X East European Conference of the International Society for Invertebrate Neurobiology

"Simpler Nervous Systems"

Moscow, Russia, September 6-10 , 2012

Program

6 SEPTEMBER
from 12.00 Hotel accommodation.
16.00 - 17.00 Registration (Institute Higher Nervous Activity and Neurophysiology, RAS, Butlerova 5A).
Chairs: Ito E. (Japan), P.M.Balaban. (Russia)
17.00 - 17.10 Balaban P.M. (Moscow, Russia), Ito E. (Sanuki, Japan)
OPENING ADDRESS.
17.10 - 17.30 Sakharov D.A. (Moscow, Russia)
"THE ROOTS OF THE INTERNATIONAL SOCIETY FOR INVERTEBRATE NEUROBIOLOGY (ISIN)"
17.30 – 18.10 Reznikova Zhanna (Novosibirsk, Russia)
WHAT "SIMPLE" BRAINS ARE CAPABLE OF: COGNITIVE SPECIALIZATION IN ANTS
18.30 Welcome Party

7 SEPTEMBER
Chairs: Gillette R., Zakharov I.S.
10.30 - 11.00 Posters mounting (in alphabet order of first author names from A to M inclusive)
11.00 - 11.50 Kemenes G. (Brighton, UK)
DYNAMIC CELLULAR AND MOLECULAR MECHANISMS OF MEMORY: NEW INSIGHTS FROM MOLLUSCAN MODELS
11.50 - 12.30 Alania M. (Tbilisi State University, Tbilisi, Georgia)
THE STOMATOGASTRIC NERVOUS SYSTEM OF THE MEDICINAL LEECH
12.30 - 13.00 Nikitina E.A. Dolgaya Yu.F., Utesheva N.K., Savvateeva-Popova E.V. (St.Petersburg, Russia)
HSP70 INTRACELLULAR LOCALIZATION IN DROSOPHILA cd MUTANT OF THE KYNURENINE PATHWAY
13.00 - 14.00 Lunch
14.00 - 16.00 Poster session 1-30

Chairs: Reznikova Z. (Novosibirsk, Russia), Puthanveettil S. (Florida, USA)
16.00 - 16.50 Frasnelli E. (Altenberg, Austria)
LATERALIZATION IN THE INVERTEBRATE NERVOUS SYSTEM
16.50 - 17.20 Zapara T.A., Romashthenko A.V., Ratushnyak A.S. (Novosibirsk, Russia)
EXPERIMENTAL ANALYSIS OF TICK SYNGANGLIA (IXODES PERSULCATUS) NEUROTRANSMITTER SYSTEMS
17.20 - 17.50 Nikitin ES, Korshunova T, Balaban PM, Kemenes G. (Moscow, Russia; Brighton, UK).
LEARNING-INDUCED ALTERATIONS OF AXONAL SPIKE PROCESSING AND LONG-TERM ASSOCIATIVE MEMORY IN LYMNAEA
17.50 - 18.10 Young investigator talk. Atsarkina N.V., Iakovlev I.K., Reznikova Zh.I. (Moscow- Novosibirsk, Russia).
COGNITIVE POTENTIAL OF THE "INTELLECTUAL ELITE" IN AN ANT COLONY: A BEHAVIOURAL PORTRAIT OF A SCOUT

8 SEPTEMBER
10.30 - 11.00 Posters mounting (N to Z)
Chairs: Mozzachiodi R. (Texas, USA), Kamyshev N.G. (St.Petersburg, Russia)
11.00 - 11.50 Puthanveettil Sathya (The Scripps Research Institute, Florida, USA)
MOLECULAR MECHANISMS OF INITIATION AND PERSISTENCE OF MEMORY STORAGE.
11.50 - 12.30 Sidorov A.V. (Minsk, Belorussia)
REACTIVE OXYGEN SPECIES MODIFY OPERANT CONDITIONING OF AERIAL RESPIRATORY BEHAVIOR IN MOLLUSK LYMNAEA STAGNALIS
12.30 - 12.50 Young investigator talk. Dorosheva E.A., Reznikova Zh. I.
CATALOG LEARNING IN INVERTEBRATES
13.00 - 14.00 Lunch
14.00 - 16.00 Poster session (30-60)
Chairs: Prof. Kemenes G. (Brighton, UK), Prof. Sakharov D.A. (Moscow, Russia)
16.00 -16.50 Gillette R. (Urbana, USA).
A BASIC MODULE FOR COST-BENEFIT DECISION.
16.50 - 17.30 Vedenina V., Pollack G. (Moscow, Russia; Montreal, Canada).
FIXED AND FLEXIBLE TRAITS IN MATING SIGNALS: EVOLUTIONARY AND PHYSIOLOGICAL BACKGROUNDS.
17.30 - 17.50 Young investigator talk. Panova A. A. (St.Petersburg, Russia).
INFLUENCE OF GROUP KEEPING ON LOCOMOTOR ACTIVITY AND COURTSHIP BEHAVIOR OF Drosophila MALES
18.00 - 18.30 Balaban P.M. (Moscow, Russia). MEMORY AND GENOMICS.

9 SEPTEMBER
Chairs: Frasnelli E. (Altenberg, Austria), Alania M. (Tbilisi, Georgia)

11.00 - 11.50 Mozzachiodi R. (Texas, USA).
CONSEQUENCES OF AVERSIVE EXPERIENCE IN APLYSIA: PLASTICITY BEYOND DEFENSIVE NEURAL CIRCUITS.
11.50 - 12.20 Malyshev A. (Moscow, Russia).
EVOLUTIONARY ORIGIN OF THE HOOKS IN THE BUCCAL APPARATUS OF CLIONE LIMACINA: NEUROPHYSIOLOGICAL EVIDENCES.
12.20 - 12.40 Young investigator talk. Tolstenkov O.O., Prokofiev V.V., Sungatulina A.A., Sizhiuhina S., Terenina N.B., Gustafsson M.K.S. (Moscow, Russia; Abo, Finland).
EFFECT OF SEROTONIN ON MOTILITY OF CRYPTOCOTYLE LINGUA CERCARIAE (TREMATODA).
12.40 - 13.00 Young investigator talk. A.B. Zuzina, M.S. Lemak, A. Timoshenko, A.Y. Malyshev, P.M. Balaban (Moscow, Russia).
ROLE OF ATYPICAL ISOFORM OF PROTEIN KINASE C IN MAINTENANCE OF LONG-TERM SYNAPTIC POTENTIATION IN THE SNAIL NEURONS
13.00 - 15.00 Lunch
Chairs: Sidorov A. (Minsk, Belorussia), Malyshev A. (Moscow, Russia)
15.00 - 15.50 Ito E. (Sanuki, Japan).
ROLE OF MOLLUSCAN INSULIN-RELATED PEPTIDES IN FORMATION OF LONG-TERM MEMORY OF THE POND SNAIL LYMNAEA
15.50 - 16.40 Kamyshev N.G. (St.Petersburg, Russia).
FORWARD GENETIC APPROACH: IS IT EXHAUSTED?
19.00 sharp! Start of Boat excursion.

10 SEPTEMBER
DEPARTURE.

ABSTRACTS

IN ALPHABETICAL ORDER

STOMATOGASTRIC NERVOUS SYSTEM OF MEDICINAL LEECH

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The stomatogastric (STG) nervous system of decapod crustaceans has contributed significantly to the present understanding of rhythmic motor circuits. Little is however known of the cephalic STG nervous system of another important invertebrate preparation for neuroscience - the medicinal leech, *Hirudo medicinalis*. Feeding is in the dominant position of behavioral hierarchy in the medicinal leech and at least two decision-making modules mediate suppression of competing behaviors within the nervous system. The stereotyped movements and glandular activities underlying feeding of the medicinal leech were described earlier in detail and integrative function of serotonin was suggested for this behavioral pattern (Lent, 1985, *Brain Res. Bull.* 14, 643–655). Recent research demonstrates that the generation of feeding behavior in the adult medicinal leech is attributed to the STG system (Mesce, Alania et al., 2008, Soc. Neurosci. Abst. 574.8). Specifically, two novel ganglia were identified in the STG nerve ring; localization of biogenic amines serotonin and dopamine in the STG nerve ring was defined; the earlier described pair of serotonergic LL neurons from the headbrain was characterized in relation to the STG nerve ring; electrophysiological recordings and visualization of intrinsic interneurons in the STG ganglia were performed for the first time. More detailed characterization of STG nerve system of medicinal leech could add new insights in the comprehension of motor pattern generation in general.

COGNITIVE POTENTIAL OF THE “INTELLECTUAL ELITE” IN AN ANT COLONY: A BEHAVIOURAL PORTRAIT OF A SCOUT

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In red wood ants division of labour within a colony is based on behavioural rather than morphological features. Recently, deep specialization has been revealed within groups of aphid tenders: they include members of different "professions" (shepherds, guards, transporters and scouts) which stay in stable cohesion for days and weeks (Reznikova, 2011). A laboratory model of this vital situation is reflected in the "binary tree" experimental paradigm: red wood ants enjoy sophisticated communication based on task allocation between scouts and foragers; scouts are able to memorise and pass the information about a sequence of turns on the way to a feeder, whereas foragers are not (Reznikova, Ryabko, 1994, 2011). What makes an ant a scout is still enigmatic.

In laboratory experiments on *Formica aquilonia* we studied at the individual level 11 scouts, 30 foragers and 20 control (randomly selected) ants using a round maze and a binary tree maze for testing ants' ability to memorize their way home, and pieces of "artificial world" for examining their exploratory activity. We also tested individual ants' aggressiveness towards ground beetles, their natural enemies (Reznikova, Dorosheva, 2004). In comparison with the control, the foragers and, especially, the scouts appeared to be more mobile, fearless and aggressive. The most expressive features of scouts include the highest level of exploratory activity towards novel items and situations, and the tendency to switch between different types of activity. Our preliminary data suggest that the scouts form spatial memory faster and keep the information longer and more precisely than foragers.

BIOLISTIC DELIVERY OF VOLTAGE-SENSITIVE DYES FOR FAST RECORDING OF MEMBRANE POTENTIAL CHANGES IN INDIVIDUAL NEURONS.

Nikolay Aseyev, Matvey Roshchin, Victor N. Ierusalimsky, Pavel M. Balaban, Evgeny S. Nikitin

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Optical recording of membrane potential changes with fast voltage-sensitive dyes (VSD) in neurons is one of the very few available methods for studying generation and propagation of electrical signals to the distant compartments of excitable cells. The more lipophilic VSD is, the better signal-to-noise ratio of the optical signal can be achieved. However, there are no effective ways to deliver water-insoluble dyes into the membranes of live cells. Here, we report a possibility to stain individual live neurons with highly lipophilicVSDs in acute brain slices using biolistic delivery. We tested a range of four ANEP-based VSDs with different lipophilic properties and revealed their ability to stain single neurons in a slice area of up to 150 □m in diameter after being delivered by a biolistic apparatus. In the

slices of neocortex and hippocampus, the two most lipophilic dyes, di-8-ANEPPS and di-12-ANEPPQ showed cell-specific loading and Golgi-like staining patterns with minimal background intensities. Simultaneous patch-clamp and optical recording of biolistically stained neurons demonstrated good match of optical and electrical signals both for spontaneous APs (action potentials) and stimulus-evoked events. Our results suggest high efficiency of fast and targeted method of biolistic delivery of lipophilic VSDs for optical signals recording from mammalian neurons in vitro.

