yohimbine, a blocker of tyramine-receptors, led to selective inhibition of the depressor system. In both experiments, the elevator system remained unaffected.

Mutant fruit flies (*Drosophila melanogaster*), which cannot produce octopamine, are able to fly but exhibit severe difficulties in long flight performances. This is consistent with the idea that octopamine is necessary for long lasting, energy demanding behaviours and affects both the central nervous system and a variety of peripheral target organs in a concerted manner.

## ANALYSIS OF FEEDING AND WITHDRAWAL CIRCUITS REVEALS NEURAL MECHANISMS OF DECISION MAKING.

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The feeding behaviour of the pond snail (*Lymnaea stagnalis*) is well-characterised and the underlying neuronal network is thoroughly mapped. Critical neuronal elements of the withdrawal network are also known. However the interactions between the two neural systems have not been studied before. Using intracellular electrophysiological recordings we investigated the connections among key withdrawal and feeding neurons and discovered points of convergence between the two opposing systems. An identified pleural coordinating interneuron (PIB), appears to have a profound influence on both systems. Injection of a small depolarizing current into the cell and aversive mechanical or chemical stimulation causes high frequency firing in PIB that hyperpolarises key feeding interneurons (CV1 and CGC) and stops feeding, while injection of a maintained negative current supports the feeding response. Sucrose stimulation induces the feeding response and causes hyperpolarization of PIB. Furthermore, the activity of this neuron is also dependent on the strength of the stimulation; a weak tactile stimulus to the lip inhibits PIB and enhances feeding while strong stimulation excites PIB and inhibits the feeding response. We also investigated the connections of another coordinating withdrawal interneuron (PeD11) with PIB as well as with identified withdrawal motoneurons and feeding interneurons. PeD11 is not synaptically connected to PIB but, like PIB, causes inhibition in CV1, a command-like interneuron of the feeding network. Neither of these two coordinating interneurons is involved directly in the initiation of muscle contraction. However motoneurons (CeAlip, CeAcm, PeGcm, PeL1, etc) that directly innervate the lip- and columellar muscles are electrotonically coupled with PeD11 and indirect coupled with PIB interneurons. Since these interneurons indirectly also influence the feeding system their activity can mediate the interaction between the feeding and withdrawal networks acting as key elements in non-cognitive decision making (i.e. decision between feeding and withdrawal).

## PACAP - A PHYLOGENETICALLY CONSERVED NEUROPEPTIDE

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Since its discovery in 1989, pituitary adenylate cyclase-activating polypeptide (PACAP) has become increasingly recognized for its important and diversified roles in the central and peripheral nervous system and in several peripheral organs of a variety of vertebrate and invertebrate species. The primary structure of PACAP has proved to be remarkably conserved during evolution, not only in higher and lower vertebrates but also in invertebrates. Molecular analysis of PACAP revealed a >89 % homology of amino acid sequences in variety of vertebrate and invertebrate species. Based on earlier data, the purpose of this presentation is to highlight the role of PACAP in a range of physiological and behavioural processes of gastropod molluscs. This presentation surveys the importance of PACAP and/or PACAP-like peptides in the pulmonate snails *Helix pomatia* and *Lymnaea stagnalis*.

In our experiments we used a variety of different techniques for the investigation of PACAP, such as immunohistochemistry, western-blot and MALDI mass spectrometry for peptide identification, and intracellular recording, bioassays, behaviour tests for physiological and behavioural processes.

We observed the occurrence, localization and distribution of a PACAP-like peptide and its receptor in *Helix* and *Lymnaea*. It is speculated that a 15 kDa protein band detected by a PACAP antibody using WB in molluscs represents a PACAP-like molluscan peptide of larger molecular weight than the vertebrate 4-6 kDa PACAP molecules. Western blot experiments revealed three binding sites of which two corresponded well to the VPAC1 (~45 kDa) and PAC1 (~60 kDa) of vertebrates and further various *in vivo* pharmacological treatments along with immuno-assay experiments confirmed their corresponding presence around 45 kDa and 55 kDa in snail brain homogenates. These findings favour the presence of a specific PACAP receptor in the snail. We also identified several biological effects of PACAP in snails: 1) it has an anti-apoptotic effect in the salivary gland cells; 2) it has a possible role in the maintenance of active state, because in active snails the amount of PACAP is four-fold higher compared to the brain of hibernating snails; 3) it may elicit membrane potential changes in several neurons leading to changes in action potential frequency; 4) it is both necessary for and accelerates the formation of long-term memory after food-reward classical conditioning; 5) and finally, the cardioactive nature of PACAP is an entirely novel finding.

Based on our results we conclude that both the molecular structure and function of PACAP and its receptors are evolutionarily conserved between gastropod mollusks and vertebrates.

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# INFLUENCE OF SEROTONIN AND DOPAMINE ON FUNCTIONING OF NEURAL NETWORK INVOLVED IN OLFACTION AND TENTACLE MOVEMENTS IN *HELIX* SNAIL

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In the present study we have investigated the role of serotonin and dopamine in regulation of olfactory system functioning and odor-evoked tentacle movements in *Helix* snail.

