XI EAST EUROPEAN CONFERENCE OF THE INTERNATIONAL SOCIETY FOR INVERTEBRATE NEUROBIOLOGY

XI РЕГИОНАЛЬНАЯ КОНФЕРЕНЦИЯ МЕЖДУНАРОДНОГО ОБЩЕСТВА НЕЙРОБИОЛОГИИ БЕСПОЗВОНОЧНЫХ

ABSTRACTS

Simpler Nervous Systems

Простые нервные системы

May 15-19
Zvenigorod 2016

XI East European Conference
of the International Society for Invertebrate Neurobiology

"Simpler Nervous Systems"

May 15-19
Zvenigorod 2016

Supported by International Brain Research Organization, Russian Foundation for Basic Research, International Society for Invertebrate Neurobiology, Russian Academy of Sciences, I.P.Pavlov Russian Physiological Society, Institute of Higher Nervous Activity and Neurophysiology RAS, N.K. Koltsov Institute of Developmental Biology RAS

XI РЕГИОНАЛЬНАЯ КОНФЕРЕНЦИЯ МЕЖДУНАРОДНОГО ОБЩЕСТВА НЕЙРОБИОЛОГИИ БЕСПОЗВОНОЧНЫХ

ПРОСТЫЕ НЕРВНЫЕ СИСТЕМЫ 2016

15-19 мая 2016, Москва-Звенигород

Конференцию поддержали:
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РОССИЙСКОЕ ФИЗИОЛОГИЧЕСКОЕ ОБЩЕСТВО ИМ. И.П.ПАВЛОВА
ИНСТИТУТ ВЫСШЕЙ НЕРВНОЙ ДЕЯТЕЛЬНОСТИ И НЕЙРОФИЗИОЛОГИИ РАН
ИНСТИТУТ БИОЛОГИИ РАЗВИТИЯ ИМ. Н.К. КОЛЬЦОВА РАН
МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО ИССЛЕДОВАНИЮ МОЗГА (IBRO)

Organizing Committee:

Co-chairman: Prof. Balaban, Pavel (Russian Academy of Sciences, Russian Physiological Society)
Co-chairman: Prof. Dr. Zakharov, Igor (Moscow, Russia)
iszakharov@yandex.ru

Program

15, May, 16.00 – Departure from Institute of Higher Nervous Activity (Moscow, Butlerova str. 5a) on the bus to the biological station of M.V. Lomonosov Moscow State University (Moscow area, *latitude* 55°41′13″N (55.687064), *longitude* 36°44′54″E (36.748466), nearest town Zvenigorod).

16, May

9.30-12.30
Opening of the Conference

Comparative neurophysiology of memory. **Balaban P.M.** (Moscow, Russia).

From neurons to behaviour: complex neuronal changes of Lymnaea to exposure of progestogens pharmaceuticals, **Zsolt Pirger** (Balaton Limnological Institute, TIHANY, Hungary)

Nervous system miniaturization in smallest insects. **A.A. Polilov**, (Lomonosov Moscow State University, Biological faculty)

13.30-14.30 Lunch
14.30-16.30 Poster session (posters A-K)
16.30-17.00 Tea time
17.00- 19.00

From peripheral sensory cells to the integrative nerve centers: phylogenetic and ontogenetic morphodynamics of sensory cells. **Zaitseva O.V., Voronezhskaya E.E.** (St.Petersbourg, Moscow, Russia).

Serotonin and neuropeptide FMRFa in the nervous system of flatworms **Kreshchenko N.D.(1), Terenina N. B.(2), Zaripova F.F.(3)** (1) Insitute of Cell Biophysics of Russian Academy of Sciences, Pushchino, Moscow Region, (2) Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Center of Parasitology, Moscow, (3) Private Educational Institution of Higher Professional Education "St. Petersburg Medico-Social Institute", St. Petersburg

Innervation of the lophophore in ctenostome ectoprocts uncovers the bryozoan bauplan. **Temereva E.N., Kosevich I.A**. (Russia, Moscow, Moscow State University, Biological Faculty, Dept. Invertebrate Zoology)

17 May

9.30-12.30

Behavioral patterns and patterns of neurospecific gene expression in embryonic and postembryonic development in snails. **Zakharov I.**[(1)], **Boguslavsky D.**[(1)], **Balaban P**.[(2)]. [(1)]Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Laboratory of neurobiology of development; [(2)]Institute of High Nervous Activity of Russian Academy of Sciences, Moscow, Laboratory of cellular neurobiology of learning

New thermogenetic and biosensory technologies in neurobiology. **Belousov V.** (Moscow, Russia).

Organization of senso-efferent systems in the pond snail (Lymnaea stagnalis) **Réka Horváth, Izabella Battonyai, Károly Elekes (**Department of Experimental Zoology, Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary)

13.30-14.30 Lunch

14.30-16.30 Poster session (posters L-R)

16.30-17.00 Tea time

17.00- 19.00

Encoding of High Frequencies by Snail and Rat Neurons: Correlation with an Action Potential Initiation Dynamics. **Malyshev A. (**Moscow, Russia).

Detection of the newly synthesized RNA in nervous system of the terrestrial snail *Helix*. **Ierusalimsky V.N.** (Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences, Moscow)

Electrical properties of identified neuron (RPeD1) after operant conditioning of aerial respiration in Lymnaea. **Sidorov A.** (Minsk University, Belorussia).

18 May

9.30-12.30

Serotonin and dopamine are both active during the pre-nervous emvryonic development of vertebrates. **Nikishin D.A.**[(1, 2)], **Khramova Yu.V.**[(2)], **Bagaeva T.S.**[(2)], **Kremnyov S.V.**[(2)], **Shmukler Yu.B.**[(1)]. [(1)]Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Laboratory of Problems of Regeneration; [(2)]Lomonosov Moscow State University, Moscow, Biology Faculty, Department of Embryology.

Does fear exist in insects? **Kamyshev N.G., Goncharova A.A.** (St.Petersbourg, Russia).

UV-light-induced freezing in cockroach Periplaneta Americana: sleep or passive avoidance? **Novikova E.S., Zhukovskaya M.I. (**Sechenov

Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint-Petersburg)

13.30-14.30 Lunch
14.30-16.30 Poster session (posters S-Z)
16.30-17.00 Tea time
17.00- 19.00

The earliest Bilaterian nervous systems in Nemertodermatida (Xenacoelomorpha): an example of plasticity **Raikova O.I.[1, 2], Meyer-Wachsmuth I.[3, 4], Jondelius U.[3]** [1]Zoological Institute of the Russian Academy of Sciences, St.-Petersburg; [2]Saint-Petersburg State University, Chair of invertebrate zoology; [3]Swedish Museum of Natural History, Stockholm, Sweden; E-[4]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic;

Philosophy, vegetables and nicknames of Pavlov's dogs: How the names of Drosophila mutations tell us the story of understanding learning and memory. **Savvateeva-Popova E**. (Pavlov Inst. Of Physiology, St.Petersbourg, Russia).

Coding of sound frequency in the mosquito auditory system. **Vorontsov D.D.[1], Lapshin D.N**.[2], [1]Koltzov Institute of Developmental Biology Russian Academy of Sciences, Moscow, Laboratory of neurobiology of development; [2]Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), Moscow, Laboratory of Sensory Information Processing;

19 May

8.00 Breakfast
9.00- ***Departure to Moscow***

ABSTRACTS

IN ALPHABETICAL ORDER

CHARACTERIZATION OF SNAILS' GRAVITAXIS AND ITS CHANGES AFTER EXPOSURE TO GRAVITATIONAL STIMULI.

Aseyev N., Vinarskaya A., Roschin M., Zyuzina A., Korshunova T. [(1)], Lemak M., Malyshev A., Ierusalimsky V. N., Zakharov I. S. [(1)], Balaban P. M., Popova Y. [(2)], Boyle R. [(2)]

Institute of Higher Nervous Activity & Neurophysiology RAS, Moscow
(1) Koltzov Institute of Developmental Biology, Moscow
(2) Life Sciences Division of NASA Ames Research Center, USA

We investigated the behavioral response of terrestrial gastropod snail *Helix lucorum* L. to sudden fall of horizontal platform which snail crawled to vertical position 'head down'. Behavior was filmed by digital camera, and records of all 5 groups of snails were analyzed later by blind protocol. We have studied naïve snails (n=15), also as additional 3 groups of controls to space biology satellite Bion M1 (launched at May, 2013, 30 days of microgravity exposure), and postflight group (12 h after landing, n=15) as well. One of control group was affected by simulated short-term overloads in centrifuge (10G, 2 s; n=21), providing vestibular stimulus in direction opposite to weightlessness; two other groups were aimed to additionally control extraneous factors of unmanned flight: starvation (n=17) and light cycle disruption (n=20).

Most of the snails of all groups show negative geotaxis and turned its body to position head above the shell (82 of 88 snails — 93%). We distinguish few phases of this gravitaxis reaction: 0 — immediately after platform fall, snails withdraw its tentacles in fear reaction (less then 1 s); T0 — snails protract tentacles back to almost maximal length, eyes appears at the tentacles tips, and head movements began; T1 — snails scanning space around by movements of head and turn head upward to level of inferior margin of the shell; T2 — snails accomplish body turn to level head at the superior margin of the shell; T3 — snails accomplish U-turn, its head on the level of the rear tip of the foot; T4 — full body turn accomplished, snails moving upward (zenith±15°).

We found significant differences of Bion M1 postflight group in latency of early gravitaxis phase T0 (Kruskal-Wallis test, Dunn's method of *post-hoc* pairwise tests, $p_{adj} \leq 0.001$) but no statistically evident differences for later phases latencies or durations. Retracted tentacles length at phase 0 was significantly smaller in control groups in comparison to postflight group (KW $p = 0.009$, Dunn's $p_{adj} < 0.035$ for 3 of 4 control groups, and insignificant $p_{adj} = 0.1$ for comparison with starvation group). Tentacles length at T4 in postflight group was smaller, then 3 controls, and in group of simulated overloads were higher then 3 controls, what probably reflects previous opposite gravitational expositions of these groups. No significant differences between group were found in amount of head-scanning movements and inter-tentacle angles at different gravitaxis phases. Our data suggests microgravitational exposure affects snail gravitaxis behavior, and our physiological records of statocyst nerves in postflight and control groups confirm it mediated at least partly by vestibular system.

ADAPTIVE PLASTICITY OF HELIX STATOCYST TO CHANGES IN GRAVITATION.

Aseyev N., Malyshev A., Roschin M., Zyuzina A., Vinarskaya A., Lemak M., Korshunova T. (#), Ierusalimsky V. N., Zakharov I. S.(#), Balaban P. M., Popova Y.(*), Boyle R.(*)

Institute of Higher Nervous Activity & Neurophysiology RAS
(#) Koltzov Institute of Developmental Biology
(*) Life Sciences Division of NASA Ames Research Center

We investigated the adaptive plasticity of vestibular apparatus in frame of the space biology project Bion M-1 (launched at May, 2013, 30 days duration), and compared results with data of Foton M-3 (2007, 14 days). In terrestrial gastropod snail *Helix lucorum* L. we performed extracellular recordings from *n. vestibularis* during series of the semi-intact preparation tilts up to 19 degrees in different orientations and with different speed. Spike discrimination allowed us to analyze separately responses of up to 11 primary receptor neurons from the 13 that comprise the statocyst in the snail.
We found ON- and OFF-responses in activity of individual neurons of snail statocyst to tilts in certain direction. Analysis of neural responses to tilts with different directions revealed that single receptor cells can respond to vestibular stimuli in all directions, but is specifically tuned for only one. Motion in this direction evokes strongest ON-responses of the neuron; motion in opposite direction evokes strongest OFF-responses.
Animals tested after space flight showed normal spontaneous activity in vestibular nerve, but respond strongly higher to vestibular stimuli up to 20 h after landing. After that critical time statocyst responses have less magnitude than control due to readaptation processes. Our results suggest that plasticity of snails' vestibular system to changes in gravitation originates in primary sensory neurons of the statocyst.

NITRIC OXIDE TRIGGERS PROTEIN DEGRADATION IN NEURONS.

Bal NV, Roshchin MV, Salozhin S, Balaban PM
Institute of Higher Nervous Activity and Neurophysiology of RAS

We demonstrated earlier that simultaneous blockade of protein synthesis and NOS led to LTP formation similar to control conditions, whereas blockade only of the NOS impaired late phase of LTP. The effect of L-NAME (nitric oxide synthase inhibitor) on LTP in our experiments is similar to protein degradation inhibition influence showed by other groups (Fonseca R. et al., 2006). These data suggest that nitric oxide can trigger protein degradation during synaptic plasticity.

To test this suggestion we transduced cultured hippocampal neurons by lentivirus consisting GFP with proteasomal degradation signal ubiquitin. Fluorescence recording was carried out with confocal microscope at the 18-23 day in vitro. Before experiment we added picrotoxin 30 mkM to increase neuronal activity and anisomycin (50mkM) to inhibit protein synthesis. Picrotoxin was washed before start of recording. We analyzed GFP fluorescence decay rate in neuronal processes during 40 min and revealed that blockade of nitric oxide synthase by L-NAME significantly decrease the fluorescence decay rate in this condition. This data also support our idea that nitric oxide can trigger protein degradation during neuronal activity.

ACTIVITY OF SEROTONERGIC NEURONS IS NECESSARY FOR RECONSOLIDATION OF MEMORY.

P.M. Balaban, M. Roshchin, A.B. Zuzina, A. Timoshenko, A.Y.Malyshev

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences

Retrieval of memory (reminding) is followed either by reconsolidation that maintains the memory, or by extinction that results in weakening of existing memory or formation of a competing memory. In our study we analyzed the behavior and responses of identified neurons involved in the network underlying food-aversion associative learning in terrestrial snail *Helix*, and made an attempt to describe the conditions in which the retrieval of memory leads either to extinction or reconsolidation.

Using a specific for serotonergic neurons neurotoxin 5,7-DiHT it was shown previously that the serotonergic system is necessary for induction of learning, but is not necessary for retrieval of the memory. These results suggest that serotonergic neurons that are necessary as part of reinforcement for developing the associative changes in the network may be not participating in the retrieval of memory. The hypothesis tested in the present study is whether the activity of the "reinforcing" serotonergic neurons is the gate condition for the choice between extinction/reconsolidation triggered by memory retrieval: if these serotonergic neurons do not respond during the retrieval due to adaptation, habituation, changes in environment, etc., then we will observe the extinction; while if these neurons respond to the CS during memory retrieval, we will observe the reconsolidation phenomenon.

