ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
令和１年　９月　３日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人 日本学術振興会 理事長　殿

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（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：ロンドン　　（国名：英国）

2. 研究課題名（和文）
単一神経細胞内における局所情報処理機構の光学的解析

3. 派遣期間：平成２９年　７月　１８日　～　令和　１年　７月　１７日

4. 受入機関名及び部局名
University College London, Wolfson Institute of Biomedical Research

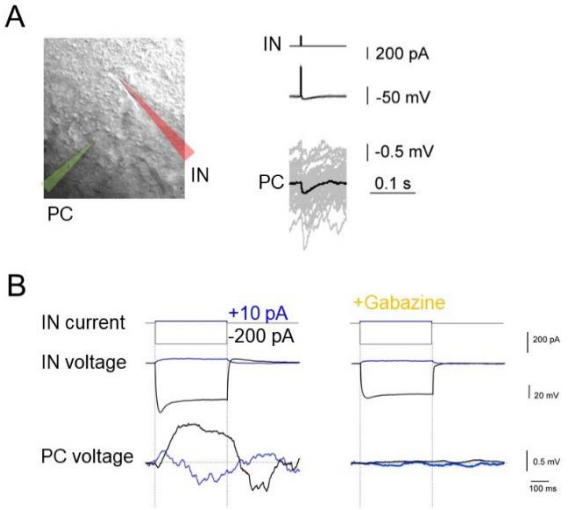
5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**

（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

With the JSPS oversea fellowship, I worked at University College London, Wolfson Institute of Biomedical Research, Neural computation laboratory. My research project was to understand how neuronal activity is modulated by local network of inhibitory interneuros. Inhibitory signals are essential elements of the nervous system and the interaction between excitation and inhibition underlies fundamental operations performed by neural circuits. However, the exact role and mode of operation of inhibition on principal cells is still poorly understood. One reason for our limited understanding is the lack of knowledge about the organization of inhibitory circuit.

In the cerebellum, the sole output is provided by Purkinje cells (PCs), which are regulated by synaptic inhibition provided by molecular layer interneurons (MLIs). MLIs are interconnected by both chemical and electrical synapses, and the complexity of this circuit structure provides diverse insights of functional role of inhibitory circuits on principal cells. While action potential-driven, chemical synaptic signalling is unidirectional and necessarily involves a threshold, electrical synaptic signalling is bidirectional and does not involve a threshold. How these circuits interact to shape the functional output of a circuit is not well understood.

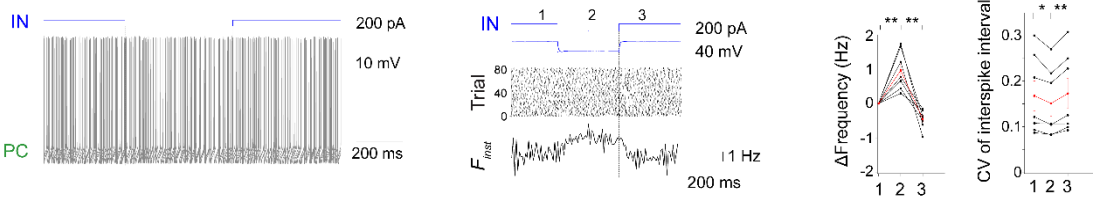
To understand the functional interactions between INs and PCs, we made paired recordings from MLIs and PCs in cerebellar sagittal slices at physiological temperatures (A). Triggering an action potential in the MLI evoked an inhibitory postsynaptic event in PCs (A, right). In addition to this usual GABAergic response, we observed an unexpected effect of MLIs on PCs. A steady state hyperpolarization of the MLI, in response to a current step (400 ms, -100 pA), caused a depolarization of the PC, while a steady state subthreshold depolarization of the MLI (400 ms, +10 pA) caused a hyperpolarization in the PC (B). As the effect was blocked by $GABA_A$ receptor antagonist, gabazine (SR95531), GABAergic input to the PC is likely to be involved. Remarkably, this effect was observed in both monosynaptically connected and unconnected MLI-PC pairs. This finding suggested that the manipulation of individual interneuron engaged other MLIs that were directly inhibiting the PCs.



MLIs are electrically coupled via gap junctions. A steady state hyperpolarization in an MLI is expected to affect the coupled MLIs. We performed triple recording from a pair of electrically coupled MLIs and PC. When IN1 is hyperpolarized, a spontaneous spiking of the coupled MLI (IN2) was largely reduced, reducing the inhibition of the PC and causing it to depolarize. The high probability of an electrical connection between MLIs (probability = 0.4, Rieubland et al., 2014) suggested that most MLIs could influence the activity of at least one of their coupled neighbours.

