Supporting Information

Analysis of acetylcholinesterase activity in cell membrane microarrays of brain areas as screening tool to identify tissue specific inhibitors

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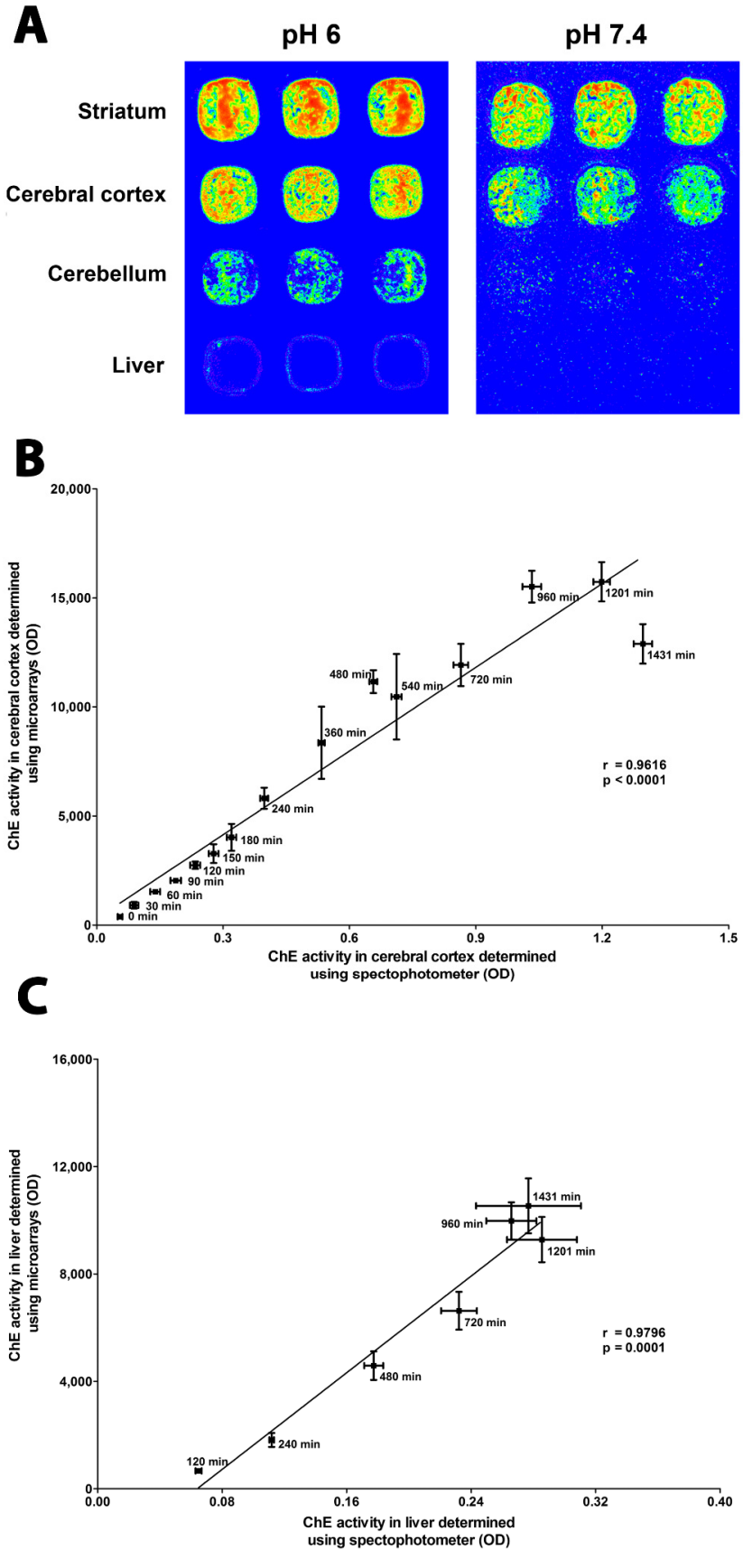
A
pH 6
pH 7.4
Striatum
Cerebral cortex
Cerebellum
Liver
B
ChE activity in cerebral cortex determined using microarrays (OD)
ChE activity in cerebral cortex determined using spectophotometer (OD)
r = 0.9616
p < 0.0001
0 min
30 min
60 min
90 min
120 min
150 min
180 min
240 min
360 min
480 min
540 min
720 min
960 min
1201 min
1431 min
C
ChE activity in liver determined using microarrays (OD)
ChE activity in liver determined using spectophotometer (OD)
r = 0.9796
p = 0.0001
120 min
240 min
480 min
720 min
960 min
1201 min
1431 min

Figure S1. False color representative images of cholinesterase activity determined at different pH in cell membrane microarrays consisted of rat membranes homogenates isolated from striatum, cerebral cortex cerebellum and liver (A). An increase in the deposition of unspecific precipitates and a decrease in the cholinesterase activity were observed at pH 7.4 in comparison with the same assay performed at pH 6. Correlation between cholinesterase activities quantified using the spectrophotometer or cell membrane microarrays in rat membrane homogenates isolated from cerebral cortex (B) and liver (C). Data are mean ± SEM values from 3 experiments performed in different CMM.

