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# A visible light-sensitive caged Serotonin.

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Kevin J. Bender: Electrophysiological Experiments, Discussion, Writing.

Kira E. Poskanzer: Electrophysiological Experimental Design, Discussion, Writing.

Roberto Etchenique: Chemical setups, Data Analysis, Discussion, Writing.

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ABSTRACT

Serotonin, or 5-hydroxytryptamine (5HT), is an important neurotransmitter in the nervous system of both vertebrates and invertebrates. Deficits in 5HT signaling are responsible for many disabling psychiatric conditions, and its molecular machinery is the target of many pharmaceuticals. We present a new 5HT phototrigger, the compound  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{2+}$ , where  $\text{PMe}_3$  is trimethylphosphine. As with other ruthenium-bipyridyl based caged compounds,  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{2+}$  presents activity in the visible region of the spectrum. We characterize and discuss the photochemical properties of the caged compound, and demonstrate its use by modulating the excitability of mouse prefrontal principal neurons.

KEYWORDS Ruthenium complex, Serotonin, Phototriggers, Neurophysiology.

## 1. Introduction

A caged compound, or phototrigger, is a molecule reversibly bound to or "caged within" a protecting group that restricts its interactions with cell-membrane receptors, proteins, or other molecules. Entities that have been caged include ions, [1] acids, [2] bases, [3] neurotransmitters, [4] fluorophores, [5,6] small-molecule drugs, [7] gene inducers [8,9] and even nucleic acids [10] and proteins [11,12]. Phototriggers are light-sensitive, and will release the caged molecule upon irradiation with light of the proper wavelength, [13] thus removing the restrictions for the caged entity to interact normally with its targets. As the uncaging spot size can be precisely determined by focusing a low-power laser, this technique can have very high spatial and temporal resolution, making it of great advantage in biological research, for it allows the delivery of molecules without mechanically acting on the preparation, a problem usually associated with more invasive techniques like microinjection.

1 Most common caged compounds comprise an organic protecting group, usually a nitrobenzyl [14] or  
2 nitroindonyl [15] which are active in the UV region and therefore can damage the biological tissue.  
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4 Therefore, new kinds of organic-based protecting groups have been developed, such as coumarine  
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6 derivatives, which can extend the photoactivity to the violet region, thus reducing cell damage. [16]  
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11 As we have shown in recent years, ruthenium-bipyridyl coordination complexes yield excellent  
12 phototriggers suitable for neurophysiology studies. They present unique properties, including  
13 nanosecond photoreaction kinetics through heterolytic photocleavage with no secondary reaction  
14 products, [7,17] and sensitivity to visible light in the blue and green regions. [3,6,7,18-20] As an  
15 important advantage over other photoprotective groups, Ru-bipyridyl phototriggers can be used in a two-  
16 photon regime. [17, 21-24] Ruthenium phototriggers were also used for general delivery of drugs,  
17 toward application in therapy. Turro and Bonnet have shown the versatility of Ru(II) polypyridines for  
18 caging nitriles and thioethers.[25-27] A similar strategy using the photoredox properties of the Ru(II)  
19 polypyridyl chemistry was used by Ford and Santana da Silva to release nitric oxide (NO) by visible  
20 light, both directly or mediated by energy transfer processes from molecular antennas.[28-29]  
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35 Ruthenium phototriggers are very stable at room temperature, both as a solid or in physiological  
36 solutions when kept in the dark, minimizing dark leakage of the caged biomolecule, one of the most  
37 common drawbacks of nitrobenzyls and similar caging groups.  
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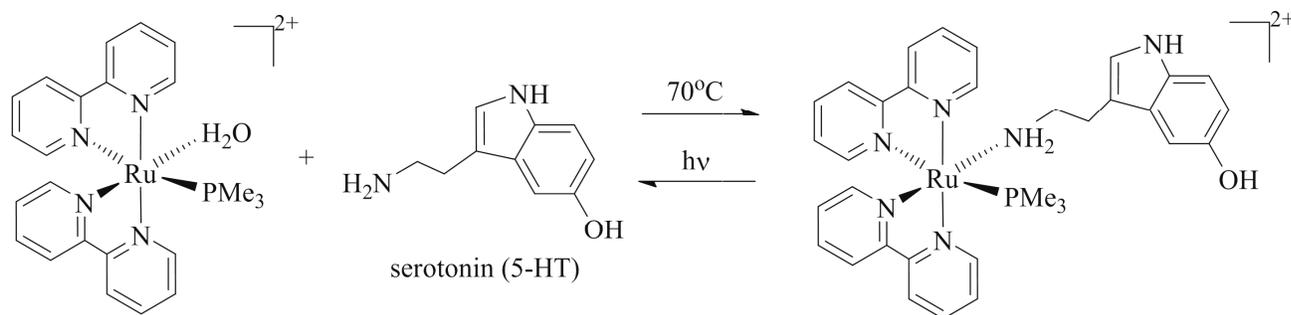
45 Serotonin is one of the most important neuromodulators in a very wide range of animal phyla. It  
46 modulates basic behaviours such as feeding and mating, [30] and imbalances in its physiology lead to  
47 debilitating psychiatric conditions in humans, such as obsessive compulsive disorder, panic disorder, or  
48 major depression. [31] For this reason, the serotonergic system is the target of many pharmaceuticals  
49 such as tricyclic antidepressants (TCAs) selective serotonin reuptake inhibitors (SSRIs) and  
50 monoamine oxidase inhibitors (MAOIs), and even many recreational drugs. [32] Discerning the effects  
51 of 5HT on its various receptor and cellular subtypes and understanding its interactions with other  
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1 systems of neurotransmitters would be greatly aided by a caged 5HT. As serotonin does not exhibit a  
2 carboxylate group, most strategies used to cage neuroactive aminoacids cannot be extended directly to  
3 cage this amine. Currently, two of the commercially available caged serotonins are NPEC-5HT and  
4 BHQ-O-5HT, which use the phenol group of the 5HT for anchoring the photoremovable protecting  
5 group and are active under UV light (365 nm). While the first shows negligible absorption in two-  
6 photon regime [33] and has a very slow response time in the hundreds of milliseconds, [34] BHQ-O-  
7 5HT can also be used at 740 nm (2P) with good quantum yields. [35,36] Other caged 5HT compounds  
8 have been described, showing dissimilar performances. [36-40]