It was shown in semi-intact and isolated CNS preparations that several serotonin applications effectively increased amplitude of synaptic inputs (CS) to the withdrawal interneurons, and that intracellular activation of a single identified giant serotonergic pedal cell #4 was effective as a reinforcement in *Helix*. Electrophysiological experiments were performed in semi-intact preparations that allowed to deliver a drop of juice to the lip and simultaneously record intracellularly from identified interneurons involved in feeding behavior and withdrawal behavior before and after associative training sessions. It was shown that after food-aversion

training, consisting of pairing of food presentation with electric shock to the nerve, the serotonergic cerebral interneurons involved in feeding have not changed significantly the responses to food (CS), while the premotor interneurons (FMRFa-containing) involved in triggering the withdrawal started to respond with a spike discharge to previously subthreshold food stimuli. Serotonergic pedal neurons, whose intracellularly induced activity was shown to be capable to elicit associative changes in tentacle-withdrawal network (reinforcing neurons), did not respond before training to the non-noxious stimuli. After the associative training these serotonergic neurons started to respond during retrieval to previously ineffective stimuli, thus participating in triggering the reconsolidation. Impairing functioning of the serotonergic system with the neurotoxin 5,7-DiHT resulted in extinction of memory in behavioral experiments. Thus, participation of the "reinforcing" serotonergic neurons in memory retrieval can be the gate condition for the choice between extinction/reconsolidation.

POSTSYNAPTIC MECHANISMS OF MEMORY MAINTENANCE

Pavel M. Balaban

Institute of Higher Nervous Activity and Neurophysiology, Russian

Academy of Sciences,

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 and a possibility of its modification during the late phase of LTP when neurogenetical mechanisms play an essential role is unknown.

Nitric oxide (NO) is synthesized as needed by NO synthase (NOS) and does not react with receptors but diffuses into adjacent cells. In place of reversible interactions with targets, NO forms covalent linkages to a multiplicity of targets which may be enzymes, such as guanylyl cyclase (GC) or other protein or nonprotein targets. One of possible ways of NO influence on nerve cell activity is S-nitrosylation, the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine, conveys a large part of the ubiquitous influence of NO on post-translational regulation of most or all main classes of proteins. S-nitrosylation changes physiological function of existing proteins, mostly inhibiting their normal role, in some way changing the "past" of the nervous system. Influence of NO via GC activates intracellular signaling cascades and triggers increased synthesis of proteins, influncing the "future".

In our experiments we tested the idea that NO is involved in labilization of memory during reactivation of memory. We investigated the influence of blocking NO function on reconsolidation of context memory in terrestrial snails Helix lucorum L. After a 10-day session of electric shocks in one context only, context memory in snails was observed in test sessions as the significant difference of amplitudes of withdrawal responses to tactile stimuli in two different contexts. After a 1 day rest, a session of "reminding" was performed, preceded by injection in different groups of

the snails either with vehicle, or combination of a protein synthesis blocker anisomycin (ANI) with one of the following drugs: the NO scavenger PTIO, or the NO-synthase inhibitors L-NNA, nitroindazole, L-NAME, or an NO donor SNAP.

Testing the context memory at different time intervals after the reminder under ANI injection has shown that the context memory was impaired 24 hrs and later, while the reminder under combined injection of ANI and each of the NO-synthase inhibitors used or the NO scavenger showed no impairment of long-term context memory. Injection of the NO donor SNAP with or without reminder had no effect on context memory. Obtained results demonstrate that NO is necessary for labilization of a consolidated context memory.

PKMZETA IS NECESSARY FOR MAINTAINING THE LONG-TERM FACILITATION OF GLUTAMATERGIC SYNAPTIC INPUTS BUT NOT OF SOMATIC GLUTAMATE RESPONSES IN THE SNAIL NEURONS

P.M. Balaban, M.S. Lemak, T. Korshunova, M. Roshchin A.B. Zuzina, A. Vinarskaya, A.Yu. Malyshev

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences

In behavioral experiments it was shown previously that selective inhibitor of PKMζ Zeta Inhibitory Peptide (ZIP) impairs aversive context memory in terrestrial snail *Helix lucorum*.

Long-term facilitation of excitatory (presumably glutamatergic) 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 underlying aversive tentacle withdrawal. Long-term facilitation of excitatory synaptic inputs to premotor interneurons was induced by high-frequency stimulation combined with 5 serotonin bath applications and lasted at least four hours. We found that bath application of 2*10-6 M ZIP at 90 min after tetanization reduced EPSP amplitude almost to the non-tetanized EPSP values. Application of scrambled ZIP peptide at the same concentration didn't affect the EPSP amplitude in comparison with saline or scrambled ZIP applications. Results support the idea of PKMζ involvement in post-induction maintenance of long-term synaptic plasticity in CNS of *Helix*.

It was shown previously that repeated (1 per min) pressure applications of glutamate on the somatic membrane ("artificial synapse") of the giant premotor interneurons triggering tentacle withdrawal in the snail elicit local potentials that can be facilitated for hours by 5 2-min serotonin applications (Balaban et al.. Eur J Neurosci. 2004 Jan; 19(2): 227-33). We repeated similar experiments under ZIP/scrZIP added 120 min after the facilitating procedure. Obtained results showed that in conditions of "artificial synapse", facilitated responses to glutamate were not influenced by ZIP or scrZIP application. These results suggest that PKMzeta is not involved in postsynaptic plasticity of somatic glutamate responses in the snail neurons.

Supported by RFBR, Council for grants of RF President, grants of RSCF.

5-HYDROXYTRYPTAMINE AND ASSOCIATIVE LEARNING IN SNAIL

Bogodvid T.Kh. (1,2), Andrianov V.V. (1), Deryabina I.B. (1), Muranova L.N. (1), Vinarskaya A.Kh. (3), Gainutdinov Kh.L. (1,4)
(1) Kazan Federal University, Kazan, Laboratory of Neurobiology; *E-mail: tat-gain@mail.ru*
(2) Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan;
(3) Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Moscow;
(4) Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan.

One of the widespread and well-investigated transmitters of the nervous system is serotonin (5-HT). The 5-HT neurons innervate specific central pattern generators and other circuits of the nerve system, receive feedback from them, and support general behavioral arousal. It is well known that learning on the basis of the defensive reflexes of molluscs is mediated by 5-HT. In connection to the discovery of the relationship between the level of 5-HT in the hemolymph of mollusks and the sensitization of reflexes, a lot of experiments have been conducted using manipulation of the 5-HT system to investigate cellular analogues of learning. These findings and questions motivated us to investigate the role of 5-HT in the mechanisms of learning by behavioural and electrophysiological methods, using its "neurotoxic" analogues 5,6- or 5,7-dihydroxytryptamine (5,6/5,7-DHT), and the precursor of 5-HT synthesis, 5-hydroxytryptophan (5-HTP). In the present work we also studied the changes of excitability of premotor interneurons under the application of 5-HT in preparations made from intact and learned snails.

The terrestrial snails *Helix lucorum*, the nervous system of which has been well described, were used for experiments. We elaborated three conditioned reflexes (CR): defensive conditioned reflex to the tapping on the shell, defensive conditioned food aversion and context conditioning. Context conditioning was considered elaborated if the reaction on the ball (i.e., standard conditions) was significantly higher than that on a flat surface. The results show that both 5,7-DHT and blocker of 5-HT synthesis p-chlor-phenylalanin themselves don't affect contextual memory after a reminder, but they inhibit the effect of blocker of protein synthesis anisomycin on disrupting reconsolidation. In other experiments the snails were trained after the injection of 5-HT, as a control, produced training after a saline injection. To produce a 5-HT deficit, 5,7-DHT were used. Injection of 5,6/5,7-DHT was found to disrupt CR, within two weeks of neurotoxin application the ability to learn had recovered. Daily injection of 5-HT before a training session accelerated CR and daily injections of 5-HTP in snails with a deficiency of 5-HT induced by 5,7-DHT restored the snail's ability to learn. We found that injection of 5-HT caused a decrease in the resting potential (Vm) of the premotor interneurons LPa3 and RPa3 on 4.5 mV in snails that received only an injection of 5-HT and on 5.5 mV in snails, which were injected by 5-HT before training. A significant decrease of threshold potential (Vt) on 4.0 mV and 4.5 mV, respectively, was found also. In third series of experiments the changes of Vm and Vt in the premotor interneurons in response to application of 5-HT (at a concentration of 1 micromol/l) in solution washing the nerve system in intact and trained snails were studied. In some experiments methiothepin was preliminarily added to solution for blockade of the 5-HT 1st type receptors. It was found that application of 5-HT causes a reliable decrease of Vm of premotor interneurons of both naive and trained snails. Also a reliable increase of Vt of these neurons of trained snails in contrast to the naive animals on application of 5-HT was found. Methiothepin, added to the washing solution for 30 minutes before application of 5-HT, removes its effect on Vm in intact snails and reduces – in trained. The results obtained demonstrate also the participation of the 1st type 5-HT receptors in this reaction.

This work is supported by RFBR, grant nr. 15-04-05487_a.

Proteasome activity in procerebral ganglion *Helix aspersa* in the period of active neurogenesis.

Boguslavskiy D.V., Zakharov I.S., Karpova Y.D.
Koltzov Institute of Developmental Biology
of Russian Academy of Sciences IDB RAS, Moscow; *E-mail:*
boguslavsky@rambler.ru

Proteasome plays the main role in the proteolysis of the proteins in the Metazoa's cells, whereas lysosomes specialize mainly in the hydrolysis of the structural components of the cell and protein aggregates. The proteolytic activity of the proteasome, their polypeptide composition and physico-chemical properties are regulated in accordance with the state of the cells and extracellular signals, that enable animals adaptation changing environmental conditions. Organization of the nervous system of *Helix aspersa* mollusc shellfish allows analyzing functional relationships and the formation of neuronal networks during in individual development, as well as changing's during development adults. Over the past years, we explore the subtle regulation mechanisms of proteolytic ubiquitin-proteasome system (UPS) and the interaction of the UPS with the system of chaperone proteins (heat shock proteins - HSP) in the nerve cells of the mollusk *Helix aspersa* in certain periods of their development associated with the new forms of behavior in condition of animal functional state. The data are showing the change of chymotrypsin-like activity of the proteasome and caspase (in homogenates of samples bleached by hydrolysis of fluorogenic oligopeptide Suc-LLVY-AMC and Z-LLG-AMC, respectively) and total proteasome pool procerebrum in juvenile Helix aspersa species in the period of active neurogenesis. Proteasomal activity procerebrume is 1.92 times higher compared with visceral ganglion. High proteasomal procerebrume activity is also shown by Western blot analysis and immunohistochemical staining of nervous system with antibodies to the alpha subunit of the proteasome.

The study was supported by RFBR grants 14-04-00537 and 14-04-00875.

CHRONICALLY EXPRESSED BDNF AND PROBDNF REGULATE PRE- AND POSTSYNAPTIC STRUCTURES DIFFERENTLY IN PRIMARY NEURAL CULTURE.

Borodinova A.A., Spivak Yu.S., Volobueva M.N.

Institute of High Nervous Activity of Russian Academy of Sciences, Moscow; Laboratory of molecular neurobiology; *E-mail: borodinova.msu@mail.ru*

Brain-derived neurotrophic factor (BDNF) is believed to be the key regulator of synaptic structure and plasticity. It is synthesized as a precursor protein (proBDNF) that undergoes proteolytic cleavage in order to become a mature molecule. Recent evidences suggest that proBDNF has its own biological role, frequently in opposition to BDNF functions. We are testing the hypothesis that BDNF and its precursor can differently regulate pre- and postsynaptic characteristics in cortical neurons.

Lentivirus constructs, carrying plasmid with coding sequence for either proBDNF (pCSC-proBDNF) or cleavage-resistant proBDNF (pCSC-proBDNF-CR), were created for continuous BDNF and proBDNF expression. Rat primary neuron cultures were transduced by lentivirus constructs on the 7th day *in vitro* (7 DIV). One week later (14 DIV) cell lysates were gathered for subsequent Western blotting analysis. It was shown, that proBDNF-CR overexpression markedly decreased the level of the key postsynaptic protein PSD95 as compared to control (pCSC), whereas BDNF overexpression led to the weak changes in PSD95 expression. It is well known that neuronal activity can determine intracellular transport, secretion and proteolysis of neurotrophins. According to our electrophysiological experiments, cortical neurons demonstrate strong spontaneous activity after 20 DIV, when cultures are supposed to be mature and synaptic connections should be formed. We found out, that two-week proBDNF-CR overexpression (22 DIV) leads to much more profound suppression of PSD95 protein level in cortical neurons as compared to control neurons transduced by lentivirus construct with pCSC.

Next, we focused on the presynaptic effects of neurotrophins in primary neuron culture. VGlut protein was considered as the possible presynaptic target for proBDNF and BDNF action. According to the immunocytochemical staining data, BDNF significantly increased the number of VGlut puncta in the presynaptic terminals of glutamatergic primary neurons (22 DIV), whereas cleavage-resistant BDNF precursor had no effect on VGlut distribution.

Therefore, our results suggest, that chronically expressed neurotrophins have spatial preferences in regulation of synaptic structure and plasticity. Thus, proBDNF is responsible for postsynaptic suppression via PSD95 regulation, whereas mature neurotrophin acts mainly presynaptically, potentiating synapses formation.

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No independent promotor for PKMζ kinase was found in terrestrial snail *Helix lucorum*

Chesnokova E.A. [1]***, Roschin M.V.*** [1]***, Kanygina A.V.*** [1]***, Kolosov P.M.*** [1]

[1]*Institute of Higher Nervous Activity and Neurophysiology of RAS, Moscow, Russia*

Objectives: Protein kinase M zeta (PKMζ) is shown to be crucial for LTP, learning and memory in both vertebrate and invertebrate species. Technically, PKMζ represents an independent catalytic domain of brain-specific isoform of PKCζ kinase. There is a specific promotor for PKMζ within the PKCζ gene in mammals. Nevertheless, there is still no evidence of existence of such alternative promotor in the PKCζ gene in Turkish snail (*Helix lucorum*), which is a popular model organism in neuroscience. The aim of our study was to locate an alternative promotor for PKMζ protein within PKCζ gene in *Helix lucorum.*
Methods & Results: We extracted total RNA from the brain of 4 snails, performed reverse transcription of RNA and sequenced it using ion semiconductor sequencing technique. The transcriptome of *Helix lucorum* brain was assembled *de novo* using Trinity application. Since there is no annotated genome of *Helix lucorum* available in databases, genomes of allied species *Lymnaea stagnalis* and *Aplysia californica* were used as references. An ORF upstream of the catalytic domain was located, and the primers were designed for 5' RACE-PCR. Step-out PCR approach was used to perform 5' RACE-PCR. PCR products were isolated, cloned and sequenced. It was found that there was only one 5' RACE-PCR product.
Conclusions: We can conclude that there's no alternative promotor for PKMζ in snail. Moreover, there is a single splice isoform of PKCζ mRNA in this species. We may suppose that in snail, unlike mammals, PKMζ protein is formed as a result of PKCζ proteolysis.

STIMILATION OF MOTOR RHYTHMS IN THE BUCCAL MASS AND GUT OF *Lymnaea stagnalis*

Chistopolskiy I.A., Dyakonova V.E.

Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia. Laboratory of neurobiology of development; *E-mail:*leophotos@mail.ru

The feeding behavior of *Lymnaea stagnalis* is a popular model system for studying the mechanisms of motor program generation. We analyzed the radula movements and gut contractions in semi-intact preparations of *L. stagnalis* using video registration. Analysis of the buccal mass activity in 16 animals has revealed various modifications of the standard three phase rhythm. The foregut contractions could occur in the absence of the buccal rhythm. During the buccal rhythm, they were coordinated with the feeding cycles. In ten preparations demonstrating 3-5 feeding cycles per minute, strong contraction of the foregut occurred after the third phase (swallowing). In two preparations demonstrating fast feeding (12 cycles per minute), foregut contracted weakly only during the second phase of the buccal rhythm (radula retraction). The transection of dorsal buccal nerves connecting the buccal ganglia with salivary glands and gut resulted in the loss of coordination between the buccal mass movements and gut contractions.

We propose this experimental model as suitable for studying central-peripheral interactions and coordination of motor rhythms within the feeding system of *Lymnaea*. It is likely that the buccal system of *Lymnaea* is a multifuncional neuronal ensemble, which rhythmic activity depends upon various factors including signals from rostral and distal parts of the gut. Supported by RFBR grants 14-04-00537 and 14-04-00875.

RECONSOLIDATION OF CONTEXT CONDITIONING IN TERRESTRIAL SNAILS: EFFECTS OF NEUROTOXIC ANALOGUE OF SEROTONIN

Deryabina I.B. (1), Bogodvid T.Kh. (1, 2), Muranova L.N. (1), Andrianov V.V. (1), Gainutdinov Kh.L. (1,3)
(1) Kazan Federal University, Kazan, Laboratory of Neurobiology; *E-mail: ira-kan@yandex.ru*
(2) Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan;
(3) Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan.

At present a considerable experimental material pointing to the association between functioning of serotoninergic system and the ability for learning has been accumulated. The ‘neurotoxic’ analogues of serotonin (5-HT) 5.6- and 5.7-dihydroxytryptamine (5.6- and 5.7 DHT) destroying 5-HT vesicles in terminals are used for investigation of serotoninergic system’s role in behavior. Therefore, we conducted a study of the role of 5-HT in context conditioning (CC) and its reconsolidation using the "neurotoxic" serotonin analogue 5,7-DHT and blocker of 5-HT synthesis by p-chlor-phenylalanin (pCPA). For development of the CC, 5 electrical stimulations per day were presented to snails within 5 days. Then after 2 days we tested CC. To do this, we measured the amplitude of retraction of ommatophores in response to tactile stimulation of the anterior part of foot. Testing the behavioural reactions were performed: 1) on the ball (i.e., standard conditions), 2) on a flat surface (i.e., under conditions different from the standard). CC was considered elaborated if the reaction on the ball was significantly higher than that on a flat surface. The next day, after testing confirming CC, snails were placed for 20 minutes on the ball that served as a reminder, and then blocked the protein biosynthesis by injection of anisomycin (ANI) of "Sigma" (0.4 mg in 0.2 ml of saline) per snail and the next day the maintenance of CC was tested. Disruption of memory demonstrated the process of reconsolidation. To study the role of 5-HT in the reconsolidation and its disruption the snails were injected with 5,7-DHT at doses of 20 mg/kg 3 days before reminder or pCPA at doses of 300 mg/kg after reminder. The results show that both pCPA and 5,7-DHT themselves don’t affect contextual memory after a reminder. In the case of ANI injection after reminder both pCPA and 5,7-DHT inhibit the effect of ANI on disrupting reconsolidation. Probably, the obtained results point to the necessity of 5-HT for the process of reconsolidation of memory.
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TOWARDS MATH MODELS OF MULTI-TRANSMITTER NEURONAL ENSEMBLES

Dyakonova V.E. (1), Kuznetsov O.P. (2), Sakharov D.A. (1) Zhilyakova L.Yu. (2),

(1) Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia, *Email: dyakonova.varvara@gmail.com*
(2) Institute of Control Sciences, Russian Academy of Sciences, Moscow, Russia, *Email: zhilyakova.ludmila@gmail.com*

In the conceptual foundation of neuroscience, the basic elements of the nervous system have long been considered to be substantially homogeneous. The latest version of such approach, the connectomics, describes the substrate of nervous activity in terms of wiring diagram. The connectome adherents declare that an understanding of the mechanisms of brain and behavior is reachable by a research strategy aimed at a complete description of all constituent cells and synapses. Homogeneous elements became the structural units for mathematical and technical models of artificial neural network and artificial intelligence. Meanwhile, the biological nervous systems are built of nerve cells with different transmitter phenotypes. A large amount of data has been accumulated, evidencing that the diversity of chemical signals and non-synaptic communication are essential for operation of well investigated neuronal ensembles, such as central pattern generators (CPG). The literature has repeatedly emphasized a sharp discrepancy between existing theoretical approaches and experimental data on chemical heterogeneity of neurons. For this reason there is a sharp need in alternative approaches. Here, we announce the start of a collaborative work of our two groups in this direction. Possible approaches to math modeling of multi-transmitter neuronal ensembles will be discussed.

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THE RESPONSE OF ISOLATED NEURONS TO NEUROTRANSMITTERS DEPENDS UPON CHEMICAL MICROENVIRONMENT OF GANGLIA

Dyakonova T. L, Dyakonova V.E.

Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

Email: dyakonova.varvara@gmail.com

There is now growing evidence that extrasynaptic neurotransmitter release plays an important role in interneuronal communication in the mammalian brain and in invertebrates of various taxa. Tonic extrasynaptic release of neurotransmitters from the buccal and pedal ganglia of the mollusc *Lymnaea stagnalis* has been demonstrated by pharmacological experiments and the use of biosensors in our laboratory (Chistopolsky and Sakharov, 2003; Dyakonova and Dyakonova 2010). Chemical microenvironment of neurons was suggested to play a 'socializing' role within the network and to represent the current behavioral state of an animal (Dyakonova et al., 2015). At the same time, the behavioral state is known to affect the neuronal responses to chemical signals. Here, we tested whether responses of identified buccal and pedal neurons to glutamate and serotonin are affected by the chemical microenvironment of buccal or pedal ganglia, respectively. To exclude synaptic and electrical influences, the neurons were isolated from the nervous system, and the effects of neurontransmitters were studied on isolated cell placed either near its initial localization in the ganglia or moved away from the ganglia, where no chemical effects of ganglia could be detected. Chemical microenvironment decreased the response of neurons to neurotransmitters as was revealed by statistical analysis of changes in the membrame potential and the firing rate of isolated neurons after bath application of glutamate and serotonin. In some cases (B4 neuron + glutamate), cell responses to the same concentration of neurontransmitter were opposite near and at a distance from the ganglia. The results are in line with the suggestion that chemical microenvironment plays an important neuromodulatory and socializing role in neuronal ensembles.

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SOME ASPECTS OF THE FUNCTIONAL ORGANIZATION OF THE EYE IN *LYMNAEA STAGNALIS*.

Fedorenko A.D., Tuchina O.P., Zhukov V.V.

Immanuel Kant Baltic Federal University, Kaliningrad; *E-mail: valerzhukov@mail.ru*

Two points were in focus of the present study:
1) do axons of photoreceptor cells form a direct pathway to cerebral ganglia in *L.stagnalis*?
2) is there FMRFamide-ergic retinopetal control?

Response to light stimulation of the eye consists of slow electrical waves (ERG) and action potentials. Both components could be elicited in the presence of Mg^{2+} in bath solution up to 15 mM and depressed by 1 mM EDTA or 10 mM Mn^{2+}. Since it was not possible to obtain any alteration of impulse responses without related changes in ERG amplitude, we concluded that not only ERG but also spikes are the result of photoreceptor cells activation. The absence of blocking effect of high Mg^{2+} indicates propagation of spikes from photoreceptors to the cerebral ganglia without interruption in chemical synapses. The inhibitory effect provided by chelation of divalent cations or Mn^{2+} on the light responses could be result of disrupted phototransduction.

The presence of FMRF-immunoreactivity in the optic nerve fibers, eye capsule and nuclear layer of the retina was detected immunohistochemically. Both ERG and impulse activity of the optic nerve fibers were inhibited in the presence of FMRF-amid 10^{-8} M. One can suppose that the FMRFergic neurotransmitter system is involved in the efferent control of the photoreceptor cells in *L.stagnalis* retina.

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NEGATIVE REATION OF ADULT DINOPHILID CILIATED LOCOMOTION TO INCREASED 5-HT LEVEL

Fofanova E.G., Mayorova T.D., Voronezhskaya E.E.
Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia, Department of Developmental Neurobiology
e-mail: lizchenbio@mail.ru; mayorova@wsbs-msu.ru

Dinophilid worms belong to the Archiannelida group and are represented by two species: *Dinophilus gyrociliatus* (Schmidt, 1857) and *Dinophilus taeniatus* (Harmer, 1889). The structure of the 5-HT system differs significantly in these species. *D. taeniatus* has orthogon-like serotonergic system with ganglion-like groups of cells located along the ventral nerve cord while *D. gyrociliatus* has irregular 5-HT system with solitary cell bodies scattered randomly. Juveniles demonstrate the same pattern as adults in both species.

We investigated the locomotion of juveniles and adults of both species after incubation in 5-HT and 5-HT precursor (5-HTP).Whole-mount immunocytochemistry with anti-5-HT antibodies was performed to control the level of 5-HT in worm nervous system.

Pharmacological experiments demonstrated an alternative reaction of juveniles and adults. While 5-HT and 5-HTP application increased locomotion speed in juveniles, the same application does not affect or even retarded locomotion of adults. Immunochemical analysis confirmed increases level of 5-HT within nervous structures in all cases.

Thus, adult diniphilids represent the first example of the animals with ciliated locomotion expressing the negative reaction to increased 5-HT level. Different reactions obtained for juveniles and adults may indicate the difference in 5-HT receptors expression involved in regulation of ciliary locomotion.

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EFFECTS OF SIMULTANEOUS INJECTIONS OF INHIBITORY PEPTIDE ZIP WITH NITRIC OXIDE DONOR AND INHIBITOR OF NO-SYNTHASE ON RECONSOLIDATION OF CONTEXT CONDITIONING IN TERRESTRIAL SNAILS

Gainutdinov Kh.L. (1,2), Deryabina I.B. (1), Muranova L.N. (1), Andrianov V.V. (1), Bogodvid T.Kh. (1,3)
(1) Kazan Federal University, Kazan, Laboratory of Neurobiology; *E-mail: kh_gainutdinov@mail.ru*
(2) Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan;
(3) Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan.

In recent years while studying the mechanisms of memory it was found that a protein called protein kinase (PK) Mzeta was critical at formation of memory. It was found that PKMzeta was necessary and key for the retention of late-phase of long-term potentiation in hippocampal slices. It was also shown that zeta inhibitory peptide, ZIP, led to disruption of long-term memory (LTM) for several forms of learning. An opinion was expressed that PKMzeta was a component of evolutionary conserved molecular mechanism of the LTM storage. But recently, however, there have been reports from studies, performed using knockout animals, the results of which contradict to a determined role of PKMzeta in memory processes. Thus, it was shown that PKC/PKM- knockout mice didn't have deficits in the formation of long-term potentiation. According to the authors neither PKMzeta nor the related protein kinase PKC were not directly related to memory, and all previously studied effects were associated simply with the insufficient selectivity of blocker, ZIP. Thus, there are contradictory data on the role of PKMzeta in the processes of memory formation. Therefore, we conducted a comparative study of the effects of ZIP and the protein synthesis inhibitor anisomycin (ANI) on the possibility of reconsolidation in terrestrial snails, taking into account that ZIP peptide inhibits the activity not only of PKMzeta, but also of several other protein kinases.
We elaborated a context memory in terrestrial snails, when the animals could distinguish the test signals used in different contexts (on ball and a flat surface). Context learning was considered to be elaborated if the response to tactile stimulation on the ball was significantly higher than that on a flat surface. Next day, after a test confirming context learning, snails were injected by inhibitor of protein biosynthesis ANI (0.4 mg in 0.2 ml of saline) per snail or were injected by ZIP (0.1 mg in 0.1 ml of saline) per snail after the session of reminding or without the reminding. Memory storage testing showed that injections of ANI and ZIP without reminding didn’t disrupt the contextual memory on the first and second days of testing, however they caused disrupting of memory if they were injected simultaneously with the reminder. Thus, we observe here the disruption of reconsolidation. Then to study the role of NO in reconsolidation we used injections of NO donor SNP at a concentration of 10^{-4} mol/l and the inhibitor of NO-synthase L-NAME at a doses of 100 mg/kg. It was shown that after the injection of L-NAME with ANI the number of positive responses on tactile stimulation decreases from 80% to 38% and 18% on the first and second day of testing, respectively, and injection with ZIP immediately reduced the number of positive responses on tactile stimulation to 25-30% on both days. After injection of SNP with ANI the number of positive responses decreased from 80% to 50% and 40% for the first and second day of testing, respectively, and its injection with ZIP immediately reduced the number of positive responses on tactile stimulation to 20-30% on both days. Thus, the NO as well asdonor SNP and the inhibitor L-NAME don’t affect the disruption of the reconsolidation process, caused by ZIP, but at the same time inhibit the disruption of the reconsolidation process, caused by ANI.
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EFFECTS OF INJECTION OF SEROTONIN ON DEFENSIVE REFLEX ELABORATION IN TERRESTRIAL SNAILS

Golovchenko A.N. (1), Andrianov V.V. (1,2), Bogodvid T.Kh. (1,3), Deryabina I.B. (1), Muranova L.N. (1), Gainutdinov Kh.L. (1,2)

(1) Kazan Federal University, Kazan, Laboratory of Neurobiology; *E-mail: galexsnik@inbox.ru*
(2) Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan;
(3) Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan.

One of the widespread and well-investigated transmitters in the nervous system is serotonin (5-HT). It is accepted that 5-HT is a basic neurotransmitter for plasticity of defensive behavior in mollusks, therefore, the role of the serotoninergic system in elaboration of defensive conditioned reflexes in mollusks it is difficult to overestimate. These findings and questions motivated us to investigate the role of 5-HT in the mechanisms of learning using injections in animal's body of 5-HT, its "neurotoxic" analogue 5,7-dihydroxytryptamine (5,7-DHT), and the precursor of 5-HT synthesis, 5-hydroxytryptophan (5-HTP). For this purpose, we elaborated two conditioned reflexes: defensive conditioned reflex to the tapping on the shell and defensive conditioned food aversion. The snails were trained after the injection of 5-HT, as a control, produced training after a saline injection. To produce a 5-HT deficit, its "neurotoxic" analogue, 5,7-DHT were used. It was found that daily injection of 5-HT before a training session accelerated both reflexes conditioning, and daily injections of 5-HTP in snails with a deficiency of 5-HT induced by 5,7-DHT restored the snail's ability to learn. We found that injection of 5-HT caused a decrease in the resting (Vm) and threshold (Vt) potentials of the premotor interneurons LPa3 and RPa3. It was shown that Vm decreased on 4.5 mV in snails that received only an injection of 5-HT and on 5.5 mV in snails, which were injected by 5-HT before training. A significant decrease of Vt on 4.0 mV and 4.5 mV, respectively, was found also

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NEW FUNCTION OF *DROSOPHILA* PHEROMONE CIS-VACCENYL ACETATE

Goncharova A.A.