NEUROGENETICAL REGULATION OF SYNAPTIC PLASTICITY

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The maintenance mechanism of the late, protein synthesis dependent phase of long-term potentiation (LTP) of synaptic effectivity is critical for the storage of long-term memory. Although the expression of the early induction phase of LTP has been studied extensively (Bliss and Collingridge, 1993), the mechanism for synaptic enhancement during the late phase of LTP when neurogenetical mechanisms play an essential role is unknown. One approach to identify the expression mechanism of synaptic enhancement during late-LTP is toexamine how the molecular mechanism that maintains late-LTP enhances synaptic transmission. A prime candidate for a core molecule maintaining late-LTP is protein kinaseMZ (PKMZ), an autonomously active isozyme of protein kinase C (PKC). PKMZ maintains synaptic enhancement during late-LTP through its second-messenger-independent and thus persistent kinase activity. PKMZ consists of an independent PKCZ catalytic domain produced from a brain-specific PKMZ mRNA, which, lacking an autoinhibitory PKCZ regulatory domain, is constitutively active (Sacktor et al., 1993; Hernandez et al., 2003). During LTP induction, afferent tetanic stimulation increases the synthesis of PKM_ from its mRNA(Hernandez et al., 2003; Kelly et al., 2007), and the resulting persistent increase in the autonomously active kinase is both necessary and sufficient for maintaining LTP (Ling et al., 2002). Postsynaptic perfusion of PKMZ increases the efficacy of AMPA receptor (AMPAR)-mediated synaptic transmission (Ling et al., 2006), whereas inhibitors of the kinase activity of PKMZ reverse LTP even when applied hours after the tetanization, without affecting baseline synaptic transmission.

We have investigated involvement of PKMzeta in maintenance of contextual memory in the terrestrial snail Helix. Results suggest that blockade of PKMzeta impairs memory with latency about 1 hr. Reminding addresses memory and starts the process of reconsolidation of memory even if the “old” memory later is impaired by ZIP.

Long-term facilitation of excitatory synaptic inputs from sensory neurons to giant premotor interneurons triggering tentacle withdrawal is supposed to be a

basis of aversion learning and memory in terrestrial snails. We investigated whether PKMzeta takes part in maintenance of long-term facilitation in neural circuit of tentacle and body withdrawal. Long-term facilitation of excitatory synaptic inputs to premotor interneurons was induced by 5 high-frequency stimulations combined with 5 serotonin bath applications and lasted at least four hours. We found that bath application of 2*10-6M ZIP at 90 min after tetanization reduced EPSP amplitude almost to the non-tetanized EPSP values (57±7%, n=12 vs control 44±6%, n=25, NS). Application of scrambled ZIP peptide at the same concentration didn't affect the amplitude of potentiated EPSPs (135±28%, n=13 vs control 44±6%, n=12, $p<0.01$). Results supported the idea of PKMzeta involvement in post-induction maintenance of long-term plasticity in withdrawal circuit of Helix lucorum. Changes in synaptic effectivity of functionally identified neurons observed during LTF may underlie behavioral changes during context learning.

Thus, late-LTP persistence can be explained by a plasticity mechanism, in which the synthesis of an autonomously active kinase continually drives receptors to postsynaptic sites. This persistent but dynamic neurogenetical mechanism may allow for stable yet flexible storage of information at synapses to be the physiological substrate of long-term memory.

IMMUNE PROTEOSOMES IN SNAIL CNS

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The proteasome is a multicatalytic protein complex that plays an important role in intracellular protein degradation from archaebacteria to eukaryotes. This complex is made up of two copies each of seven different alpha and seven different beta subunits arranged into four stacked rings (alpha7beta7beta7alpha7). Proteolysis by the ubiquitin-proteasome pathway (UPP) is now widely recognized as a molecular mechanism controlling normal functions in the nervous system. Immunoproteasome alpha-subunits expression in snail (Helix lucorum L.) was investigated. Using Western-blot analysis, the alpha-subunits were detected in the ganglions of CNS. Immunofluorescence revealed that proteasome-immunopositive materials were mainly located in cells of procerebrum - important processing centre for olfaction in snail. Previously we revealed neurogenesis in procerebrum in juvenile and adult animals. The immunoproteasome expression in cells of procerebrum might indicate important functions of this ganglion in CNS and correspond with reorganization of morphology of neurons. Supported by RFBR grant 10-04-01726

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REVERBERATION OF NERVE IMPULSES IN CRAYFISH NERVE CORD: THE ROLE OF LATERAL GIANT AXON SYSTEM

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The neuronal circuitry responsible for the escape tail-flip of the crayfish has been extensively studied, and it is now one of the best-known "simple nervous systems" in the animal kingdom. We would like to call attention to one puzzling feature revealed in those neural networks, which remained unsearched for many years. In the isolated abdominal nerve cord of the crayfish, a single short electrical pulse may evoke the repetitive high-frequency discharge. This phenomenon never was commented, though it seems hardly compatible with recent evidence concerning the lateral giant axon circuitry underlying tail-flip escape reaction in the crayfish. We attempt to explain the repetitive discharge of the lateral giant axons in the light of recent data about their structure and function. This phenomenon is caused by circulation of nerve impulses in loops of the lateral giant axon system resulting from their ladder-like structure. One may suggest that it is a remnant of evolutionary past when the lateral axons had chemical connections with their output neurons (fast flexor and swimmeret motor neurons). This easily reproduced phenomenon may be used for experimental study of functional abilities of commissural junctions as well as for model studies of reverberatory processes in various structures.

POSTTRANSLATIONAL HISTONE'S MODIFICATION IN EPIGENETIC MECHANISMS OF MEMORY FORMATION

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Epigenetic mechanisms of memory are posttranslational histone and DNA modifications, leading to the induction of genes expression important for plastic rearrangements of nervous system under environmental changes. The goal of this study was the investigation of the role of histone acetylation and methylation in food aversion reflex formation in mollusk Helix. To the current knowledge, acetylation of histones only activates gene expression, while methylation can both activate expression of the genes and repress it.

Here we found, that in CNS of Helix during learning (food aversion reflex formation) there is induction of acetylation and methylation of Histone H3 on 2 different sites (trimethylation of histone H3 on lysine 4) and (dimethylation on lysine 9). It is known, that dimethylation of histone H3 on lysine 9 leads to the repression of transcription, and trimethylation on lysine 4 leads to induction of transcription.

Our data suggest that epigenetic changes on the chromatin during food aversion reflex formation can be the reason for induction and repression of the genes and result in memory consolidation. At the same time repression of inhibitory transcription factors can be achieved through the induction of methylation of inhibitory sites. On the other hand, methylation on Lysin 9 can actively influence habituation processes, which, as was shown by I.P. Pavlov, play an important role in CNS functioning and are poorly studied on molecular level.

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ROLE OF ADENYLATE CYCLASE SYSTEM IN RECONSOLIDATION OF CONTEXT CONDITIONING IN TERRESTRIAL SNAILS

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In the present work we studied a role of the adenylate cyclase system in mechanisms of reconsolidation of the long-term memory, as well as duration and stability of the retention of conditioned environmental reflex and amnesia, appearing at the disruption of reactivation of the memory. Process of the elaboration of conditioned environmental reflex (CER) in snails Helix lucorum conducted in standard conditions on the ball. Before the beginning of learning snails were tested, for this measure an amplitude of draw back of ommatophores in response to tactile stimulation of front part of legs and value of an ommatophores reactions changes. Procedure of learning lasted 5 days, snails of experimental group each day received on 5 electrical stimuli with the interval 15-20 minutes (1-2 mA, 1 sec, 50 Hz). On the following day after testing, confirmatory elaboration of CER, snails were placed on 20 min. on the ball (in the situation, in which conducted learning) that serve a reminder of terms of the elaboration of CER, but then they were immediately injected in the region of sinus by 8Br-cAMP in concentrations $2x10^{-4}$ Mol/l and anisomycine (AN), soluble in 0.2 ml of saline solution (SS) in the dose 0.4 mg to one snail. Other group for the comparison after the reminder was vehicle injected.

The injection of SS to control snails after the reminder did not elicit any changes in the defensive reactions. In snails, injected with AN, the reminder blocks the CER (Gainutdinova et al., 2003). Such forgetting reflects the presence of the

process of memory reconsolidation. It was found that after injection of 8Br-cAMP before the AN, a significant increase in amplitude of the defensive reactions was observed demonstrating prevention of the memory disruption by the protein biosyntheses blockade using 8Br-cAMP during the process of reconsolidation. The retention of the long-term memory was found in the second series of experiments on CER under injections of SS and its disruption under protein synthesis blockade by AN. The memory disrupted by AN applied after the reminder was not restored.
This work is supported by RFBR, grant 12-04-00235.

CATALOG LEARNING IN INVERTEBRATES

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In our experiments with insects we have revealed a relatively simple, universal and quite "natural" form of learning. The idea is that sometimes animals do not learn to do something really new in order to gain advantage from their environment; instead, they learn to select more and more quickly among several innate behavioral patterns. In this study we simulated territorial interferences between beetles and red wood ants and revealed that four carabid species modified their behaviour in order to avoid damages from aggressive ants. Beetles learned to select more and more successfully the relevant patterns from the (apparently innate) set of seven patterns, common to all four species. It is likely that this form of learning, which we call "catalog learning", is present in a wide variety of species, both invertebrates and vertebrates, and, together with associative learning, can be considered a basic element of animal cognition. It should be noted that whereas latent learning, learning set formation, rule extraction, and social learning can be attributed to cognitive abilities, catalog learning, guided learning (Gould. Marler, 1987), and imprinting (Lorenz, 1935) are based on innate predisposition to build up one set of associations more readily than another. Among these more or less "pre-programmed" forms of learning, "catalog learning" is based on a "stimulus – pattern" model (Reznikova, 2012).

The study was supported by RFBR (11-04-00536).

DNA-DEPENDENT MECHANISMS OF AVERSIVE MEMORY IN MOLLUSK HELIX LUCORUM

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Synaptic plasticity underlying long-term memory requires new protein synthesis. But how a short-lived molecules can produce a long-term changes still remains unclear. Here we used the terrestrial snail, Helix lucorum (Gastopoda, Pulmonata), to assess whether DNA synthesis in neurons correlates with long-term memory, by means of behavioral single-trial conditioning together with injections of nucleoside analogs, which incorporate into newly synthesizing DNA, and its immunohistochemical detection. We show that after single-trial aversion conditioning on a new food (paired with 10% quinine application) or a new taste (paired with 0.3M LiCl injection) long-term memory lasting no less than 14 days is established, and injection of 3'-azido-3'-deoxythymidine (AZT), reversed transcription inhibitor, 30 min before training disrupted memory tested at 24 h. Attenuation of taste neophobia was prominent at 24 h, but not at 14 days after training, and was not impaired by AZT. 5-bromo-2'-deoxyuridine (BrdU) immunohistochemistry 24 h after food aversion have shown BrdU incorporation into nuclei of giant pleural and parietal interneurons and rostro-medial region of pedal ganglia known to be crucial for aversive learning. These observations indicate, that a new DNA synthesis is critical for aversive memory in mollusks, and activation of reversed transcription may be involved in these mechanisms.

Genetic determinants of generating the motor pattern of rhythmic movements in *Drosophila melanogaster*

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The aim of our research is identification of *Drosophila* genes responsible for central pattern generator (CPG) functioning. In 2011, the list of 22 candidate genes for these neuronal processes was generated by methods of forward genetics. Mutations of candidate genes result in locomotor and courtship song deviations. To determine what candidate genes really impact the CPG development and functioning we tested locomotor parameters in lines with post transcriptional gene silencing in *Drosophila* central nervous system (CNS). Silencing was provided by synthesis of interfering RNA by means of GAL4/UAS system under control of CNS-specific gene promoters (*elav, appl, nrv2, tsh*).