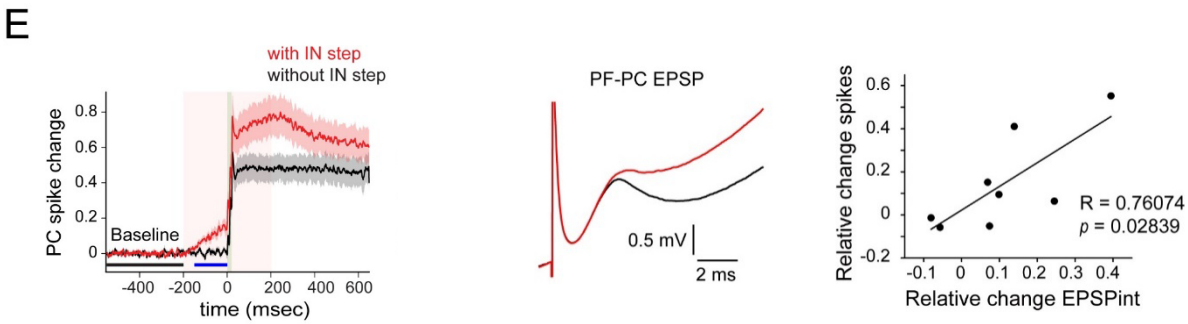
Next, I evaluated the effect of this disinhibition on spontaneous firing on PCs by comparing the period of disinhibition with pre and post period (D). The firing frequency of PCs was significantly increased by disinhibition. Also, irregular spiking pattern became more regular, defined by a lower coefficient of variation (CV) of the inter spike intervals (ISI) (D, right). The effect of inhibition on the PC spiking irregularity has been demonstrated before, in particularly removing inhibition causes more regular firing (Hausser & Clark, 1997).

D

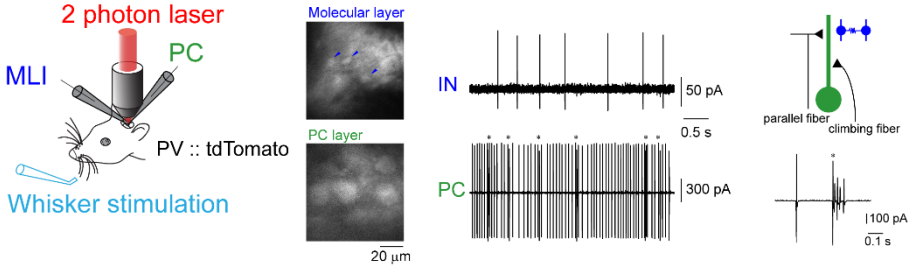


How can this indirect connection that we found be functioned in a physiological network? Excitatory synaptic inputs of parallel fibers (PFs) activate both PCs and MLIs. Thus, in addition to tonic inhibition of MLIs to PCs (Hausser & Clark, 1997), PC is also inhibited via PF evoked feed-forward inhibition (FFI) (Mittmann et al, 2004). We found that, on top of the increase in spontaneous PC spikes, PF-evoked PC spikes were significantly increased when the indirect inhibition was removed (E). We also found that the change in PF-evoked spikes is positively correlated with change in PF-evoked EPSP area (E, lower panels). These results suggest that the PF-evoked FFI is regulated by coupled MLIs network.

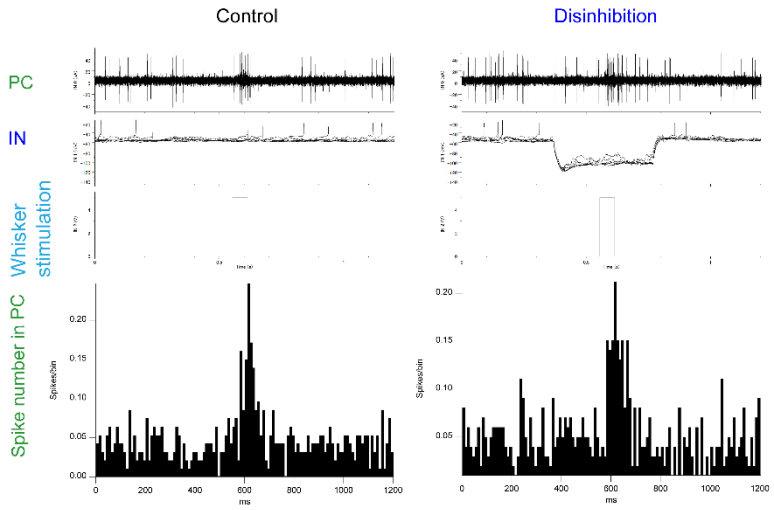
E



Finally, we explored how these mechanisms influence *in vivo* functional connectivity between MLIs network and PCs. I performed dual target patch-clamp recording from IN and PC under guidance of 2photon images. We used transgenic mice that express tdTomato in both INs and PCs.



I recorded spontaneous spiking in cell-attached mode in PCs. To see an effect of coupled networks in INs, whole cell recording was performed in IN and membrane potential of the IN was manipulated in the same way as experiments in slice condition. Whisker was stimulated by air puff to evoke a sensory input to cerebellar cortex. Excitatory inputs to parallel fibers (PFs) activate both PCs and INs, and thus FFI is recruited onto PCs. We found that spike number in PCs was enhanced when FFI was withdrew by hyperpolarization of the IN. This result suggest that coupled network of MLIs strongly influence cerebellar output in vivo.



In conclusion, I demonstrated the existence of a di-synaptic connectivity that may have a highly relevant functional role in the cerebellar cortex in vitro and in vivo. These results help us understand the principles about the output of interneuron network and how inhibition is delivered to principal neurons at a network level.