Pavlov Institute of Physiology of Russian Academy of Sciences, St. Petersburg, Laboratory of Comparative Behavioral Genetics; *E-mail: AnemoneNemorosa@yandex.ru*

Pheromones play an important role in regulation of *Drosophila* behavior. A volatile male-specific pheromone, cis-vaccenyl acetate (cVA), participates in attraction of flies into aggregations, regulation of aggression and courtship. We found that it also suppresses locomotor activity of *Drosophila* males.

Drosophila melanogaster males collected soon after eclosion were kept either in a group of 20 flies (experiment) or individually (control). On the third day locomotor activity of experimental and control individuals was tested. Keeping males of the wild-type strain Canton-S in a group led to a suppression of their locomotion. The mutant males *Orco* (*odorant receptor co-receptor*), which are not able to smell, did not suppress their locomotion after group housing. The presence of containing pheromones hexane extract of mature males did not alter the activity of the wild-type experimental males, but reduced the activity of control males to the level of experimental ones, suggesting that the effect of group housing is mediated by pheromones. We repeated this experiment replacing the hexane extract by synthetic cVA and obtained the same result: males housed individually decreased their locomotion in the presence of cVA.

For maintenance of aggregation the fruit flies must suppress their activities: aggression, courtship and locomotion. Our data show that the main agent regulating interactions between flies in a group is cVA.

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Organization of senso-efferent systems in the pond snail *(Lymnaea stagnalis)*

Réka Horváth, Izabella Battonyai, Károly Elekes

Department of Experimental Zoology, Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary

The pond snail, *Lymnaea stagnalis* has a simple central and peripheral nervous system, which is highly advantageous, among others, for analyzing fundamental principles of neural integration related to the perception of the environment. Previous functional studies of identifiable sensory neurons have focused on those located in the gastropod central nervous system (CNS), whereas peripheral sensory neurons (PSNs), which are pivotal in controlling behavior as members of different reflex arches, attracted less attention. Applying histochemical and/or immunohistochemical (IHC) methods a number of putative PSNs have been described in different gastropod species, including catecholaminergic, nitrergic and glutamatergic cells (e.g. Croll et al., 1999; Hatakeyama et al., 2007; Wyeth and Croll, 2011; Carrigan et al., 2015). However, their spatial and possible functional relationship to each other as well as other neural and non-neural elements is not known. Therefore, in order to get a better insight into the organization of peripheral senso-efferent systems of gastropods, we studied it in different peripheral tissues in *Lymnaea stagnalis*, using antibodies raised against serotonin (5-HT), histamine (HA), glutamate (Glu) and FMRFa (Fa), all being key members of the signaling systems of gastropods. We focused on the lips and tentacles as primary regions of the chemical sensory system. At the same time, we also extended our investigations on the foot as a major mechanosensory and efferent target. Numerous HA-immunoreactive (HA-IR), Fa-IR and Glu-IR sensory cells were found both in the lips, tentacles and foot. The sensory cells displayed typical bipolar anatomy with sensory dendrites to the epithelial surface and sensory axons forming bundles projecting towards the CNS. In case of HA and Fa containing elements a rich subepithelial varicose network could also be observed. A rich 5-HT-IR innervation of central origin was prominent in all the three peripheral tissues studied, consisting of a dense network of varicose fibers located both under the epithelium and in the deeper muscular layer. Double labeling experiments revealed in most case a distinct but closely related localization of the four signaling systems. In addition, co-localization of 5-HT with Fa occurred in the foot and lips. Based on it, a complex functional organization of sensory and efferent systems is suggested in the periphery of *Lymnaea*, including possible local decision making, in the course of which interactions between the different neuronal elements containing the different signal molecules studied may occur. The interactions may involve parallel/simultaneous actions and/or sequential modulation of the signaling events. Our results provide a firm basis for further studies to understand the functional relevance of the senso-motor systems in the control of behavior responding to environmental challenges in *Lymnaea*.

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DETECTION OF THE NEWLY-SYNTHESIZED RNA IN NEURVOUS SYSTEM OF THE TERRESTRIAL SNAIL *Helix*

Ierusalimsky V.N. (1), Balaban P. M. (1,2)

(1) Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences, Moscow, Laboratory of cellular neurobiology of learning; *E-mail:* ierusalimsky@yahoo.com

(2) Lomonosov Moscow State University, Moscow.

For the first time, Click-iT method was applied to study RNA synthesis in neurons of terrestrial snail *Helix*. RNA synthesis was detected by means of the in vivo incorporation of 5-ethynyluridine (EU) in newly-synthesized RNA in the CNS tissue of adult and juvenile snails. First labeled neurons were detected in the adult CNS after 4-hrs of isolated CNS incubation in EU solution (nuclear and nucleolar staining). Extensive labeling of most CNS neurons was evident after 12-hrs of incubation. 2-3 days of CNS washout led to the tag appearance in neuron's cytoplasm. Immersion of the intact juvenile animals in EU solution for 1 h, as well as the similar incubation of isolated CNS, caused the staining of apical region in the cerebral ganglia. The maximum pattern of staining in juvenile CNS was obtained after 4-hrs of incubation in EU solution. The data suggest that sensitivity of the Click-iT method is not enough to detect the early events in the RNA synthesis. The synthesis is more active in juvenile snails than in adults. EU readily penetrates into the bodies of juvenile snails immersed in EU solution. RNA species can be stable in neurons for several days.

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DETECTION OF THE NEURONAL POPULATIONS EXPRESSING THE SELECTED EARLY GENES IN NEURVOUS SYSTEM OF THE TERRESTRIAL SNAIL *Helix*

Ierusalimsky V.N. (1), Vinarskaya A. Ch. (1), Balaban P. M. (1,2)

(1) Institute of Higher Nervous Activity and Neurophysiology Russian Academy of Sciences, Moscow, Laboratory of cellular neurobiology of learning; *E-mail:* ierusalimsky@yahoo.com

(2) Lomonosov Moscow State University, Moscow.

The search of RNA transcripts encoding the immediate early genes in CNS of terrestrial snail *Helix* revealed several sequences potentially belonging to the early genes family. The DNA antisense probes to RNA of potential early genes were designed and commercially produced against three of them: C/EBP, cFos, Jun. In situ hybridization experiments were performed in whole-mounts of CNS after 2-h stimulation of CNS via *nervus intestinalis*. The pattern of RNA expression was compared with the population of neurons sending processes in this nerve (nerve backfill). All three probes revealed the specific set of neurons, while control probes showed no staining. The number of cells expressing the studied RNA was less than the actual number of cells projecting to the stimulated nerve, though most of the detected cells belonged to the stimulated population of neurons. Nevertheless, some of the neurons detected by the *in situ* hybridization method were not the ones projecting to the *nervus intestinalis*. Probably, these neurons were synaptically connected to the electrically stimulated cells. Interestingly, the pattern of early genes RNA expression varied between three studied genes. The data suggest that electrical stimulation of appropriate cell population can cause the expression of several early genes in the neurons, the cell sub-populations (for different early genes) coinciding only partly.

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INDUCTION OF LONG-TERM POTENTIATION WITH K^+ AS A MAJOR CATION IN THE INTRACELLULAR SOLUTION

V.O. Ivanova[1], N. V. Bal[2], Balaban P.M.[2]
[1]Sholokhov Moscow State University for the Humanities;
[2]Institute of Higher Nervous Activity and Neurophysiology of RAS

Long-term potentiation (LTP) of the Schaffer collateral synapses in the CA1 region of the hippocampus is the primary model for the study of the associative synaptic plasticity thought to underlie learning and memory. A commonly used protocol for inducing LTP is to give "tetanic" stimulation in which a large number of axonal inputs is stimulated at a high frequency (100 Hz) for 1 s. Because of the complexity of the postsynaptic processes that affect depolarization during tetanus-induced LTP, many investigators have sought to evoke LTP in a simpler way by using a "pairing protocol" using a low-frequency synaptic stimulation (100–200 pulses, 1–2 Hz) under a depolarizing voltage-clamp pulse (1–3 min in duration).

Typically, Cs^+ is used as the major internal cation because it blocks K^+ channels and makes it possible to achieve a dendritic depolarization. Recent studies have shown that using K^+ as the major cation allows to induce LTP by brief pairing protocol (high-frequency stimulation (50 Hz) with a brief (15 s) depolarization to 0 mV). We have examined whether it is possible to induce LTP by standard pairing protocol using K^+ and found that under the abovementioned conditions the evoked potentiation is 189 ± 17%, n=4. Although according to other researchers' results the amplitude of potentiation is much higher using Cs^+ (400 ± 20%), recording of neuronal electrical activity using K^+ corresponds better to physiological conditions and is preferable in some paradigms.

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COMPARATIVE PHYSIOLOGICAL STUDIES OF THE DEFENSIVE REFLEXES IN THE *LAMELLIBRANCHES* AND *GASTROPODA* MOLLUSKS.

Kamardin N.N.

St. Petersburg Scientific Research Center for Ecological Safety, RAS
nik-kamardin@yandex.ru

It is known that neurons of viscero-parietal arc of ganglia are involved in the generation and regulation of important physiological functions of the animal. They form a stable neuronal network capable of generating rhythmic breathing movements, regulate the heart rate and defensive behavior. Marine *Lamellibranches* demonstrate the bradycardia upon rapid decrease in the salinity of 50%. At the same time the heart rate (HR) is reduced, it remains stable low to the start of the increase in salinity. With increasing salinity, after a short latency period, HR returns to a high background level. This evolution of the HR usually follows the opening and closing the valves. Defensive reactions caused by external influences can be found in gastropods: retraction of the siphon and gills of Aplysia, closing and opening the pneumostome at the Helix, the retraction body and foot at Lymnaea. Obviously, there is a single coordinated neuronal network for defensive protective behavior, respiration and heart regulation. This is observed in experiments with registration of movement's of mussels valves and cardiac activity. Collaterals of the same nerve excite cholinergic motor neurons of adductor muscles causing inhibition of the heart, both directly and through the corresponding motor neurons. Another part of the inhibitory processes involves serotonergic motoneurons of muscles-adductor going to the heart muscle, or in contact with the heart motor neurons, causing excitation of the mollusk heart. This model of reciprocal inhibition is controlled by certain central part of the central nervous system, causing sometimes a paradoxical reaction. The attack of the predator mollusk - Buccinum causes closing of the mussel's valves, but also increase of HR, which continues all time of the predator's attack. The reaction of groups of neurons of the respiratory chain (RPeDl, A-cluster, RPDl, RPD2, VD1, VD2, VD4, H-cluster) of Lymnaea have been investigated to the chemical stimulation of osphradium - sensory organ associated with respiration and osmoregulation.

DOES FEAR EXIST IN INSECTS?

Kamyshev N.G., Goncharova A.A.

Pavlov Institute of Physiology of Russian Academy of Sciences, St. Petersburg, Laboratory of Comparative Behavioral Genetics; *E-mail: nkam@pavlov.infran.ru*

Fear is a primary emotion, which is directly caused by emerging threat and provokes immediate responses, such as escape or aggression. The existence of emotions, including fear, in insects is denied by most people. However, the human fear has deep phylogenetic roots, and insects occupy an intermediate position in its evolution. The evidence of fear-like states in insects is discussed.

We found a fear-like state in *Drosophila melanogaster* when examined the consequences of housing males in groups. One of them consists in a reduction of male courtship, which is accompanied with a rise in locomotion, but not with a rise in other non-courtship behaviors. After group housing a male prevents encounters with a female by running away, demonstrating the startle response. Importantly, the objects evoking the escape response in experimental males may be mobile or immobile, fertilized or virgin females, as well as mature males. We have supposed that this effect of previous social experience reflects generalized conditioned fear acquired during aggressive interactions of males in a group. However, the effect is also present in known memory mutants. So, a mechanism producing potentiation of the startle response to courtship objects, other than fear conditioning, should exist. At the first place, possible hormonal shifts induced by a stressful group situation must be taken into account.

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SEASONAL TUNING OF DEVELOPMENTAL AND BEHAVIORAL PROGRAMS: INVOLVEMENT OF MONOAMINES IN FRESHWATER MOLLUSCS

Khabarova M.Yu., Voronezhskaya E.E.

Institute of Developmental Biology Russian Academy of Sciences, Moscow, Laboratory of developmental neurobiology; *E-mail: m.khabarova@yandex.ru*

How the developing embryo can adapt to the environment which it will be faced only after birth or hatching? We demonstrated earlier that level of serotonin (5-HT) within mother reproductive system affects expression of developmental and behavioral programs of the progeny in freshwater molluscs (Ivashkin, Khabarova et al., 2016). In the present work we described in details the developmental tempo of *Lymnaea stagnalis* (Mollusca, Pulmonata) at premetamorphic and postmetamorphic embryonic stages as well as after hatching until adulthood, seasonal (year- and month- round) egg laying; seasonal developmental tempo of *Helisoma trivolvis* (Mollusca, Pulmonata) in normal condition and after activation of various 5-HT receptors. In parallel, seasonal variations of 5-HT, DA, and their metabolites levels were measured within female and male reproductive systems as well as in the central nervous system using HPLC method. All data were collected during three years period.

Our results demonstrate that 5-HT and DA content within specialized tissues are under strong seasonal and moon cycle control. Correspondingly, the main characteristics of the embryonic growth, embryonic and juvenile locomotion, egg laying of mature snails are perfectly tuned to the respective season. Moreover, the expression of different types of 5-HT receptors in embryo also changed according to the season.

Obtained results allow to suggest the unambiguous seasonal correlation between monoamines level (and 5-HT and DA ratio) and expression of developmental and behavioral patterns of both embryonic and adult molluscs.

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Oscillatory Activity of Simple Nervous Systems of Mollusks

Lyudmila K. Khludova
Lomonosov Moscow State University, Department of Psychophysiology
Faculty of Psychology Moscow, Russia
Khludova@yandex.ru

A significant part of ganglion neurons of a grape snail has an endogenous pacemaker mechanism of action potential generation, which can produces rhythmic activity in a wide range of frequencies, including alpha and gamma oscillations. The neuronal pacemaker activity of these neurons is determined by two types of calcium channels with low and high thresholds of the start of the action potentials. The same mechanism for generating rhythms was investigated in higher vertebrates.

Summary electrical activity from different parts of the parietal ganglia was registered using glass micropipettes in experiments on semi-intact preparation of a grape snail. Background electrical activity of mollusk ganglia is represented by frequencies in the 0.3-35 Hz range. High-frequency activity was recorded in the abdominal ganglion and represented by action potential groups with varying amplitude and fluctuations in the range of 0.5-7 Hz. The parietal ganglia demonstrated oscillations from low 0.2 to high-frequency 35 Hz. The pleural ganglia also demonstrated high-frequency activity of action potential of 20-40 Hz with varying amplitude and short intervals in between. Different in-phase slow oscillations of 2 and 5 Hz or 0.4 and 6 Hz and other combinations were observed in case of registration of background activity of two ganglia. There was a brief depression (0.5 s) of oscillations and subsequent recovery of activity in case of tactile stimulations of the pallium.

It can be concluded that the neuronal pacemaker activity of a mollusk can serve as a model for studying the molecular mechanisms of oscillatory brain activity.