*In vitro* experiments have revealed the effects of serotonin and dopamine on the spontaneous and odor-evoked activity in *Helix* olfactory system (olfactory nerve and procerebrum) and identified retractor motoneuron of the posterior tentacle MtC3. Reduced nose-brain (Nikitin et al., 2005) or isolated posterior tentacle preparations were exposed to cineole odor and to different concentrations of serotonin, dopamine, serotonin precursor 5-hydroxytryptophan and dopamine precursor L-DOPA. Serotonin bath applications reduced the responses to odor recorded in the olfactory nerve and hyperpolarized the MtC3 motoneuron; dopamine applications led to the opposite changes. The effects of precursors (5-hydroxytryptophan and L-DOPA) on MtC3 were of the same direction as the effects of serotonin and dopamine, correspondingly. The amplitude of spontaneous procerebral oscillations decreased under the dopamine action, but no significant changes in the odor responses were found.

*In vivo* studies have shown that injection of 5-hydroxytryptophan in freely moving snails reduced tentacle withdrawal in response to aversive odor (ethyl acetate) and tactile stimulation of the skin, while L-DOPA intensified withdrawal reaction to cineole and ethyl acetate odors. Thus, the rises of the serotonin or dopamine content evoked by injections of precursors have elicited the opposite shifts in behavioral state of the snails.

Our data suggest that the balance of serotonin and dopamine concentrations in cerebral ganglia and tentacles influences the odor perception and behavioral performance. This mechanism of behavioral control may be related to the level of arousal and/or different stages of feeding behavior in *Helix*.

# FIGHTING BEHAVIOR OF WHITE-EYE MUTANTS IN THE CRICKET *GRYLLUS BIMACULATUS*

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Male crickets *Gryllus bimaculatus* show intensive aggressive behavior when they come across another male and start to fight each other [1]. It has been reported that they use many sensory cues, including chemical, visual and tactile cues, during fights to express adequate behavior to the opponent. Shutting off the visual cue from the opponent by painting the compound eyes or darkening the arena causes significantly more intensive fights [2, 3], and this suggests that they estimate the opponent's strength by their appearance, for instance, size of the body or display of mandible flare. In this study, we examined fighting behavior of the white-eye mutants to clarify whether they are able to use the visual cue during the fight or not.

We first investigated the fighting behavior in the dark to clarify how visual input from the opponent affected their fight. The fight in the dark lasted significantly longer than in the light, and the level of the aggression of the fight was also significantly higher. Darkening made the fight longer not only during the phase including mandible flare display but also during antennal fencing indicating that they use visual cues from the beginning of the fight. Unusually longer fights were also observed when the compound eyes of the winner or loser were painted. This result suggests that both winner and loser need visual information to terminate the fight.

Next, we construct ethograms of fighting behavior of the cricket under the following three conditions: 1) intact pairs in the light, 2) intact pairs in the dark and 3) pairs of the white-eye mutants in the light. Pairs of the white-eye mutants could show typical sequentially escalating fight, and the time course of the fight was quite similar with that of the intact pair in the dark suggesting that they were not able to use visual information during the fight.

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## THE OLFACTORY SYSTEM OF THE RED FLOWER BEETLE, *TRIBOLIUM CASTANEUM*: ARCHITECTURE AND POSTMETAMORPHIC PLASTICITY

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With the fully sequenced genome, its susceptibility for transgenic approaches such as directed gene expression and powerful reverse genetics based on systemic RNA interference, and its longevity, *T. castaneum* offers an excellent system to study plasticity of the olfactory system. A prerequisite to understand changes in the olfactory pathway occurring either naturally or which are induced by manipulation is a thorough analysis of the system. In *Tribolium*, olfactory sensory neurons (OSN) based in sensilla of the outer three antennal segments project via the antennal nerve into about 70 olfactory glomeruli of the antennal lobe (AL). Anatomical analysis of adult beetles revealed no sexual dimorphism on the level of the antenna (general morphology, number and types of sensilla) and the AL (number and size of glomeruli). Analysis of a Gal4-UAS line, expressing tGFP in all OSNs containing the Orco (olfactory receptor co-receptor), revealed OSN projections into about 50 % of the glomeruli, all located in the lateral AL. Volume measurements of brain neuropils incl. AL and mushroom bodies (MB) of freshly eclosed (A0), four and seven day (A7) old beetles revealed a pronounced volume increase in all analyzed neuropils. Two main possibilities may explain the adult neuropilar growth: first, it might represent the final maturation processes, which are possibly hormonally controlled, or second, it might be a manifestation of early adult neural plasticity depending on sensory input. During early insect life, these two possibilities may build on each other. To reveal mechanisms of postmetamorphic plasticity of the olfactory pathway, we started several experimental approaches including e.g. behavioral, neuroanatomical, neurochemical and physiological studies using e.g. 3D reconstructions, immunocytochemistry, mass spectrometry, electrophysiology and imaging in combination with genetical manipulation and/or odor deprivation or stimulation. Preliminary results suggest the involvement of olfactory input in the postmetamorphic shaping of the olfactory pathway. For example, first knockdowns of Orco resulted in smaller ALs but not OLs. Comparing the numbers of tachykinin-ir (TK-ir) AL neurons of females and males at A0 and A7 revealed a sexual dimorphism at A0, with females having more TK-ir AL neurons than males. In both sexes, the numbers of AL TK-ir cells increased from A0 to A7. Females, isolated shortly before adult molt, showed no increase in the number of TK-ir cells, in contrast to males kept under same conditions. In females, this phenotype can be rescued by addition of the aggregation and sex pheromone 4,8-Dimethyldecanal (DMD). Our findings suggest that the increase of TK-ir cells between A0 and A7 depends on the perception of the pheromone signal and thus favors an activity dependent mechanism for the maturation of the TK-system.

