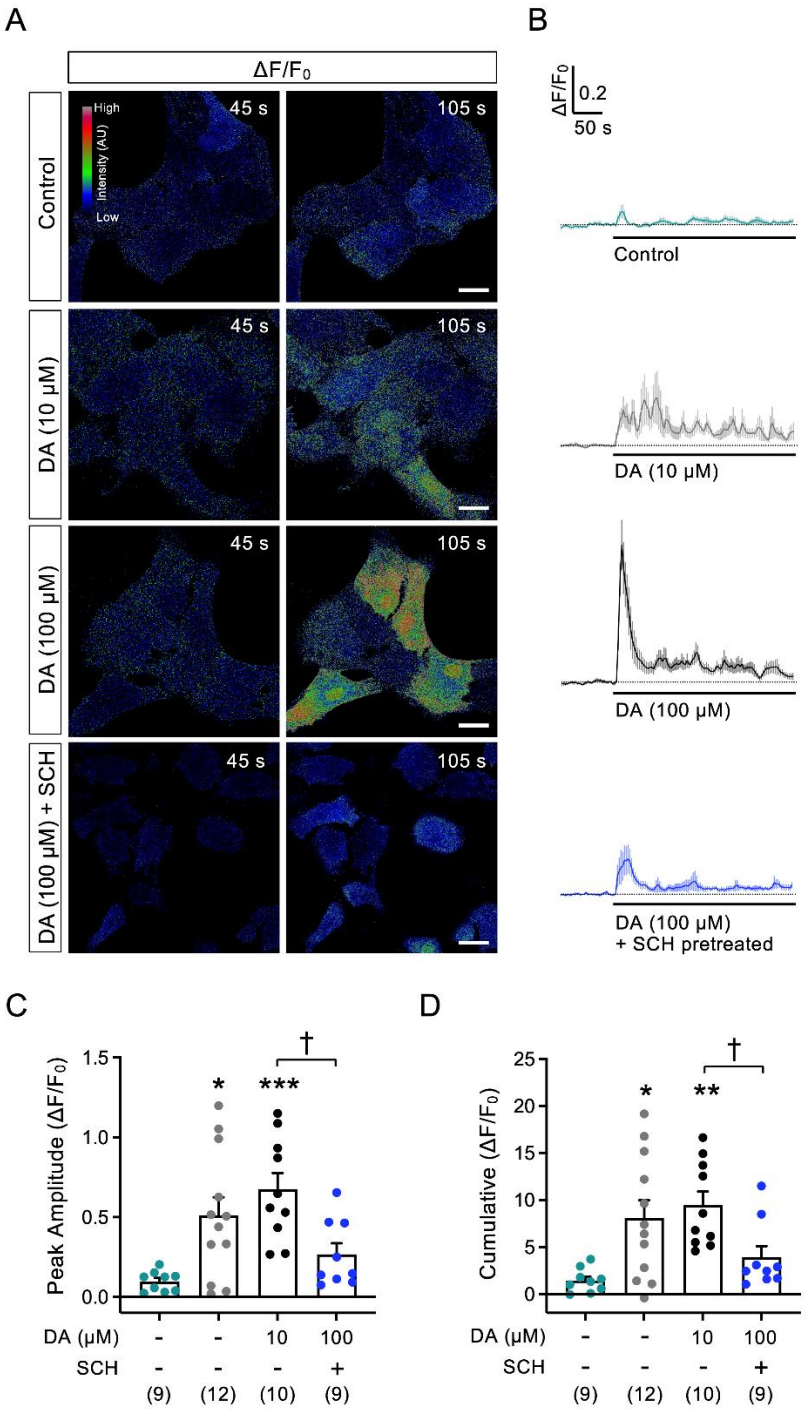
SUPPLEMENTARY DATA

Dopamine-Induced L-Lactate Production in Cortical Astrocytes Cross-Reacts with β_1-Adrenoceptor-Mediated cAMP Signalling

Keita Sugiyama, Anemari Horvat, Klemen Dolinar, Sergej Pirkmajer, Borut Furlani, Jernej Jorgačevski, Nina Vardjan, Robert Zorec

© 2026. Sugiyama K et al. Published online at http://www.aginganddisease.org/EN/10.14336/AD.2025.1442