SWIMMING IN THE PTEROPOD MOLLUSC IS DETERMINED BY THE DOPAMINE-SEROTONIN BALANCE

Korshunova T.A, Zakharov I.S.

Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Laboratory of neurobiology of development; *E-mail: korshun_tanya@mail.ru*

Natural behavioral states in the swimming system of the pteropod mollusc *Clione limacina* include passive sinking, slow swimming and fast swimming. Serotonin (5-HT) switches the behavior program from slow to fast swimming. Conversely, the injection of dopamine (DA) results in inhibition of swimming. We examined how the locomotor pattern of the swim central pattern generator (CPG) is determined by the balance of both 5-HT and DA neurotransmitters.

The fictive rhythmic motor program was recorded in isolated CNS from the identified swim CPG neurons. 5-HT applied to the isolated CNS switched the locomotor rhythm from slow to fast in the whole range of concentrations. DA inhibited locomotor rhythm in the CPG neurons in a dose-dependent manner and this inhibitory effect of DA depended on initial frequency of the spontaneous locomotor rhythm. Results of the simultaneous DA and 5-HT action depended on the neurotransmitters concentration in the mixture and on the initial frequency of the locomotor rhythm.

We suggest that the locomotor CPG switched to one of programs (passive sinking, slow or fast swimming) depending on the balance of 5-HT and DA concentrations. We expect existence of mechanism of the stabilization of the motor programs in a range of concentrations.

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COMPARATIVE ANALYSIS OF DROSOPHILA MELANOGASTER LOCUS WHITE ON PHOTOTAXIS

Kostenko V.V
Kazan Federal University, Kazan, Department of Genetics
E-mail: vvkostenko1@gmail.com

The *white* (*w*) gene *Drosophila* encodes a subunit of a heterodimer transmembrane protein. The White protein is involved in the uptake of guanine and tryptophan, which are indispensable precursors in the synthesis of *Drosophila* pigments. Since guanine is further required for the synthesis of dopamine, and serotonin and tryptophan are also a precursor of serotonin, *w* mutants display altered levels and distributions of these neurotransmitters. Studies of fly eyes development have been particularly revealing because the development of the fly visual system shares much in common with the developing visual system and CNS in vertebrate systems. We used 4 mutant stocks: $w^{1}_{C\text{-}S}$, $w^{t}_{C\text{-}S}$, $w^{a}_{C\text{-}S}$, $w^{sat}_{C\text{-}S}$ containing corresponded *white* alleles in the genetic background of wild type *Canton-S.* Adult phototaxis was assessed by the T-maze method separately for males and females. Results have shown that, $w^{sat}_{C\text{-}S}$ mutants are characterized by the maximum number of individuals with photopositive reaction (female: 73,3±1,2, male: 77,9±1,1) and minimal level of this reaction was found in $w^{1}_{C\text{-}S}$ mutants (female: 15,7±1,1, male: 19,8±1,6). $w^{t}_{C\text{-}S}$ and $w^{a}_{C\text{-}S}$ mutants showed intermediate characteristic values. According to ANOVA it can be concluded that the phototaxis depends on allele (η^2 = 45,3%, $p<0,05$), sex (η^2 = 14,8 %, $p<0,01$). Correlation analysis of positive phototaxis and pigment level has shown a direct relationship (female: r_s = 0.92, male: r_s = 0,71, $p<0,05$). Thus, photopositive reaction depends on the degree of eye pigmentation and is a result of the levels of neurotransmitters dopamine and serotonin.

SEROTONIN AND NEUROPEPTIDE FMRFAMIDE IN THE NERVOUS SYSTEM OF FLATWORMS

Kreshchenko N.D.(1), Terenina N. B.(2), Zaripova F.F.(3)

(1) Insitute of Cell Biophysics of Russian Academy of Sciences, Pushchino, Moscow Region, *E-mail: nkreshch@rambler.ru*
(2) Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Center of Parasitology, Moscow, *E-mail: terenina_n@mail.ru*
(3) Private Educational Institution of Higher Professional Education "St. Petersburg Medico-Social Institute", St. Petersburg, *E-mail: faliabio@yandex.ru*

The nervous system of parasitic and free living flatworms contains a wide spectrum of signaling molecules, including serotonin and neuropeptides. Neurotransmitters participate in the regulation of various parasitic functions — migration, behavioral reactions, carbohydrate metabolism, muscular contractility etc. To further investigate neural signaling substances in flatworms, the occurrence and distribution patterns of serotoninergic and FMRFamidergic components were examined in trematodes *Opisthorchis felineus* (Opisthorchiidae) and *Opisthioglyphe ranae* (Plagiorchiidae). FMRFamidergic components were studied also in free living planarian *Schmidtea mediterranea* (Dugesiidae).

The serotonin-immunoreactive nerve cells and fibers were revealed in the head ganglia, circular commissure, longitudinal nerve cords and their connective commissures of trematodes, as well as around the oral and ventral suckers, oesophagus and genital pore. FMRF-specific immunoreactivity was observed in head ganglia, longitudinal nerve cords and terminal parts of its reproductive system. In planarian FMRF-like immunoreactivity was observed in cephalic brain, a pair of longitudinal nerve cords, transversal commissures, pronounced immunostaining to FMRF was found in group of large neurons situated in the auricles on the head end.

We may conclude that data obtained support the idea that presence of serotoninergic and FMRFamidergic components in Platyhelminthes nervous system is a conservative trait characterizing the helminthes which belong to different taxa and have different hosts as well as localization inside them. Functional roles which the neurotransmitters play are related to the regulation of muscle function of reproductive and digestive tracts and attachment organs, the morphogenetic action of FMRF-like peptides and its possible role in sensory function is also proposed.

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RHYTHMIC OSCILLATIONS IN HETERORHABDITIS MEGIDIS NEMATODE GUT

Kuznetsov V.P.(1,2), Slivko-Koltchik G.A.(1,2), Popova L.B.(3), Panchin Y.V.(1,3)

(1) Institute for Information Transmission Problems of Russian Academy of Sciences, Moscow, Russia;

(2) Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow, Russia;

(3) A.N. Belozersky Institute Of Physico-Chemical Biology MSU, Moscow, Russia;

E-mail: scikvp@gmail.com; s.koltchik@gmail.com; ypanchin@yahoo.com

Caenorhabditis elegans is one of the most investigated organisms in the world. This nematode is continuously eating. While eating it is rhythmically defecating with the stable period of 45-50 seconds. The nervous system usually controls rhythmic behaviors, but the defecation program of *C. elegans* is a distinguished exclusion. A signal is generated and spread through the gut cells. Intestine cells release protons to the lumen, activating and recruiting muscle cells into the process that cause defecation. All signaling functions of this process are produced by endoderm cells without the participation of the nervous system. The small size of *C. elegans* cells impairs the use of standard electrophysiological methods. We suggest a new model organism *Heterorhabditis megidis* that is closely related to C. elegans but has bigger gut cells suitable for electrophysiological methods. By using microelectrode techniques, we have recorded rhythmical changes of membrane potential related to the defecation cycle in the gut cells of H. megidis isolated intestine preparation. Our study demonstrates that intestinal cycling in H. megidis is associated with unusual all-or-none hyperpolarization action potential with a fixed duration of about one minute and a period of up to 15 minutes and amplitude about 60 mV. In addition, it was shown that gut cells are closely electrically coupled via gap junctions.

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

Aleksey Y. Malyshev[1], Alexander V. Martynov[2] and Pavel M. Balaban[1]

[1] Institute of Higher Nervous Activity and Neurophysiology of RAS, Butlerova 5A, Moscow, Russia, malyshev@ihna.ru

[2] Zoological Museum of Moscow State University, Bolshaya Nikitskaya str. 6, Moscow,
Russia

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.

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ENCODING OF HIGH FREQUENCIES BY SNAIL AND RAT NEURONS: CORRELATION WITH AN ACTION POTENTIAL INITIATION DYNAMICS

Aleksey Malyshev[1] and Maxim Volgushev[1,2]

[1]*Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia*

[2]*Department of Psychology, University of Connecticut, Storrs, Connecticut, United States of America*

Theoretical analysis predicts that dynamics of action potential initiation is a key factor determining the ability of neuronal populations to encode high frequencies in changes of their firing rate. Indeed, in our prior work we have demonstrated that this relation holds for pyramidal neurons from neocortex in mammals. However, comparison of the predictions from theoretical analyses of point-processes to experimental results from neocortical neurons is restricted for two reasons. First, action potentials in neocortical neurons are initiated not in the soma but in the axon initial segment. It is unclear how distal initiation influences encoding and to what extent predictions from point process analysis hold for distally-initiated spikes. Second, absence of an established model for action potential initiation in cortical neurons hinders direct comparison of experimental results to predictions from computer models. To circumvent these limitations, we studied the relationship of dynamics of action potential initiation and encoding abilities of neurons in pulmonate snail *Helix lucorum*. Our prior work demonstrated that generation of action potentials in these neurons is captured perfectly well by the Hodgkin-Huxley formalism. To characterize encoding in snail neurons we measured their frequency transfer function. The transfer function was assessed using spike responses to injection of fluctuating current. The transfer function was calculated as the ratio of Fourier transforms of spike-triggered average and autocorrelation of the injected current. We first measured transfer function of intact snail neurons in preparation of isolated CNS. Since the generation of action potentials in intact molluscan neurons occurs in the neurite, at some distance from the soma, this may result in filtering of the fast components of the signal during their propagation to the site of action potential initiation, thus impairing the precision of measurement of encoding properties of the spike generation mechanism. To circumvent this limitation, we have grown a culture of snail neurons in conditions which suppresses cell branching. Neurons in these cultures had a spherical shape, thus providing an experimental model which can be approximated most closely by point-process formalism. The action potentials recorded in these cells had slow exponential onset dynamics, which was significantly different from step-like onset of action potentials in mammalian neurons, and was perfectly captured by Hodgkin-Huxley equations. Transfer function of the cultured spherical snail neurons expressed the cutoff frequency around 50 Hz. Therefore, the population of such neurons is capable of encoding signal frequencies only below 50 Hz, but cannot encode faster signals. This low cutoff frequency was in a market contrast to the encoding abilities of cortical neurons of vertebrates, which generate fast-onset spikes and can encode frequencies up to 300-600 Hz in their population firing. These results demonstrate, for the first time, that the tight relation between dynamics of action potential initiation and encoding capabilities of the neuron is a universal principle in the animal kingdom and is valid for both invertebrate animals (molluscs) and mammals.

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COMPARATIVE STUDY OF EFFECTS OF SEROTONIN APPLICATION ON ELECTRICAL CHARACTERISTICS OF PREMOTOR INTERNEURONS OF INTACT AND LEARNED SNAILS

Muranova L.N. (1), Bogodvid T.Kh. (1,2), Deryabina I.B. (1), Vinarskaya A.Kh. (3), Andrianov V.V. (1,4), Gainutdinov Kh.L. (1,4)
(1) Kazan Federal University, Kazan, Laboratory of Neurobiology; *E-mail: m.luda@rambler.ru*
(2) Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan;
(3) Institute of High Nerve Activity and Neurophysiology of the Russian Academy of Sciences, Moscow;
(4) Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan.

In the present work we studied the changes of excitability (sensitivity) of premotor interneurons under the application of serotonin (5-HT) in preparations made from intact and learned snails. The terrestrial snails *Helix lucorum*, the nervous system of which has been well described, were used for experiments. For the elaboration of the conditioned defensive reflex, as a conditioned stimulus was presented the tapping on the shell. As an unconditioned stimulus an air blow into the lung cavity orifice (pneumostome), which produced a defensive reaction of pneumostome closure in animals, was used. The changes of membrane (Vm) and threshold (Vt) potentials of premotor interneurons in response to application of 5-HT (at a concentration of 1 mmol/l) in solution, washing the nerve system, of intact and trained snails were studied. In some experiments methiothepin was preliminarily added to solution for blockade of 5-HT the 1st type receptors.
It was found that application of 5-HT causes a reliable decrease of Vm of premotor interneurons of both naive (from -60.3±0.6 mV to -58.1±0.7 mV) and trained (from -56.3±0.3 mV to -53.4±0.8 mV) snails. Also it was found a reliable increase of Vt of these neurons of trained snails in contrast to the naive animals on application of 5-HT: from 15.9±0.4 mV to 18.9±0.6 mV. Methiothepin, added to the washing solution for 30 minutes before application of 5-HT, removes its effect on Vm in intact snails and reduces – in trained. Methiothepin also reduces the effect of 5-HT on Vt in trained snails. Thus, it was shown that the threshold potential in trained snails in response to application of 5-HT increases, in contrast to naive, this result shows a decrease of excitability of premotor interneurons in response to extracellular 5-HT after training. The obtained results demonstrate also the participation of the 1st type 5-HT receptors in this reaction.
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SEROTONIN AND DOPAMINE ARE BOTH ACTIVE DURING THE PRE-NERVOUS EMBRYONIC DEVELOPMENT OF VERTEBRATES

Nikishin D.A. (1, 2), Khramova Yu.V. (2), Bagaeva T.S. (2), Kremnyov S.V. (2), Shmukler Yu.B. (1)

(1) Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Laboratory of Problems of Regeneration; *E-mail:* denisnikishin@gmail.com

(2) Lomonosov Moscow State University, Moscow, Biology Faculty, Department of Emryology.

Monoaminergic transmitters are present in eggs and early embryos of a wide variety of animal groups and regulate early embryonic development long before the appearance of the nervous system. Using molecular genetic techniques, we investigated the composition of the serotonergic and dopaminergic systems of early embryos of Xenopus and mouse.
Several serotonin receptors are expressed simultaneously at early developmental stages of Xenopus and mouse. These receptors are coupled to the same second messenger system (adenylate cyclase) but in the opposite ways. At the same time, two dopamine receptor, that are expressed at early stages of Xenopus development, both inhibit cAMP-signaling. This may be associated with complex concentration-dependent mechanisms and spatio-temporal organization of transmitters system regulations. Membrane, as well as vesicular transporters of dopamine and serotonin are expressed at early stages of Xenopus and mouse embryonic development. Thus, both transmitter systems have all the components necessary for their activity as signaling molecules during the earliest stages of embryogenesis.
Presence and functional activity of serotonin and dopamine system components at the ealy stages of development suggested that the primary function of these substances was humoral regulation of the functional state of the cell, and neurotransmitter function arose secondarily in nerve cells. Our results demonstrate that the multiplicity of possible mechanisms of action is one of the characteristics of pre-nervous embryonic serotonergic and dopaminergic systems.

COMPARTMENTALIZED REMOTE-CONTROLLED INCREASE IN SYNAPTIC EFFICACY .

Nikitin ES, Balaban PM.