It was found that locomotor activity was essentially lower in transgenic control and experimental lines in comparison to the wild type. However, we revealed that knockdown of candidate gene CG15630 led to opposite deviations in run duration under control of different promoters. While RNA interference (RNAi) directed by *appl* and *tsh* promoters reduces the run duration, RNAi under *elav* promoter control, conversely, increases run duration. *elav* is expressed exclusively in CNS and therefore these results indicate that CG15630 is participant of neural mechanism responsible for locomotor parameters. *tsh* is expressed mostly in thoracic-abdominal ganglia, where CPG structures function. These results demonstrate the high probability of involving the CG15630 gene product in motor pattern generation and indicate the good perspectives in exploration of role of the other genes in realizing the motor functions.

The study was supported by grant from Ministry of Education and Science of Russian Federation (contract P316), the programs of Presidium of the Russian Academy of Sciences "The Living Nature" and "Mechanisms of integration of molecular systems for realization of physiological functions".

LATERALIZATION IN THE INVERTEBRATE NERVOUS SYSTEM

Elisa Frasnelli

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Traditionally, only humans were thought to have strong left-right asymmetries in brain and behaviour, but recent studies have revealed that most vertebrates and invertebrates are indeed lateralized. Further, it has become apparent that two patterns of lateralization exist across species. In "individual-level" lateralization an equal number of left- and right-biased individuals coexist in the species, while in "population-level" lateralization a majority of individuals is right- or left-biased. The latter is the case of humans, as exemplified by handedness. While individual-level lateralization may have evolved because it increases individual efficiency, population-level lateralization is unrelated to individual efficiency, and remained unexplained until a few years ago, when it was suggested that the alignment of lateralization at the population level might evolve as an Evolutionary Stable Strategy when individually asymmetrical organisms must coordinate their behaviour with that of other asymmetrical organisms. Game-theoretical models developing this idea and considering group-living individuals engaging in intraspecific and interspecific interactions predict that lateralization at the population-level is more likely to characterize social rather than solitary species. Empirical data supporting this hypothesis has been recently obtained comparing olfactory responses of the right and the left antenna using behavioural (conditioning of the Proboscis Extension Reflex, PER), physiological (ElectroAntennoGraphy, EAG) and morphological (Scanning Electron Microscopy, SEM) measurements in three species of Hymenoptera Apoidea:

eusocial honeybees (*Apis mellifera* L.), solitary mason bees (*Osmia cornuta* L.) and bumble bees (*Bombus terrestris* L.), an annual eusocial species. In honeybees, the extent of the asymmetry has been investigated also in the volume of the primary olfactory centres of the central nervous system, the antennal lobes, using two-photon microscopy.
Moreover, evidences about the role of antennal asymmetries in social interactions have been provided in highly social ant species belonging to *Formica rufa* group. The analyses of "feeding" contacts where a "donor" ant is exchanging food with a "receiver" ant through trophallaxis revealed a population-level asymmetrical use of the right and left antenna. Overall, these results seem to support the hypothesis that brain and behavioural lateralization at the population level has evolved under social selective pressures as a strategy to optimize coordination among asymmetrical individuals.

CHANGES IN EXCITABILITY OF COMMAND NEURONS OF TERRESTRIAL SNAIL UNDER EXTRACELLULAR SEROTONIN DURING ELABORATION OF THE CONDITIONED REFLEX

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Serotonin is one of widespread and well-studied transmitters of the nervous system. Last years many experiments were carried out on cellular analogues of learning, in which application of serotonin was used for reproducing the plasticity phenomena. These experiments have shown a determinating function of serotonin in synaptic facilitation. We studied the role of serotonergic system in mechanisms of elaboration of conditioned reflex in identified neurons of terrestrial snails.

We studied the changes of characteristics of command neurons (CN, changes of membrane and threshold potentials) under the application of serotonin in concentrations 1 microMol/l in preparations made from intact and learned snails. It was found that the application of serotonin decreased the membrane potential of CN in intact and in learned snails. In learned, but not in intact snails the application of serotonin increased the threshold potential, indicating a decrease of excitability of CN. It is known that one of the ways of action of serotonin is an activation of the adenylatecyclase system. We studied the direct effects on this system and system of phosphodiesterases in experiments on the isolated preparations. After application of 8-Br-cAMP and forskolin, the depolarizing shift of membrane potential in CN was found. This effect was similar both in intact and learned snails. Under the action of nonspecific inhibitor of phosphodiesterases IBMX, we observed the depolarizing shift of membrane potential in learned snails, but not in intact.

This work is supported by RFBR, grant 12-04-00235.

NEW FORM OF FEEDING BEHAVIOUR IN THE FRESHWATER SNAIL *Lymnaea stagnalis* (L.).

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Monitoring of behavior of the juvenile freshwater snail *Lymnaea stagnalis* (L.) reveals previously not described form of behavior. It is known that the pond snail can often be observed moving just below the surface of the water with the foot on the surface. We found that in this position the juvenile animal periodically generates a mucous web that can be near the size of their bodies. Food particles dispensed in water are trapped within those webs by accomplished ciliary activity on the foot. Then this mucous boll is transferred to the mouth via suction movement of the buccal mass. This type of feeding behavior is typical for small juveniles but time–to time can be observed even in adult snails in addition to usual forms of feeding/biting movements. Previously such type of feeding was described for euthecosome pteropods (*Limacina helicina),* which capture the planktonic food particles by means of an external mucous web and for the sedentary mollusks vermetids which are attached to hard surfaces.
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A BASIC MODULE FOR COST-BENEFIT DECISION

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Concrete examples of computation and implementation of cost-benefit decisions at the level of neuronal network circuits are largely lacking. Such decisions are based on appetitive state, which is a moment-to-moment integration of sensation, internal state and memory. From that computation emerge stimulus valuation and risk assessment to be expressed in behavioral choice. Value-based decisions are accessible in neuronal circuitry of the predatory sea-slug *Pleurobranchaea,* a simple model system in which appetite is readily quantified in behavior and related to decisions for approach-avoidance. *W*e found that the excitation state of the feeding motor network of *Pleurobranchaea* both manifested appetitive state and controlled expression of orienting vs. avoidance turns. Isolated CNS from hungry and non-hungry donors conserved appetitive state in spontaneous feeding network activity directly proportional to donor feeding thresholds. Moreover, CNS from hungry and non-hungry donors expressed fictive orienting or avoidance turn motor output, respectively, in response to brief

stimulation of sensory nerves. Behavioral choice could be controlled by artificially changing appetitive state. Thus, fictive avoidance turns could be reversibly replaced with orienting turns by directly increasing the excitation state of the feeding network, through stimulation of command neurons and feeding nerves. A resulting circuit model and a computational simulation suggest a basic cost-benefit decision module from which to consider the elaboration of the circuitry in evolution to serve more intricate valuation processes in complex animals.

DYNAMICS OF CHANGES OF EXCITABILITY OF PREMOTOR INTERNEURONS OF DEFENSIVE BEHAVIOR DURING THE ASSOCIATIVE LEARNING IN TERRESTRIAL SNAIL

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The processes at the level of the membrane of nerve cells contribute to the mechanisms of associative learning. Excitability - a property of cellular membrane to respond to the external stimuli by specific changes in ion permeability and membrane potential. Excitability provides a possibility to nerve cells to transform the information of signals from the external environment and respond to them, and underlies the transductive and integrative neural functions. The membrane correlates of learning at different stages are a subject of our studies.

In our experiments, as an associative learning was chosen the conditioned food aversion. We presented 10-20 combinations of conditional and unconditional stimuli in one day. There were chosen 2 arbitrary points, corresponding to achievement of 30-40% and 60% of positive responses to 10 test stimuli. The analysis of electrical characteristics was carried out in isolated preparations of the nervous system. Results of experiments have shown that already at a stage of 30-40% of positive responses the decrease of membrane and threshold potentials can be observed, i.e. the excitability increases. Similar changes of membrane and threshold potentials were observed after achievement of 60% of positive responses. Thus, we show that already at early stages of learning an increase of excitability in premotor interneurons of the defensive behavior can be observed, what suggests that those changes of membrane characteristics can possibly explain facilitation of input signals on early stages of learning.

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OCTOPAMINE INCREASES AGGRESSIVE BEHAVIOUR IN RED WOOD ANTS

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It is known that octopamine is a key factor in controlling intraspecific aggressive behavior in crickets and fruit flies, while its effect on aggression in ants and other social insects remains poorly studied. In this work, we investigated the role of octopamine in modulation of aggressive behavior of red wood ants *Formica aquilonia*. In laboratory we studied the effect of chronic oral treatment with octopamine (5 mg/ml in sugar solution) on the individual ants' aggressiveness towards ground beetles, their natural enemies (Reznikova, Dorosheva, 2004). For this purpose, groups of 20 ants were collected after 1 and 2 weeks of octopamine treatment from the treated colony and the control one. It was found that octopamine treatment significantly increased frequency of ants' attacks and decreased occurrence of non-aggressive reactions, such as antennal touching with the beetle, ignoring and avoiding. The aggression-enhancing effect became more obvious after 2 weeks of octopamine treatment. It should be noted that octopamine did not influence ants' locomotor activity. We speculate that octomapinergetic regulation of aggressive behaviour in ants can be involved in the process of their behavioural specialization within a colony as guards or hunters (Reznikova, Iakovlev, 2008). The study was supported by RFBR (11-04-00536).

ROLE OF MOLLUSCAN INSULIN-RELATED PEPTIDES IN FORMATION OF LONG-TERM MEMORY OF THE POND SNAIL *Lymnaea*

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The pond snail *Lymnaea stagnalis* is capable of learning taste aversion and consolidating this learning into long-term memory (LTM) that is called ‘conditioned taste aversion’ (CTA). Previous studies showed that some molluscan insulin-related peptides (MIPs) were up-regulated in snails exhibiting CTA. We thus hypothesized that MIPs play an important role in neurons underlying the CTA-LTM consolidation process. To examine this hypothesis, we first applied exogenous mammalian insulin or secretions from MIPs-containing cells to the isolated CNS, we observed a long-term change in synaptic efficacy (i.e. enhancement) of the synaptic connection between the cerebral giant cell (a key interneuron for CTA) and the B1 motoneuron (a buccal motoneuron). This synaptic enhancement was blocked by application of an insulin receptor antibody to the isolated CNS. Next, injection of the insulin receptor antibody into the snail before CTA training, while not blocking the acquisition of taste aversion learning, blocked the memory consolidation process, thus LTM was not observed. These data suggest that MIPs trigger changes in synaptic connectivity that may be

correlated with the consolidation of taste aversion learning into CTA-LTM in the *Lymnaea* CNS.
This work was performed with Jun Murakami, Ryuichi Okada, Hisayo Sadamoto, Suguru Kobayashi, Koichi Mita, Yuki Naganuma, Mika Morikawa, Miki Yamagishi, Dai Hatakeyama, Emi Otsuka, Akiko Okuta, Hiroshi Sunada, Satoshi Takigami, Manabu Sakakibara, Yutaka Fujito, Masahiko Awaji, Shunsuke Moriyama and Ken Lukowiak.

