require the models be revised. Next, we will use this technique to measure fluid motion in scala media, the fluid compartment that contains the structures that directly stimulate the hair cells.

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Graphene Oxide Nanosheets Target Excitatory Synapses in the Hippocampus: Reversible Down Regulation of Glutamate Neurotransmission In-*Vivo*

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Neurological disorders significantly impact the quality of life of patients. Promising therapeutic paradigms take advantage of nanomaterials to manipulate the activity of target cells against these diseases. Graphene offers a range of unique physicochemical properties that can be tuned for biomedical needs. In cultured hippocampal neurons, small graphene oxide nanosheets (s-GO) were reported to interfere selectively with excitatory synapses, sizing down glutamatergic activity. Glutamate is the main excitatory neurotransmitter in the central nervous system, and apart from neurodegenerative diseases, growing evidence suggests that glutamate is involved in psychiatric and neurological disorders. Localized targeting of the glutamatergic system is attractive objective, but a mechanistic understanding of s-GO interference with synapses and the translation *in vivo* of these evidences are lacking. Here we used cultured neurons and acute hippocampal slices to demonstrate that s-GO flakes directly impair synaptic vesicle release in excitatory synapses, inducing a vesicle depletion that results, in the long term, in a down regulation of the glutamatergic activity, leaving the inhibitory GABAergic system intact. We further tested *in vivo* the injection of s-GO in the hippocampus of juvenile rats and patch-clamped single neurons to measure glutamatergic synaptic activity from brain slices isolated after 48 and 72 hours from brain injections. 48h after surgery, acute hippocampal slices isolated from sGO-treated rats showed a significant reduction in glutamatergic synaptic frequency, while GABAregic one was unchanged, in respect to controls (saline injections). These effects were reversed in slices isolated 72h post injections. Tissue reactivity, quantified by astrocyte and microglia labelling, was equal or even lower than in controls. s-GO ability to target and silence glutamatergic synapses may have special importance for experimental neuroscience research, because in specific brain regions such an activity may contribute to neuropathologies.

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Graphene Oxide Nanosheets and Neural System: From Synaptic Modulation to Neuroinflammation

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Graphene possess extraordinary properties and is extensively explored for biomedical applications. In neurology, such developments are focusing on novel neuro-devices, including regenerative interfaces or drug delivery vector systems, based on few-layer graphene sheets. Any further exploitation in the central nervous system of biomedical devices based on these 2D planar nanostructures requires deep understanding of their interactions with the neuronal tissue. We recently reported the ability of graphene oxide nanosheets (s-GO) to interfere specifically with synapses and to alter glia reactivity without affecting cell viability, in cultured hippocampal networks. While the specificity of the synaptic alteration might pose the basis for exploiting s-GO for target theranostics applications, the potential tissue reactivity raises concerns from a toxicity point of view. To tailor s-GO safe developments we need to model in vitro glial cell responses, and in particular microglial reactivity in complex tissues challenged by chronic s-GO delivery at high doses. In this work, we design long-term exposure to s-GO in 3D tissue cultures. We use mouse organotypic spinal cultures that are ideally suited for studies involving long-term interference with cues at controlled times and concentrations. In this preparation, the normal presence, distribution and maturation of anatomically distinct classes of neurons and glial cells are preserved to reconstruct the basic cytoarchitecture of spinal cord segments. We address the impact of treatments with s-GO on premotor synaptic activity monitored by single cell patch clamp recordings from ventral interneurons. We further investigate by immunofluorescence labeling and confocal microscopy the accompanying glial responses. We focus on microglia either in organotypic spinal cord slices or in isolated primary glial cultures and pure primary microglial cultures, treated with s-GO. In these conditions we test the role of microglial microvescicles (MVs) release in mediating their response to s-GO.

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The MEMS μHammer: Impacting Neuroscience One Cell at a Time

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Approximately 1.7 million people sustain Traumatic Brain Injuries (TBI) annually, whether through physical impacts on sports fields or percussive blasts on the battlefield. Yet while the response to TBI-level forces and subsequent recovery has been extensively studied on the tissue level, less is known on the cellular level. Investigating the damage and recovery mechanisms of NSCs (neural stem cells) will improve the current understanding of neuroplasticity and may lead to new therapies, as these cells are vital to the regenerative capabilities of the brain. Current systems for applying forces to individual cells are often limited to low force (sub-μN) and strain-rate (1-10 s^{-1}) ranges, and all are low throughput (tens of cells per experiment), making them ill-suited for gathering enough data for biologically-relevant analysis. We have developed a microfluidic MEMS device, the μHammer, to subject individual cells to TBI-relevant impacts with high throughput, allowing us to investigate NSCs' responses to impact as a function of force and duration. The μHammer can impact up to a rate of 80,000 cells per hour at strain rates ranging from 20,000 to 200,000 s^{-1} and 30 to 60% strain. Using a simple Hertz contact model, we estimate the force generation to be in the 1-100 μN range, which falls within characteristic TBI parameters. Initial experiments explored the effects of impact on NSCs at various strain rates and demonstrated inhibited proliferation commensurate to the rate of strain applied. Further, the influence of strain rates on induced cell necrosis and apoptosis is being explored as well as the effect of impact on NSC differentiation. In conclusion, the μHammer's capability of impacting thousands of cells at controlled TBI-relevant force magnitudes will open doors to unprecedented analysis of damage and recovery mechanisms of NSCs and other neural cells.

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Epileptiform Activities in Cultured Human IPSC-Derived Neuronal Networks

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The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating drug toxicity. Epileptiform activity is one of phenomena in neuronal toxicology. To evaluate the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons, we used the high-throughput multielectrode array (MEA) system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates. We examined chemically evoked epileptiform activity. Epileptiform activities were induced by 4-Aminopyridine (4-AP), pilocarpine, chlorpromazine, and pentylenetetrazole (PTZ) . The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. On the other hand, the duration and spikes in a synchronized burst were increased at PTZ administration. Phenytoin used in anti-convulsant drug suppressed electrophysiological activities. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using MEA system has the potential to investigate the neuronal toxicity in drug screening.

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3D Micro Scaffolds for Tailor-Made Three-Dimensional Neural Network Studies

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One of the most important scientific tasks of our time is to understand the complex activities in neural networks (for example in the US BRAIN Initiative and the EU Human Brain Projekt). Until recently, it was difficult to simulate neural networks at laboratory scale, since conventional circuits based on 2D-contacts differ strongly from biological data processors like the brain, which is a truly three-dimensional (3D) circuit. Huge advances in the additive manufacturing at the nano and micro scale, especially direct laser writing by 2-photon polymerization, now make the construction of complex resist-based 3D substrates