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia

Although synaptic plasticity is widely regarded as the primary mechanism of memory, it has now been widely recognized that non-synaptic plasticity, such as persistent somal depolarization makes contributions to the storage of the long-term memory in both vertebrates and invertebrates. There is however little information on particular neuronal compartments if they are subjected to maintained membrane-level changes and can specifically contribute to the storage and retrieval of the memory trace with regard to functional morphology of individual neurons. Here we show that somal depolarization of CGCs (Cerebral Giant Cells) can spread to the distal end of axonal side branch to enhance synaptic outputs of the cell. We recorded optically with calcium-sensitive and voltage-sensitive probes the activity of the axonal side branch of the CGC as its tiny neurites are out of scope of electrophysiological techniques. Amplitudes of measured 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 CGC soma by current injection resulted in increase in the AP area and amplitude which was more pronounced in the proximal segments of the side branch. Bath application of 4AP increased the amplitude of APs and calcium transients at the distal end of the side branch but not in the proximal locations, suggesting that A-type potassium current regulates the AP propagation along the side branch. Our experiments with a single-trial classical reward conditioning demonstrated that the amplitude of AP-evoked calcium transient at the distal end of the side branch correlated with the persistent depolarization and increased in 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 insight 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.

UV-LIGHT-INDUCED FREEZING IN COCKROACH *PERIPLANETA AMERICANA*: SLEEP OR PASSIVE AVOIDANCE?

Novikova E.S., Zhukovskaya M.I.
Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint-Petersburg, Lab. Evolution of Sense Organs,
E-mail: os_sacrum@list.ru

Choice of behavioral program in response to aversive environmental stimuli depends on its type and intensity and is species specific. Nocturnal insect, American cockroach (Periplaneta americana L.), actively avoids aversive cues. Unexpected behavioral reaction, freezing, was observed when a cockroach was illuminated with compact fluorescent cold white lamp. We classified freezing in response to bright light as passive avoidance reaction in contrast with escape as an active avoidance one. On the other hand, masking effect as a phenomenon of switching to daylight behavior under sudden illumination during the dark phase of day-and night cycle probably took place in freezing response to light. Since cockroach eye contains two types of photoreceptors, green and ultraviolet sensitive, further experiments were done to explore spectral sensitivity of freezing reaction. UV light of low intensity caused freezing in almost every animal while green light of low and intermediate intensity induced an increase in locomotor activity. We hypothesize that activation of short wavelength sensitive photoreceptors induce sleep-like passive avoidance while long wavelength sensitive ones are responsible for active avoidance reaction.
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MONOAMINE-CONTAINING CELLS APPEARANCE AND THEIR INVOLVEMENT IN REACTION TO BIOTIC STIMULI IN SEA URCHIN LARVAE

Obukhova A.L., Khabarova M.Yu. and Voronezhskaya E.E.

Institute of Developmental Biology Russian Academy of Sciences, Moscow, Laboratory of developmental neurobiology; *E-mail:* allobukhova@gmail.com

Monoamine-dependent developmental regulation is prominent feature in various animal groups. While involvement of serotonin-containing (5-HT) nerve elements is known for mollusc and annelid larvae, the dopamine-containing (DA) system needs detailed investigation. We used larvae of *Mesocentrotus nudus* (synonymited name of *Strongylocentrotus nudus,* Echinodermata) at developmental stages from blastula till 4 arms pluteus for analysis of 5-HT and DA synthesis and uptake. In addition, correlation between brightness of monoamine -containing elements and specific biotic stimuli (chemical components of water conditioned by conspecific adults, CW) was examined. Both immunochemical detection of monoamines and FaGlu method for DA visualization were applied.

While 5-HT and DA cells appear at 2-4 arm pluteus stage only in the course of normal development, the system of monoamines synthesis and uptake is already functioning as early as prism stage and can be detected after incubation in 5-HT and DA. Three 5-HT cells comprise the apical organ and two DA cells appeared at the base of arm rudiment. By 4 arm pluteus more 5-HT cells differentiate within apical organ and around mouth; two pairs of DA cells located at the base of each arm, two cells located at the base of the lateral lobe. Their fibers projected underneath the arm ciliary bundles. Incubation in CW resulted in changes in proportion of larval body. No corresponding changes in brightness were detected for 5-HT apical cells while DA cells modify their brightness.

Thus we revealed early appearance of monoaminergic system in sea urchin larvae and suggest involvement of DA cells in reception of external biotic stimuli.

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INFLUENCE OF MONODIET ON NEURONAL ACTIVITY OF THE EDIBLE SNAIL

Palikhova T.A.
Department of psychophysiology, faculty of psychology, M.V. Lomonosov Moscow State University. Russia, Moscow. E-mail: palikhova@mail.ru

We have opened our experiments on the mono-diet for the edible snails *Helix pomatia and lucorum* in order to see a long-lasting influence of exogenous serotonin precursor on morphology, behavior and neuronal activity of the snails. The bananas had been chosen because it contains more 5-hydroxitriptamine in comparison with carrot and have very similar concentration of other compounds. It had been found that at first the snails successfully survived (1-2 years) while eat only one of these products. Second, the snails of two groups differed by color of the shell, "carrot" snails were more dark, but the snails of both diet groups differ significantly from initial coloring of their shells (intensive coloring of Crimea *Helix lucorum* changed to very similar as for Moscow region *Helix pomatia*). The snails of both mono-diet group and of control didn't change in behavior. It was surprisingly that snails' neuronal activity did not differ too. Background activity was similar and typical for comparing identified neurons in different preparation as for synaptic potentials as for spikes patterns. Absence of differences might be a result of small difference in the content of serotonin precursors in the diet products. Another possibility is a compensatory system for constant level of serotonin to use this modulatory system for response to environmental life factors.

FROM NEURONS TO BEHAVIOUR: COMPLEX NEURONAL CHANGES OF *LYMNAEA* TO EXPOSURE OF PROGESTOGENS PHARMACEUTICALS

Zsolt Pirger

MTA-ÖK BLI NAP_B Adaptive Neuroethological Research Group, Experimental Zoology

Increasing concentration of priority pollutant hormones aquatic ecosystems is a priority issue of environmental protection. Nothing is more indicative of this than the fact that these agents have been put on the European Union watch list of emerging pollutants. Animals living under aquatic conditions are more sensitive against these pollutants or their residues capable of damaging the metabolism of endogenous hormones functioning in the CNS, consequently affecting the homeostasis of the animal.

Following the determination of the word wide relevant concentrations (1-10 ng/L) of synthetic oral contraceptives in the catchment area of Lake Balaton and River Zala, we investigated adaptive changes at cellular and system level, induced by these agents. Using the *Lymnaea* experimental model, we performed electrophysiological recordings from various components (modulatory interneurons, motoneurons, CPG cells) of identified networks responsible for controlling feeding and learning and memory. Hormone treatment decreased the firing frequency of the feeding and learning modulatory interneurons via increasing the amplitude of their $Ca2^{+}$ currents. This effect seemed to be cell type specific, since in the case of the respiratory CPG cells there were no such amplitude changes. Feeding motoneurons also showed decreased activity, since their responsiveness to external sucrose stimulus was reduced. Consequences of cellular changes were further examined at behavioural level, showing that hormone treated snails had a lower feeding (rasping) activity to feeding stimulus compared to control. Using different behaviour tests we have also observed that similar to feeding, the locomotion activity, learning ability and memory pathways are also influence by progestogens exposure compared to control animals. Parallel with these observation, the signal transduction pathways have also investigated by phospho-MAPK array kit.

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NERVOUS SYSTEM MINIATURIZATION IN SMALLEST INSECTS

A.A. Polilov

Lomonosov Moscow State University, Biological faculty

Miniaturization leads to considerable reorganization of structures in insects, affecting almost all organs and tissues. In the smallest insects, comparable in size to the unicellular organisms, modifications arise not only at the level of organs, but also at the cellular level. Miniaturization is accompanied by allometric changes in many organ systems. The consequences of miniaturization displayed by different insect taxa include both common and unique changes.

All microinsects display strong oligomerization and concentration of ganglia. The highest degree of compaction and concentration of the nervous system among microinsects is found in beetles. The central nervous systems of Corylophidae are represented by a single formation with poorly discernible ganglia. Another important peculiar feature of the central nervous system of microinsects is asymmetry. Miniaturization is accompanied by a considerable decrease in the size of cells in the nervous system of insects. Microinsects also have considerably fewer neurons in comparison with larger members of related taxa. Accordingly to received data, the smallest insects have only about 10 000 cells in the brain, with the average diameter only about 1.5 — 2 μm. The decrease in neuron size in the smallest insects is accompanied by considerable changes in the nuclear-cytoplasmic ratio. A unique structure of the nervous system has been found in one of the smallest members of the family Trichogrammatidae, *M. mymaripenne.* At the final stages of pupal development, the nuclei and cell bodies of more than 95% of the neurons undergo lysis, prior to the emergence of the adults from the pupa. As a result, the adult central nervous system consists almost entirely of neuropil with only 360 nuclei.

Because the smallest insects are among the smallest metazoans and have the most complex organization of nervous system among organisms of the same size, their peculiar structural features and the factors that limit their miniaturization are of considerable theoretical interest to general biology.

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THE EARLIEST BILATERIAN NERVOUS SYSTEMS IN NEMERTODERMATIDA (XENACOELOMORPHA): AN EXAMPLE OF PLASTICITY

Raikova O.I. [1, 2], Meyer-Wachsmuth I. [3, 4], Jondelius U. [3]

(1) Zoological Institute of the Russian Academy of Sciences, St.-Petersburg; *E-mail:* ORaikova@gmail.com

(2) Saint-Petersburg State University, Chair of invertebrate zoology; *E-mail:* ORaikova@gmail.com

(3) Swedish Museum of Natural History, Stockholm, Sweden; *E-mail:*Ulf.Jondelius@nrm.se

(4) Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; *E-mail*: Inga.MW@googlemail.com

The earliest extant branch of bilaterian animals, Xenacoelomorpha, includes a small group of microscopic marine worms, Nemertodermatida, in some aspects more primitive than Acoela. The nervous system (NS) of four species of Nemertodermatida has been investigated by immunohistochemistry and confocal microscopy using anti-tubulin, anti-5-HT and anti-FMRFamide antibodies. The NS of *Flagellophora* is composed of a large neuropile and a loose brain at the level of the statocyst with several nerve fibres innervating the enigmatic frontal broom organ. *Sterreria* sp. shows a commissural-like brain and several neurite bundles going in frontal and caudal directions from this. The NS of *Nemertoderma* consists of a brain ring lying outside the body wall musculature at the level of the statocyst and a pair of ventro-lateral neurite bundles. The NS of *Meara* has no anterior brain-like concentration, but consists only of a pair of basiepidermal lateral neurite bundles. The NS of the nemertodermatids displays considerable plasticity. The innervation of the gut in Nemertodermatida is apparently absent similar to the condition in *Xenoturbella*. The NS evolution within Xenacoelomorpha is discussed.
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MOLECULAR MECHANISMS UNDERLYING UNDERLYING BASIC FUNCTIONS IN NEURONS

Ratushnyak A.S., Zapara T.A., Proskura A.L., Sorokoumov E.D.
Design Technological Institute of Digital Techniques SB RAS,
Novosibirsk, E-mail: ratushniak.alex@gmail.com

To solve the key problems of neurobiology it is important to integrate the knowledge about the molecular mechanisms of the main functions of all living systems - maintaining the vital functions on the base of prognostics. At the molecular and the cellular levels those properties were evolutionally formed and later became the basis of higher information functions of the brain. At this level, biologically active substances, pharmacological drugs and exposures are acting to restore, preserve and increase the brain resources. Therefore, the most urgent task at this stage of brain research is to integrate the existing knowledge about molecular organization and neuron functional architecture dynamics. This will create the prerequisites for the formation of a generalized theory of functioned of the neuronal systems as a physical phenomenon of decreasing the entropy on the base of informational and forecasting processes in the complexes of molecular assemblies.

The ability to create such a theory based on the negentropic principle seems now problematic because it is unreal to integrate a huge amount of research results in the field of neuroscience which have appeared in recent years. However, there is a hope that this problem can be solved on the basis of the limited range of conceptual studies. Preconditions for its implementation are the subject of this work which is focused on the theoretical and experimental analysis of the molecular architecture characteristics, information properties and molecular structures of basic functional systems oriented on performance of prediction tasks.

Presented in the data obtained in the performance of the base project for Fundamental Research RAS VI.35.1.5, RFBR grant № 15-29-04875

CALCIUM ENTRY INTO THE PROXIMAL SYNAPTIC BOUTONS AND 1ST RANVIER NODE OF L5 PYRAMIDAL NEURONS SHAPES ACTION POTENTIALS.

Roshchin M, Balaban PM, Nikitin ES
Institute of Higher Nervous Activity & Neurophysiology RAS

Synaptic plasticity is widely regarded as the primary mechanism of learning. Little is known, however, about how the activation of efficient synapses can change the neuronal excitability of the presynaptic neuron in short-term at subcellular level. In central pyramidal neurons the first axonal collaterals with presynaptic boutons emerge at the first node of Ranvier, ~100 mkm from the axon initial segment (AIS) where inputs are converted into the action potentials (APs). Our experiments with a synaptophysin-based genetically encoded probe demonstrated that the most proximal synaptic boutons of neocortical layer 5 neurons were located at ~8 mkm from the 1st node. Imaging of evoked by APs calcium transients in the node and bouton revealed a gradient of calcium concentration elevation which peaked at the proximal bouton. Calcium elevation by local Ca^{2+} uncaging in the bouton or node produced significant narrowing of the following somatic APs. Intracellular Ca^{2+} elevation was sufficient for the Ca^{2+}-induced AP narrowing and did not require additional network-dependent mechanisms or presynaptic transmitter release from the recorded neuron. Presynaptic calcium elevation is known to regulate release of neurotransmitters at synapses and to exert powerful influence over the synaptic strength. Here we propose a novel mechanism of how the presynaptic plasticity and changes in calcium levels in proximal boutons can regulate the backpropagation of APs and tune the excitability of the presynaptic neuron through Ca^{2+} binding to the Ca^{2+}-dependent potassium channels.
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TOPOGRAPHIC ANATOMY OF NEURONS OF SUPRAESOPHAGEAL, MESO- AND METATHORACIC GANGLIA IN PALEO- AND NEOPTEROUS INSECTS

Severina I.Yu., Isavnina I.L., Shchekanov E.E., Knyazev A.N.
Sechenov Institute of Evolutionary Physiology and Biochemistry, RAN;
Sankt-Peterburg, E-mail: severinaira@mail.ru; isavnina@iephb.ru

Ascending (AN) and descending (DN) neurons of the supraesophageal and thoracic ganglia in the nervous system of winged insects (Pterygota) – representatives of infraclasses Palaeoptera (Odonata, *Aeschna grandis*, dragonfly) and Neoptera (Blattoptera, *Periplaneta americana*, cockroach) were studied. These insects are different in ecological niches, lifestyles, sets of behavioral complexes, levels of locomotor system development, evolutionary age and systematic position. Neuronal bodies and processes were stained with nickel chloride ($NiCl_2$), their topography was studied on total ganglia preparations. Unlike cockroaches, in dragonfly protocerebrum DNs sending their processes to ocelli were found. Dragonfly DN processes show a specific type of arborization in thoracic ganglia, with collaterals directed both ipsi- and contralaterally. AN bodies lie similarly in respect to the ascending process. In cockroaches collaterals of DN processes are arranged ipsilaterally whereas AN bodies in the same ganglia are predominantly localized contralaterally. Essential differences in allocation of DNs and ANs in insects dissimilar in locomotion manner reflect a different extent of supraesophageal ganglion control over segmental centers activity. It seems related to neither the evolutionary age of insects, nor the antiquity of origin, nor their systematic position. Probably, a different degree of locomotion control depends on the way of getting food - catching prey in air by “paleopterous” dragonflies unlike maneuverable walking or running across a solid substrate by “neopterous” cockroaches.

