

# Euro Neuropharmacology 2018: PET imaging of ischemia-induced impairment of mitochondrial complex I function in living brain - Hideo Tsukada - Hamamatsu Photonics K K.

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## Abstract:

Entorhinal cortex considered as the gateway mediating the connections between the hippocampus and other cortical areas. Sensory inputs from olfactory structures, parasubiculum, perirhinal cortex, claustrum, and amygdala converge onto the two and three superficial layers of the EC, that sends projections onto the hippocampus; the axons of the stellate neurons in layer II of the EC form the perforant path that innervates the dentate gyrus and Cornu Ammonis, whereas those of the pyramidal neurons in layer III form a temporoammonic pathway which synapses onto the distal dendrites of pyramidal neurons in CA1 and the subiculum. Furthermore, neurons in the deep five to six layers of the EC that relay a large portion of hippocampal output projections back to the superficial layers of the EC and to other cortical areas. Dopamine and norepinephrine are neurotransmitters or neuromodulators involved in the modulation of a variety of physiological functions such as working memory and neurological and psychiatric disorders. DA activates 5 types of G protein-coupled receptors which are classified as D<sub>1</sub>- (D<sub>1</sub> and D<sub>5</sub>) and D<sub>2</sub>-like (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>) receptors, whereas norepinephrine interacts with α<sub>1</sub>, α<sub>2</sub>, β<sub>1</sub>, β<sub>2</sub>, and β<sub>3</sub> adrenergic receptors. Studies reveal that there are promiscuous interactions among dopaminergic and adrenergic receptors. Current-clamp mode made from stellate neurons in layer II of the EC. The recording electrodes were filled with cesium gluconate, ethylene glycol-bisN,N,N',N'-tetraacetic acid, 5 MgCl<sub>2</sub>, 8 NaCl, 2 ATPNa<sub>2</sub>, 0.3 GTPNa, 40 4-(2-Hydroxyethyl piperazine-1-ethanesulfonic acid (HEPES), and 1 QX-314 (pH 7.3). The extracellular solution comprises NaCl, NaHCO<sub>3</sub>, KCl, NaH<sub>2</sub>PO<sub>4</sub>, MgCl<sub>2</sub>, CaCl<sub>2</sub>, and glucose, saturated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. To record GABA<sub>A</sub> receptor-mediated sIPSCs, the external solution was added with dl-2-Amino-5-phosphonopentanoic acid and 6,7-Dinitroquinoxaline-2,3-dione. Synaptic currents recorded at a holding potential of +30 mV. mIPSCs recorded due to the including tetrodotoxin in the external solution. eIPSCs recorded from stellate neurons internal and external solution by placing a stimulation electrode. Data filtered at 2 kHz, digitized at 10 kHz, and acquired on-line using the pCLAMP 9. The recorded sIPSCs and mIPSCs were analyzed by Mini Analysis. Each detected data inspected visually to exclude obvious artifacts before analysis. As recorded in an event-free stretch of data, threshold for detection was set to 3 times the standard deviation of the noise. Mean amplitude, frequency, cumulative amplitude, and frequency histograms were calculated using this program. The basal frequency of the events varied considerably for individual cells, normalized the average of the frequency of the events recorded for 5 min before the application of dopamine for better comparison. The recorded eIPSCs analyzed by pClamp 9. Resting membrane potentials, action potentials, and holding currents were recorded from interneurons in layer III of the EC with the intracellular

solution containing 100 potassium gluconate, 0.6 EGTA, 5 MgCl<sub>2</sub>, 8 NaCl, 2 ATPNa<sub>2</sub>, 0.3 GTPNa, phosphocreatine 7, and 33 HEPES. Interneurons identified morphologically.

The sizes of interneurons are smaller compared with the principal neurons in layer III. The shapes of interneurons are bipolar, spindle, or ovoid. Orientation was typically perpendicular to the axis of the surrounding principal cells in layer III and parallel to the pial axis of the slice. The properties of interneurons were confirmed electrophysiologically and showed fast spikes, whereas the principal neurons in layer III demonstrated slow spikes. After 10–15 min, the establishment of whole-cell configuration to record stable responses. N-methyl-d-glucamine (NMDG), the extracellular NaCl concentration was replaced by the same concentration of NMDG and HCl was adjusted pH to 7.4. Current–voltage curves constructed from the interneurons in layer III. K<sup>+</sup>-gluconate internal solution used and the extracellular solution was supplemented with 0.5 TTX, 100 CdCl<sub>2</sub>, 200 NiCl<sub>2</sub>, 10 DNQX, 50 dl-APV, and 10 bicuculline. Current–voltage relationship using a ramp protocol from -110 to -50 mV. Due to the maximal effect of DA usually occurred at 5–10 min, the current–voltage curves recorded before and when the maximal effect of DA was observed. Stock DA solution at 100 mM prepared, aliquoted, and frozen till use. For each cell, 15 μL of the frozen DA stock solution was freshly dissolved in 15 mL of the extracellular solution and then applied to the cells in about 8 min to prevent oxidation of DA. Change of the color of the solution, suggested that there was no apparent oxidation of DA. Data calculated as the means ± SEM. The concentration–response curve of Dopamine was fit by Hill equation:  $I = I_{max} \times \{1/[1 + (EC_{50}/[ligand])^n]\}$ , where  $I_{max}$  is the maximum response, EC<sub>50</sub>, the concentration of ligand producing a half-maximal response, and  $n$ , the Hill coefficient. Analysis of variance was used for statistical analysis as appropriate. Statistical analysis was performed using Origin 7 and GraphPad Prism 4.  $P$ -values are reported throughout the text and significance was set as  $P < 0.05$ . For sIPSC cumulative probability plots, events recorded for 2 min before DA application and 2 min of the maximal effect of DA were selected. Same bin sizes were used in the analysis of data from control and DA treatment.

The Kolmogorov–Smirnoff test used to assess the significance of the cumulative probability plots.  $N$  in the text represents the number of cells examined. SCH23390, LE300, SKF38393, SKF81297, sulpiride, corynanthine, mibepradil, ZD7288, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), thapsigargin, doxazosin, and TTX purchased from Tocris Cookson Inc. Other chemical reagents were Sigma-Aldrich Products. All the drugs prepared as a stock solution that was frozen below -20°C till use. The stock solution diluted in the extracellular solution to make the final concentrations applied

to the slices. When dimethyl sulfoxide required to dissolve drugs, the final concentration of the vehicles was kept <0.1%. For experiments involving inhibitors, slices pretreated with the extracellular solution containing the inhibitors for at least 20 min and the same concentration of the drugs continuously applied in the bath to ensure a complete inhibition of the targets. Dopamine increases frequency without affecting the amplitude of sIPSCs and mIPSCs in the EC. The effects of Dopamine are not mediated by DA receptors, but by  $\alpha_1$  adrenoreceptors. Endogenously released DA exerts the same effects on GABAergic transmission. DA-induced increases in the frequencies of sIPSCs and mIPSCs are due to DA-mediated depolarization of GABAergic interneurons resulting in the facilitation of AP firing frequency and the activation of T-type  $\text{Ca}^{2+}$  channels. DA-mediated depolarization of interneurons caused by the inhibition of Kirs.