Supporting information

Synthesis of the complex $[Ru(bpy)_2(PMe_3)(Dopa)](PF_6)_2$

The precursor complex $[Ru(bpy)_2(PMe_3)Cl)]PF_6$ was synthesized as reported elsewere[23]. To solubilize the complex in water, 100 mg of $[Ru(bpy)_2(PMe_3)Cl)]PF_6$ were dissolved in 3 mL acetone, and 3 mL of an aqueous suspension of 1 gram of anionic resin Dowex-22 (chloride form) was added. After 10 minutes to allow PF_6/Cl exchange the acetone was removed by rotary evaporation.

Then, 100 mg of dopamine.HCl were dissolved in the precursor complex solution under nitrogen atmosphere. After 15 minutes of N_2 bubbling, 2.5 equivalents (50 mg) of solid NaOH were added. The solution was heated under N_2 during 16 hours to prevent dopamine oxidation, cooled to 0ºC and neutralized with 120 μL of acetic acid. It was filtered to remove insoluble material, and poured over excess of saturated solution of KPF_6. The product was purified by redissolving three times in an aqueous suspension of Dowex-Cl and further reprecipitation with KPF_6. Yield: 62% Anal.Calcd: C, 41.16; H, 4.01; N, 4.65. Found C, 40.2; H, 4.4; N, 4.5. After three redissolutions no further precipitation was performed, and the aqueous solution was lyophilized in dark, yielding 36 mg of a very hygroscopic orange powder. NMR (D_2O): 1H δ 0.95 (d, 9H), 1.78 (m, 2H), 2.30 (m, 2H), 2.70 (t, 1H), 3.52 (t, 1H), 6.15 (s, 1H), 6.23 (d, 1H), 6.58 (d, 1H), 7.02 (t, 1H), 7.20 (t, 7H), 7.36 (d, 1H), 7.42 (d, 1H), 7.57 (t, 1H), 7.67 (t, 1H), 7.71 (t, 1H), 7.89 (t, 1H), 8.08 (t, 1H), 8.16 (t, 1H), 8.19 (d,1H), 8.29 (d,1H), 8.37 (d,1H), 8.39 (d,1H), 8.73 (d,1H), 8.76 (d,1H)

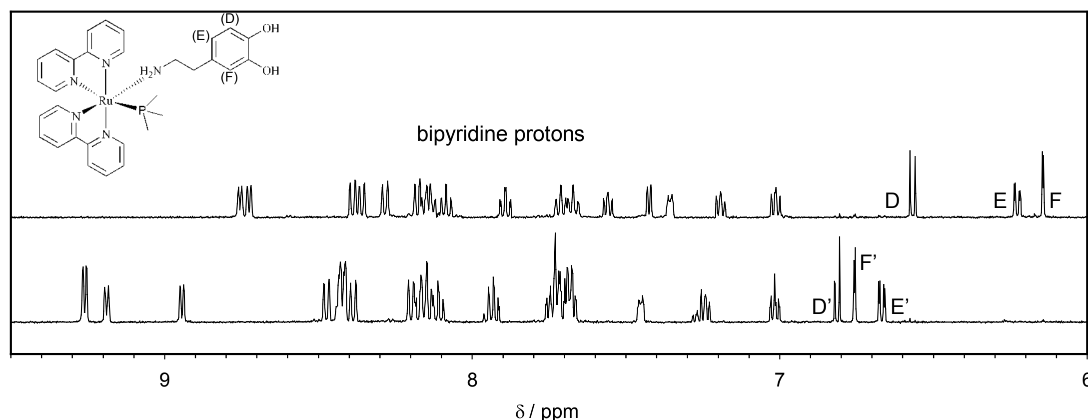

Figure S1. Aromatic part of the NMR spectra of RuBi-Dopa in D_2O before (top) and after (bottom) irradiation into the NMR tube with a 525 nm green LED. The signals of coordinated dopamine (D, E and F) and of free dopamine (D’, E’ and F’) are apparent. The signals at δ > 6.9 ppm correspond to the bipyridine protons of RuBi-Dopa (top) and of the photoproduct $[Ru(bpy)_2(PMe_3)(H_2O)]^{2+}$ (bottom).