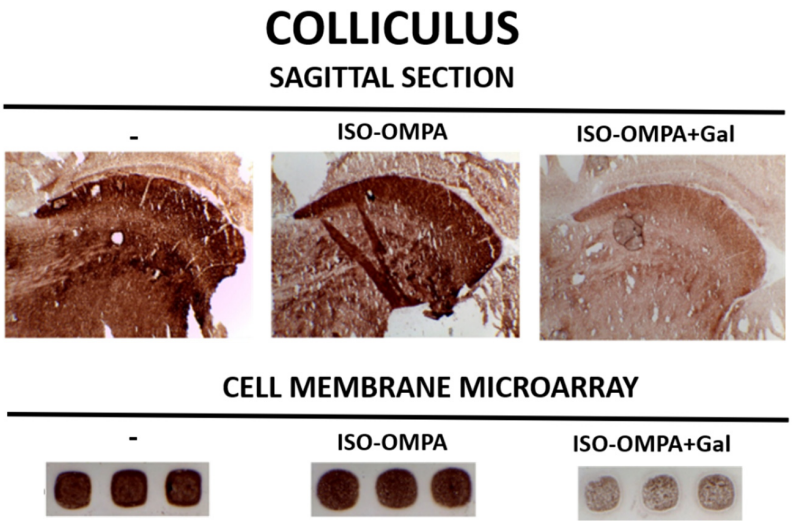

Figure S2. Cholinesterase effects of specific inhibitors of BuChE and AChE on the rat colliculus. Sagittal sections (A) and cell membrane microarrays (B) were incubated in absence or in presence of 100 µM Iso-OMPA alone or together with 100 µM galantamine. Note the decrease in the colorimetric signal evoked by the AChE inhibitor, galantamine, on sagittal tissue sections and cell membranes microarrays of rat colliculus.