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12 We report the synthesis and chemical properties of the complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{+2}$ , which  
13 releases 5HT upon absorption of visible light, and demonstrate its use by modulating the excitability of  
14 mouse prefrontal principal cells that have previously been shown to be regulated by 5HT. [41]  
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## 19 2. Results and discussion

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32 The complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{2+}$  is yellow-orange in color. Its water solubility is well above  
33 the tens of millimolar range. No decomposition was detected in samples stored in the dark for months at  
34 room temperature either dry or in solution. Water solutions of this complex present a metal to ligand  
35 charge transfer (MLCT) band with a molar extinction coefficient ( $\epsilon$ ) of  $6718 \text{ M}^{-1} \text{ cm}^{-1}$  and an absorption  
36 maximum in the visible range at 447 nm. Its synthesis is a simple one-pot reaction and the photolysis is  
37 fast and clean, as depicted in Scheme 1.  
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Scheme 1. Coordination reaction between serotonin and  $[Ru(bpy)_2(PMe_3)(H_2O)]^{2+}$  (forward) and the clean, one step photorelease reaction (reverse) releasing serotonin upon light absorption.

Figure 1 shows the  $^1H$ -NMR spectra of the complex (top) and of its partial photolysis (bottom). In the first, unphotolyzed spectrum, the aromatic region shows the expected 8 doublets and 8 triplets that are characteristic of the bipyridines in a *cis* octahedral complex of the form  $[Ru(bpy)_2(L_1)(L_2)]^{n+}$ , and the four aromatic signals of 5HT. In the aliphatic region (Figure 1b), besides the  $PMe_3$  signal at 0.92 ppm the two aliphatic signals for the amine and methylenes group of coordinated 5HT can be seen between 3.60 and 1.71 ppm. The fact that proton signals of amine group can be seen in a  $D_2O$  solution at 3.57 ppm and 2.65 ppm and the shift and splitting of the methylene signals indicates the coordination of serotonin to the metal atom through its amine nitrogen. Also visible in the first spectrum are the set of signals corresponding to the *trans* configuration of the complex, although in much smaller proportion. The subtle differences in the photochemical behaviour of the *cis* and *trans* configurations of the complex are negligible in the scope of this work, and no further distinction between these isomers will be made, although a full characterization of both isomers should be needed for any biomedical uses.