RESPONSES OF IDENTIFIED NEURONS WITHIN *LYMNAEA*'S CNS ON CHRONIC ACTION OF SODIUM NITRITE

Shahrani M., Sidorov A.V.
Department of Physiology, Belarusian State University, Minsk, Belarus;
E-mail: sidorov@bsu.by

Different sources of pollution contribute to increased inorganic nitrogen concentrations in freshwater ecosystems. Only few studies have produced acute nitrite or nitrate toxicity data. Meanwhile cell and tissue concentration of NO_2^- and NO_3^- are crucial in the regulation of many metabolic functions (Cruz e.a., 1997). Fresh-water pond snail *Lymnaea stagnalis* was use as a model system to study the effects of sodium nitrite on electrical properties of its identified central neurons. Mollusks were preliminary incubated in 10 mM solution of $NaNO_2$ for the period of 1, 3 and 7 days. After this, intracellular recordings of giant dopamine- and serotonin-containing neurons of right and left pedal ganglions (RPeD1 and LPeD1 respectively) were done by means of glass microelectrodes (3.5 M KCl, 10–15 MΩ). We revealed substantial differences in the responses of mentioned above cells. RPeD1 membrane (n = 10) was slightly depolarized from -56±2 to -45±3 and -46±4 mV on the 1st and 7 day after the impact respectively. Voltage-current (VI) curve show the decrease of membrane excitability (1st day) and its gradual restoration to the control level on the 3 and 7 day of the experiment. LPeD1 rest potential didn't undergo any significant changes. Its VI curve shifts give the evidence for excitability increase starting from the 3d day after the impact. Thus, nitrites level could affect the activity of neuronal networks constructing from dopamine- and serotonin-containing neurons.

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WHY STUDENTS-PSYCHOPHYSIOLOGISTS NEED TO STUDY EDIBLE SNAILS

Shirenova S.D., Kostanyan D.G., Vukolova D.E., Palikhova T.A.
Moscow State University named by M.V. Lomonosov, faculty of psychology, department of psychophysiology. Russia, Moscow; *E-mail:* shirenova.jr@gmail.com.

Internet today presents multiple impressive variants of prepared for eating edible snails. Such popular name has mollusk *Helix pomatia (or H. lucorum)*. These snails live today at the psychophysiological department of Moscow State University but not for eating. They are used for investigation of physiological base of psychic processes. Students know from the first year of learning at the faculty of psychology the general strategy of psychophysiology "Human-Neuron-Model". To use this strategy one has to understand how a single neuron functions, how they interact. The snails present a good possibility to study "neuron" from this trial, because, first, their neurons are in general similar to human's neurons and, second, they are identified neurons. For neuron to be identified means a possibility to compare the same neurons in different snails. The peculiarity of simple nervous systems is the small amount of neurons, for instance some ganglia of *Helix pomatia* have less than a thousand neurons. The students begin to study the snails at the practical course only at fourth year of studying. At the beginning the students know psychophysiology as a practical science. Such classical psychophysiology is limited by polygraph recordings of physiological processes in correlation with psychological processes and functions in *Human*. Studying *Helix pomatia* opens up new possibilities. It allows for registration ERPs directly from a neuron. This way less artefacts are present. Moreover, this kind of research makes it possible to observe neural signal on the bio-chemical level. The modern science direct to understanding of physiological base of psychic processes as it was suggested by professor E. N. Sokolov. This is where the snails might help us. Future researches need to fill in on how the data obtained by polygraph recordings is linked to the data gained by studying simple nervous systems.

ELECTRICAL PROPERTIES OF IDENTIFIED NEURON (RPED1) AFTER OPERANT CONDITIONING OF AERIAL RESPIRATION IN *LYMNAEA*

Sidorov A.V., ElRahal A.
Department of Physiology, Belarusian State University, Minsk, Belarus;
E-mail: sidorov@bsu.by

Giant dopamine-containing neuron of right pedal ganglion (RPeD1) plays a key role in operant conditioning of aerial respiratory behaviour in mollusc *Lymnaea stagnalis* being involved in the structure of its central pattern generator (CPG). Electrophysiological recordings from the isolated CNSs after operant conditioning showed that the spontaneous patterned respiratory activity of the CPG neurons was significantly reduced (Spencer e.a., 1999). For operant conditioning, animals were trained as described previously by Lukowiak et al. (1996) with modifications. Intracellular recordings of RPeD1 were done by means of glass microelectrodes (3.5 M KCl, 10–15 MΩ) both in normal and zero Ca^{2+} physiological solutions. The activity of superoxide dismutase (SOD), a key enzyme involved in reactive oxygen species degradation, was analyzed in total CNS preparations. This study demonstrates the decrease of an electrical excitability of RPeD1. We revealed substantial differences ($P < 0.05$) in resting membrane potential – -69±2 mV *vs* -61±3 mV for operant ($n = 10$) and control ($n = 15$) groups respectively. In these case duration of depolarization phase of action potential also differ significantly: 6.9±0.4 ms *vs* 8.9±0.7 ms. Action potential, threshold and undershoot amplitudes did not change statistically after operant conditioning. Learning results in twofold increase of SOD activity within CNS: 31.2±3.4 U/ml (operant) *vs* 15.4±1.7 U/ml (control). Overall, our results support the hypothesis that intrinsic membrane properties of RPeD1 seem to be a target of operant conditioning changes of *Lymnaea* pulmonary respiration.

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INHIBITION OF RECEPTORS OF DIFFERENT NEUROTRANSMITTERS DURING RECONSOLIDATION LEADS TO THE DEVELOPMENT OF DIFFERENT AMNESIA TYPES

Solntseva S.V. (1), Efimova O.I. (2), Nikitin P.V. (3), Kozyrev S.A. (3), Nikitin V.P. (3)

(1) V. P. Serbsky State Research Center for Social and Forensic Psychiatry, Moscow, *E-mail:* sols3@yandex.ru

(2) National Research Center «Kurchatov Institute»; *E-mail: efimova_oi@nrcki.ru*

(3) P.K. Anokhin Institute of Normal Physiology, Moscow; *E-mail: nikitin.vp@mail.ru*

Dynamics of amnesia development after memory reconsolidation disruption by NMDA glutamate receptors antagonist (MK-801) or 5-HT receptor antagonist (methiothepin) in trained to conditional food aversion snails was studied. In 2 days after learning, MK-801 or methiothepin administration before the reminder by conditioned stimulus resulted in the amnesia progression. In 3 days after amnesia induction by the MK-801/reminder or methiothepin/reminder the procedure of repeated training caused faster formation of the aversion memory than in the initial training. In 10 days after the methiothepin/reminder application in session of the repeated training the memory formation dynamics was similar to that in the initial training. However, the repeated training in 10 days after the MK-801/reminder application have not resulted in formation of long term memory. Thus, the conditional food aversion memory reconsolidation disruption by serotonin receptor antagonist or NMDA glutamate receptors antagonist led to development of different types of amnesia that had the similar time gradient of development at their early stage, but were different by the possibility of memory formation after repeated training at the late stage.

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INNERVATION OF THE LOPHOPHORE IN CTENOSTOME ECTOPROCTS UNCOVERS THE BRYOZOAN BAUPLAN

Temereva E.N. (1), Kosevich I.A. (1)
(1) Russia, Moscow, Moscow State University, Biological Faculty, Dept. Invertebrate Zoology; *E-mail: temereva@mail.ru, ikosevich@gmail.com*

Because ctenostome bryozoans retain some ancestral features of organization, the investigation of their nervous systems may contribute to the reconstruction of the bryozoan nervous system bauplan. A detailed investigation of the polypid nervous system of the ctenostome bryozoan *Amathia gracilis* is reported here. The cerebral ganglion displays prominent zonality and has at least three zones: proximal, central, and distal. The neuroepithelial organization of the cerebral ganglion is revealed. The cerebral ganglion gives rise to five groups of main neurite bundles. Based on the characteristics of their innervations, the tentacles can be subdivided into two groups: four that are near the anus and six that are near the mouth. Two longitudinal neurite bundles—medio-frontal and abfrontal—innervate each tentacle. The zonality of the cerebral ganglion, the presence of three commissures, and location of the main nerves emanating from each zone might have resulted from the fusion of the three ganglia of the three body parts in the bryozoan ancestor: epistomal, lophophoral, and truncal. The presence of a remnant lumen in the cerebral ganglion of *A. gracilis* supports the idea of its ancestral state among all bryozoans. The presence of lophophoral dorso-lateral nerves, described here for the first time, suggests the reduction of the complex ancestral horseshoe-like lophophore within bryozoans. The six nerves within each tentacle is the ancestral state for all bryozoans. The presence of the outer nerve ring makes the lophophore innervation within the group (clade) of lophophorates similar and provides morphological evidence of the lophophorates monophyly.

NEW TYPE OF LARVAL NERVOUS SYSTEM IN VIVIPAROUS PHORONID

Temereva E.N. (1)

(1) Russia, Moscow, Moscow State University, Biological Faculty, Dept. Invertebrate Zoology; *E-mail: temereva@mail.ru*

Phoronids are marine invertebrates with biphasic life cycle. Planktotrophic larvae of phoronids have complex nervous system, which is different in holopelagic and brooding phoronid species. We have discovered new type of larval nervous system in a new phoronid species that has unique for phoronids type of development – viviparity, in which mothers release fully developed early larvae into the environment. Early larva has apical organ and two main nerves. These nerves form two circles around the remnant of the blastopore. First of these nerves underlies the preoral and postoral ciliated bands. Such a unity of the preoral and postoral ciliated band is known in deuterostomian larvae. Second nerve extends along the preoral ciliated band and gives rise to the neurites of the oral field. The main nerve ring, which is the first neurite bundle for appear in other phoronid larvae, is absent in early larvae of viviparous phoronids. Although the main nerve ring is presence in competent larvae of viviparous phoronids, it does not give rise to the T-like intertentacular neurite bundles, which are characteristic of larvae of other phoronids. The minor nerve ring, which is prominent in all planktotrophic phoronid larvae, is not developed in larvae of viviparous phoronids. In competent larvae of viviparous species, the apical organ does not contain basal neurons as it does in larvae of other phoronids. The development and simple organization of larval nervous system in viviparous phoronids may correlate with specific type of development as well as it may reflect the ancestral state of larval nervous system in phoronids.

LATERAL MOBILITY OF ACETYLCHOLINE RECEPTORS IN POSTSYNAPTIC MECHANISM OF HABITUATION

Vasilyeva N.A.(1,2), Murzina G.B.(2), Pivovarov A.S.(1)

(1) Moscow Lomonosov State University; Moscow, E-mail: as_pivovarov@mail.ru

(2) Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, E-mail: gbmurzina@mail.ru

Theoretical analysis based on an original kinetic model has revealed that a depression sensitivity t*o* acetylcholine in *Helix* neurons in cellular correlate of habituation depends not only on exocytosis and endocytosis, but also on the lateral diffusion of Ach-receptors. Lateral diffusion of Ach-receptors under experimental conditions was assessed by applying electrophysiological methods. The following drugs were used: MbCD (depletes cholesterol in the cell membrane), Ro 48-8071 (cholesterol synthesis inhibitor), antibodies spectrin and merlin (against actin-binding proteins). In the presence of MbCD (1 мМ) and Ro 48-8071 (2 мкМ) the rate of Ach-induced current depression was slower. The steady-state level of peak current was achieved in case of MbCD, but not of Ro 48-8071. In the presence of antibodies spectrin (1:100) and merlin (1:50) the rate of Ach-induced current depression was faster and recovery of peak current was reduced. Fitting a kinetic model to electrophysiological data shown that MbCD causes a decrease the diffusion rate and constants of endocytosis and exocytosis. Whereas antibodies causes an increase of the diffusion rate and constants of endo - and exocytosis. These results indicate that lateral diffusion of Ach-receptors participates during depression of Ach-induced current in the cellular correlate of habituation.

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CELLULAR AND BEHAVIOURAL ALTERATIONS INDUCED BY NEONICOTINOID INSECTICIDES IN A NON-TARGET AQUATIC ORGANISM, THE POND SNAIL *LYMNAEA STAGNALIS*

[1]Vehovszky, Á., [1]Farkas, A., [2] Székács, A., [2]Mörtl, M., [1]Győri, J.

1. Department of Experimental Zoology, MTA Centre for Ecological Research, Balaton Limnological Institute, H-8237 Tihany, POB 35, Hungary
2. Department of Environmental Analysis, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, H-20122 Budapest, Herman O. u. 15, Hungary

Neonicotinoids are widely used agrochemicals, but their systemic nature and water solubility also suggest the potential exposure of aquatic animals in the local environment. We used the pond snail *Lymnaea stagnalis* as a model organism for testing insecticides in commercially available formulations, which contain neonicotinoids as their active ingredients: acetamiprid, imidacloprid, thiamethoxam, clothianidin and thiacloprid. In the isolated central nervous system (CNS) the identified cholinergic (VD4-RPeD1) synapse was reversibly inhibited by all the tested neonicotinoids in the bath. Thiacloprid (10 µg/ml) was the most effective insecticide by blocking almost 90 percent of the postsynaptic potentials, while the less effective thiamethoxam (100 µg/ml) reduced the synaptic responses by about 15 percent. Intact specimens of *Lymnaea stagnalis* were also treated by (1 – 100 µg/ml) neonicotinoids while the feeding and locomotory activity were tested as behavioural endpoints of toxicity assays. After 30 min exposure acetamiprid blocked the sucrose-evoked feeding response while thiacloprid reduced the feeding rate to about 10% of its control value (this effect lasted for 24 hours). Acetamiprid and thiacloprid also reduced the spontaneous locomotion (10 – 15 %), while imidacloprid proved to be the less effective blocker of both behaviours. Electrophysiological studies, therefore, confirmed the involvement of acetylcholine receptors (AChRs) as primary target of neonicotinoids in the snail CNS, while behavioural data suggest additional, probably neuromuscular modulation in the periphery. These data suggest a complex (multitarget) neuronal effect of neonicotinoids in molluscs and also confirm that these insecticides may result alteration of behavioural/neuronal mechanisms in non-target invertebrates.

SYNAPTOTAGMIN 1, SYNTAXIN 1, N-TYPE Ca^{2+}-CHANNEL, NICOTINIC CHOLINORECEPTOR AND ACETYLCHOLINE IN THE NEUROMUSCULAR JUNCTIONS OF EARTHWORM SOMATIC MUSCLE *LUMBRICUS TERRESTRIS*

Volkov M.E., Volkov E.M*.

