

<b>Models/Materials</b>	Transgenic animals	CX3CR1-GFP KI mice, expressing GFP in myeloid cells (microglia, macrophages)
	Cell lines	Primary neuronal cultures, astrocyte and microglial cultures. N9 microglia cell line
	Other	
<b>Protocols and Methods</b>	<b>Cell culture</b>	
	<b>Primary cultures of neurons, astrocytes and microglia:</b>	cortical and hippocampal astrocytes are obtained from 2 day-old C57BL/6 mice. Cortex and hippocampus are freshly dissected, cut into small sections and washed in Hank's Balanced Salt Solution supplemented with 10 mM Hepes /Na pH 7.4, 12 mM MgSO <sub>4</sub> , 50 U/ml Penicillin and 50 µg/ml Streptomycin. The tissue is then dissociated with 2.5 mg/ml trypsin type IX in presence of 1 mg/ml deoxyribonuclease (DNase, Calbiochem) for 10 min at 37 °C in two subsequent steps and the supernatants obtained were diluted 1:1 in medium containing 10% fetal bovine serum (FBS). The cells are plated in MEM (Life Technologies) supplemented with 10% FBS, 33 mM glucose, 100 mM Na <sup>2+</sup> / Pyruvate (Lonza), 50 U/ml penicillin-G and 50 ug/ml streptomycin and maintained in 75 cm <sup>2</sup> flasks (1 for pup) at 37 °C in a humidified 5% CO <sub>2</sub> incubator. Pure cultures (> 99.5%) of astrocytes are obtained by shaking flasks at 220 rpm for 24 h at 37 °C at day 2 and 6 after plating. Shaking medium (5 ml/flask) was Minimum Essential Medium with Hank's salts, supplemented with 10% horse serum, 33 mM glucose, 200 mM Ultraglutamine (Lonza), 10 mM Hepes/ Na pH 7.4, 50 U/ml Penicillin and 50 µg/ml Streptomycin. Primary microglia cells are obtained from astrocytic layers by shaking the flasks for 45' at 230 rpm, 10–12 days after dissection. Detached cells (about 90% microglia with a 10% astrocytic contamination) are plated in multiwells (150,000 cells per well in 12 well plates) coated with poly-L-ornithyne hydrobromyde (100 µg/ml). Cultures usually contain 95% microglial (CD11b+) cells.
	<b>Methodologies</b>	Western blot analysis Immunofluorescence analysis and confocal microscopy Single cell calcium imaging Relatime PCR. Genes investigated include P2X <sub>7</sub> , P2X <sub>4</sub> , GFAP, IBA1, M1 and M2 microglial markers and inflammatory and antiinflammatory cytokines . ATP detection by luciferin/luciferase-based kit Access to quantification of extracellular microvesicles by Nanosight instrument
<b>Non commercial antibodies</b>	P1 receptors	
	P2 receptors	
<b>Non commercial drugs</b>		
<b>Fluorescent probes</b>	plasmids encoding farnesyl-GFP, SNAP-25-GFP/ red tag , P2X <sub>7</sub> -GFP, channelrhodopsin-red tag	
<b>Investigational diagnostic test</b>		
<b>Biosamples</b>	Human origin	
	Animal origin	