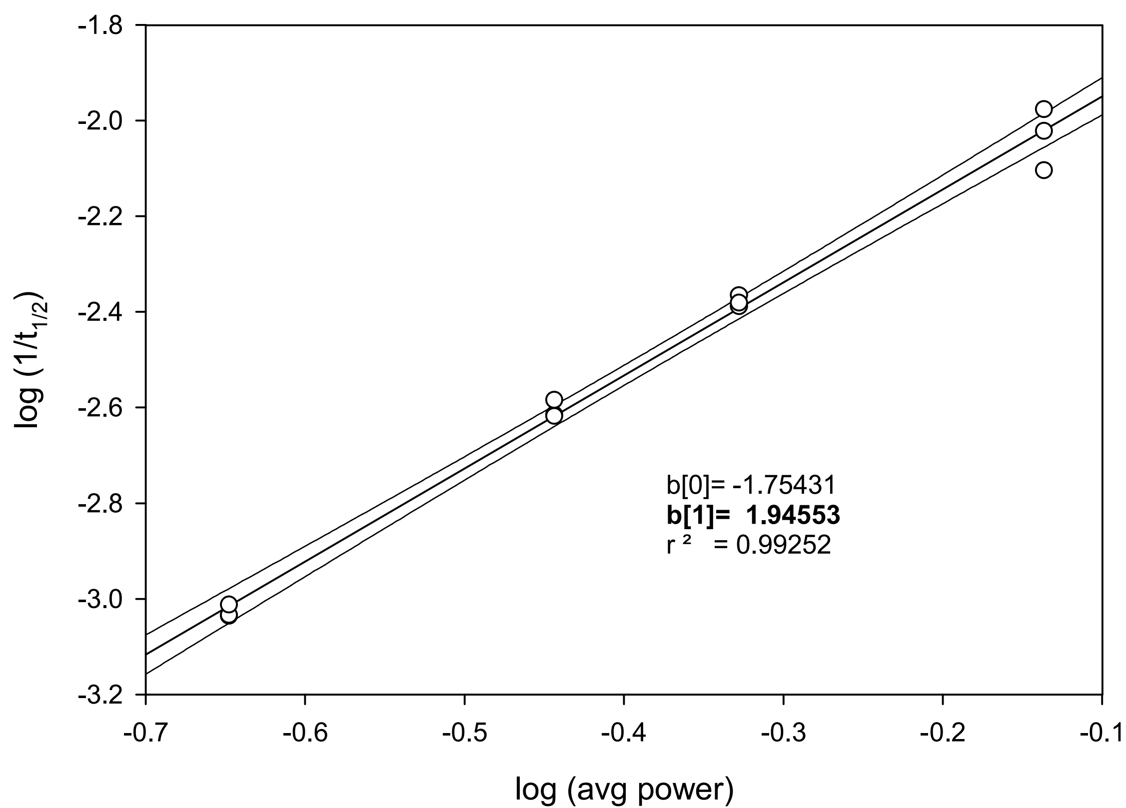

Figure S2. Dependence of the photo-dissociation half time of RuBi-Dopa ($t_{1/2}$) with the pulsed laser power. Note that the log-log plot has a slope **b[1])** = 1.94 (≈2), indicative of a quadratic response with light intensity, characteristic of a two-photon process. Confidence intervals (95%) are depicted.

Brain Slice Preparation and Electrophysiology

Brains from postnatal day 14-16 C57BL/6 mice were removed and immersed in cold cutting solution: 27 $NaHCO_3$, 1.5 NaH_2PO_4, 222 Sucrose, 2.6 KCl, 1 $CaCl_2$, 3 $MgSO_4$ and equilibrated with 95% O_2/5% CO_2. 300 µm thick coronal slices were prepared using a Leica VT1000-S vibratome using cutting solution. Slices were then incubated at 36°C in ACSF containing (in mM): 126 NaCl, 26 NaHCO3, 10 Dextrose, 1.15 NaH2PO4, 3 KCL, 2 CaCl2, 2 MgSO4 and oxygenated with 95%O2/5% CO2 for 30 min and then kept at room temperature for at least 30 min before transferring them to the recording chamber. The recording chamber was bathed in ACSF (pH 7.4). Electrophysiological recordings were done in whole cell current–clamp configuration using Multiclamp 700b amplifiers (Axon). Whole-cell patch electrodes (4–7 MΩ) were filled with internal solution containing (in mM): 135 $KMeSO_4$, 10 KCl, 10 HEPES, 5 NaCl, 2.5 Mg-ATP, 0.3 Na-GTP, and 0.2 Alexa Fluor 488 and 0.2 Fluo-4 (see below). Neurons were held at a resting membrane potential of −65 mV. All experiments were conducted at 37°C.

