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ABSTRACT

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Abstracts

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S2.200. The deviation from the rule "one neuron - one receptor" in the expression of chemoreceptor genes in olfactory neurons of vertebrates: occasional or determined phenomenon

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The neuron population of the vertebrate olfactory epithelium may be considered as a matrix of chemosensory elements capable of detecting a wide range of compounds. The ability to differentiate substances diverse in chemical nature is provided by the fine turning of sensory cells at the level of olfactory receptors (OR) expression predetermining in fact correct afferent projections in corresponding bulbar zone. It remains generally accepted that the rule "one neuron – one receptor" lies in the basis of selective recognition of odorant stimuli. Mechanisms regulating the expression of unique gene from the set of multiple genes encoding olfactory receptors (1360 for mouse, 816 for human, 811 for the dog) are still not known in details being one of the most intriguing problems regarding managing of genome functions during maturation of neurons. Few ways are employed for OR selection and switching: heterochromatin modification by means of metilase, recruitment of transcription factors Lhx2 and Ebf , formation of inter- and intra-chromosomal contacts making certain genetic loci available for transcription. Nevertheless, the scRNAseq data allow to suppose the expression of 2 - 9 OR genes in mature olfactory neurons. These facts incompatible with dominating hypothesis could be considered as a consequence of automatic cell sorting, resulting in capturing of mRNA originated from neighbouring cells during sample preparation. Special efforts allowing avoiding the implication of concomitant RNA in the reactions could substantially improve the results. In present work we refined the procedure of isolation of single olfactory neurons from mouse olfactory epithelium. Mature neural cells were selected by the presence of OMP marker, subjected to preparation and total cDNA was synthesized from single cell lysate. cDNA was used as a template for PCR with degenerated oligonucleotide primers specified to broad range of OR.PCR fragments obtained from each cell were cloned in pJET1.2 vector used for transformation of E.coli, so that plasmid library is thought to contain protein-coding regions of all OR species expressed in given cell. Plasmids purified from 20 to 40 colonies containing the insert, were sequenced and the type of OR gene was determined using BLAST in the genome of Mus musculus (C57BL/6J, RefSeq GCF_000001635.27).
Totally 10 individual olfactory neurons were taken into analysis and three types of transcript distribution was observed. In the first case the expression of the singular OR was detected unambiguously. In the second one two OR transcripts appeared to be expressed with obvious prevalence of the major one. In the third case from two to four OR transcripts were detected. For the latter type major multiple transcripts were present in the following combinations: Or8k35 and Or4c109 (60,7 and 32,14 % correspondently); Or6z6 and Or4c116 (57,7 and 34,6%); Or4p8 and Or6z6 (68,4 and 21,1%); Or4e1 and Or4c113 (48 and 36%). In those cases when one prevalent OR gene was detected, it may belong to the group of highly expressed genes according to populational RNAseq available from Ibarra-Soria and co-authors, - Or6p1, Or4c117, Or4e1, Or6z7, as well as tends to possess only trace expression in the case of Or4c113. The detection of weakly transcribed mRNA confirms rather high sensitivity of the approach used in this study. Or6z6 transcript, being co-expressed with Or4c116 in single cell, exceeds it's counterpart 2-fold whereas in mixed population it's level is shown to be 25-fold higher. For the pair Or4p8/Or6z6 the opposite ratio is observed: Or6z6 being more active in mixed population, upon concomitant expression with Or4p8 is presented as less abundant product. In both cases transcription of Or6z6 located on Chromosome 7 is obviously coupled with activity of genes Or4c116 or Or4p8 nested in Chromosome 2. The latter is enriched by OR genes subgroupped in 3 remote clusters. One cannot exclude that locus of Chromosome 7 bearing Or6z6 is able to form physical contacts with Chromosome 2 in the area of long cluster 38 containing 269 OR genes including Or4c116 and Or4p8. These genes separated by 213 927 bp may participate in the formation of the same structural domain by means of inter-chromosomal contacts between Chromosome 2 and Chromosome 7 and due to spatial proximity may be expressed along with Or6z6. Individual pattern of OR co-expression observed in given cell is determined by DNA structural features in the vicinity of inter-chromosomal nodes and alternative modes of concomitant expression with the prevalence one or another OR gene may arise in different cells. When more than one OR gene is involved in transcription in certain cell the ratio of corresponding mRNA species depends likely on transcriptional status of simultaneously expressing genes rather than on individual properties of each one per se. This effect may be relayed on transcription-driven dynamical rearrangements of chromatin structure in the regions of inter-chromosomal contacts and regulatory factor's binding. The results of this study allow to suggest that olfactory neurons are capable to express one as well as few OR genes and in multy-receptor cells the choice of the partner gene seems to be not occasional.
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S2.201. The difference between the responses of premotor interneurons to serotonin and the precursor of its synthesis 5-HTP in intact and sensitized snails