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## INSECT BRAIN NEURONS LABELLED BY AN ACTIN ISOFORM

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Actins are major components of the cytoskeleton. They are involved in controlling cell shape, growth and motility. Actin occurs in polymerized filamentous (F-actin) or unpolymerized globular (G-actin) state. Actins are found in all compartments of vertebrate and invertebrate neurons.

Insect brain neuropil shows in most areas F-actin, exclusively demonstrated by phalloidin/actin binding. Previous investigations, using multiple marking of neuron types and confocal microscopy, located F-actin in pre- and postsynaptic neuronal specializations (Rössler et al., *Chem Senses* 2002, vol. 27, 803-810; Frambach et al., *JCN* 2004, vol. 475, 303-314). In mushroom body calyces of adult crickets and of several other species, F-actin appears to be attached to the subsynaptic membrane of dendritic tips, presumably part of the subsynaptic density in identified intrinsic nerve cell types (Kenyon cells). Kenyon cells receive in microglomeruli excitatory cholinergic input by boutons of identified projection neurons. No F-actin could be found in these presynaptic boutons. Tracts of projection neurons lacked F-actin. Kenyon cell dendrites, coupled to presynaptic GABA-elements, did not show F-actin, nor could it be assigned to synaptic spots in local microcircuits with reciprocal synapses between GABA- and Kenyon cell fibres of inner parts of anterior calyces and upper stalk region. F-actin could so far only be attributed to Kenyon cell dendrites.

Staining of insect brain neuropil by an antibody directed against the isoform  $\beta$ -actin allowed a marking of Kenyon cell somata, neurites, dendrites and their axon like fibres. Interestingly, most intense labelling was stated for type III Kenyon cell clusters ( $\gamma$ -lobe neurons), receiving input from tritocerebral projection neurons. Mature type II Kenyon cell somata and fibres of the anterior calyx were less intensely stained. Comparing labelling by phalloidin/F-actin and of globular  $\beta$ -actin by immunocytochemistry, we found overlap of both actin forms at the subsynaptic dendritic tips of Kenyon cells. Preliminary results indicate similar relations for groups of central body neurons. Mushroom bodies of adult insects undergo specific morphological changes such as increase or decrease of neuron types and total mushroom body volume, related to behavioural changes during life. We suggest that  $\beta$ -actin labelling identifies neuron types capable of structural plasticity or even reflects their state of remodelling.

# NO-INDUCING SIGNALS ARE ESSENTIAL FOR THE REGENERATION OF THE SNAIL TENTACULAR GANGLION

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The tentacular ganglion is the primary center of processing different sensory information like odor, light, and touch in stylommatophoran terrestrial snails. Regeneration of the tentacle after mechanical ablation takes 15 weeks, in the course of which the whole organ including the ganglion is morphologically and functionally rebuilt from epithelial stem cells. Serotonin-containing neurons are known to command the reorganization of newly differentiated neurons, but other molecular details of the regeneration is not known. In other invertebrate models the gaseous transmitter nitric oxide (NO) was found to be a key factor for neural regeneration, by modulating the pathfinding of neural growth cones. Based on it we studied the possible contribution of NO to the regeneration of the tentacular ganglion of the snail, *Helix pomatia*, by following the distribution and activity of NO synthase (NOS), NO-induced cyclic guanosine monophosphate (cGMP), and protein kinase G (PKG) activity.

