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Nanodiamonds-induced effects on neuronal firing of mouse hippocampal microcircuits

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Fluorescent nanodiamonds (FND) are carbon-based nanomaterials that can efficiently incorporate optically active photoluminescent centers such as the nitrogen-vacancy complex, thus making them promising candidates as optical biolabels and drug-delivery agents. FNDs exhibit bright fluorescence without photobleaching combined with high uptake rate and low cytotoxicity. Focusing on FNDs interference with neuronal function, here we examined their effect on cultured hippocampal neurons, monitoring the whole network development as well as the electrophysiological properties of single neurons. We observed that FNDs drastically decreased the frequency of inhibitory (from 1.81 Hz to 0.86 Hz) and excitatory (from 1.61 to 0.68 Hz) miniature postsynaptic currents, and consistently reduced action potential (AP) firing frequency (by 36%), as measured by microelectrode arrays. On the contrary, bursts synchronization was preserved, as well as the amplitude of spontaneous inhibitory and excitatory events. Current-clamp recordings revealed that the ratio of neurons responding with AP trains of high-frequency (fast-spiking) versus neurons responding with trains of low-frequency (slow-spiking) was unaltered, suggesting that FNDs exerted a comparable action on neuronal subpopulations. At the single cell level, rapid onset of the somatic AP ("kink") was drastically reduced in FND-treated neurons, suggesting a reduced contribution of axonal and dendritic components while preserving neuronal excitability.

Among the multitude of fairly novel materials for scientific, technological and clinical applications, diamond nanocrystals (or nanodiamonds, NDs) earned a solid reputation and vast interest due to their unique features, such as low cytotoxicity[1], the possibility of stable chemical functionalization and extreme mechanical properties (robustness, low friction coefficient)[2]. The diamond lattice can host a large number of optically active defects[3,4], the most common and widely employed of which is represented by the negatively charged nitrogen vacancy complex (also referred as NV^- center). This system is characterized by a wide excitation spectrum (500–600) nm, emission in the red range (600–800 nm) with zero phonon line (ZPL) at 638 nm. The neutral charge state of the defect (i.e. the so-called NV° center, with ZPL emission at 575 nm) is not equally appealing, due to a less convenient electronic structure to implement protocols of local sensing of electromagnetic fields.

NDs incorporating NV^- centers provide a stable luminescent label suitable for different types of bio-imaging and bio-sensing applications[5–15]. A significant advantage of fluorescent nanodiamond (FNDs) is related to their photostability, resistance to bleaching or quenching phenomena[16], that allows their monitoring along neuronal branches with high spatio-temporal resolution[14], as well as to perform long-term cell tracking[12,17]. More specifically, the peculiar structure of the spin-dependent radiative transitions of the NV^- centers allows the optical detection of weak electro-magnetic fields and small temperature variations within the biological samples under exams, by means of Optically Detected Magnetic Resonance (ODMR), thus disclosing a range of new perspectives in cell sensing with unprecedented spatial resolution and sensitivity[5,8,10,18,19]. Patterned ND networks and

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