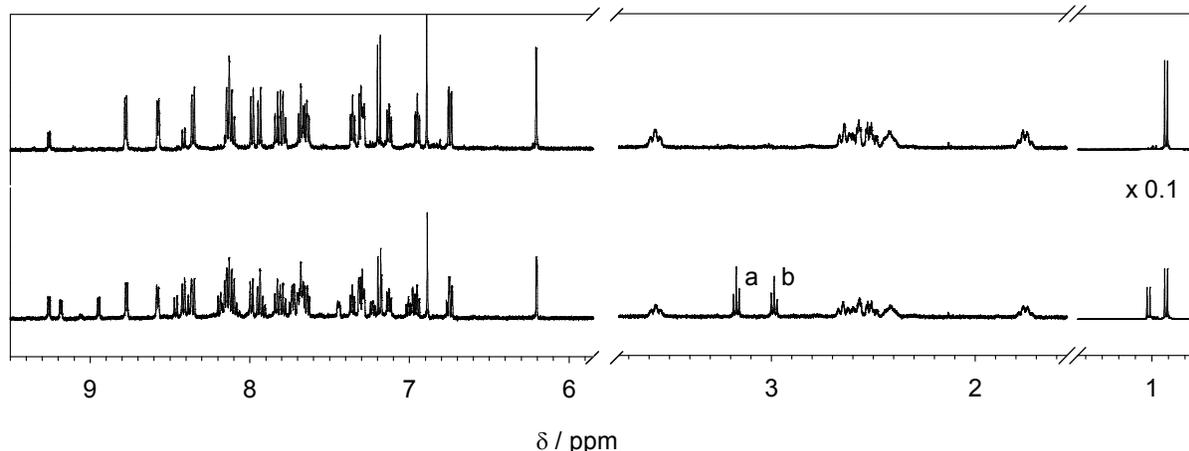


Figure 1.  $^1\text{H}$ -NMR spectra of a 5 mg/mL solution of  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]\text{Cl}$  in  $\text{D}_2\text{O}$  before irradiation (upper trace); and after 60 s irradiation with 525 nm Green LED inside the NMR tube (lower trace). Note that after irradiation appear the triplets **a** and **b**, characteristics of free serotonin.

Once irradiated inside the NMR tube for 60 seconds, the photolysis progressed around 40%. The signals of the bipyridines in the original complex appear diminished, while a new set of signals corresponding to the aquo-complex are evident. In the aliphatic region, the two characteristic triplets of the methylenes of free 5HT appear at 3.17 ppm and 2.98 ppm (Figure 1, signals a and b). Furthermore, amine protons are rapidly exchanged for deuterium and these signals are not present in the free 5HT. The signal from  $\text{PMe}_3$  protons is now shifted to higher fields and corresponds to that of the newly formed aquo complex.

To measure the quantum efficiency of photolysis, a water solution of the complex was irradiated with a 8.56 mW, 405 nm (violet) diode laser at  $\text{pH} = 7$  and  $T = 24\text{ }^\circ\text{C}$ . Figure 2 shows the UV-Vis spectra during the progression of the photolysis reaction. As is deduced from the presence of an isosbestic point at 434 nm, the reaction yields just one absorbing product and proceeds to completion in around 12 minutes. The spectrum of the photoproduct corresponds to that of the aquo complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{H}_2\text{O})]^{2+}$  accordingly with the known photochemistry of the complexes of the form  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{L})]^{n+}$  in water at neutral pH. [17, 22, 23] At  $\text{pH} > 12$  a broader and red-shifted

absorption spectrum is obtained, which corresponds in this case to the hydroxo complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{OH})]^+$ .