In the intact tentacular ganglion, NOS, visualized by NADPH-d histochemical reaction, was localized both in neural somata sending projections towards the tentacular nerve, and in the neuropil receiving axon fibers from the sensory epithelium. From the 10th week of regeneration intensive NADPH-d activity resembling that of the control reappeared in the tentacular ganglion which sustained until the end of regeneration. Western-blot of NOS protein demonstrated a single band at 150 kDa mw which could be detected from the 9th week of the regeneration. The changes of NOS protein level correlated well with that found by histochemistry. In *ex vivo* NOS assay a six-fold increase of the control value ( $1.8 \pm 0.3$  nmol nitrite/ $\mu$ g protein/h) was detected at the 13th week of regeneration and then it returned to the control level by the 15th week. cGMP immunoassay showed  $12.3 \pm 4.5$  pmol/mg protein cGMP content in the intact tentacle which increased following incubation with 1mM SNAP or 10  $\mu$ M YC-1. In the regenerating tentacle the cGMP level was peaking at the 13th week with  $38.4 \pm 8.4$  pmol/mg protein. ELISA assay of the phosphorylated PKG substrate Akt revealed the highest PKG activity during the time intervals of 1-5 and 11-15 weeks of regeneration. Application of 1  $\mu$ M PKG inhibitor peptide RKRARKE indicated that other kinases also have a significant activity at the early phase of regeneration. Weekly injection of 10 mg/kg L-NAME into the body cavity decreased *ex vivo* NOS activity by  $42 \pm 15\%$  at the 15th week, and prolonged the morphological regeneration of the tentacular ganglion by 4-5 weeks. Taking together, our observations seem to provide evidence for the contribution of the NO-cGMP-PKG signal pathway in the neural regeneration of the *Helix* tentacular ganglion.

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## NEUROANATOMY FOR PHOTOPERIODISM IN INSECTS

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Photoperiodism controls seasonal development like diapause, seasonal morphs and migration in various insects. The minimal requirements for photoperiodism are photoreceptors, a photoperiodic clock and hormonal effectors. Although photoreceptors and hormonal effectors have been studied in many species, the neural mechanism underlying the photoperiodic clock has not been understood. Bünning first pointed out in 1936 that endogenous circadian rhythms are involved in the measurement of day or night length in the photoperiodic clock. Recently mutant analysis and RNA interference studies have shown in several species that circadian clock genes are involved in photoperiodism. Yet circadian clock genes are expressed in many kinds of cells. To understand neural mechanisms underlying photoperiodism identification of circadian clock neurons and other key neurons, and their neural circuitry is necessary. In *Drosophila melanogaster* circadian clock genes and neurons are well studied. Unfortunately, however, its photoperiodic response is very shallow and difficult to assay. Therefore we are focusing on photoperiodism in the blowfly *Protophormia terraenovae*.

Ablation experiments showed that pars lateralis neurons in the dorsal protocerebrum are important for diapause induction under short-days and low temperature, the pars intercerebralis neurons for ovarian development under long-days and high temperature. Five types of PERIOD-immunoreactive cells were found. When a type of PERIOD cells (s-LN<sub>v</sub>s) was bilaterally ablated, flies became arrhythmic in locomotor activities, and did not discriminate photoperiod for diapause induction, suggesting that s-LN<sub>v</sub>s are important as circadian clock neurons and play some role in photoperiodism. In the s-LN<sub>v</sub>s, PERIOD-immunoreactivity in the nucleus was highest at 12 h after lights-off and lowest 12 h after lights-on regardless of photoperiod. Thus, as in *D. melanogaster*, PERIOD nuclear translocation entrains to photoperiod and day-length information seems to be encoded in clock cells. Immuno-electronmicroscopy revealed synaptic connections from s-LN<sub>v</sub> to the pars lateralis neurons indicating that clock neurons could directly affect diapause control.

Our results suggest that circadian clock neurons, s-LN<sub>v</sub>s, are involved in time measurements and may synaptically signal day-length information to the pars lateralis neurons. We then have to determine how the clock neurons switch the activity of pars lateralis neurons, and how the number of day-length is counted or stored in the brain.

# **ARTHROPOD NERVOUS SYSTEMS: THEIR HISTORICAL IMPACT ON IDEAS ABOUT THE COMMON ORIGIN OF BRAINS, AND A MODERN REJECTION OF CONVERGENT EVOLUTION.**

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In 1850, Felix Dujardin proposed that parts of the insect brain were analogous to the human cerebral cortex. For many years after, the *idée fixe* was that any similarities between arthropod and vertebrate brains were due to convergent evolution. However, even before 1859 when Charles Darwin published “On the Origin of Species” there was intense debate about whether chordate and invertebrate nervous systems originated from a common ancestor. After 1859, Thomas Huxley gave serious thought to the problem, as did E. Ray Lankester who proposed a segmental ancestor of Annelida and Arthropoda. In 1875 Anton Dohrn, the founder of the Naples Zoological Station (and the originator of international collaboration) wrote a monograph addressed to the great embryologist Karl von Baer proposing an annelid origin of the chordates. This did not go down well in Europe, whereas in the English-speaking World the doyen of vertebrate morphology, Richard Owen, adopted Dohrn’s ideas lock stock and barrel

Descriptions of comparable organization in arthropod and mammalian brains have an honorable tradition: in 1883 by Bellonci, with reference to olfactory glomeruli in eels and crickets; in the 1890s by Gustav Retzius, with reference to neural organization in lamprey spinal cord and arthropod and annelid ganglia; by Ramón y Cajal in 1908, with respect to insect, vertebrate, and cephalopod visual system organization; and in the 1920s by the great Russian neuroanatomist Alexei Zavarzin, with cross phyletic reference to visual systems, segmental ganglia and spinal cord. Neural based phylogenies in the early 1920s by Nils Holmgren and Bertil Hanström claimed a deep segmental ancestor to Arthropoda but there was still hesitancy in claiming nervous system homology across Phyla. Most similarities suffered by being explained away as results of evolutionary convergence.