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One of the manifestations of long-term memory is long-term sensitization (LTS). Sensitization is a form of non-associative learning in which the animal experiences a significant increase in the magnitude of the evoked response to a previously neutral stimulus following the application of a strong (damaging) stimulation [1,2]. If a single strong stimulus causes short-term sensitization lasting minutes, then the repetition of such stimulation causes LTS lasting days and weeks [3]. It has been shown that the developed LTS persists from 2 weeks to 1 month [1]. The long-term nature of the phenomenon is also proved by the fact that LTS is not produced when blockers of protein biosynthesis and transcription blockers are used. These results demonstrate that LTS, despite the non-associative nature of its production, needs protein synthesis; it is a form of long-term memory. It was found that when Aplysia receives a dangerous (strong) stimulus, for example, a tail electric shock, the network of serotonergic neurons of the animal releases endogenous serotonin [4]. This released serotonin (5-HT) induces a series of cellular changes that lead to an increase in the defensive reflex. Evidence

of the need for 5-HT for the formation of LTS came from experiments using a neurotoxin that depletes serotonin.

In addition to the well-known role of 5-HT as a mediator in synaptic transmission, it was shown that it can perform integrative functions when released into the extracellular environment [5]. These results provided the basis for the application of 5-HT bathing solution as a reinforcer to create cellular analogues of learning. It is known that the application of 5-HT causes effects similar to the facilitation of dehabituating and sensitizing stimuli on the neural network underlying the defensive response. By means of applications of 5-HT in the solution bathing the central nervous system, it is also possible to reproduce the electrophysiological correlates of plasticity [6]. Previously, we found that applications of 5-HT and 5-hydroxytryptophan (5-HTP) into a solution washing the preparation of the nervous system caused a decrease in the membrane potential of premotor interneurons in both intact and trained snails [7]. At the same time, in trained snails, in contrast to intact ones, applications of 5-HT and 5-HTP caused an increase in the threshold potentials of LPa3 and RPa3 premotor interneurons. In this work, we studied changes in the excitability of premotor interneurons in response to the application of 5-HT and 5-HTP in preparations of intact snails and snails after LTS.

The experiments were carried out on isolated preparations of the nervous system of the mollusk Helix lucorum. To develop the LTS of the defensive reflex, the animals were presented with electrical stimuli in the head area 4 times a day for 4 days at an interval of 1.5-2 hours. Registration of electrical characteristics was carried out on the premotor interneurons of the defence reflex LPa3 and RPa3; to evoke an action potential, a rectangular current pulse with a duration of one second was applied through the recording electrode. Membrane potential (Vm) and action potential generation threshold (Vt) values were analyzed in response to the application of 5-HT and 5-HTP solutions in preparations of intact snails and snails after LTS. It was found that the application of 5-HT and 5-HTP significantly reduced the membrane potential in the groups of both intact and sensitized snails (by 4 mV). The action potential generation threshold, on the contrary, increased insignificantly. The results obtained indicate changes in the properties of various 5-HT receptors during the formation of LTS.

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S2.202. The effect of acute phase inflammatory proteins, C-reactive protein, serum amyloid A, alpha-1-acid glycoprotein, fibrinogen and ceruloplasmin, on the activity of peripheral blood neutrophils

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Neutrophils are the leading cells of the innate immune system and the main population of leukocytes responsible for the primary reaction of the body to various infectious particles. The latter are destroyed by neutrophils due to the processes of phagocytosis and a cascade of reactions, including the respiratory burst reaction (RBR). As a result of RBR, neutrophils produce reactive oxygen species (ROS) and reactive halogen species, powerful cytotoxic agents that destroy particles in the phagolysosome. All of these processes require regulation, since excessive activation of neutrophils can lead to ROS-mediated damage to the tissues surrounding the focus of inflammation, and proteins of the acute phase of inflammation (APP) claim to be regulators of inflammation. We have previously shown the participation of ceruloplasmin in inhibiting RBR of neutrophils in blood samples [1], and fibrinogen, on the contrary, increased the intensity of RBR [2]. The effect on neutrophil functions has not been studied in detail for all APPs and especially their combinations. In this paper, for the first time, the effect of a number of APPs, C-reactive protein (CRP), serum amyloid A (SAA), alpha-1-acid glycoprotein (a1AGP) and fibrinogen on the ability of peripheral blood neutrophils to RBR using flow cytometry with registration of ROS production in cells as part of peripheral blood samples was investigated [3]. Significant changes in the ability of neutrophils to produce ROS were found for a number of combinations of the studied APPs. The study of the interaction of ceruloplasmin and fibrinogen with peripheral blood neutrophils on a confocal microscope revealed their membrane localization. It seems promising to identify receptors for these APPs on the neutrophil membrane, as well as to study their influence on the biomechanical characteristics of peripheral blood neutrophils.

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S2.203. The effect of combined magnetic fields on the growth of NCTC clone L929 cells

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The aim of this work is to study the combined magnetic fields tuned to parametric resonance for Ca2+ (Ca2+-CMF), K+ (K+-CMF) and Mg2+ (Mg2+-CMF) ions on the growth of cells of the NCTC clone L929 fibroblast line.

Several series of experiments were conducted to select optimal conditions for the cultivation of selected cells. During the experiments, the cells of the NCTC clone L929 line were in thermostatically controlled