Kazan Medical State University, Russia *E-mail: euroworm@mail.ru

The somatic muscle of earthworm contains myoneural synapses forming clusters of «synaptic buttons». In these «buttons», the proteins syntaxin 1, synaptotagmin 1 and alpha 1B subunit of the Ca^{2+}-channel of N-type were identified. We suppose that «synaptic buttons» contain a limited number of active zones due to their small size (1—2 mm) and the pattern of distribution of proteins of exo-endocytotic cycle. The postsynaptic membrane of cholinergic synapses contains nicotinic acetylcholine receptors capable to bind alpha-bungarotoxin. The area of location of receptors on postsynaptic membrane is strictly limited to the region of synaptic contact. Experiments with fluorescent dyes showed that high concentrations of K^+ ions in the medium depolarize the membrane and enhance exo-endocytosis in nerve structures, which is accompanied by an increase in acetylcholine concentration in the somatic muscle of earthworm. In the presence of BAPTA and without Ca^{2+} exo-endocytosis is sharply decelerated, the level of acetylcholine in the muscle decreases, but remains relatively high.

CODING OF SOUND FREQUENCY IN THE MOSQUITO AUDITORY SYSTEM

Vorontsov D.D. (1), Lapshin D.N. (2)

(1) Koltzov Institute of Developmental Biology Russian Academy of Sciences, Moskow, Laboratory of neurobiology of development;
E-mail: colupaika@gmail.com
(2) Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), Moskow, Laboratory of Sensory Information Processing;
E-mail: lapshin@iitp.ru

Swarming male mosquitoes detect a female entering the swarm by the sound of her flight. Males use their highly sensitive feather-like antennae with the Johnston organs (JO) at their bases to receive the acoustic waves. Each JO contains several thousands of mechanosensory cells. These cells transform the oscillations of the antenna into the electric potentials which follow the waveform of acoustic signal.
Recording from the axons of sensory cells, we found that many cells are functionally paired, that is in good agreement with the morphological structure of the JO where the cells are combined by two (or, rarely, three) into the sensillae. In a pair, two cells are tuned to different frequencies and have the opposite (mirror-like) directional diagrams. This means that one and the same deflection of the antenna elicits anti-phase signals in the two cells. We speculate that such a kind of signal coding allows to represent a frequency of sound as a normalized difference between the two signals, in other words, to implement the principle of opponent coding. The latter, until recently being known primarily for color vision, can be in fact a more common feature of different sensory systems.

FROM PERIPHERAL SENSORY CELLS TO THE INTEGRATIVE NERVE CENTERS: PHYLOGENETIC AND ONTOGENETIC MORPHODYNAMICS OF SENSORY CELLS

Zaitseva O.V.(1), Voronezhskaya E.E.(2)

(1) Zoological Institute of Russian Academy of Sciences, St-Petersburg, Laboratory of evolutionary morphology; *E-mail: ovzaitseva@inbox.ru*

(2) Institute of Developmental Biology Russian Academy of Sciences, Moscow, Laboratory of developmental neurobiology; *E-mail: lenavor@gmail.com*

Sensory cells are the first nerve elements appear in the course of development and evolution of multicellular organisms. Prerequisite of their appearance is the separation of the inner space of organism from the external environment by the layer of tightly connected cells (epithelium rudiment). Such morphology corresponds to the blastula stage during development or to the trochozooid organism considered to represent hypothetical ancestor for bilateria. Consequently, the emerging gradient between apical and basal parts of the epithelial cell allowed receptor cell differentiation. The way how the solitary receptor cells clustered to the sensory nerve structures and then latter combined to the high integrative centers is the main mystery attracting attention of scientists for centuries.

In the present lecture we will combine the morphological analysis of polymodal sensory structures found within external epithelia, internal epithelium of gastrointestinal system, olfactory and visual systems. The representatives of various phylogenetic groups (gastropods, plathelminthes, nemertini, annelids, bryozoans and ascidia) both adults and larval forms will be analyzed. Throughout the whole our investigations we applied the similar morphological methodology thus allowing to compare obtained results.

The pattern of evolutional and morphogenetic dynamics of receptor cells such as shape changes, emergence of processes, and position of the receptor cell within epithelial layer will be described. We hypothesize the leading role of sensory-associative cells and their individual evolution in the way of formation of earliest nerve centers independently from the receptor modality. Thus, the on-screen composition of integrative centers of high invertebrates may be based on evolution of both visual and olfactory modalities.

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BEHAVIORAL PATTERNS AND PATTERNS OF NEUROSPECIFIC GENE EXPRESSION IN EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT IN SNAIL

Zakharov I. (1), Boguslavsky D. (1), Balaban P. (2).

(1) Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Laboratory of neurobiology of development; *E-mail: iszakharov@yandex.ru*

(2) Institute of High Nervous Activity of Russian Academy of Sciences, Moscow, Laboratory of cellular neurobiology of learning; *E-mail: balaban@ihna.msk.ru*

A search for genes specifically expressed in the functionally identified cells of the snail resulted in description of genes with characteristic changes in pattern of expression during embryo- and postembryogenesis. Gene HCS2 was shown to be expressed in the neurons involved in the network underlying withdrawal behavior, and ontogenetic development of this behavior (appearance of plasticity) corresponded with appearance of new cellular clusters expressing this gene. The HelSFamide gene was shown to belong to the gastrine-cholecictokinine family involved in control of feeding behavior. Appearance of additional clusters in specific areas of nervous system (involved in olfaction) during postnatal development also corresponded with appearance of changes in feeding behavior: transition from feeding on shells and non-developed eggs in the nest to vegetable food. Similar results were obtained when we compared dynamics of sexual behavior development with expression pattern of the preproGFAD gene. The pattern changes significantly with maturation of the animal from juvenile to adult.

Correlation analysis of gene expression pattern and behavioral development of the animal may result in understanding of mechanisms of ontogenetic launching of behavior via regulation of gene expression in specific neural populations.

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CORRECTION OF GLUTAMATERGIC NEUROTRANSMITTER SYSTEMS HYPERACTIVITY BY LAMBERTIANIC ACID AMIDES

Zapara T.A., Vechkapova S.O., Proskura A.L., Ratushnyak A.S.

Design Technological Institute of Digital Techniques SB RAS, Novosibirsk, E-mail: zapara.tatiana@gmail.com

The neurotoxic effect conditioned by hyperactivity of glutamatergic mediator system, became the subject of intensive researches when the glutamatergic nature of many neuroinduced pathologies was revealed. Classic neurodegenerative disorders (Alzheimer's, Parkinson's and many other) although are caused by different mechanisms, but may share a common path - hyperactivation of ionotropic glutamate receptors, particularly of NMDA subtype. NMDA receptor mediates $Ca2^{+}$ influx, required for many types of synaptic plasticity. Excessive NMDA-dependent $Ca2^{+}$ influx can lead to excitotoxic cell death.

Extremely limited list and poor efficiency of drugs for correcting these pathologies invites searching the new pharmacologically promising substances. In this paper we investigated the biological activity of lambertianic acid amide (AmLK). On the glutamatergic synapses of pyramidal neurons in CA3-CA1 fields of hippocampus the AmLK neuroprotective effect was detected. It normalized the developed epileptiform activity of CA1 pyramidal neurons caused by the absence in the environment of endogenous blocker (magnesium ions) of calcium-conductive channel of NMDA type receptors. When slices were pretreated by AmLK, it blocked the development of such activity, and probably associated with it the redundant entry of Ca^{2+} into neurons. The analysis of the principles and mechanisms of functioning the glutamatergic neurons, led to the assumption that lambertianic acid may possess the glutamatergic mechanism of action and is pharmacologically promising compound.

Presented in the data obtained in the performance of the base project for Fundamental Research RAS VI.35.1.5, RFBR grant № 15-29-04875

PRIMARY PROJECTION AREAS OF ANTENNAL AFFERENTS IN THE BRAIN AND SUBOESOPHAGEAL GANGLION OF *ACHETA DOMESTICA* L.

Zhemchuzhnikov M.K., Shchekanov E.E., Knyazev A.N.
Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences
e-mail: ank50@mail.ru, mihaland@mail.ru

Insect antennae have a large number of chemo- and mechanoreceptors. This study was conducted to obtain information about primary projection areas (PPA) of antennal afferents in the central nervous system of the cricket Acheta domestica (Orthoptera). Similarly to the previous findings on Gryllus bimaculatus (Yoritsune and Aonuma, 2012), we identified several PPA. The first one is antennal lobe (AL) which is commonly connected with chemosensory information. The second one is antennal motor- and mechanosensory center of the deutocerebrum. This center, known as the primary projection area of the basal segments of antenna, contains also motoneurons of antennal muscles. The last one is located in the suboesophageal ganglion. This one is of special interest because theoretically it may be connected with the grooming center or other integration centers. It is interesting that no afferents pass via the cervical connectives into the ventral cord, likewise it was found previously in other orders: Diptera (flies) and Lepidoptera (moths). This may be related to lower importance of the antennae for flight in orthopteran species as compared to other orders.

ANTIOXIDATIVE PROPERTIES OF KYNURENINES, THE REGULATORS OF BEHAVIOR AND NEURODEGENERATIVE PROCESSES IN DROSOPHILA

Zhuravlev A.V.[1], Nikitina E.A.[1,2], Tokmacheva E.V.[1], Savvateeva-Popova E.V.[1]

[1]Pavlov Institute of Physiology RAS, St. Petersburg, Laboratory of neurogenetics. E-mail: azhur@pavlov.infran.ru, [2]Herzen State Pedagogical University, St. Petersburg, Laboratory of anatomy and physiology. E-mail: 21074@mail.ru

Kynurenines, the tryptophan catabolites, have multiple neuroactive properties and participate in the development of neurological disorders, such as Huntington and Parkinson diseases. The accumulation of 3-hydroxykynurenine (3-HOK) and 3-hydroxyanthranilic acid (3-HAA) in cell leads to the hyperproduction of toxic reactive oxygen species (ROS). 3HOK and 3HAA were also shown to be ROS scavengers, more powerful than xanthurenic acid (XAA). Their antioxidant activity is related to the aromatic OH group which easily abstracts H-atom and electron. Both the absence of kynurenines and the excess of kynurenic acid (KYNA) and 3HOK in the *Drosophila* kynurenic mutants impair long-term memory formation in the conditioned courtship suppression paradigm.

The redox properties for kynurenines were calculated using density functional theory. O-H bond dissociation enthalpy and ionization potential for 3HOK and 3HAA are lower than for XAA, several phenolic antioxidants, and ascorbic acid. The reaction rate for H donation to phenoxyl and methyl peroxy radicals decreases in the order: 3HOK ~ 3HAA > XAA(oxo) > XAA(enol). Thus, the high antioxidant activity of 3HAA and 3HOK is the result of the high rate of H transfer to free radicals. This may partly explain the positive effects of kynurenines on nervous system in mammals and *Drosophila*.

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PKMZETA IS NECESSARY FOR MAINTAINING THE LONG-TERM FACILITATION OF GLUTAMATERGIC SYNAPTIC INPUTS BUT NOT OF SOMATIC GLUTAMATE RESPONSES IN THE SNAIL NEURONS

A.B. Ziuzina, A. Timoshenko, P.M. Balaban

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences

In behavioral experiments it was shown previously that selective inhibitor of PKMζ - Zeta Inhibitory Peptide (ZIP) - impairs aversive context memory in terrestrial snail *Helix lucorum*.

Long-term facilitation of excitatory (presumably glutamatergic) 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 (LTF) in neural circuit underlying aversive tentacle withdrawal. Long-term facilitation of excitatory synaptic inputs to premotor interneurons was induced by high-frequency stimulation combined with 5 serotonin bath applications and lasted at least four hours. We found that bath application of 2*10-6 M ZIP at 90th min after tetanization reduced the EPSP amplitude almost to the non-tetanized EPSP values. Application of scrambled ZIP peptide at the same concentration didn't affect the EPSP amplitude. Results support the idea of PKMζ involvement in post-induction maintenance of long-term synaptic plasticity in CNS of *Helix*.

Earlier it was shown in *Helix* that in “artificial synapse” experiments (the experiments in which the transmitter - glutamate - was directly applied to the somatic membrane of “synaptically isolated" identified giant withdrawal interneurons Pa2 or Pa3) applications of serotonin lead to LTF of Glu-induced responses (Balaban et. al., 2004). We repeated similar experiments under ZIP/scrZIP added 120 min after the facilitating procedure. Obtained results showed that in conditions of "artificial synapse", facilitated responses to glutamate were not influenced by ZIP or

scrZIP application. These results suggest that PKMzeta is not involved in postsynaptic plasticity of somatic glutamate responses in the snail neurons.

However, it was shown that applications of ZIP and scrambled ZIP in the conditions of “artificial synapse” resulted in an abrupt and short-term transient decrease of the Glu responses amplitude followed by their slow increase. It is possible that the transient inhibiting effect of ZIP/scrZIP application is an artifact of this type of preparation. In order to clarify this situation we carried out a special series of experiments on isolated CNS with preserved synaptic inputs. We have found that applications of ZIP or scrambled ZIP produced a transient drop in Glu-responses in parietal interneurons but no changes in the amplitude of complex EPSPs elicited in the same cell by the cutaneal nerve stimulation. Therefore, it appears that both peptides (ZIP and scrambled ZIP) have some non-specific membrane effects on the responses induced by the application of glutamate on the somatic membrane of parietal withdrawal interneurons.

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TWO DISTINCT MECHANISMS IN AN ULTRADIAN RHYTHM GENERATOR FOR NEMATODE DEFECATION

Slivko-Koltchik G.A.(1,2), Kuznetsov V.P.(1,2), Panchin Y.V.(1,3)
(4) Institute for Information Transmission Problems of Russian Academy of Sciences, Moscow
(5) Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow
(6) A.N. Belozersky Institute Of Physico-Chemical Biology MSU, Moscow

E-mail: s.koltchik@gmail.com, scikvp@gmail.com, ypanchin@yahoo.com

Slow circadian central pattern generators (CPGs) can function within a single cell and depend on positive and negative autoregulatory feedback loops of transcription and translation. On the other hand, well studied CPGs for the “fast” rhythmical behaviours such as running, scratching are shown to be driven by neuronal networks while voltage gated channels in individual cells from these networks play crucial role in rhythm generation. Calcium wave CPG in the nematode *Caenorhabditis elegans* gut is a leading biological model for ultradian rhythms studies and the role of different cellular and molecular mechanisms underlying its function are currently under investigation. The small size of *C.elegans* intestinal cells complicated the use of classical electro-physiological approaches in this species. We introduced a different yet related to *C.elegans* nematode species (*Heterorhabditis megidis*) with noticeably bigger gut cells in order to facilitate electro-physiological studies in nematodes and to elucidate some intestine CPG features that are difficult to resolve in *C.elegans*.

We show that intestine CPG cycling could be perturbed by shifting gut cells membrane potential, suggesting participation of plasma membrane

voltage gated channels. At the same time, we demonstrate, that CPG cycling persist in experiments were membrane potential was continuously clamped at steady voltage levels, that excludes the involvement of plasma membrane voltage gated mechanisms by definition. We suggest that two distinct pacemakers, one based on plasma membrane channels and another based on intrinsic calcium release mechanisms coordinate intestinal CPG cycling.