In 1996, Eddy De Robertis and Yoshiki Sasai showed that genes in frogs and flies sharing the same nucleotide sequences regulate the development of dorso-ventrality in the frog, and the reverse in the fruit fly. Six years later transphyletic rescue experiments by Heinrich Reichert and Katsuo Furukubo-Tokunaga showed that the human gene *Otx*, necessary for the development of the mammalian forebrain, rescued the development of the fly’s forebrain (protocerebrum) when it was engineered into the genome of a mutant fly lacking the homologous gene *otd* (*orthodenticle*). The reverse experiment worked in rescuing the mouse forebrain. These and similar experiments showed arthropod and vertebrate brains to share segmental homology. And, in 2010, Detlev Arendt and colleagues at the European Molecular Biology Laboratory showed that a suite of regulatory genes expressed in mushroom bodies of the developing polychaete brain corresponds to an identical suite expressed in the spatio-temporal pattern in the developing mouse pallium.

Comparisons of arthropod brains reveal corresponding ground pattern organization in numerous centers, and equivalence of organization in centers in vertebrate brains. However, “equivalence” is likely to be a misnomer: there is optimism that such similarities indeed represent genealogical correspondences and thus common ancestry. Insects in the fields and crayfish in the nearby streams are much more closely related to our species than many of its members would like to admit.

## IN-VITRO CONDITIONING IN *LYMNAEA*

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Stress alters adaptive behaviors including vigilance behaviors. In *Lymnaea* one of these vigilance behaviors is a heightened withdrawal response to a shadow. The shadow withdrawal response (SWR) is mediated by dermal photoreceptors located primarily on the foot, mantle cavity, and skin around the pneumostome area. Here we asked whether we could obtain a neural correlate of the heightened SWR following traumatic stress. We measured the electrophysiological properties of 'Right Pedal Dorsal 11 (RPeD11)', the interneuron that plays a major role in mediating the whole-body withdrawal response. In naïve animals the shadow stimulus elicited a rapidly habituating withdrawal response and a depolarizing response of 8 mV in RPeD11. However, in traumatized snails 24 hours after the trauma they responded to a shadow stimulus with an augmented withdrawal response. Their augmented response to a shadow lasted at least one week. Accompanying the behavioral change in these 'traumatized' preparations there are a number of significant changes in the neuronal properties of RPeD11 compared to naive preparations. For example, RPeD11 is significantly more depolarized (~10mV) has significantly larger input resistance, and the duration of the response elicited by the shadow persists longer. All these changes result in an increased RPeD11 response.

## IN-VITRO AVERSION CONDITIONING IN *LYMNAEA*

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Conditioned taste aversion (CTA) was carried out on the pond snail, *Lymnaea stagnalis*. The conditioned stimulus (CS) was sucrose which elicits feeding behavior, while the unconditional stimulus was a tactile stimulus to the head which causes feeding to be suppressed. The neuronal circuit that drives feeding behavior in *Lymnaea* is well work out. We therefore compared the electrophysiological responses elicited by the CS in conditioned vs. naive snails in 3 classes of neurons involved with feeding behavior. The cerebral giant cell (CGC) modulates feeding behavior, N1 medial neuron (N1M) is one of the central pattern generator neuron that organizes feeding behavior, whilst B3 is a motor neuron active during the rasp phase of feeding. We found no change in the resting membrane potential and spontaneous generating impulse frequency of the CGC between trained vs. naive snails. There was, however, a significant increase in spontaneous activity; a disappearance of bursting activity typically initiated by the CS. These neuronal modifications are consistent with the behavioral phenotype elicited by the CS following conditioning. In the CTA it has not yet established neuronal correlation between generation of fictive feeding rhythm and control of withdrawal behavior, we examined the neuronal connection between CGC and right pedal dorsall1 neuron (RPeD11) with electrophysiological techniques. RPeD11, which controls the withdrawal behavior, had monosynaptic inhibitory-chemical synapse with CGC. This synaptic connection was thought to be crucial for the CTA, we next examined whether the CGC, N1M and B3 behaved in the same way as observed in-vivo conditioning using semi-intact preparation.

# IMMUNOHISTOCHEMICAL STUDY ON DERMAL PHOTORECEPTOR IN *LYMNAEA*

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It is well known that gastropod mollusks have non-ocular dermal photoreceptors distributed around the body surface. Dermal photoreceptors in *Lymnaea stagnalis* play the key role for the whole body withdraw behavior from their predator in response to shadow stimulus. Physiological and behavioral characteristics on the dermal photoreceptor have been revealed (Chono et al., 2002; Pankey et al., 2010). Main findings from our previous study are followings; 1) photoresponse originated from dermal photoreceptor is mediated via right pedal dorsal 11 neuron (RPeD11) which is the key inter-neuron to control withdrawal behavior; 2) phototransduction in the photoreceptor is involved with cyclic nucleotide-gated channel (CNG gated channel); 3) phototransduction is selectively abolished with cis-diltiazem nor 2-APB (Pankey et al., 2010). These characteristics are good contrast to that of the ocular photoreceptors involved in TRP channel in *Lymnaea* (Sakakibara, 2006).