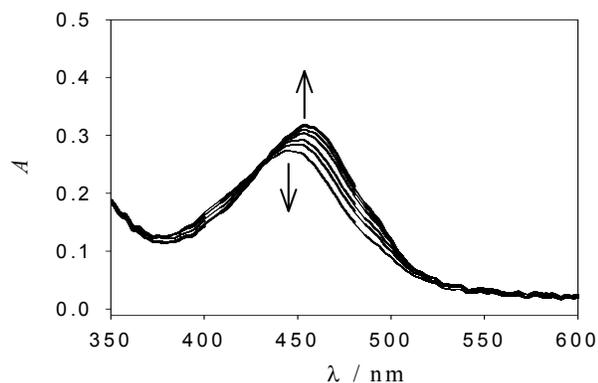


Figure 2: Subset of UV-Vis spectra of  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{2+}$  acquired in aqueous solution at pH=7 while irradiating with a 405 nm laser diode. The arrows indicate photolysis progress.

The progression of the photoreaction is shown in Figure 3 as the amount of photoreleased 5HT versus the total number of photons absorbed. Although the quantum efficiency of the phototrigger can be directly obtained from the initial slope of this graph, a more precise value was obtained by fitting the complete curve, as described below.

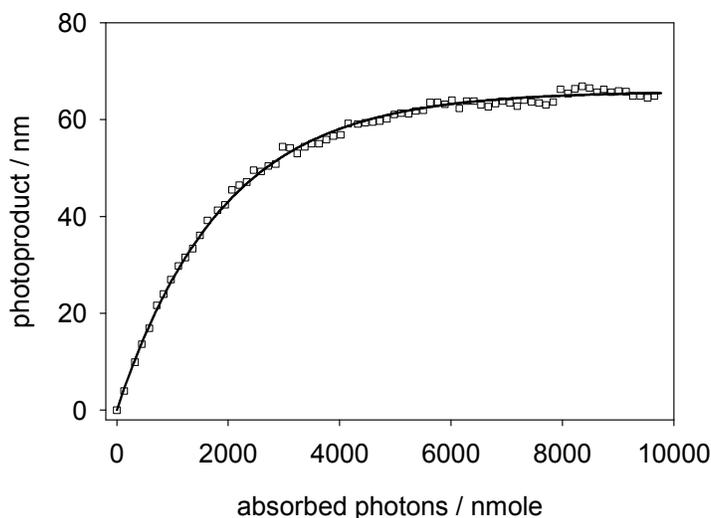


Figure 3: Progression of the UV-Vis spectra during a photolysis reaction against absorbed photons. The continuous line is the theoretical fit according to equation 1. The initial slope of the curve represents the quantum yield  $\phi_{PD} = 0.034$ .

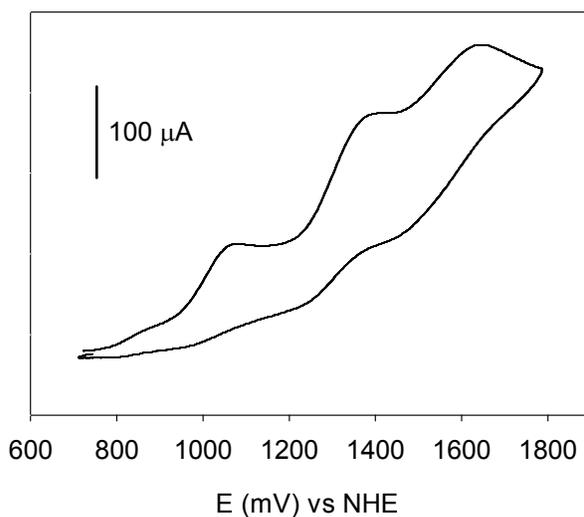
Given the power of the irradiation beam, its optical path, and the amount of complex in the solution, it is possible to calculate the differential amount of product as:

$$\frac{dn_p}{dt} = I_{beam} \cdot (1 - 10^{-Abs_T}) \cdot \frac{Abs_R}{Abs_T} \cdot \phi_{PD} \quad (1)$$

where  $n_p$  is the number of the moles of uncaged product,  $I_{beam}$  is the intensity of the incident light in Einsteins/s,  $Abs_T$  and  $Abs_R$  are the total solution's absorbance and the reactant's absorbance, respectively, and  $\phi_{PD}$  is the quantum yield of photouncaging. The expression has nonlinear factors, and adjusting the value of  $\phi_{PD}$  by iterating a finite differences algorithm over the entire obtained spectra converges to the unique solution for the integrated expression of equation 1.