POLYMORPHISM OF LIMK1 IS NECESSARY FOR *DROSOPHILA MELANOGASTER* ADAPTATION

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One of the crucial regulators of cytoskeleton remodeling is LIMK1 which phosphorylates cofilin and affects actin filament dynamics leading to dendritic spine reorganization, learning and memory. As shown previously by means of Western blot analysis, a different ratio of D and C isoforms LIMK1 in the heads of *Drosophila* males from wild type strains – *Berlin*, *Oregon-R* and *Canton-S* accompanied with similar total content of LIMK1, while mutant agn^{ts3} had high activities of LIMK1. Using quantitative real-time PCR we found lower level of mRNA LIMK1 in *Berlin*, *Oregon-R* and agn^{ts3} in comparison with *Canton-S*. Using setup for automatic registration of courtship song, we evaluated learning ability in two types of training condition - 30 min and 5 hours. Learning indices (LIs) was calculated based only on singing index (wing vibration produced by a male during courtship before and after learning). *Berlin* and *Oregon-R* demonstrated lower learning indices than *Canton-S* after 30 min of training, while after 5 hours - had LIs similar to *Canton-S*. agn^{ts3} had lower learning indices after both training conditions in comparison to *Canton-S*. Polymorphism LIMK1 seems to lead to the best way for adaptation in different populations.

FORWARD GENETIC APPROACH: IS IT EXHAUSTED?

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The forward genetic approach consists in looking for genes, which disfunction or overexpression strongly influence on manifestation of some trait. The researcher has no initial idea, what these genes may code. The aim of this approach is to find out gene products - unknown players in the circuit leading to the final trait, in case of neurobiology this is, of course, the behavior itself or some trait (such as brain anatomy) that could obviously influence the behavior (sideways genetics).

The best examples of forward genetics approach, applied to neurobiology of *Drosophila,* are presented. The techniques, mainly used in the past (chemical mutagenesis) and which have been used recently (transposon mutagenesis) are considered. The methods of site(gene)-specific mutagenesis are discussed.

The main question – is the forward genetic approach is still actual in neurobiology of *Drosophila* or it may be fully substituted by studying gene expression patterns under various conditions followed by methods of the reverse genetics (knockout or knockdown of the known genes).

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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 categorization 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 signaling molecules involved in early and later phases of the consolidation as well as the reconsolidation of memory. Recent research on molluscan model systems, particularly (but not exclusively) *Aplysia* and *Lymnaea*, has 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. An exciting new discovery has been that epigenetic changes, such as histone modifications also play key roles in the process of memory formation in molluscs. 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. I will put recent findings from these two molluscan model systems into the context of new findings from other molluscs (e.g., *Helix*) and well-known insect and mammalian models of learning and memory. Acknowledgements: GK's original research has been funded by the MRC, BBSRC and the Royal Society (UK).

Biorhythmicity of adaptive adult-to-embryo chemical signaling in *Lymnaea stagnalis* (Mollusca: Pulmonata)

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Biphasic life cycle of freshwater molluscs represents a suitable model to study adaptive mechanisms underlying regulation of embryonic development. Earlier we described that chemical signal released by starved adults affected both the speed of embryonic development and expression of serotonin-dependent motor programs such as feeding, locomotion and heart beating. Noteworthy that described chemical signal retarded embryonic development during premetamorphic stages and accelerated postmetamorphic stages.

In this work we examined the rhythmicity in the chemical signal emission using laboratory culture of *Lymnaea stagnalis*. The length of *Lymnaea* embryo was used to monitor their developmental speed. Activity of water conditioned by starved adults (CW) and direction of the CW-induced effect were tested at pre- and postmetamorphic *Lymnaea* embryos. The minimal time interval for signal emission was 12 hours of adult's starvation and the effect reached plateau after 24 hours. The circadian day-night rhythm with statistically stronger effect for daily conditioned water was observed. The production of emitted factor depends also from the moon cycle: water conditioned during full moon was 1,5-2 times more

effective than collected under the same conditions during new moon. The maximal retardation effect was found for the water collected during July-August and October-November. The water collected during April-May expressed the maximal acceleration effect. The observed facts suggested that chemical system of adult-to-embryo communication expresses diurnal, moon and seasonal rhythmicity. We hypothesized that rhythmical changes in 5-HT level in adult *Lymnaea* may underlay such rhythmicity.

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INHIBITION OF NITRIC OXIDE SYNTHESIS INFLUENCES SYNAPTIC PLASTICITY AND MEMORY FORMATION IN SNAILS

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In behavioral and electrophysiological experiments we investigated the contribution of NO in synaptic plasticity and memory formation in *Helix lucorum.* In isolated brain preparations the influence of NO-synthase inhibitor L-NNA on amplitude of complex EPSPs evoked in withdrawal interneurons by stimulation of nerves were investigated. It was shown that 5 nerve tetani combined with serotonin bath applications induced a long-term increase of the EPSPs. L-NNA bath applications after tetanization decreased the EPSPs. L-NNA bath applications before nerve tetanization prevented tetanus-induced long-term increase of EPSPs.

The withdrawal interneurons are the key elements in network underlying avoidance reactions. We suggested that L-NNA injections in snails can influence the fear conditioning learning. Snails were one or five days trained to remember the context in which they were shocked. In one day training expeniments the snails received 5 electrical shocks in one context. Group #1 was sham-injected, group #2 was L-NNA injected before, and group #3 was L-NNA injected after training. Immediately after training groups #1 and #3 demonstrated the significant increase of behavioral responses versus responses of group #2. Testing 1h20 min after training revealed significantly weaker responses of groups #2 and #3. Next day only snails of group #1 had high amplitude behavioral responses but no differences depending on context were observed. These results show good correlation with electrophysiological results. However, one day training was not sufficient for long-term context learning. In another experiments snails for five days received electrical shocks. Each training day the snails of group #1 were sham-injected and snails of group #2 were injected with L-NNA. A day after training only the group #1 demonstrated selective aversive context memory whereas the responses of group #2 were weak.

Results demonstrated that inhibition of NO synthesis influences synaptic plasticity in withdrawal interneurones, that may underlie behavioral changes. Inhibition of NO synthesis prevents development of new memory as well as influences formation of a long-term memory. Supported Ministery of Education grant P608, grant RFBR 10-04-00554-a.

DIFFERENTIATION OF THE FMRFERGIC AND SEROTONINERGIC COMPONENTS OF PLANARIAN NERVOUS SYSTEM IN COURSE OF ANTERIOR REGENERATION.

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According to previous reports, the nervous system of flatworms comprises neurons of different chemical phenotypes. Here, using immunostaining to FMRF and serotonin and confocal laser scanning microscopy, we have followed the details of differentiation of neuronal elements during regeneration of anterior end in dissected tail fragments in the planarian *Girardia tigrina*. The gradual development of the nervous system complexity has been revealed during the regeneration process, specifically, the appearance of new cells (neurons), the outgrowth of nervous fibres, the formation of the pattern of new cephalic ganglion and peripheral nervous plexus, and intensification of immunostaining reflecting the increase in the production of serotonin and peptide(s) during the maturation of respective neurons. The formation of the new cephalic ganglion has been completed by the day 7 of regeneration, with its pattern corresponding to that of the intact ganglion and its size remaining smaller than in intact planarians. In addition, we have mapped FMRF-like immunoreactive cells and fibres in two other planarian species, *Polycelis tenuis* and *Schmidtea mediterranean.* Supported by RFBR grant 12-04-01086a

NATURAL AND EXPERIMENTAL SYNCITIAL FUSION OF THE MOLLUSK NEURONS

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The process of formation of the syncytial connection between the shoots and the bodies of different neurons has been revealed by means of computer time-lapse video filming of the dissociated mollusk neuron culture. It has been shown, that nerve shoots of one neuron establish connection with another one. After the destruction of the cell body (the trophic centre) these neurons do not undergo the

wallerian degeneration. In other cases within several hours it is possible to observe the translocation of the cytoplasmic varicosities through the branches from one neuron into another. The fusion of the nerve cell bodies preliminarily detached by the known methods from the Lymnaea stagnalis ganglions was also carried out in the course of the work. The nerve cells were placed into the culture medium for 2 days. On the 2nd day of the cultivation shoots start to grow from the neurons, with their help they contact and contracting bring together the cell bodies. The contacting neuron bodies form the 8-shaped structures generating on their borders the fusion bridges and similar to vacuole formations of the amplified intercellular cleft.

The ultrastructural researches prove, that the cytoplasmas of the adjoining cells fuse, i.e. the cells unite in syncytium. The majority of the membranes differentiating the cytoplasm of the neighboring neurons in the area of the bridges of fusion, become destroyed. Only their short residual fragments can be found instead of external cellular membranes. Thus, the neuroplasmas of the adjoinig cells pass directly each into other. Binuclear cells or multinuclear symplasts are forming. It's the first time when spontaneously appeared binuclear neurons were found at a mollusk.

The results of these experiments, in our opinion, solve the main issue of the discussion on the fundamental possibility of the syncytial connection of neurons. It's not the initial small interneuronic membrane pores and perforations that have been shown but an almost total destruction of the twin neuron membranes and the fusion of their cytoplasm.

ROLE OF ATP-SENSITIVE POTASSIUM CURRENT IN INTRINSIC ACTIVITY OF ENTORHINAL CORTEX LAYER III NEURONS DURING POSTNATAL MATURATION

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The medial temporal lobe serves important functions in learning and memory formation which go along with coherent network oscillations. Intrinsic properties of the participating neurons play an important role in the emerging patterns at the network level. Principal neurons from layer III (LIII) of the immature medial entorhinal cortex (mEC) display at least two distinct patterns of intrinsic firing behavior: spontaneous Ca^{2+}- and voltage-dependent prolonged intrinsic bursts and P15) all mEC LIII neurons display exclusively regular firing. Here, we report that expression of ATP-

sensitive potassium currents (I_{KATP}) is developmentally regulated and goes in parallel with the transition from bursting to regular firing patterns. Western blot and RT-PCR analysis showed U-shaped expression of Kir6.2 protein and mRNA between P0 and P25 with minimal levels at P7-13. The functional role of I_{KATP} was assessed by whole-cell and cell-attached recordings from mEC LIII neurons in horizontal brain slices obtained from rats at P7-13 and P18-25. The I_{KATP} blockers tolbutamide (300 μM) and glibenclamide (1 μM) reversibly induced a strong prolongation of bursts in the younger group. Furthermore, bursting activity was strongly suppressed by diazoxide (100-400 μM) and NN414 (5-10 μM), drugs that activate I_{KATP}. Regular firing was impaired by diazoxide and NN414 in both groups. We suggest that developmental regulation of Kir6.2 contributes to the functional maturation of discharge properties of neurons in the mEC. Supported by Ministry of Education and Science of Russia (ГК-16.740.11.0350) and BMBF (IB/RUS 11/015 and BCCN) Germany.

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.

NEURAL NET IN CLADONEMA CALIFORNICUM POLYPS (HYDROZOA, CNIDARIA)

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Cnidaria are thought to be the most primitive animal phylum possessing neural system. Their neurons contain different neuropeptides, catecholamines or neurotransmitters as neuroactive substances. Serotonin is biogenic monoamine belonging to classical neurotransmitters. RF-amides are a widespread neuropeptide family, all members of which have -Arg-Phe-NH2 sequence. Both substances were detected in cnidarian neurons. However, their role is not completely revealed yet. The present study showed serotonin and RF-amide immune-positive cells and processes throughout Cladonema polyp including gastral region, oral and aboral tentacles. Generally, all the processes are parallel to each other and to the main polyp axis. They pass above epiderm muscle processes and enter the tentacles. The area of hypostome and oral tentacles has more dense neural net in comparison with the gastral region. Serotonin- and RF-amide-positive neural ring encloses hypostome. Ultrastructurally, specific vesicles with electron-dense or electron-translucent content and microtubule bundles characterize the neural processes. Probably, the vesicles with electron-dense and electron-translucent content found in Cladonema neurites bear RF-amides and serotonin respectively. The pharmacological experiments with serotonin and its blockers propose serotonin involvement in the contractions of muscle processes in Cladonema polyps. For example, exogenous serotonin provokes prolonged tentacles contraction.