Concerning the morphological characteristics nothing has known yet on the dermal photoreceptor, we tried to identify the photoreceptor cell by immunohistochemical technique. We used the retinal rod photoreceptor CNG-anti body (anti-CNGA3), TRP antibody and rhodopsin antibody to the mantle. We found round-shape neuronal architectures of 50µm in diameter are selectively positive to anti-CNGA3 antibody at the periphery of both side of the mantle. To probe the functional role of these architectures we will show the light-off response electrophysiologically.

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## **PATTERNS OF SEROTONIN, FMRFAMIDE AND NO IN THE COMPLEX LIFE CYCLE OF DIGENEA.**

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Parasites within the Class Trematoda (Subclass Digenea) are characterized by a complex life cycle comprising several developmental stages. Little is known about the development of neuronal signal systems during the life cycle of trematodes.

The localization of serotonin (5-HT), peptide FMRFamide, nitric oxide and  $\alpha$ -tubulin in the nervous system (NS) of twenty six species of the Digenea has been investigated by indirect immunofluorescence in combination with confocal scanning laser microscopy.

In our studies we observed a successive increase in complexity of the NS during the life cycle of the different species of the Digenea. In redia and sporocysts, the NS is simple and extends in only two dimensions and shows minor difference between species. Their NS consists of a bilobed brain and two main nerve cords (MCs) and minor cords. In cercariae, a typical orthogon occurs. During the development from cercaria to metacercaria and adult, the number of transverse commissures increases significantly, however, the number of marker neurons remains almost the same.

Analysis of the number and relation of 5HT-immunoreactive (IR) marker neurons and the general map of the trematode NS stained with anti-FMRFamide revealed the same trends. Cephalization, i.e. the concentration of neurons in the bilobed brain and the differentiation of neurons both structurally and by position, appears to be the major trends in the evolution of the NS within the Trematoda. In cercaria, the number of 5HT-IR neurons in the CNS correlates mainly to their size. In all orders of the Trematoda, forms with low level of neuronal differentiation were found, which indicates that the organization of the NS is not dependent of the systematic position. The evolution of NS has proceeded independently in the major taxons of Digenea although the trends are the same.

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## PHYSIOLOGICAL, BEHAVIORAL, AND LIFE-SPAN PLASTICITY IN *DROSOPHILA*

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Genetic studies in different organisms, from unicellular eukaryotes to humans, have revealed many evolutionarily conserved genes that subserve similar cellular functions. The fruit fly *Drosophila* offers a unique genetic model system in which well-established forward genetic methodologies can be combined with genomic analysis to facilitate high through-put discoveries of relevant genes that interact with previously identified key genes in classical mutant analysis of nervous system development and function. We demonstrate that this combined approach can now be applied to complex phenotypes that have been elusive in genetic or genomic studies. Social isolation in *Drosophila* can induce aggressive behaviors not common in group-reared individuals. Combined genetic and electrophysiological studies indicate that coupled with aggression, nerve and muscle excitability are greatly enhanced along with accumulation of intracellular reactive oxygen species (ROS) in isolated flies (Ueda and Wu, 2009). Conversely, we found that social interaction can be beneficial to certain short-lived mutant flies that are defective in ROS regulation. Our experiments demonstrate that interactive activity with co-housed younger “helpers” of different genotypes significantly extends the lifespan of the mutant flies of the Superoxide dismutase (*Sod1*) gene (Ruan and Wu, 2008). Further genomic analysis will help identify candidate key genes underlying such plasticity of physiological processes and behavioral phenotypes that are susceptible to modification by social interaction.

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## PHARMACOLOGICAL CHARACTERIZATION OF NEUROTOXIC EFFECTS OF CYANOBACTERIAL EXTRACTS IN MOLLUSCAN (CNS, HEART) MODELS

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The responses of the central neurons (*Helix pomatia*, *Lymnaea stagnalis*) and the isolated *Helix* heart were characterized after application of aqueous extracts made from strains of cyanobacteria isolated during natural algal blooms in the Balaton region (Hungary).

The nicotinic ACh receptors (both excitatory and inhibitory) in the CNS are similarly blocked by the extracts of some *Cylindrospermopsis* strains (ACT 9505) and the pure cyanotoxin (anatoxin-a), while enhancement of the ACh responses suggest acetylcholine esterase inhibitory effect of other strains (*Cylindrospermopsis*, ACT 9504). The neuronal effects of the *Aphanizomenon* extracts, moreover, suggested pharmacologically different receptors involved.