We performed recordings from layer 5 pyramidal cells somas, and acquired the signals through a National Instruments PCI 6259 board using either custom software developed with LABView or Matlab. SCH 23390 (10 µM, Tocris) was used to block D1 receptors (Supplementary Figure 3) while recording spine calcium responses at the spine head, and the excitatory post-synaptic potentials (EPSPs) at the soma. Calcium measurements after two-photon uncaging were purely optical and therefore less invasive than standard electrical techniques. Moreover, simultaneous current-clamp measurements demonstrated that the Ca^{2+} release in the spine does not induce important voltage changes into the cell soma (n = 13 spines, 5 experiments; Peak amplitude at a 100 ms time window after uncaging = 0.21 ± 0.1 mV, and 0.06 ± 0.08 mV of peak amplitude at a 30 ms time window after uncaging, n = 253 uncaging events), and thus the dopaminergic activation of spines would go unnoticed with electrophysiological techniques.

Off-line analysis was conducted using Matlab or IGOR Pro with the Neuromatic v2.0 package. For consistency throughout the study, all measurements are expressed as mean ± SEM. Statistical significance was assessed using Student's t-test.

Two-photon RuBi-Dopa Uncaging and spine calcium measurements

$Ru(bpy)_2(PMe_3)(Dopa)](PF_6)_2$ (RuBi-Dopa, 300 µM) was bath-applied, and a Dynamax peristaltic pump (Rainin Instruments Inc., Woburn, MA) was used to control bath perfusion, recirculating the media. The concentration chosen for two-photon experiments, 300 µM, was selected based on previous experiments with a RuBi-Glutamate (1). Two- photon imaging of spines was performed at 725 nm and two-photon uncaging of RuBi-Dopa anc Ca^{2+} imaging at 820 nm. The two-photon laser was positioned at approximately 0.2 µm from spine heads of layer-5 pyramidal neurons that were filled with 200 µM Alexa 488 (to uncover spine structure) and 200 µM Fluor-4 (Molecular Probes, to record Ca^{2+} responses at the spine head) through recording pipettes. Pipette solution was otherwise the same as described above. The two-photon Ti-Sapphire laser power was controlled by a Pockels cell (Quantum Technology, Lake Mary, FL), gated with square voltage pulses (Master-8; AMPI, Jerusalem, Israel). At approximately 30 min after break-in, spines from basal dendrites of layer 5 pyramidal neurons were selected for imaging (725 nm) and uncaging (820 nm). Then, the laser (set at 820 nm to uncage RuBi-Dopa and image spine Ca2+ responses) was positioned at the spine head for 500 ms at a low imaging power level (at approx. 5-8 mW laser power on sample) (Figure 4A green dot, Laser on spine head), then a 4-ms high-power (at approx. 25 mW on sample), uncaging laser pulse (Fig. 4 A, Dopa uncaging) was generated at the position indicated by a red dot in Figure 2A, and this was immediately followed by repositioning the laser for 1 s at the same spine head location for calcium imaging again at low power levels. Spine Ca^{2+} fluorescence signals were collected with a photomultiplier tube (H7422P-40; Hamamatsu, Hamamatsu City, Japan) as described (**2).** The fluorescent signal detection, was blocked for a few ms before, during (middle trace) and after the uncaging pulse with the use of an ultrafast shutter (top trace) to prevent any potential PMT saturation and errors in signal detection (Figure 4C). Averaged calcium signals from spine heads were calculated with 4-ms window smoothing and used to calculate the percentage change in basal fluorescence, expressed as ΔF/F

Images were acquired using a custom-made two-photon laser scanning microscope based on the Olympus FV-200 system (side-mounted to a BX50WI microscope with a 60× , 0.9NA, water immersion objective) and a Ti:sapphire laser (Chameleon Ultra II, Coherent, >3 W at 800nm, 140 fs pulses, 80 MHz repetition rate). The PMT was connected to a signal amplifier (Signal Recovery AMETEK Advanced Measurement Technology, Wokingham, UK) whose output was connected to the Fluoview system.

(1) Fino, E.; Araya, R. ;Peterka, D.S.; Salierno, M.; Etchenique, R.; Yuste, R. Frontiers in Neural Circuits 2009 3:2, doi: 10.3389/neuro.04.002.2009

(2) Araya, R.; Eisenthal, K. B.; Yuste, R. Proc. Natl. Acad. Sci. U.S.A 2006, 103, 18799.