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CONSEQUENCES OF AVERSIVE EXPERIENCE IN APLYSIA: PLASTICITY BEYOND DEFENSIVE NEURAL CIRCUITS

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Although the mechanisms by which aversive experience modifies defensive neural circuits have been extensively explored in invertebrate models, those altering non-defensive behaviors remain largely unknown. We are currently examining the effects of aversive stimuli, which induce enhancement of defensive responses (i.e.,

sensitization) in Aplysia, on the expression of a non-defensive behavior (feeding) and its underlying neural circuit. Training, consisting of repeated noxious electrical stimuli, produced concurrent long-term sensitization (LTS) and suppression of feeding, lasting at least 24 h. At the cellular level, LTS training decreased the excitability of B51, a decision-making neuron in the feeding neural circuit, whose activity influences the expression of ingestive motor programs. B51 decreased excitability would diminish the neuron's ability to become active and reduce the likelihood of expression of ingestive motor programs, which is consistent with the suppression of feeding. When feeding and B51 excitability were examined at a time point in which LTS is no longer observed (72 h post treatment), no differences were measured between trained and untrained animals, strengthening the role of B51 as a locus of plasticity underlying feeding suppression. Because serotonin mediates sensitization in Aplysia, we examined whether it could also play a role in the suppression of feeding. Serotonin bath application induced LTS, but did not alter either feeding or B51 excitability 24 h after treatment. In summary, these findings indicate that, in Aplysia, aversive stimuli concurrently modify the activity of both defensive and non-defensive neural circuits, and also suggest that these changes are mediated by distinct biochemical pathways.
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EFFECTS OF THE INFLUENCE OF NITRIC OXIDE DONOR SODIUM NITROPRUSSIDE AND NO-SYNTHASE BLOCKER L-NAME ON THE LEARNING AND RECONSOLIDATION IN TERRESTRIAL SNAILS

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Recently it was shown that the NO is necessary not only for learning, but for deleting the memory (Balaban et al, 2011). It was interesting to investigate the influence of the NO on reconsolidation of memory and on different forms of learning, leading to elaboration of declarative (conditioned environmental reflex), and procedural memories (conditioned food aversion).

We have shown that chronic injection of NO donor sodium nitroprusside accelerates elaboration of the conditioned food aversion in snails in contrast with control snails, but inhibition of NO-synthase (L-NAME injections) decreased elaboration of this reflex. It was also shown that injection of sodium nitroprusside did not influence learning (conditioned environmental reflex was formed in the same way as in control snails, injected by saline solution), but injections of L-NAME led to decrease of elaboration of the conditioned food aversion. These data confirm necessity of NO for learning.

Influence of NO on reconsolidation of memory waqs investigated. In these experiments, snails after the reminder were injected by L-NAME, L-NAME + anisomycin (blocker of protein syntheses) or saline solution. The following testing has shown retention of conditioned environmental reflex in all groups of snails, except the group, injected only by anisomycin. Consequently, NO does not influence the retention of this reflex, but is needed for forgetting (deleting of memory). Anisomycin blocks a process of reconsolidation, and if simultaneously with anisomycin the synthesis of NO is blocked, the forgetting of old memory does not occur. This result shows that NO is necessary for the process of reconsolidation itself. This work is supported by RFBR, grant 12-04-00235.

TRANSMITTERS IN THE EMBRYOGENESIS OF XENOPUS LAEVIS

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The presence of monoamine transmitter substances in embryogenesis of clawed frog X.laevis was investigated by HPLC. It was shown that serotonin, dopamine and noradrenaline are present in cells of embryos and larvae. During cleavage divisions the levels of serotonin was $2 \pm 0{,}4$ x 10-15 mol/embryo and then increased significantly at neurula and hatching stages (20 - 100 fold). Preincubation of the embryos with 100 μM 5-hydroxytryptophan during cleavage divisions evoked about tenfold increase of serotonin levels. Noradrenaline was present in the concentration of 439 ± 7 x 10-15 mol/embryo during cleavage divisions and then decreased to hatching stage in contrast to serotonin. Dopamine levels remained more or less constant during embryonic development ($5 \pm 0{,}6$ x 10-15 mol/embryo). It is noteworthy that the levels of the transmitters could differ greatly in embryos from different females.

Thus, three transmitters were present in the cells of X.laevis embryos simultaneously, although the list of functionally active embryonic transmitters may not be limited by them. At least as concerns serotonin, the data obtained evidences in favor of the activity of system of serotonin synthesis (aromatic aminoacids decarboxylase) already during embryonic stages.

LEARNING-INDUCED ALTERATIONS OF 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 somatic depolarization also makes contributions to the storage of long-term memory in both vertebrates and invertebrates. There is however, quite sparse 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 somatic 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 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 neurite. Bath application of 4-aminopyridine increased the amplitude of APs and calcium transients at the distal end of the neurite 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 μm 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.
Supported by RFBR and MCB RAS grants (ESN, TK, PMB) and GK's laboratory is funded by the BBSRC, UK.

HSP70 INTRACELLULAR LOCALIZATION IN *DROSOPHILA cd* MUTANT OF THE KYNURENINE PATHWAY

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The structure and function of many genes are similar in *Drosophila* and humans. Therefore, a study of pathological process in *Drosophila*, especially neurodegenerative processes accompanied by progressive memory loss, helps to understand the etiology of neurodegenerative human disorders and to develop therapeutic strategies. A large number of neurodegenerative diseases are known to share a common pathological feature of abnormal brain deposits. It results from the alterations in the functioning of heat shock/chaperone machinery. Moreover, neurodegenerative disorders are characterized by altered content of the intermediates of the kynurenine pathway. The *Drosophila* mutant *cardinal* (*cd*, excess of 3-hydroxykynurenine (3-HOK), the generator of oxidative stress), can serve as a model for dementia due to progressive decline in learning and memory, accompanied by synaptic pathology. We investigated HSP70 intracellular localization in *Drosophila* mutant *cd* under heat shock (HS) treatment using confocal microscopy. At normal conditions HSP70 is located on nuclear surface both in *cd* and CS (wild type) larvae. In both stocks HS treatment results in HSP70 relocation to the central nuclear regions. We determined HSP70 decrease in *cd* larvae in comparison to wild type at normal and stress conditions. Found differences can result from an accumulation of 3-HOK in *cd* mutant.

INFLUENCE OF MONODIET ON ACTIVITY STATE OF TERRESTRIAL SNAILS

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Influence of quantity of food to humans' functional and emotional state is a focus of attention of modern dietetics. Neuroscience data are very useful in this aspect to understand the mechanisms of these influences. We have a possibility to compare activity state of snails *Helix* which been on two different monodiets during half a year. We chose carrot (*Daucus carota*) and bananas (*Musa*) because, first, these are natural dietaries for the snails and, second, they are similar in nutritional value per 100g (http://en.wikipedia.org). Carrot and bananas differ in

concentration of amino acids tyrosine and tryptophan, these dopamine and serotonin precursors present two times more in bananas. Two groups of animals, each consisted from 10 snails, were maintained in similar conditions and had free access to food, water and possibility to move. Evaluation of active state by subjective criteria was 100% exact for snails from bananas group. Activity state was estimated using two criteria: number of active snails in each group and levels of activity for every snail in a 5 levels scale. The results obtained did not show statistically meaningful differences between the two groups of snails.

TETANIC INTESTINAL NERVE STIMULATION INFLUENCES QUANTITY BUT NOT AMPLITUDES OF SYNAPTIC POTENTIALS IN BACKGROUND ACTIVITY OF THE PARIETAL COMMAND NEURONS OF THE SNAILS

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Post tetanic potentiation (PTP) is one of the best studied phenomena of synaptic plasticity. This is well known for identified command neurons of the parietal ganglia of terrestrial snail *Helix lucorum*. Evoked synaptic responses were used as the test in these investigations. Serotoninergic hypothesis was suggested as the mechanism of PTP in the snail's command neurons (Balaban, Zakharov, 1992). We studied PTP for compound excitatory postsynaptic potentials (EPSPs) evoked by stimulation of viscera and for elementary EPSPs evoked by spikes in the identified parietal sensory neurons (Sokolov, Palikhova, 1999). In presented study we used the possibilities of "Mini Analysis Program by Justin Lee" (Synaptosoft Inc., 2001; http://www.synaptosoft.com) for testing influence of tetanic stimulation of intestinal nerve (INS: 2min, 50msec, 2Hz) to background synaptic activity recorded in the snail's command neurons. The results were unexpected. We suggested that amplitudes of EPSPs averaged for every 5 min. would rise after INS during 1.5 hours of traced time as it was for evoked EPSPs. The results obtained were that background EPSPs did not changed in amplitudes but rise about two times in quantity of EPSPs after INS (N=9). Results obtained show that serotoninergic mechanism of post tetanic potentiation is not universal as it's suggested.

INFLUENCE OF GROUP KEEPING ON LOCOMOTOR ACTIVITY AND COURTSHIP BEHAVIOR OF DROSOPHILA MALES

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The modification of *Drosophila melanogaster* behavior after sexual and aggressive experience is well studied. But there have been only few studies investigating the influence of maintenance of the males in a group on their further behavior. We have assessed the influence of group experience on locomotor behavior of males, intensity of courtship and parameters of their song production.

Males of *Drosophila melanogaster*, wild-type strain *Canton-S*, were collected without any anesthesia soon after eclosion and kept under standard conditions 3-5 days of age. There were two groups of males: housed individually and in a group of 10-20 flies.

It has been shown that males tested individually after group experience show reduced locomotor activity (due to a decrease in run duration and run frequency) in comparison to males kept individually from the moment of their eclosion. This effect was observed up to 3 hours after isolation from a group. Also, males previously kept in a group demonstrated lower courtship and song production intensities than males kept individually (levels of locomotor activity were equally high).

Observed changes in behavior can be interpreted as an example of operant learning. For the first time, this learning was found in *Drosophila* females. It was shown that in a group the flies try to avoid close unpleasant contacts accompanied by kicks and wing threats from other insects. Therefore, they run from the individuals, which came too near, and stop their running to prevent meeting with another female. Trial and error learning forces them to suppress their locomotor activity and stay at rest; as a result they have less unpleasant contacts. But in case of females this learning leads to no after-effects in absence of the appropriate contextual cues. Males, in contrast, possibly due to more intensive aggression, retain the altered behavior for a long time independently of the context, in which they experienced aggression from other individuals. Being tested alone, a male, which previously was kept in a group situation, maintains the low locomotor activity, but, when it is paired with a female, he tries to avoid any contacts with her that leads to high locomotor and low courtship activity.

The study was supported by grant from Ministry of Education and Science of Russian Federation (contract P316), the programs of Presidium of the Russian Academy of Sciences “The Living Nature” and “Mechanisms of integration of molecular systems for realization of physiological functions”.