On the isolated *Helix* heart the excitatory effects of cyanobacterial extracts (frequency increase and transient twitch contraction), did not mimic the ACh evoked heart inhibition, suggesting that the spontaneous rhythmic activity is rather modulated by non-cholinergic receptors, or directly affecting the electrical or contractile mechanisms of the myogenic activity.

Analytical results, however, did not confirm the presence of any of the already identified cholinergic cyanotoxins (anatoxin-a, homoanatoxin-a, anatoxin-a(s)) in any of the extracts. Diversity of the cyanobacterial effects in the CNS and heart, therefore, suggest that some unidentified toxic metabolites produced by the cyanobacterial strains are acting on different receptors.

## NEURONAL MODULATION OF LARVAL GROWTH IN BAY MUSSEL *MYTILUS TROSSILUS* DEVELOPED IN VARIOUS SALINITIES

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It's known that among various abiotic factors salinity is one of the most essential influencing larval development. We demonstrated earlier that larval neurons expressing peptide FMRFamide (FMRFa) are involved in osmoregulation in gastropod larvae and neomycin (NM) modulate expression of FMRFa. In its turn, increased level of FMRFa induces changes in serotonin (5-HT) system (Chaban and Voronezhskaya, 2008). In recent work we investigated growth and expression of FMRFa and 5-HT within larval neurons of the bay mussel *Mytilus trossilus* cultivated in various salinities with addition of neomycin. Ampicillin was used as a positive control of antibiotic activity. Larvae were cultivated in normal (33‰), decreased (10‰) and increased (40‰) salinity from 2,5 days to 10 days after fertilization. Immunocytochemistry combined with LCSM was used to visualize larval neuronal structures.

Both neomycin and ampicillin resulted in 2-3 folds better survival of larvae in changed salinities. Note, that survival in 10 ‰ combined with NM was approximately 5,5 times high than in control. We observe the common trade in average veliger size: larvae became bigger in parallel with increased salinity. However, in 10 ‰ with NM 3,6% of animals had unexpectedly two times bigger shell size than control, and their shape resembled that of pediveligers. No abnormalities were found in morphology of either FMRFa- or 5-HT- immunoreactive neurons under all studied conditions. Immunostaining revealed the lowest FMRFa level within the larval nervous system in 10‰ while 33‰ and 40‰ looked equal. Incubation in NM increased general level of FMRFa while ampicillin did not affect it. Survival rate was positively correlated with low 5-HT expression in apical neurons and was minimal in 33‰.

Our results demonstrated that salinity affects larval growth and survival as well as expression of FMRFa and 5-HT by larval nervous system. We suggested that changes in neurotransmitter level reflect the process of larval adaptation to this environmental factor.

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## MECHANISM OF DELAYED ACTION OF SEROTONIN OVERPRODUCTION IN EARLY MOLLUSCAN DEVELOPMENT

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Serotonin (5-HT) is known to induce wide range of short-term and long-term (or delayed) effects. While the mechanisms of short-term 5-HT action are investigated in great detail in both vertebrate and invertebrate preparations, delayed actions of 5-HT are still *terra incognita*.

Earlier we demonstrated the phenomenon of 5-HT-induced exogastrulation (5-HT-EG) in development of pulmonate mollusc *Lymnaea stagnalis* (Voronezhskaya et al., 2005). The short time-window application of serotonin precursor (5-hydroxytryptophan, 5-HTP) during early cleavage stages resulted in distinct morphological malformation at the third day after fertilization. In the present study we utilized 5-HT-EG model to find out the intracellular mechanisms involved in delayed action of 5-HT in Molluscs.

Pharmacological analysis revealed that simultaneous enhancement of 5-HT level inside the cytoplasm and in the external medium during early cleavage stages induce exogastrulation in concentration dependent manner. Separate increasing of either inner or outer 5-HT level has no effect. Inhibition of serotonin synthesis or membrane serotonin transporter (SERT) blocked 5-HT-EG. Activation of mixed-type 5-HT receptor with pharmacological profile similar to 5-HT<sub>2</sub> receptors but with intracellular pathway which includes adenilate cyclase and PKA was necessary for 5-HT-EG induction. Immunocytochemistry demonstrated expression of 5-HT<sub>2</sub>*Lym* receptor at the surface of blastomers membrane during early cleavage stages. ELISA measurements and semi-quantitative LCS microscopy immunocytochemistry confirmed that SERT inhibition increases intracellular and reduces external level of 5-HT.

Based on the obtained data we propose a possible model of balanced 5-HT system which is sensitive to the 5-HT content inside and outside the embryo. Changes in 5-HT<sub>in</sub>/5-HT<sub>out</sub> ratio underlay the delayed actions of 5-HT in Molluscs. The possible role of such system may be regulation of early development via adult-embryo interaction conveyed through modulation of 5-HT level within the mother organism.