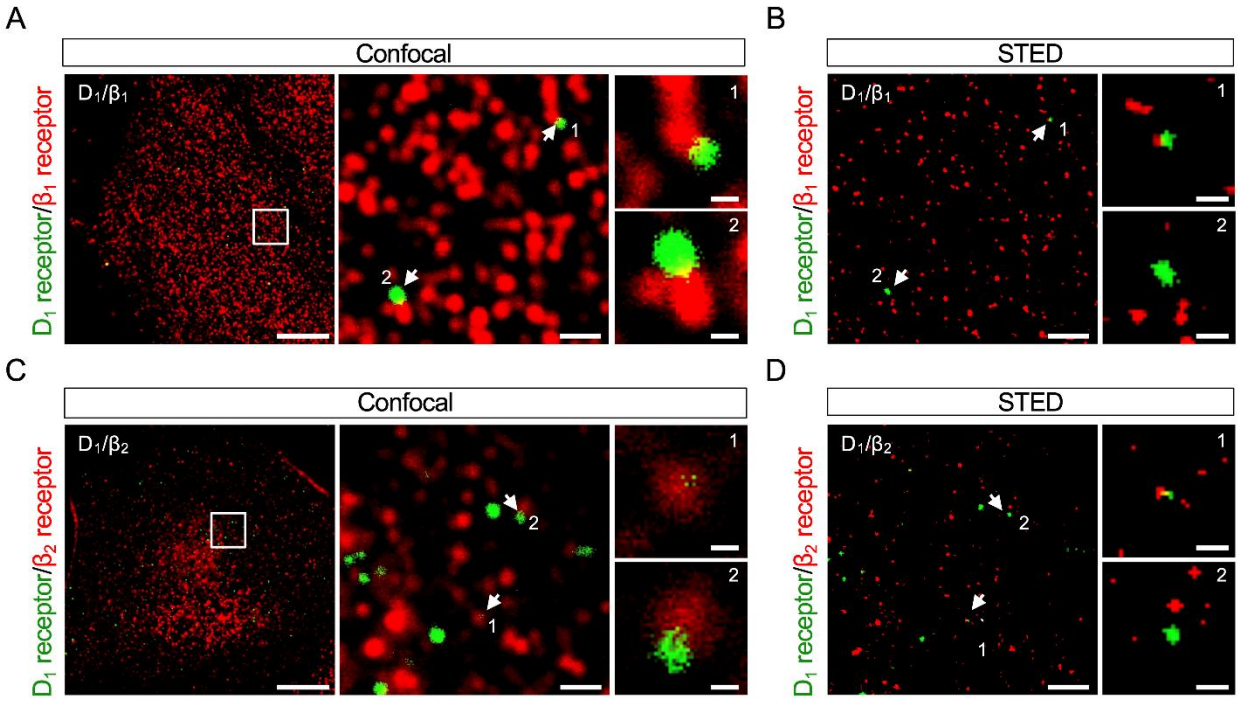
SUPPLEMENTARY DATA



Supplementary Figure 1. D1 receptor activation induces increases in cytosolic Ca^{2+} in cortical astrocytes. (**A**) Representative pseudocolour images show the relative change in Fluo-4 fluorescence (F/F_0) after stimulation with control or DA (10 or 100 μM) and after pretreatment with the D_1 receptor antagonist SCH23390 (SCH; 10 μM). Pseudocolour images were calibrated as F/F_0 and expressed in arbitrary units (AU) (see the section on calcium imagings). (**B**) Mean fluorescence intensity changes in cytosolic Ca^{2+} ($[Ca^{2+}]_i$, F/F_0) were measured in astrocytes stimulated with control, DA (10 or 100 μM), or DA (100 μM) pretreated with SCH (10 μM). (**C, D**) Mean peak amplitude (**C**) and cumulative response (**D**) in cytosolic Ca^{2+} for control, DA (10 or 100 μM), and DA (100 μM) pretreated with SCH (10 μM). Numbers in parentheses indicate the number of experiments (coverslips) analysed. Data were obtained from at least two different animals (multiple cells were recorded and averaged per coverslip). ***$p < 0.001$, **$p < 0.01$ compared with control; †$p < 0.05$ compared with DA (100 μM); Brown–Forsythe and Welch ANOVA tests followed by Dunnett's T3 multiple comparisons test.

© 2026. Sugiyama K et al. Published online at http://www.aginganddisease.org/EN/10.14336/AD.2025.1442

SUPPLEMENTARY DATA



Supplementary Figure 2. STED measurements of overlap between fluorescent signals of D_1 receptors and β_1- or β_2-adrenoceptors in cortical astrocytes. (**A**, **C**) Representative confocal images of cortical astrocytes double-immunostained for D_1 receptors and β_1- (**A**) or β_2-adrenoceptors (**C**). Centre and left panels show confocal images of the enlarged areas outlined with a white square in A and C. Scale bars, 10 µm, 1 µm, and 20 nm. (**B**, **D**) STED images correspond to the enlarged areas displayed in A and C (left panels). Arrows and numbers in the left panels correspond to the respective enlarged images in A, B, C, and D (right panels). STED microscopy reveals overlapping D_1 receptors and the β-adrenoceptors in the areas labelled as 1 in B and D (right panels): scale bars, 1 µm and 20 nm.