INVESTIGATION OF THE CONTRIBUTION OF VESICULAR-TRANSPORT IN MONOSYNAPTIC LONG-TERM POTENTIATION

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The efficiency of synaptic transmission depends on the process of membrane reorganization, accompanied by change in the number and proportion of receptors, and is the basis for neuroplasticity. Long-term potentiation (LTP) is a cellular model of synaptic plasticity and is investigated on simple neural nets. Vesicular pathways are responsible for the transmission of proteins between compartments of the vacuolar system and their transportation through the plasmatic membrane. The mature vesicles coated with receptors come out from a trans-Golgi net and incorporate into the membrane. The influence of decreasing the activity of proteins regulating the assembly of vesicles induced by brefeldin-A (BFA) was investigated on the simple neural net of a hippocampal slice. It has been shown that incubation with BFA for an hour before tetanization does not affect the basic response and induction of LTP. However, if incubated 25-30 min after tetanization, a significant decrease in the amplitude of n-spikes was observed. This decrease indicates that the phase of LTP which maintains the new neurotransmission level was impaired. It has been suggested that there is a reliable local molecular system producing the increase of neurotransmission efficiency in response to the stimulation of a distinct pattern in the synapse. Thus, maintenance of LTP is an integral cellular process requiring the participation of many cellular systems. Reconstruction of the protein network mediating the expression of LTP and the vesicle pathway was completed. In order to verify the data obtained on simple neural systems of hippocampal slices, an investigation of the neuron culture of mollusks is planned.

MOLECULAR MECHANISMS OF INITIATION AND PERSISTENCE OF MEMORY STORAGE

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As a result of advances in cellular, molecular and systems biology the mechanisms underlying the initial storage, maintenance and recall of memories are now beginning to be understood. To obtain these insights several model systems and methodologies have been used. We briefly summarize this recent progress based on study of the gill-withdrawal reflex of Aplysia. Specifically, we will discuss transcription, transport and translation in sensory and motor neurons of gill withdrawal reflex.

WHAT "SIMPLE" BRAINS ARE CAPABLE OF: COGNITIVE SPECIALIZATION IN ANTS

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The 12 000 or so ant species vary enormously in their colony size, foraging systems, communications, and in flexibility and complexity of their behaviour. Is cognitive processing possible in ant societies at the individual level? To what extent can an ant improve its innate tendency to engage in a certain job?

Mass recruiting ant species react collectively as a "super-organism", and worker allocation to tasks is unrelated to their ability to perform them. In solely foraging species individual colony members are more agile and they learn faster than members of mass recruitment species.

In group-retrieving ant species colony organisation is based on sophisticated distribution of cognitive responsibilities within stable teams of scouts and foragers. Long-term laboratory experiments revealed that scouts are able to memorise and pass "abstract" information to their team-members, and they are also capable of extracting rules in order to optimise and shorten "messages", whereas foragers are not able to transmit information. A field model of the situation in which ants act collectively to perform a specific task is the organisation of honey dew collection in the tree crown. In red wood ants groups of aphid tenders include members of different "professions" (shepherds, guards, transporters and scouts) which stay in stable cohesion for some weeks. Ants belonging to different professions possess stable sets of distinct behavioural features that can be considered a "behavioural syndrome". In general, group- retrieving *Formica* species enjoy flexible and rational communication based on cognitive specialisation at the individual level.

THE ROOTS OF THE INTERNATIONAL SOCIETY FOR INVERTEBRATE NEUROBIOLOGY (ISIN)

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Some of the key events that preceded the ISIN foundation by Prof. Janos Salanki (1989) will be briefly considered. I intend to name a few of the persons to whom we are especially indebted for the development of the idea that the comparative approach would reveal general principles of the nervous system.

NEW DATA CONCERNING THE STRUCTURE OF FRESHWATER BRYOZOAN *CRISTATELLA MUCEDO* (BRYOZOA: PHYLACTOLAEMATA) NERVOUS SYSTEM.

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Freshwater bryozoan *Cristatella mucedo* is unique species among all bryozoans which is able to move during all its life. We have conducted different immunohystochemical investigations of the central nervous system using confocal laser scanning microscopy methods (CLSM). For the first time we showed the FMRF- and 5-HT nerve position in the cerebral ganglion, body wall and basal wall of the colony. Our special attention was devoted to the colony basal wall FMRF-amidergic innervation which is appeared to be strongly associated with the musculature. Also we have compared our results with the data concerning other freshwater species.

The investigation was supported by grants RFBR № 10-04-00085-a and 10-04-01033-a. This research was done using the facilities, provided by CKU "Chromas" (Saint-Petersburg, Russia).

THE HONEYBEE BRAIN ASYMMETRY OF THE EPIGENETIC MODIFICATIONS IN CONSEQUENCE OF LEARNING

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Most researches on anatomical and functional asymmetries of brain were mainly focused on vertebrates. Evidences for brain and behavioural lateralization among invertebrates are few in number. Olfactory asymmetries in honey bees were demonstrated by Letzkus et al. (2006) in view of proboscis extension reflex (PER) paradigm. Besides, lateralization was demonstrated for mushroom bodies (insect brain structures) relating to olfactory learning and memory formation. At present the molecular and epigenetic mechanisms of long-term memory formation are not investigated enough.

We studied methylation and phosphorylation level of histone H3 in honeybee brain at 24 hours after learning (PER) by immunostaining and discovered increased methylation and phosphorylation level of histone H3. In right brain hemisphere there were more immunopositive neurons than in left hemisphere. It means that epigenetic modifications participate in associative learning.

REACTIVE OXYGEN SPECIES MODIFY OPERANT CONDITIONING OF AERIAL RESPIRATORY BEHAVIOR IN MOLLUSK *LYMNAEA STAGNALIS*

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In the past two decades, a number of authors reported the evidence for the involvement of reactive oxygen species (ROS) in both intra- and intercellular signalling. Specifically, a neuromodulatory role for hydrogen peroxide has been suggested by some groups with respect to chemical transmission in various types of synapses. It is presumed that changes in functional state of synapses determine various forms of behavioral adaptation including learning and memory. The aim of the present work was to analyse the effect of hydrogen peroxide on operant conditioning of aerial respiration and signal transduction in chemical synapses within the respiratory network of *Lymnaea*. Previously, it has been shown that *Lymnaea* is capable of operant learning (an association is made between an external stimulus and a behavioral response) and that the memory associated with the operant paradigm can persist for a long time (months). In the present experiments, addition of 100 microM hydrogen peroxide accelerated learning, while there was no significant effect with 10 and 1 microM. The observed changes were associated with the reduction of synaptic efficiency in dopaminergic synapses formed by the RPeD1 neuron (1.8-times decrease of postsynaptic potential amplitude and 1.7-times increase of synaptic delay). The peptidergic transmission between FMRF-containing neuron VD4 and its followers did not change after hydrogen peroxide application. It seems thus that ROS may be of critical importance for memory formation in *Lymnaea*.

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NERVOUS ORGANISATION OF THE POSTERIOR END IN NEREIDID AND PHYLLODOCID POLYCHAETES

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Pygidium of annelids is considered to be a simple ectodermal structure with no coelomic cavity, bearing only anus and few sensory structures (such as pygidial

cirri). However, using the immunohistochemical methods and confocal laser microscopy we have found an unpaired coelomic cavity with its own inlay in this part for Nereidid and Phyllodocid polychaetes. Also a very complex innervation was found in the pygidium. Ventral nerve cord passes through the pygidium and terminates at the pygidial cirri. In the anterior part a circumpygidial ring is situated. It is formed from a ventral pygidium commisure and some groups of tiny bilateral nerve fibres, going to the dorsal side. We have also found a group of unipolar nerve cells in the ventral part of the pygidium, besides some of these cells appeared to be FMRF-amide-positive.

The innervation of the pygidium in the Nereididae and Phyllodocidae families is appeared to be very similar, thus we can conclude that it is evolutionary conservative. Our data allow to suggest that pygidium is a highly important part of a segmented body that probably affects the subterminal growths of the worm.

The investigation was supported by grant RFBR № 10-04-01033-a. This research was made using the facilities provided by Core Facility "Chromas" (Saint-Petersburg, Russia).

EFFECT OF SEROTONIN ON MOTILITY OF *CRYPTOCOTYLE LINGUA* CERCARIAE (TREMATODA). PRELIMINARY STUDY

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Motile larval stages of trematodes – cercariae have a complex of morphological and behavioral adaptations that helps them to find and infect the next host. Cercaria *Cryptocotyle lingua* (Heterophyidae) belongs to intermittently swimming larvae with regular patterns of motility and may be used as a model to study behavioral characteristics. However the data of physiological mechanisms of behavior in cercariae is scarce.

The aim of this study was to investigate the effect of serotonin on motility of cercariae *C. lingua*. Cercariae of different age (6, 12, 18 and 24 hours) were treated with serotonin, mianserin, cyproheptadine and 5-hydroxytryptophan in concentrations of 0,01mM and 0,001mM. The motility of cercaria was registered by video recording. The number of bursts of swimming and its duration were measured. Cercariae were also stained with anti-serotonin antibodies.

Serotonin caused significant effect on the number of bursts of swimming in contrast to the duration of burst and the pause. The effect was significantly

stronger in “older” cercariae where the intensity of anti-serotonin staining was smaller compare to “younger” cercariae.
The study was supported by grants RFBR 12-04-01051-a, 12-04-01086-a, МК-1093.2011.4.

EFFECTS OF INHIBITORS OF SERINE/THREONINE AND TYROSINE PROTEIN PHOSPHATASES ON DEPRESSION OF ACETYLCHOLINE-INDUCED CURRENT AT THE CELLULAR ANALOGUE OF HABITUATION IN COMMAND HELIX LUCORUM NEURONS

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Effects of some inhibitors of serine/threonine and tyrosine protein phosphatases on depression and spontaneous recovery of acetylcholine-induced inward current (ACh-current) in command Helix lucorum neurons of defensive behavior at the cellular analogue of habituation were investigated. The following inhibitors were used in the experiments: okadaic acid (reduces activity of phosphatases PP1 and PP2A), endothall (PP2A), cyclosporine A and cypermethrin (PP2B), CCT007093 (PPM1D), dephostatin (inhibits tyrosine phosphatases). All listed inhibitors modify the depression curve, and endothall also reduces the spontaneous recovery of ACh-current. The original mathematical model, which was applied, considers the possibility of various localizations of receptors in the cell and pattern of transitions between them. The results of the electrophysiological experiments and mathematical modeling allowed to consider participation of indicated phosphatases in mobility of membrane acetylcholine receptors (endocytosis and exocytosis), ensuring ACh-current modification at the cellular analogue of habituations. These enzymes influence transport system of a neuron, cytoskeleton elements and motor proteins.

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ROLE OF CYTOSKELETON IN DEPRESSION OF ACETYLCHOLINE-INDUCED CURRENT AT THE CELLULAR ANALOGUE OF HABITUATION IN COMMAND HELIX LUCORUM NEURONS

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Код поля изменен

Код поля изменен

Previously, we showed that the cause of the decrease of acetylcholine-induced inward current (ACh-current) in command Helix lucorum neurons of defensive behavior during rhythmic local acetylcholine applications to soma (cellular analogue of habituation) is the reduction of number of membrane extrasynaptic acetylcholine receptors as a result of balance shift of endocytosis-exocytosis of acetylcholine receptors in the direction of endocytosis predominance (Makhnovsky et al., 2010, 2012). It is well known that the mobility of membrane receptors is associated with the cytoskeleton elements. The following drugs, disturbing the function of cytoskeleton elements, were used in the experiments: cytochalasin B, phalloidin (inhibitors of actin polymerization), colchicine, vinblastine (inhibitors of tubulin polymerization in microtubules), ML-7, H-RKKYKYRRK-NH2 (inhibitors of myosin light chain kinase). All these compounds reduced the depression of ACh-current. The results of current study and mathematical modeling allow to make the following conclusion: reduction of number of membrane acetylcholine receptors during the depression of ACh-induced current at the cellular analogue of habituation is connected with the participation of actin microfilaments, microtubules and motor protein myosin of neuron cytoskeleton.

