Supplementary Information

SI 1. Determination of the calcium concentration in primary cell cultures

In order to evaluate the Ca^{2+} concentration in primary hippocampal cells incubated with surface-functionalized upconversion nanoparticles (UCNPs) under test, we measured and analyzed the average fluorescence intensity of the calcium indicator Oregon Green (OGB-1) in the soma. We made use the linear dependence of the OGB-1 fluorescence intensity versus the calcium ion concentration in the concentration range of 20-200 nm, and benchmarked our measurements against the free calcium concentration of 60 nM in the cytoplasm of the nervous cells reported by Zheng et al. (Zheng et al., 2015). The incubation with tested UCNPs caused a profound decrease in the free intracellular calcium concentration. The average Ca^{2+} concentration in the cytoplasm was decreased 2.2, 1.4 and 1.45 times [respective Ca^{2+} concentrations (27 ± 1) nM, (43 ± 2) nM and (41 ± 2) nM] in cases of the 72-h cell treatment with UCNP-TMAH, UCNP-PEI and UCNP-PMAO, respectively. In addition, a significant reduction in the oscillation amplitudes in the cells, exhibiting spontaneous calcium activity was observed, as a result of the incubation with tested nanoparticles.

According to the baseline in the group of the control cultures, the amplitudes of the Ca^{2+} oscillations were measured as (35 ± 1) %, (29 ± 2) % and (26 ± 2) % in control, UCNP-TMAH and UCNP-PMAO cultures, respectively. No oscillations were registered in the UCNP-PEI treated cell cultures.

References:

K. Zheng, L. Bard, J.P. Reynolds, C. King, T.P. Jensen, A.V. Gourine et al., *Neuron,* 2015, Vol. 88, P. 277–288.

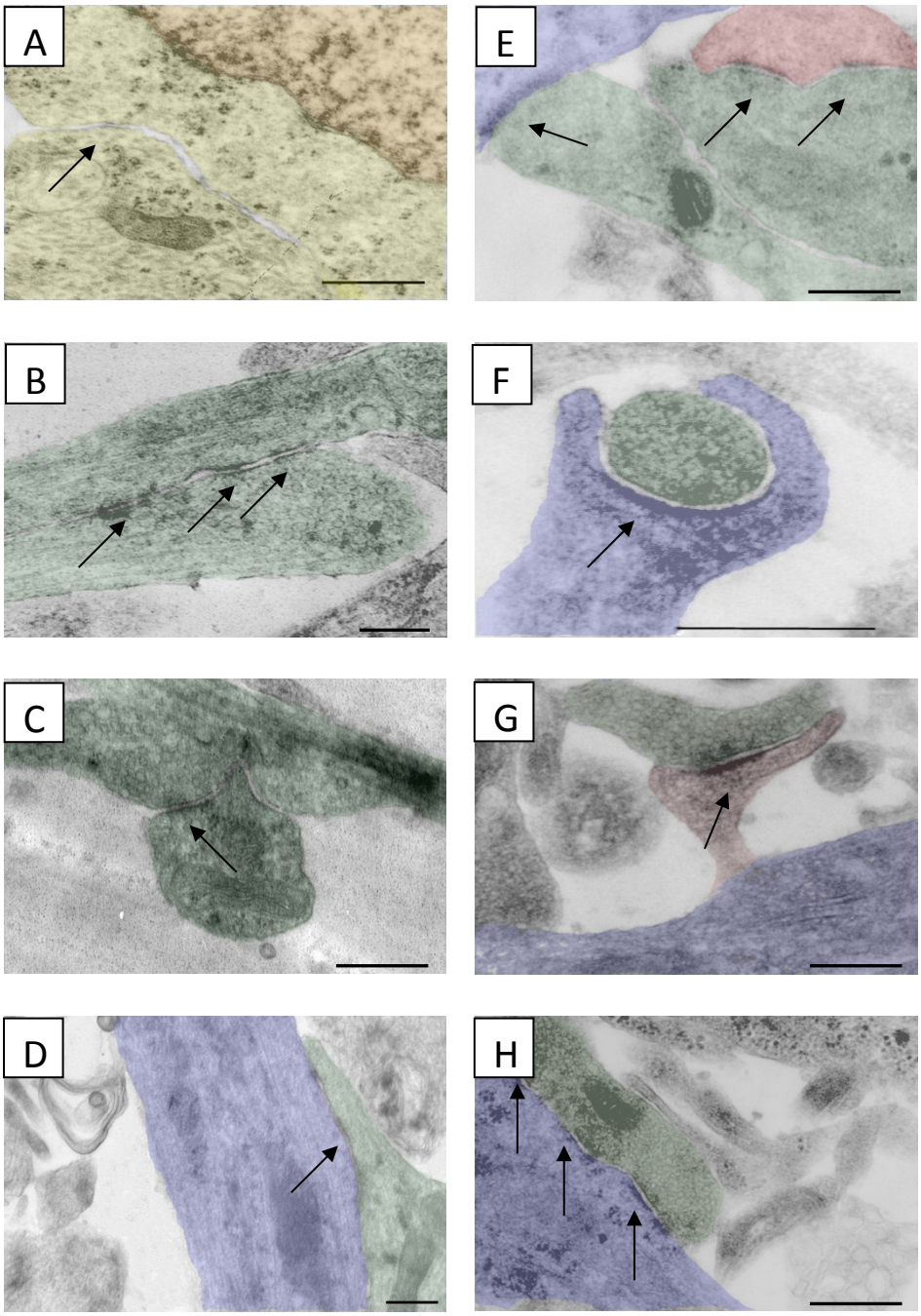
SI 2. Features of primary hippocampal cultures development