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**IDENTIFICATION AND EXPRESSION ANALYSIS OF THE GENES INVOLVED IN  
THE BIOGENIC AMINE SYSTEMS IN THE FIELD CRICKET *GRYLLUS  
BIMACULATUS***

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Biogenic amines regulate various aspects of behavior in the cricket. Pharmacological and behavioral studies have revealed the involvement of specific biogenic amines in modulating learning, circadian behavior, mating, and aggressive behaviors in the cricket. Although physiological aspects of the biogenic amine system have been extensively studied, the molecular basis of the cricket biogenic amine system has not been well investigated. To elucidate the molecular basis of the cricket biogenic amine system, we identified genes involved in biosynthesis and transduction of serotonin (5-HT), octopamine (OA), and dopamine (DA) in the field cricket *Gryllus bimaculatus* DeGeer.

We identified seven genes involved in biogenic amine synthesis including three genes involved in 5-HT synthesis (*TR*ryptophan *H*ydroxylase, *TRH*; *T*ryptophan *P*henylalanine *H*ydroxylase, *TPH*; *A*romatic *L*-*A*mino acid *D*e*C*arboxylase, *AADC*), three genes involved in OA synthesis (*T*yrosine *D*e*C*arboxylase, *TDC1* and *TDC2*; *T*yramin  $\beta$ -*H*ydroxylase, *T $\beta$ H*), and two genes involved in DA synthesis (*T*yrosine *H*ydroxylase, *TH*; *AADC*), as well as thirteen GPCR-type biogenic amine receptors including five 5-HT receptors, five OA receptors, and two DA receptors.

Analysis of tissue-specific expression of biogenic amine-related genes revealed the sites of synthesis and reception of biogenic amines in the cricket. Our study is the first comprehensive analysis of the biogenic amine-related genes in insects.

## SELECTIVE VISUAL ATTENTION IN *DROSOPHILA*

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Selective visual attention is exemplified by an animal's ability to respond selectively to only one out of two or more competing visual stimuli, while the response to the simultaneously presented residual stimuli is suppressed. During tethered flight at the torque meter *Drosophila* displays visual attention by spontaneously restricting its yaw torque response to parts of the visual field, if in the two lateral halves of the visual field two equally strong stimuli are concurrently moving in opposite directions. As is characteristic for selective attention, the fly's yaw torque tends to alternately follow the competing stimuli, resulting in a zero value for long term averages. However, the fly's yaw torque response can be shifted to one side if on this side an additional guiding stimulus is presented to the fly. The guiding stimulus can be a single visual object (vertical stripe), or a change of the background color from blue to green or vice versa in one half of the panorama, or it can be an odor. Surprisingly, no systematic torque response can be observed if the new color is presented alone, without the simultaneous presentation of the competing motion stimuli on both sides. However, if the fly is allowed to manipulate the appearance of the new color on one side of the panorama by its own yaw torque or leg posture, a preference for that new color can be observed. Depending on the features of the guiding stimulus the guidance effect can be positive (attractive) or negative (repellent). Even after a delay of up to 5 seconds between the presentation of the guiding stimulus and the onset of the test stimuli a systematic shift of the fly's attention can be observed. The mushroom bodies, which have been shown to be implemented in decision making and context generalization, are dispensable for this kind of attention.

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## CONTROL OF LOCOMOTION IN TERRESTRIAL SNAIL BY PEPTIDES

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The terrestrial snails crawls using waves of muscular contraction that spread along the sole of its foot. Injection of serotonin switches on the generation of pedal muscular waves on the sole of the foot and increases the speed of locomotion. Earlier studies indicate the role of cluster of serotonergic neurons in pedal ganglia in the control of locomotion in different mollusks. We report here that different identified serotonergic neurons of the cluster express different neurospecific genes, that encode the secreted proteins in the snail. Using *in situ* hybridization and histochemistry the expression pattern was described for 5 genes – earlier discovered genes *HCSI*, *HCS2*, *HelSFamid*, *HPep*, *GFAD* and novel gene named *HelixSFamid* also expressed in the identified group of serotonin-containing cells of pedal ganglia. Preprotein pre*HelixSFamid* have similarity with known peptides *LymnaDFamide* and *Tritonia* pedal peptide, contains at the N-terminus a hydrophobic leader and ten putative amidated neuropeptides. *In situ* hybridization experiments demonstrated that *HelixSFamid* gene is selectively expressed in several identified neurons of pedal, cerebral and pleural ganglions. Apparently, peptides of pre*HelixSFamid* participate in the organization of feeding behavior of terrestrial snail.

The peptide-cofactors under investigation have different effects on locomotion of mollusks *Helix* sp. Injection of tetrapeptide *GFAD*, encoded by gene *GFAD*, causes decrease of the speed of locomotion and even switch-off of locomotion, but “pedal peptide” – encoded by gene *HPep*, - increases the speed of locomotion of the snail. Injection of *HelSFamid* also increases the speed of locomotion and increase the instability of negative gravitaxis of the animal.

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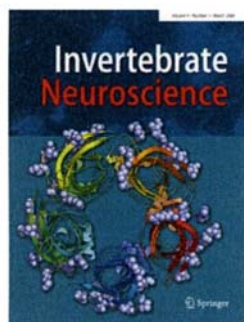
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