The reported study was partially supported by RFBR, research projects No. 09-04-00304-a, 12-04-00209-a.

FIXED AND FLEXIBLE TRAITS IN MATING SIGNALS: EVOLUTIONARY AND PHYSIOLOGICAL BACKGROUNDS

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The role of complex traits in mate choice has been much discussed. Courtship signals sometimes reach of an extreme complexity and these signals seem to provide even redundant information about their senders. Some traits are highly conserved, others are flexible. It is suggested that some cues provide species-specific identification, whereas others are important in intraspecific mate choice. How to distinguish these cues from one another? There are several ways to study this problem, in particular, testing receivers (usually females) with supernormal key stimuli in behavioural experiments.

We studied variability of courtship song of the field cricket *Gryllus assimilis*. Some elements of courtship song were quite variable (high coefficient of variation, CV) both within and between males, whereas others were more stable. Similar to

some other *Gryllus* species, the courtship song of *G. assimilis* comprised two elements: series of pulses (chirps) with low fundamental frequency (ca. 3.5 kHz) and brief high-frequency (ca.17 kHz) pulses (ticks). Chirp pulses often included substantial high-frequency components which, in some cases, could be dominant. As a result, dominant frequency of the chirps had a CV of 44% within individuals. We investigated the importance of chirp spectrum in playback experiments using synthesized courtship songs. A stimulus with 3.5 kHz chirp pulses evoked normal levels of female responses, whereas a stimulus with high-frequency (17 kHz) chirp pulses was ineffective. Surprisingly, a stimulus in which chirp pulses contained a series of higher harmonics (10.5 – 17 kHz), but lacked the low-frequency fundamental, was also effective. We investigated the neural coding of these stimuli by recording the responses of two auditory interneurons, AN1 and AN2. Chirps containing either only the fundamental frequency (3.5 kHz) or only the series of higher harmonics, elicited strong responses from both interneurons at sound intensities similar to that measured at the female during courtship. In contrast, 17-kHz chirps elicited a response from AN2, but not AN1. This suggests that activity of both neurons may be required for responses to courtship song. AN1 is much more sharply tuned than AN2, being highly sensitive to dominant frequency of the calling song. However, at higher intensities, such as occur during courtship, the frequency range of AN1 sensitivity broadens, so that even chirps lacking the fundamental frequency can result in AN1 responses. In addition, the series of higher harmonics that we used could provide excitation at the fundamental frequency through nonlinear active mechanical properties that have been described for many insect ears.

We suggest that the surprisingly lax requirements for chirp sound frequency reflect the frequency-tuning properties of AN1 and AN2, which allow both neurons to respond to chirp pulses despite their spectral variation. Thus, in contrast to the "sensory exploitation hypothesis", where a signaler's trait is favored by selection because it fits preexisting features of the receiver's sensory system, we suggest that courtship communication in *G. assimilis* is an example of "sensory permissiveness", that is, that the features of the sensory system result in a relaxation of selection pressure for precise signal structure.

MAGNETIC RESONANCE OF IRON OXIDE CRYSTALLINE PARTICLES IN NERVOUS TISSUE OF *HELIX LUCORUM*

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At present existence of biogenic nanoscaled crystals of the magnetite in nervous systems of living organisms is known. One of the methods, allowing to find and characterize the magnetic properties of such particles, is a method of the electronic magnetic resonance (MR).

The goal of our work was to find a magnetic nanoparticles in nervous system by the method of MR. We chose as a model object for the study the terestrial snail, whose hemolymph is a copper system, where contents of a ferrum is low and forming the magnetic crystals of ferric oxides is not common. By method of MR there were studied frozen samples of the nervous tissue of snail: control animals and tissues of snails, which were injected with ferric citrate. The measurements were conducted on the spectrometer EPR spectrometer Bruker-EMX of 3 centimeter range.

We discovered signals MR of particles, characterized by the dependency for directions of the magnetic field. Angular dependency of signals demonstrate that discovered particles in nervous tissues ranked, i.e. oriented along some chosen directions. This dependency is well described at the account of axial and cubic contributions to the anisotropy of MR signals. Axial contribution allows to expect that particles are builded in chains, cubic anisotropy can be characteristic by ranked arrays of particles. Similar characteristics were earlier discovered for nanoparticles of magnetite, extracted from tissue of insects.

For identifications of MR signals there were studied the tissues of snails, which were injected by ferric ions through the ferric citrate. As a result of injection of ferric ions in the system, signals of MR increase that allows to expect a participation of a protein ferritin in formation of these particles. The temperature dependence of these signals demonstrate a transformation of the crystalline structure near temperatures of 120-130K which are characteristic for crystals of the magnetite.

NON-NMDA IONOTROPIC GLUTAMATE RECEPTORS IN THE HONEYBEE BRAIN

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Glutamate plays important neuromodulatory role in central nervous system of mammals and insects. Glutamate receptors take part in neurological processes that underlie learning and memory. We have already demonstrated the role of non-NMDA ionotropic glutamate receptors in olfactory learning and memory and mushroom bodies neuronal activity in honeybees (Lopatina, 2004). We made a preliminary comparison of nucleotide and amino acid sequences between mammal non-NMDA glutamate receptors (AMPARs/KARs) and honeybee receptors and revealed high homology (>90%). In this study we investigated the honeybee brain homogenates by Western blot (anti GluR5/6/7 subunits antibody) and discovered a

band (~110 kDa). Immunostain of honeybee brain revealed the KARs presence in mushroom bodies. This result suggests that there is at least one type of non-NMDA glutamate receptors in honeybee brain.

HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY OF THE NERVOUS SYSTEM AND NEUROMUSCULAR INTERACTIONS IN NEMERTEANS

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Nemerteans are a small group of predominantly marine unsegmented worms characterized by a specialized muscular proboscis used for capturing prey. Nemerteans are a relatively poorly known group with unclear phylogenetic relationships and taxonomic structure. The nervous system and neuromuscular interactions in nemerteans are still not fully understood. The purpose of this study was to conduct a comparative histochemical and immunocytochemical analysis of the nervous and muscular systems of several nemertean species from various taxonomic groups, namely, *Poseidon (=Lineus) viridis*, *Poseidon (=Lineus) ruber* and *Cerebratulus sp.* (Heteronemertini), *Cephalotrix linearis* (Palaeonemertini), *Amphiporus lactifloreus*, *Emplectonema gracile* and *Tetrastemma cf. candidum* (Hoplonemertini). *E. gracile* was collected from the Sea of Okhotsk near Magadan; the other species were collected from the Chupa Bay in the White Sea. Fluorescent, confocal and multiphoton microscopy of serial sections and, for the first time, of the whole mounts has revealed the distribution of catecholamine-, 5HT-, FMRFamid-, and neurotensin-ergic elements in the peripheral and central nervous systems. The study has also demonstrated the distribution of acetylcholine transferase and NADPH-diaphorase in nemerteans. Phalloidin staining has been used to reconstruct the architecture of the musculature of the body wall, cephalic region and central nervous system (cephalic ganglia and lateral nerve cords). The work was supported by the Russian Foundation for Basic Research (grant 10-04-01033-a).

NEUROCHEMICAL ORGANIZATION OF PROCEREBRUM IN SNAIL *HELIX lucorum L.*

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Olfaction in terrestrial snails is a main sensory input and plays a crucial role in plasticity and development. The procerebrum of terrestrial molluscs is an important processing centre for olfaction. The neurochemical organization of procerebrum have not been investigated in detail. It is shown that serotonin- and dopaminergic systems exert modulating influences on neural activity of procerebrum by changing their responses to odors. Using histochemistry the targets of these influences in procerebrum were shown. Using *in situ* hybridization, the expression pattern was described for 3 genes, HelSFamid, HPep, GFAD that encode the secreted proteins in the snail at different stages of embryo- and postembryogenesis. For each gene was shown a specific age-dependent dynamics of the expression pattern in the procerebral neurons that may characterize the stages of maturation of corresponding forms of behavior. Development of HelSFamide-expressing neurons in procerebrum was described using immunochemistry. Supported by RFBR grant 10-04-01726-a.

EXPERIMENTAL ANALYSIS OF TICK SYNGANGLIA *(IXODES PERSULCATUS)* NEUROTRANSMITTER SYSTEMS

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Blood-sucking ectoparasitic such as ixodid ticks are the main vectors of many viruses and pathogenic bacteria. In ixodid ticks chemical sense is very important for localizing vertebrate hosts location. It is the reason why the analysis of their behaviors and nervous system organization is very urgent.

Studies were performed on the semi-intact preparations of a tick. On the dorsal surface of singanglion has been identified a zone, which was selectively activated by the air stream odor presentation to Haller's organ, tick chemosensitive organ. Electrophysiological studies revealed that in this zone the odors trigger the neuron population responses of different polarization (de-/hyper) depending on the type of stimulus (attractive/repellent/nonsignificant). Also, we showed that the activity of neurons responding to olfactory stimuli is modulated by octopamine and serotonin. Octopamine changed the polarity of the responses initiated by attractive smells and promoted the responses to nonsignificant volatile chemicals. Serotonin increased the amplitude of the invoked responses and did not influence the polarity. Behavioral tests reveal that serotonin increases the level of tick locomotor activity irrelatively of the stimulus type, while octopamine changes tick behavioral program from searching the host to searching the covert.

These data permit to come closer to understanding the interrelationship between tick neuronal organization and behavior. This will help to find the way to modulate the behavior of these ectoparasitics.

ROLE OF ATYPICAL ISOFORM OF PROTEIN KINASE C (PROTEIN KINASE M□) IN THE MAINTENANCE OF LONG-TERM SYNAPTIC POTENTIATION IN THE SNAIL NEURONS

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Neurons of neural circuit involved in withdrawal behavior of terrestrial snail were well described previously. It was shown that identified giant interneurons located in parietal ganglia of snail CNS could trigger whole aversion reactions like tentacles and head retraction. Long-term facilitation of excitatory synaptic inputs from sensory neurons to giant interneurons was supposed to be basis of aversion learning and memory of terrestrial snails. Considered previous data that PKC was required for long-term facilitation induction in snail *Helix lucorum* and that atypical PKCs were involved in long-term plasticity in sea mollusc *Aplysia*, we investigated if atypical PKCs also take part in maintenance of long-term facilitation in withdrawal neural circuit of snail *Helix lucorum*. Long-term facilitation of excitatory synaptic inputs to giant interneurons was induced by high-frequency stimulation combined with bath serotonin application and lasted at least four hours. We found that bath application of $2*10^{-6}$ M ZIP (selective peptide blocker of atipical PKC) at 90 min after tetanization reduced EPSP amplitude almost to the non-tetanized EPSP values (63±6% from initial EPSP, n=11 vs 42±8%, n=14, respectively, n.s.). Application of scrambled peptide at the same concentration didn't affect EPSP amplitude in comparison with vehicle application (153±23%, n=13 vs 142±20%, n=17 from initial EPSP respectively, n.s.). These data supported the idea of atypical PKC involvement in post-induction maintenance of long-term plasticity in CNS of *Helix lucorum*.