Cyclic voltammetry was used to further characterize the synthesized complex. Figure 4 shows a cyclic voltammogram of  $[\text{Ru}(\text{bpy})_2\text{PMe}_3(5\text{HT})]^{+2}$  recorded in acetonitrile. Ferrocene was used as internal reference. The Ru(III)/Ru(II) couple is observed at 1.07V vs. NHE (0.67 V vs. Fc/Fc<sup>+</sup>). Other two

1 irreversible processes are evident at higher potentials and correspond to the oxidation of the coordinated  
2 amine, a behaviour also found in all other similar compounds. [17]  
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25 Figure 4. Cyclic voltammogram of  $[\text{Ru}(\text{bpy})_2\text{PMe}_3(5\text{HT})]^{+2}$  recorded in acetonitrile at 100 mV/s on Pt  
26 wire electrode in  $\text{CH}_3\text{CN}$  containing 100 mM  $\text{TBAPF}_6$  as supporting electrolyte.  
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33 To confirm the biocompatibility of this complex and evaluate its performance as a serotonin  
34 phototrigger, a series of electrophysiology experiments in acute brain slices were performed.  
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40 In the mouse prefrontal cortex, separate populations of pyramidal neurons that project either  
41 subcortically to the pons (corticopontine, CPn) or to the contralateral cortex (commissural, COM)  
42 differentially express Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. HCN  
43 channel expression can be assessed electrophysiologically by hyperpolarizing neurons in current clamp  
44 recordings. HCN channels activate slowly over 10s of ms in response to neuronal hyperpolarization.  
45 Thus, cells with high HCN expression levels (e.g., CPn neurons) respond to hyperpolarization with a  
46 characteristic “sag” during and “rebound” following current pulses as the voltage overshoots steady-  
47 state membrane potential in each direction. This overshoot is indicative of the delayed activation and  
48 closure of HCN channels. [42] COM neurons express lower levels of HCN and display little to no sag,  