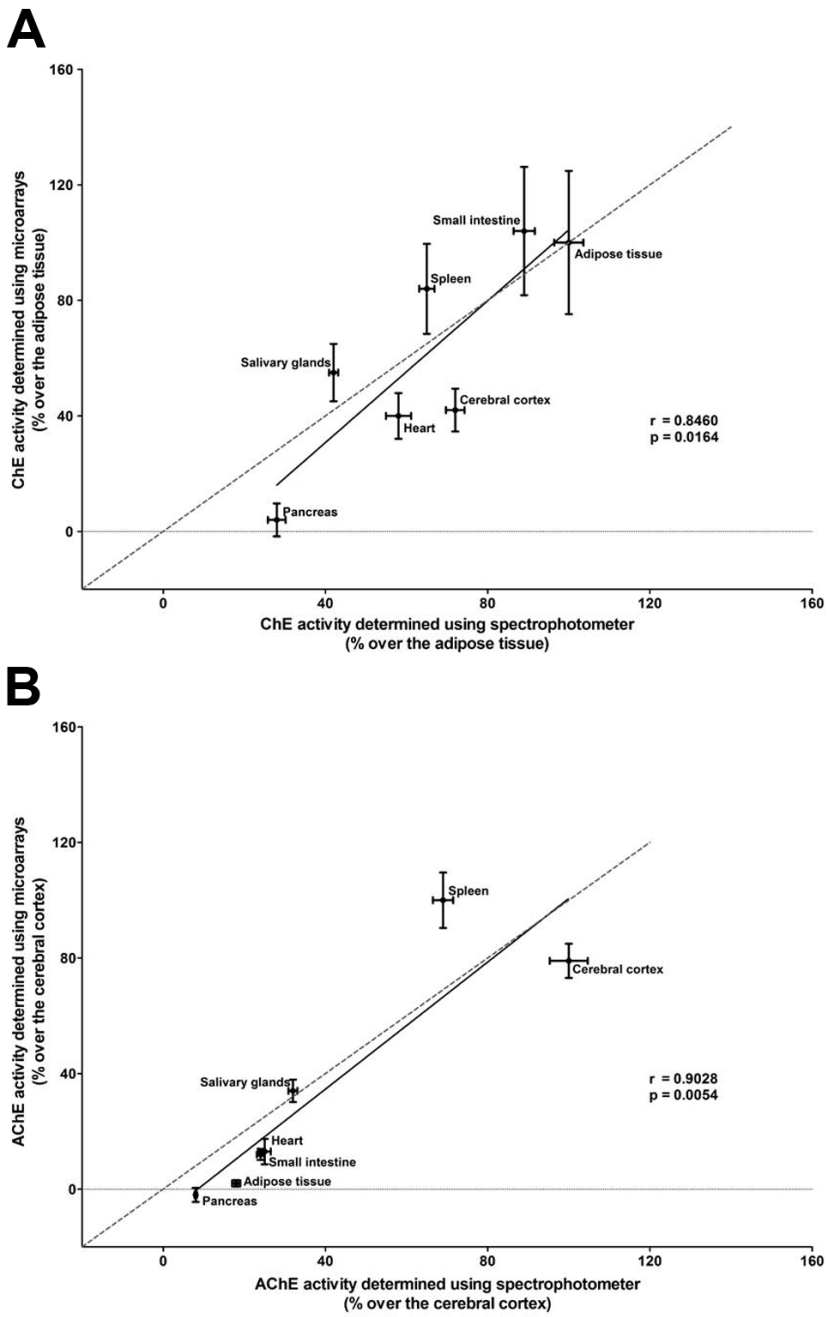

Figure S3. Correlation between cholinesterase (A) and acetylcholinesterase (B) activities quantified using the spectrophotometer or CMM in rat membrane homogenates isolated from cerebral cortex, spleen, salivary glands, heart, pancreas, small intestine and adipose tissue. Data are mean ± SEM values from 3 experiments performed in different CMM.

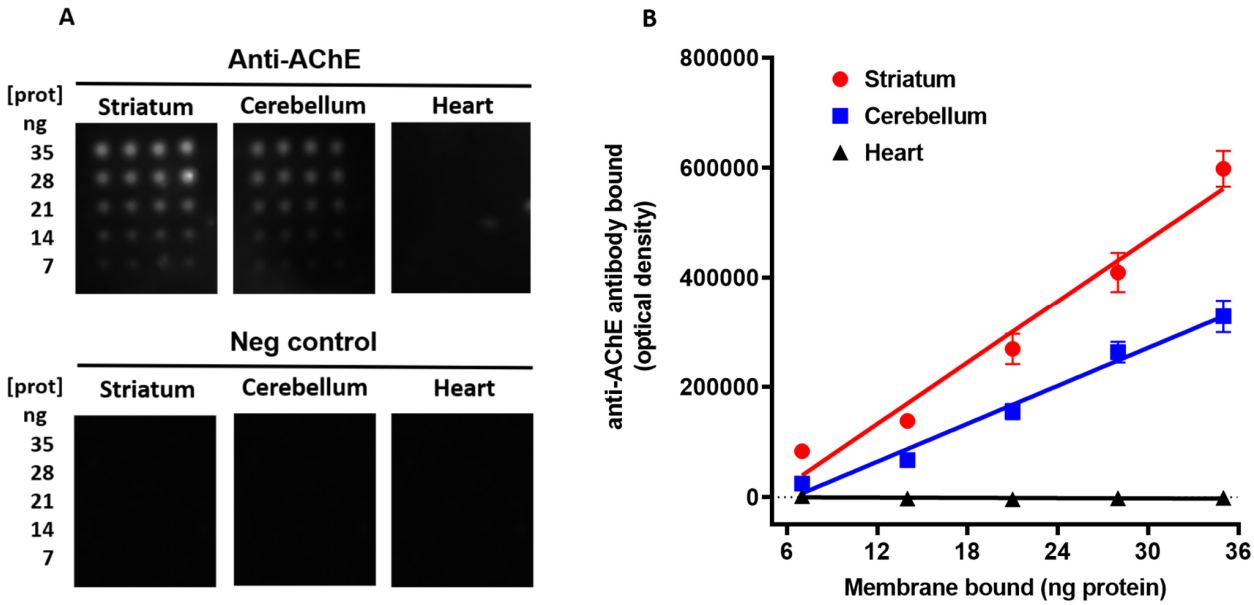

Figure S4. Representative images of the fluorescent immunodetection of AChE using rat cell membrane microarrays **(A)** and the graphic representation of the AChE signal **(B**). Cell membrane microarray consisted of increasing amount of membrane preparation isolated from a pool of striatum, cerebellum and heart of Sprage Dawnley rats (n=10). The protocol used was as follows. Microarrays were incubated with the blocking buffer (PBS, 0.3% triton and 1:200 Normal Goat Serum) for 30 min before adding the anti-AChE mouse monoclonal antibody (ref: MAB304, Merck Millipore) at 1:200 dissolved in blocking buffer. After 1 h at room temperature, microarrays were revealed with the secondary antibody goat anti-mouse Alexa Fluor 555 (Abcam) at 1:200 in the blocking buffer. Finally, microarrays were rinsed with PBS for 10 min, and the fluorescence was quantified using a ChemiDoc MP imaging system. Nonspecific immunoreactivity was determined in the absence of the primary antibody.