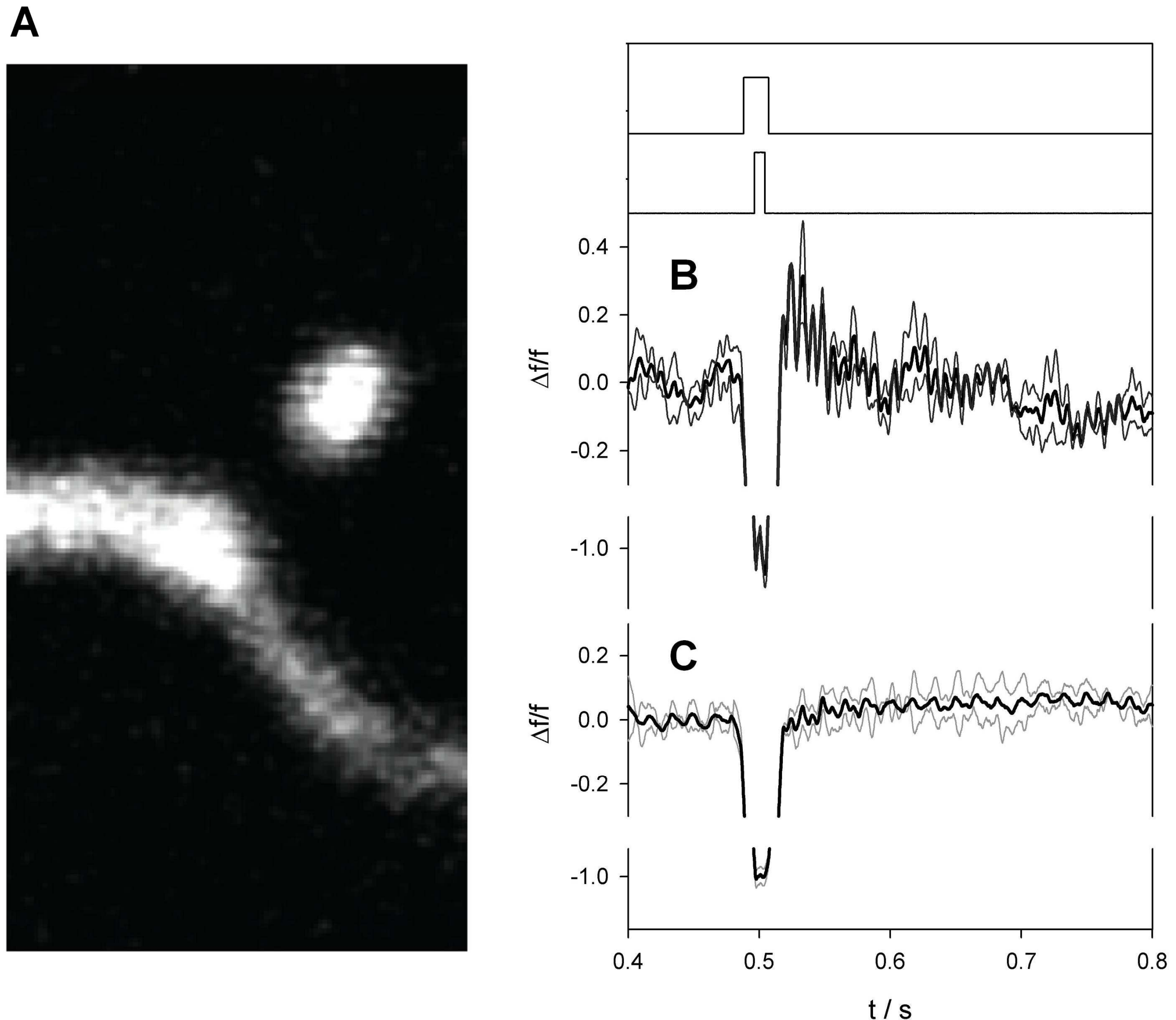

Figure S3

Two-photon activation of spine-D1 dopamine receptors triggers Ca^{2+} signals in dendritic spine heads. (A) Two-photon image of a dendritic spine from a layer 5 pyramidal neuron loaded 200 µM Fluo-4 Ca^{2+} indicator and 200 µM Alexa-488 dye. The image width in A represents 3.4 µm. The laser was parked in the spine head to monitor basal Ca^{2+} signal and then moved outside the spine head – to the edge of the head - where two-photon uncaging of RuBi-Dopa was performed. Then, the laser goes back to its initial power and location on the spine head to record the post-uncaging Ca^{2+} responses. Imaging of dendritic spine morphology was performed at 725 nm (A), and uncaging and Ca^{2+} imaging at 820 nm (B, C). The graphs in B and C depict the two-photon Ca^{2+}responses recorded at the spine head shown in A before and after two-photon uncaging of RuBi-Dopa (uncaging at 0.5 sec, pulse duration 4ms). These responses were recorded in the same spine (A) under a control condition (B) or in the presence of the bath applied dopamine D1 receptor blocker SCH-23390 (C). Note how addition of SCH-23390 completely removed the two-photon induced dopamine Ca^{2+} responses. Black traces in B correspond to the average trace (from n = 15 individual traces for control condition, and n = 30 traces with SCH-23390), and grey limits represent the ± SEM