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1 while CPn neurons express high levels of HCN and display significant sag. 5HT has differential effects  
2 on firing properties in these pyramidal neuron classes. [41] In CPn neurons, 5HT reduces neuronal  
3 excitability through 5HT1A-dependent signaling. In COM neurons, this 5HT1A-dependent reduction in  
4 excitability through 5HT1A-dependent signaling. In COM neurons, this 5HT1A-dependent reduction in  
5 excitability is often followed by a slower, 5HT2A-dependent increase in activity. [41] As such, 5HT  
6 uncaging should result in both reductions and delayed increases in these two cell classes.  
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11 To determine whether uncaging of  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]$  could alter pyramidal cell firing  
12 rates, we made recordings from COM and CPn neurons in mouse prefrontal cortex. When the caged-  
13 5HT was added to the slice in the dark, we observed no change in electrophysiological measures  
14 including resting membrane potential and input resistance, suggesting that the RuBi-5HT is not toxic in  
15 its caged form.  
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23 Consistent with previous work, firing rate was lowered by 5HT uncaging in a CPn neuron. [41] A  
24 neighboring COM neuron exhibited a characteristic biphasic modulation of firing rate after  
25  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]$  uncaging with an initial depression of firing rate followed by a brief increase in  
26 firing (Fig 5E). In a subsequent experiment, we selectively blocked the delayed excitation with the  
27 5HT2A receptor antagonist (1  $\mu\text{M}$  MDL 100907). This experimental design thus provided an internal  
28 control that 5HT photolysis occurred (by the generation of the initial decrease in firing rate) while also  
29 demonstrating that changes in firing rate were indeed due to activation of 5HT receptors (by blocking  
30 the increase in firing rate with MDL 100907).  
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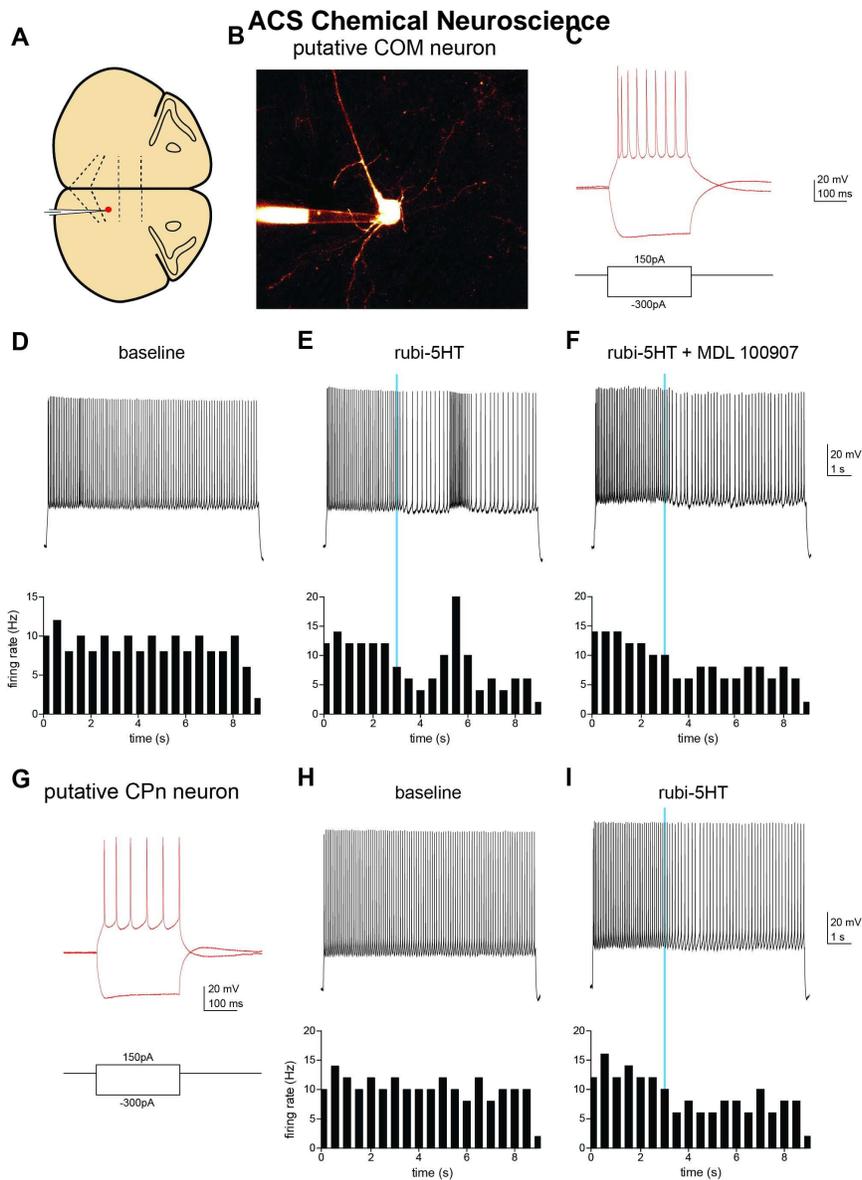


