Form 3

Abstract of Thesis

Name （Deligkaris Kosmas）	
Title	Comparison of somatic and axonal spike shape features in cortical neurons grown on a high-density microelectrode array 高密度集積型マイクロ電極アレイを用いた大脳皮質神経細胞の細胞体と軸索の活動電位波形の比較解析

Abstract of Thesis

Analysis of axonal spike shape and propagation properties can elucidate axonal mechanisms involved in learning and plasticity. Due to the thin diameter of the axon, analysis of somatic and axonal signals is technically challenging. Non-invasive extracellular recordings of axonal signals have small amplitudes, necessitating averaging of sub-threshold events. Here we present a framework for comparing somatic and axonal signals, based on a high-density microelectrode array (HDMEA), on which rat cortical neurons are grown. The HDMEA has 11,011 electrodes spaced densely enough to achieve sub-cellular resolution. We show that such a system can record large amplitude extracellular action potentials (EAPs) from both somata and axons, as identified with immunofluorescence. Areas devoid of neuronal somata showed electrical activity recorded by the HDMEA. The identified axonal spikes featured a variety of consistent waveforms and much shorter overall duration than somatic spikes. Under spontaneous activity conditions, both the amplitude and the duration of axonal EAPs were more resistant to changes induced by high-frequency activity than somatic EAPs. Our findings have the following consequences: Firstly, they lay out a framework for studies of axonal physiology, evidencing the contributions of axonal signals in overall HDMEA recordings and the differences between somatic and axonal waveshapes. Secondly, they reveal axonal firing characteristics of neurons embedded in an active network. Finally, they provide further evidence that short, triphasic spikes are axonal spikes and can be recorded in-vitro, similarly to previous in-vivo recordings.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Mr. Kosmas Deligkaris' main focus of his PhD project is on the comparison of somatic and axonal extracellular action potentials in cultures of cortical neurons. He established measurements with our circuit-based high-resolution microelectrodes arrays and initiated and innovated a method to correlate neuronal activity with the structure of the neuronal network using high-resolution electrophysiology, immunohistochemistry and fluorescent imaging. This method allowed him to assign action potentials measured extracellularly with 11,000 electrodes to either the somatic or axonal compartments of the cells. He found that axonal segments in such cortical cultures generate large extracellular signals, and that likely a larger then previously assumed fraction of extracellular signals arises from axonal sources. He showed that these large signals not only arise from the axonal initial segment, but also from more distal segments of the axons. He also showed that the reduction in the signal amplitude, or adaptation, typically observed during a burst, is more pronounced at somatic compartments as opposed to axonal segments.

Up to date, from the work done at my laboratory, Kosmas presented his studies at various international and domestic meetings (e.g. the Annual Meetings of the Japanese Neuroscience Society, International Meeting on Substrate Integrated MEAs, etc), and also co-authored a review paper published in Frontiers in Neuroscience. Kosmas also talked about his research at several RIKEN internal meetings and workshops, and for a contribution entitled "Neuronal Footprints in Dissociated Neuronal Cultures" at the RIKEN Noyori Summer School 2014, he received a best poster award.

Even though the manuscript that constitutes the main part of his thesis is not published yet, I am confident, that he will be able to actually publish it within reasonable time from now. Such a detailed study on the origin, waveform shape and variability of extracellular action potentials in *in vitro* cortical cultures will be of great value for many researchers working with such devices. Even though the results may not be ground breaking, the work itself is very solid, sound and carefully done.

With the manuscript in preparation and together with all his other disseminations, I belief Kosmas showed that he masters many aspects of scientific research, from the generation of conceptual ideas and literature studies to carefully conducting experiments obtaining solid, reproducible data and the analysis of the results.

Overall, the thesis of Kosmas Deligkaris is of high quality and represents well-done and sound work. To conclude, I recommend accepting the thesis of Kosmas Deligkaris, as submitted for a doctoral degree.