In the part of **Results** our findings are based on our previously demonstrated data (Shirokova et al., 2013):

In the first days of primary hippocampal culture development *in vitro* a plurality of neurons formed non-synaptic contacts providing morphofunctional stabilization of neuronal network at early stages of its development (Figure S1). The presence of numerous free postsynaptic density confirms the idea about the availability of postsynaptic site of neuronal membrane to form a mature contact, since Ca^{2+}-oscillations are recorded only in 1% of neurons (Figure S2). The increase of Ca^{2+}-activity on the 7th DIV appears to be associated with the formation of first chemical synapses. Since the second week in vitro the decrease of the duration of Ca2+-oscillations and the increase of their frequency coincided in time with further complication of ultrastructure of chemical synapses, among which mature axo-spinous contacts were predominated, that appears to improve the efficiency of synaptic transmission. High activity of synaptic transmission is confirmed by filling of axon terminals by synaptic vesicles. 21 DIV was characterized by the appearance of calcium "superoscillations" accompanied by the complication of bioelectrical activity of neuronal network in the form of superbursts of spikes. In addition, at ultrastructural level practically total disappearance of immature functional contacts was observed that was in agreement with the data on synaptogenesis in vivo.

References

O.M. Shirokova, L.E. Frumkina, M.V. Vedunova, E.V. Mitroshina, Y.N. Zakharov, et al., *Modern technologies in medicine*, 2013, Vol. 5(2), P. 6-13.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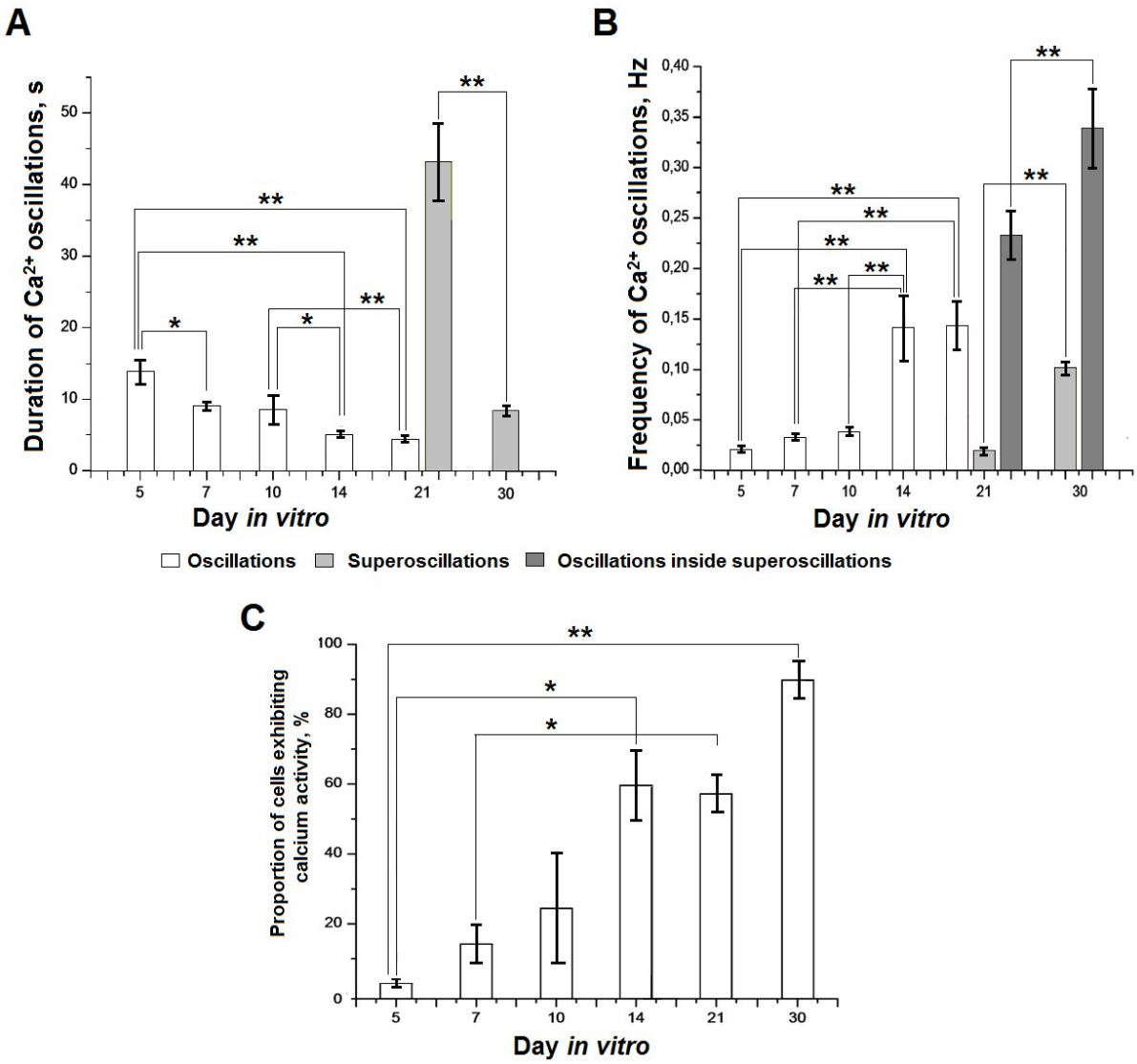
A
Duration of Ca2+ oscillations, s
0
10
20
30
40
50
5
7
10
14
21
30
Day in vitro
B
Frequency of Ca2+ oscillations, Hz
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
0,40
Day in vitro
Oscillations
Superoscillations
Oscillations inside superoscillations
C
Proportion of cells exhibiting calcium activity, %
0
20
40
60
80
100
Day in vitro

Figure S1. Electron microscopy of primary hippocampal culture on 5DIV (a,b), 7DIV(c,d), 14DIV (e), 21DIV (f), 30DIV (g,h). a - somatosomatic symmetric contact, b - axo-axonal symmetric contact, c - mixed axo-axonal contact, including desmosome and asymmetric contact, d - axodendritic desmosome-like contact, synaptic vesicles are not suitable for presynaptic density, e - perforated axospinous and axodendritic synapses, f - axodendritic asymmetric convergent contact, g - axospinous asymmetric contact, h - perforated axodendritic asymmetric synapse. Abbreviations: row - contact, false color: green - axon, blue - dendrite, pink - dendrite spine, yellow - soma, orange - nucleus. Scale 0.5 μm (adapted from Shirokova et al., 2013).

Figure S2. The main parameters of functional calcium activity in different stages of primary hippocampal cultures development: A - mean duration of Ca^{2+} oscillations, B - mean frequency of Ca^{2+} oscillations, C - proportion of cells exhibiting calcium activity; * - significant difference, $p < 0,05$, **- significant difference, $p < 0,05$, Mann–Whitney criterion, N= 28 (adapted from Shirokova et al., 2013).