Training Stay Report

Diogo M. Rombo

From January 2nd to March 30

Home Institution: Faculty of Medicine, University of Lisbon

Supervision: Dr. Ana M. Sebastião

Host Institution: Department of Pharmacology, University of Oxford

Supervision: Dr. Karri Lamsa

Project title: Modulation of selected GABAergic synapses by adenosine.

Techniques learnt: During my stay in Dr. Karri Lamsa's lab I was introduced to optogenetic technique combined with electrophysiological recordings.

I learnt stereotaxic intrahippocampal viral injection procedures to allow Channelrhodopsin expression in different lines of transgenic mice (PV-Cre and CCK-Cre mouse lines). Patch-clamp recordings combined with optogenetic stimulation were routinely performed and I learnt the procedures to anatomically analyze the postsynaptic cells recorded (immunohistochemistry techniques and cell type identification). I got familiar with new software for acquiring and analyzing electrophysiological data.

I was introduced to all technological requirements about both surgical procedures and optogenetic stimulation in order to reproduce this in my home lab.

This stay also contributed to establish important scientific collaborations between the two groups.

Report: Glutamatergic principal cell excitability in the hippocampus is regulated by local circuit neurons that release inhibitory neurotransmitter GABA. These GABAergic interneurons exhibit vast structural, physiological and biochemical diversity. They innervate both excitatory principal cells and other inhibitory interneurons (1), hence a tuned modulation of inhibitory circuits is of great importance in the control of network function.

Adenosine, acting in particular through high affinity A_1 and A_{2A} receptors, is a well-recognized endogeneous modulator of glutamatergic principal cells excitability (2). However, the role of adenosine in modulation of inhibitory transmission is much less known.

During my stay in Dr. Karri Lamsa's lab we explored modulatory actions of adenosine receptor agonists on specified hippocampal inhibitory synapses. We focused on pre- and postsynaptic actions of A_{2A} receptors agonist on two molecularly-defined inhibitory synapses, which innervate both pyramidal cells and different types of interneurons. It has been shown earlier that activation of adenosine A_{2A} receptors facilitate GABA release from presynaptic terminals in the superfused synaptosome preparation (3). However, given the huge diversity of interneuron types in the hippocampus we asked if A_{2A} receptor-mediated effect was localized in specific types of presynaptic GABAergic terminals and/or to their postsynaptic target cells. To explore this idea we used targeted optogenetic stimulation of molecularly-defined interneuron populations expressing either parvalbumin or cholecystokinin. The GABAergic synapse function was monitored in their target neurons using intracellular electrophysiological recordings.

Our experimental design allowed optical control of specified GABAergic synapse activity with laser light pulses activating expressed cation channel ChR2 in these cells (4). To achieve specific expression of ChR2we used a viral approach in association with a Cre-loxP system. Two different transgenic mouse lines expressing Cre protein in mutually exclusive populations of inhibitory neurons (parvalbumin-Cre, i.e. PV-Cre and cholecystokinin-Cre , i.e. CCK-Cre were

injected with adeno-associated viruses (AAV) carrying ChR2-eYFP genes). This way we introduced into PV-Cre and CCK-Cre mice a mammalian codon-optimised ChR2 gene fused with enhanced yellow fluorescent protein gene (ChR2–eYFP) in an AAV2/5 vector via stereotaxic injections into dorsal CA1 hippocampus. Between 10 to 15 days following virus injection, the mice were sacrificed and we prepared acute slices from both hippocampi. Then we performed whole-cell patch-clamp recordings from pyramidal cells and interneurons aligned with $eYFP^+$ axons (detected on-line under epifluorescent light). We optically stimulated the $eYFP^+$ axons with a 473nm fixed-spot laser light pulses (1-3 ms, upto 100mW) delivered via an optical fiber through the objective lens of the microscope to elicit monosynaptic inhibitory postsynaptic currents (IPSC) in their target cells. After a stable baseline, the A_{2A} receptor agonist (CGS21680, 30nM) was applied to the bathing solution, and changes in IPSC peak amplitude and paired-pulse ratio (PPR) were analyzed during 20min drug application. For all recorded target cells, neurobiotin (0.5% v/v) was added to the intracellular solution to allow their post-hoc visualization and anatomical analysis and consequent cell identification. Systematic identification of recorded cells will allow us to understand which neuron populations are targeted by PV^+ and CCK^+ fibers (5,6) and look for specificity of A_{2A} receptor actions.

Although still very preliminary, our data suggests that A_{2A} receptor actions are synapse specific. We focused our study on CCK^+ inputs to pyramidal cells (CCK^+-Pyr) and to interneurons (CCK^+-Int) and on PV^+ inputs to pyramidal cells (PV^+-Pyr) and to other interneurons (PV^+-Int). When looking to CCK^+-Pyr and CCK^+-Int synapses we observed no change on peak amplitude of IPSCs by the A_{2A} receptors agonist, CGS21680 (30nM). Until now we observed 4 pyramidal cells and 10 interneurons that did not show any change by A_{2A} receptor activation (CCK^+-Pyr : peak amplitude change to 1.0±3.4% of the baseline level; PPR change to -5.2±6.4%; n=4; CCK^+-Int : peak amplitude change to -3.4±2.2% of the baseline level; PPR changed to -3.0± 7.3%; n=10). When recording from pyramidal cells and optically stimulating PV^+ fibers (PV^+-Pyr) we similarly observed no effect by A_{2A} receptor activation in 8 cells (PV^+-Pyr: peak amplitude to -0.8±5.0% of the baseline; PPR change to 5.3±18.1%; n=8). Interestingly, when looking to PV^+-Int synapses we observed in 6 unidentified interneurons a strong facilitatory effect of A_{2A} receptor activation (peak amplitude change to 31.0±6.4% of the baseline level) together with a decrease in PPR (PPR change to -26.6±7.6% of baseline). There were 2 interneurons, however, that did not respond to A_{2A} receptor activation. Identification of these target cell types is currently under process and it is possible that the two (which showed no modulation) represent a specific cell type indicating that A2AR action is not only selective to pre- but also postsynaptic cell type.

Together, this data suggests that adenosine A_{2A} receptors modulate inhibitory synaptic transmission by acting specifically on GABA release from PV^+ terminals onto other interneurons, without affecting both PV^+ terminals to pyramidal cells or CCK^+ terminals to any type of postsynaptic neuron.

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(4) Fenno,L.,Yizhar,O.,Deisseroth,K.,2011,Annu.Rev.Neurosci, 34:389-412
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