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THE NEURONETWORK MODEL OF COMPLEX BEHAVIOR IN TERRSESTRIAL SNAIL: DEFENSIVE AND FEEDING BEHAVIOR

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The model of a neuronal network controlling the defensive and feeding behavior of snail is presented. Each network consists of minimal formal elements that imitate groups of neurons. The model contains 8 - 12 formal neurons and is functioning on the basis of a Hebb principle. Two different forms of behavior in a model are imitated by several functional subsystems. Patterns are stored in a matrix of synaptic strengths. The neuronal networks consist of three layers: sensory neurons, interneurons and motor neurons.
The analysis showed good correspondence to principle characteristics of behavioral reactions in animals and models. It shows a possibility of imitation of a surprising variety of "behavioral repertoire" with realization of the basic phenomena of complex behavior. The most interesting is a correspondence of the features describing plastic behaviors of the animal and models using one single rule – the Hebb principle. The simple model allows to simulate such forms of behavior as habituation, formation and weakening of a conditioned reflex, sensitization. To this model were added two mechanisms of information maintenance – short-term and long-term, as well as a property of the transition of short-term memory to the long-term memory. The model now possess the long-term memory capacity, as well as an independent recovery of the associative memory after a procedure of weakening of a conditioned reflex.
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EFFECTS OF CHRONIC INJECTIONS OF IBMX AND IMIDASOLE ON ELABORATION OF CONDITIONED FOOD AVERSION IN TERRESTRIAL SNAILS

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It is known that levels of cAMP depend on activities of phosphodiesterases. In our work we study the dynamics of the elaboration of the conditioned food aversion

under chronic injections of IBMX and imidasole immediately after procedure of learning. IBMX is a nonspecific inhibitor of phosphodiesterases, preventing the intracellular accumulation of cAMP, while imidasole, on the contrary, raises activity of phosphodiesterases.
For the elaboration of the conditioned food aversion, as a conditioned stimulus was presented a small piece of the cucumber, delivered to lips of the snail on a steel needle, and at a moment of first chewing movement a current that served as an unconditional stimulus was passed through the needle. The second stimulating electrode was manually put on the foot of the snail during first chewing movement. The snails were considered to elaborate the aversion memory if they reached the criterion of 10 contacts without chewing (with following aversion) or demonstrating an aversive reaction in response to food presentation.
It was shown that IBMX and imidasole influence the dynamics of learning in snails. It was found that elaboration of the conditioned food aversion was formed by 70 combined food+shock trials under chronic injections of IBMX in contrast to 80 trials under injections of saline and 100 trials under injections of imidasole. Thus, injections of phosphodiesterases inhibitor accelerates the learning, but injections of activator of phosphodiesterases, in opposite, slows it. Testing of the defensive ommatophores withdrawal before and after the experiment shows a reliable increase in defensive reactions of snails in all groups. Probably, this result indicates the presence of environmental conditioning in studied forms of the conditioned reflex.
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THE DISTRIBUTION OF P-COFILIN IN THE AREA OF DROSOPHILA LARVAL NEUROMUSCULAR JUNCTION IN THE WILD-TYPE Canton S AND THE MUTANT agnts3.

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Modern biomedical evidence indicates the important role of disturbances of the actin cytoskeleton in the genesis of age-dependent neurodegenerative (ND) diseases, prion diseases (Ramaekers, 2004). One of the manifestations of these diseases is occurrence of motor malfunctions. Elucidation of this problem is aimed to gain fundamental knowledge about the mechanisms of realization and control of

motor functions, creating new means of pharmacological and gene therapy of their malfunctions.
Remodeling of the actin cytoskeleton determines the localization of neurotransmitter receptors in synaptic densities and dendritic spines morphology affecting synaptic plasticity - the basus of learning and memory, axonal transport and phagocytosis (Birkenfeld, 2001; Meng, 2002). The signal cascade of actin remodeling transmits the signal via the chain: receptors - Rho family of small GTPases of Rho-family - LIM kinase 1 (LIMK1) - cofilin - actin. Hemizygosity for the LIM kinase 1 (LIMK1) gene for the key enzyme of actin remodeling is the cause of cognitive impairments in a genomic disease Williams syndrome (Jarvinen-Pasley, 2008). In this regard, the study of this cascade, as well as the finding of the main pathways affecting its functioning is very important.
We believe that our model fits well the molecular basis of pathological processes involving the development of neurodegenerative and genomic diseases both in higher invertebrates and in mammals. Neuromuscular junctions (NMJ) are typical for synapses mediating the interaction of motor neurons, skeletal muscle and glial cells. One of the main axon functions during the development is the capture of signals from the extracellular surrounding and the transformation of these signals into changes in the expression of nuclear genes of the neurons (Takemura et al., 2009).
The research problem is the identification of possible malfunctions in the signal cascade of actin remodeling in larval NMJ in wild-type Canton S (norm) and agnts3 mutant (Williams syndrome model).
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BUCCAL NEURONS RESPONSIBLE FOR NON-SYNAPTIC COORDINATION OF MOTOR RHYTHMS IN CNS OF LYMNAEA

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Giant hyperpolarizing waves (GHW) of the nitrergic neurons B2 controlling gut motility in Lymnaea are characteristic elements of transient coordination between the B2 rhythm and that of the buccal central pattern generator, controlling radula movements during feeding. GHWs are never observed in isolated B2s, however GHW-like changes of the B2 membrane potential can be observed in conditions excluding synaptic but permitting extrasynaptic transmission (Dyakonova and Dyakonova, 2010). Possible cell sources of volume release inhibiting the B2s have been unknown.
Here, we identified novel neurons that seem to be responsible for the occurrence of GHWs in the B2 neurons. Each of the paired buccal ganglia was found to contain

two such neurons. We called them buccal transient coordinators (Btc). Electrical stimulation (0.5 - 1.5 nA, 3 –10 s) of any single Btc neuron produces deep (tens of mV) and long lasting (tens of sec) wave of hyperpolarisation in the ipsilateral and contralateral neurons B2. The response of the B2 neuron correlated positively with duration and intensity of Btc neuron stimulation. The effects persisted in high divalent ion saline (Hi-Di), which suppresses polysynaptic transmission, therefore direct interaction between the Btc and B2 neurons seems likely. During spontaneous or NO-induced generation of the buccal rhythm, Btc neurons receive excitatory input during the third phase of the buccal rhythm. These data suggest that Btc neurons may play a role in the mechanism of non-synaptic coordination between the motor rhythm of the radula and that of the foregut.
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FIGHTHING BEHAVIOR IN MALE CRICKETS TREATED WITH SEROTONIN PRECURSOR 5-HYDROXYTRYPTOPHAN: ENHANCED AGGRESSIVE DEMONSTRATIONS AND DECREASED ABILITY TO ESTABLISH LOSER

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In male cricket Gryllus bimacultus, which has lost a fight with a contester, significant depletion of serotonin has been observed (Murakami and Itoh, 2001). In naive males, pharmacologically induced serotonin depletion reduced fight duration and win probability and enhanced the escape behavior (Dyakonova et al., 1999, Stevenson et al., 2000). There is no data, however, on whether and how upregulation of serotonergic system influences aggressive interactions in crickets. Here, we report that single injection of serotonin precursor 5-hydroxytryptophane (5-HTP, 0.1 M in 50 ul of Ringer solution, 2 –2.5 h before interaction) results in significantly extended fights between males (36□13 s versus control 5□1s, p< 0.0005) with decreased probability to produce clear winners and losers. In all control pairs (n=17, Ringer injection), fight resulted in establishment of normal winner/ loser relationships, whereas, in 5 of 18 of 5-HTP treated pairs, the loser had not been established after multiple fights until the end of observation (5 min). Multiple fights (with mean number of fights 4 and maximal 16 in a pair), interrupted by periods of chasing, were characteristic for 5-HTP treated crickets. All forms of aggressive demonstrations were obviously activated in 5-HTP-treated crickets. For example, the demonstration of spread mandibles, accompanied with unceasing rival singing in both males, could last 10-20 times longer than in the control. In contrast, 5-HTP did not affect higher levels of aggression.
These findings suggest that serotonin activates aggressive demonstrations and suppresses the decision to retreat in male crickets. They are consistent with

previous data and explain why decrease in serotonin functioning is required for production of clear fight losers. Supported by RFBR 11-04-00674.

Comparative morphology of the serotonergic system
in two related species of archiannelid worms (Annelida: Polychaeta: Dinophilidae)
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Synthetic group Dinophilidae (Archiannelida) includes small interstitial marine worms which adults have length about 1.5 mm and expresses some features of neoteny. The detailed morphology of their locomotory system and underlying 5-HT innervations is unknown for most species. We investigated the morphology of serotonergic (5-HT) system as well as their ciliary locomotory bands in two related species: Dinophilus gyrociliatus (warm water) and Dinophilus taeniatus (cold-water) using immunocytochemistry combined with laser confocal microscopy.

Tubulin immunoreactivity reveals two ciliary bands at the head region in both species. There are seven ciliary bands along the body and one continuous ventral ciliary field in D. gyrociliatus and eleven ciliary bands and one continuous ventral ciliary field in D. taeniatus. The head ciliary bands are wider at ventral side and have the dorsal breaks. The other ciliary bands are complete and join with the ventral ciliary field in both species.

In D. gyrociliatus 5-HT-antibody marks neuropile with solitary parikarya in the head region. The complex varicose network of processes with numerous irregulary located small perikarya underlay the ventral ciliary field. The paired thin nerves can be also identified underneath each body ciliary bands. To the contrary, in D. taeniatus only neuropile with no perikarya were detected in the head region. Five prominent longitudinal nerve cords: two thick and three thin, connected by five thick commissures can be identified underneath the ciliary field. Few cell bodies are located in each region there cords and commissures cross.

Our results demonstrated that two closely related species of archiannelid have the same morphology of ciliary locomotory system.

Despite of that, organization of related 5-HT system in D. gyrociliatus and D. taeniatus is principally different. Future behavior and physiological study is necessary to determine whether this distinct organization in 5-HT system reflects some physiological differences. The work was supported by RFBR grants #12-04-01510 and #12-04-10119.

CHANGES OF CARDIAC ACTIVITY DURING THE REALIZATION OF NEURVOUS MECHANISMS OF DEFENSIVE BEHAVIOR IN AQUATIC MOLLUSKS
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At slowly moving aquatic mollusks one in the most behavior important forms is the reaction of protection from predators or harmful environmental factors. The protective unconditioned reflex realizes by means of peripheral and central structures of the nervous system. Information from peripheral chemoreceptors through the nerve connections can go to the different organs and functional systems of mollusks. It was investigated the sensitivity of cellular structures of osphradium to changes in salinity or osmolarity of water, and the possibility of characteristics of cardiac activity (HR) changes under these influences. By the method of extracellular recording it was shown the rise of the impulses frequency in the pleuro-visceral nerve trunks at the chiton (Acanthopleura gemmeta), and so in osphadium nerve of mollusks Cliona limacina and Lymnaea stagnalis in response to the dilution of seawater or on NaCl solutions. Using the path-clamp method we discovered reinforcement of impulsation in the osphadium giant neurons of Lymnaea stagnalis in replay to the application of sodium chloride (10 mmol / l). Inward currents of 2-3 nA were recorded under addition the same NaCl solution to the physiological solution. The reactions of osphradium to these shifts of the water quality reflected in the characteristics of HR as was shown in our special experiments on the mollusk Littorina littorea. Dilution on 50% seawater in the tank caused the defensive reaction, accompanied by a significant decrease of heart rate. In response to the injection 1 ml diluted seawater into the mantle cavity the reaction in the form of closing the mouth of the shell was not observed, but the HR decreased on 20%. Identified reactions of the cardiac activity greatly violated after extirpation osphadium. These results were shown that stimulation of the peripheral link of chemosensory system (osphradia receptors) influences on mollusks HR and can manifest as bradycardia or tachycardia.