Supplementary Table 1. Responsiveness of single astrocytes to dopamine (DA) using FRET nanosensors

FRET nanosensor	Stimulus	Number of cells (%)		
		Responsive	Unresponsive	All
cAMP				
Figure 1	Control	2 (13.3)	13 (86.7)	15
	DA (1 nM)	0 (0)	6 (100)	6
	DA (10 nM)	2 (25)	6 (75)	8
	DA (100 nM)	3 (37.5)	5 (62.5)	8
	DA (1 µM)	10 (83.3)	2 (16.7)	12
	DA (10 µM)	7 (100)	0 (0)	7
	DA (100 µM)	10 (100)	0 (0)	10
	DA (1 mM)	9 (100)	0 (0)	9

© 2026. Sugiyama K et al. Published online at http://www.aginganddisease.org/EN/10.14336/AD.2025.1442

SUPPLEMENTARY DATA

Figure 2	SKF (10 μM)	9 (90)	1 (10)	10
Figure 3	SCH (1 μM) + DA (10 μM)	0 (100)	4 (100)	4
	Propranolol (10 μM) + DA (10 μM)	4 (40)	6 (60)	10
	SCH (1 μM) + DA (100 μM)	8 (80)	2 (20)	10
	Propranolol (10 μM) + DA (100 μM))	3 (21.4)	11 (78.6)	14
	Propranolol (10 μM) + SCH (1 μM) + DA (100 μM)	0 (0)	5 (100)	5
Lactate				
Figure 4	Control	7 (18.4)	31 (81.6)	38
	DA (10 μM)	18 (50)	18 (50)	36
	DA (100 μM)	28 (70)	12 (30)	40
	SCH (10 μM) + DA (100 μM)	30 (78.9)	8 (21.1)	38
	Propranolol (10 μM) + DA (100 μM)	6 (17.6)	28 (82.4)	34
Figure 5	siRNA NC control	4 (23.5)	13 (76.5)	17
	siRNA NC DA (100 μM)	23 (92)	2 (8)	25
	siAdrb1 control	0 (0)	23 (100)	23
	siRNA siAdrb1 DA (100 μM)	11 (57.9)	8 (42.1)	19

A cell was determined to be responsive if the signal exceeded the threshold of 3 standard deviations above the average ΔCFP/YFP or ΔmTFP/Venus ratio measured before the application of DA, as described in [45]. SKF, (±)-6-Chloro-PB hydrobromide [(±)-SKF81297]; SCH, SCH23390; n, number of cells studied.

© 2026. Sugiyama K et al. Published online at http://www.aginganddisease.org/EN/10.14336/AD.2025.1442

SUPPLEMENTARY DATA

Supplementary Table 2. Quantification of immunofluorescence signals for D_1 receptors and β_1- or β_2-adrenoceptors using stimulated emission depletion (STED) microscopy.

Pair	Immunofluorescence signals			Overlap ratio (%) (relative to D_1)	Overlap ratio (%) (relative to β)	Centroid distance of overlapping signals (nm)	Number of cells
	Overlapping pairs	Total D_1	Total β_1 or β_2				
Confocal							
D_1 - β_1	71.0 ± 10.3	1031 ± 146	73832 ± 8144	6.6 ± 0.5	0.10 ± 0.01	374.5 ± 35.9	19
D_1 - β_2	314 ± 32	2866 ± 299	66314 ± 7211	11.1 ± 0.5	0.49 ± 0.04	152.1 ± 5.5	19
STED							
D_1 - β_1	21.7 ± 3.0	289 ± 34	23734 ± 1980	7.2 ± 0.7	0.10 ± 0.01	40.4 ± 2.2***	19
D_1 - β_2	19.7 ± 3.0	860 ± 71	9279 ± 1137	2.1 ± 0.2***	0.20 ± 0.02***	47.4 ± 2.7***	19

The table shows the quantification of mean fluorescence signals measured by confocal and STED microscopy, including the number of D_1 receptor (D1) and β_1- or β_2-adrenoceptor signals (β_1 or β_2), overlapping signals, overlap percentages (relative to D_1 or β), and centroid distances of overlapping signals. Data are presented as means ± SEM and acquired from at least two different animals. Each experiment (coverslip) was performed in duplicate, and multiple cells were recorded per coverslip. ***$p < 0.001$ compared with confocal microscopy in each pair for overlap percentages or centroid distances; Student's t-test.

© 2026. Sugiyama K et al. Published online at http://www.aginganddisease.org/EN/10.14336/AD.2025.1442