Figure 5. A) Schematic indicating location of recorded neurons in coronal slice from mPFC of mouse. B) Two-photon z-stack image of recorded neuron filled with Alexa-594. C) Voltage responses of putative CPn neuron to hyperpolarizing and depolarizing current steps. D-F) A depolarizing current step was delivered to the CPn neuron to elicit spiking. Top: Firing of CPn neuron at baseline (D), using a pulse of blue light (470nm, 5ms, 3.6mW) to release 5HT (E), and with 5HT uncaging in the presence of a specific 5HT<sub>2A</sub> receptor antagonist (1  $\mu$ M MDL100907, F). Bottom: Peristimulus time histograms of firing rate of the neuron in 200ms bins. G) Voltage responses of putative COM neuron to hyperpolarizing and depolarizing current steps. H-I) Top: Firing of COM neuron at baseline (H) and with uncaging of 5HT (I). Bottom: Peristimulus time histograms of firing rate of the neuron in 200ms bins.

## Conclusions

We have developed  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]^{2+}$ , a ruthenium bipyridil-based 5HT phototrigger which has its absorption maximum well into the visible range. Due to its absorption spectrum, 5HT can be released by irradiating with readily available low-power visible wavelength laser diodes at 405, 445 and even 532 nm. The photoprocess is very clean, showing no sign of other photoproducts besides free serotonin and the corresponding aquo complexes, as is expected from the known photochemistry of this kind of compound. Its redox potential lays into the expected values for a complex of this family, and is highly related with the position of the MLCT band [43]. However, its quantum efficiency of photorelease is somewhat lower than expected. The analog complexes  $[\text{Ru}(\text{bpy})_2\text{PMe}_3(\text{Dopa})]^+$  (Dopa = dopamine)[23] and  $[\text{Ru}(\text{bpy})_2\text{PMe}_3(\text{GABA})]^+$  present the same absorption [44] but three times more activity than  $[\text{Ru}(\text{bpy})_2\text{PMe}_3(5\text{HT})]^+$ , suggesting that some degree of recaptation after uncaging may be occurring. On the other hand, the sensitivity of this new caged serotonin is high enough to allow clean and fast uncaging in biological models, as is demonstrated by confirming neuronal characterization experiments while uncaging with a 5 ms 470nm LED light source.

This phototrigger can be used with harmless visible light instead of UV light as is needed with the more traditional organic protecting groups. The water solubility of its chloride salt  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]\text{Cl}_2$  is around 200 mM, much higher than needed for biological experiments, and the excellent stability of its solutions at neutral pH has been confirmed.

## METHODS

### Syntheses

All chemicals were purchased from Sigma-Aldrich and used as received without further purification.  $\text{Ru}(\text{bpy})_2\text{Cl}_2$  was synthesized according to literature [45] and from this  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{Cl})]\text{PF}_6$  was obtained as previously described. [17]

1 Syntheses of  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]\text{Cl}_2$ : 207 mg (320  $\mu\text{mol}$ ) of  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{Cl})]\text{PF}_6$  were  
2 dissolved in 2 ml acetone and 10 ml water. The mixture was stirred with Cl<sup>-</sup>-loaded Dowex ion  
3 exchange resin during 15–30 minutes. After evaporation of acetone the resultant solution of  
4  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{Cl})]\text{Cl}$  was heated at 70 °C to obtain the aquo complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(\text{H}_2\text{O})]\text{Cl}$ .  
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10 Then 320 mg (1500  $\mu\text{mol}$ ) of serotonin hydrochloride salt were introduced under N<sub>2</sub> atmosphere in a  
11 Schlenk flask and the pH was raised to 9-10 by adding 1400  $\mu\text{L}$  of NaOH 1 M. When the formation of  
12 serotonin complex was confirmed by UV-visible spectroscopy, the mixture was cooled in an ice bath  
13 and HCl 1 M was added to lower the pH and prevent possible oxidation of serotonin by air during the  
14 subsequent procedures. The solution was filtered and after the addition of 1500  $\mu\text{L}$  of KPF<sub>6</sub> 0.5 M the  
15 complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})](\text{PF}_6)_2$  precipitates as an orange powder. Anal. Calcd. for C, 41.47; H,  
16 3.90; N, 8.79. Found: C, 41.2; H, 4.1; N, 8.5. For the purification of the serotonin complex,  
17 hexafluorophosphate anion was interchanged for chloride using Dowex-22 ion exchange resin. The  
18 complex was reprecipitated with KPF<sub>6</sub> 0.5 M at pH 3, the anion interchanged again and pH adjusted  
19 between 7–8. The lyophilized complex  $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)(5\text{HT})]\text{Cl}$  was characterized by NMR  
20 spectroscopy. <sup>1</sup>H-NMR (D<sub>2</sub>O, K<sub>2</sub>CO<sub>3</sub>)  $\delta$ , ppm = 8.78 (1H, d, bpy), 8.57 (1H, d, bpy), 8.36 (1H, d, bpy),  
21 8.13 (1H, d, bpy), 8.10 (1H, t, bpy), 7.99 (1H, d, bpy), 7.94 (1H, d, bpy), 7.83 (1H, t, bpy), 7.79 (1H, t,  
22 bpy), 7.69 (1H, t, bpy), 7.64 (1H, t, bpy), 7.36 (1H, t, bpy), 7.31 (1H, d, bpy), 7.29 (1H, d, bpy), 7.19  
23 (1H, d, J=8.5 Hz, 5HT), 7.13 (1H, t, bpy), 6.95 (1H, t, bpy), 6.89 (1H, s, 5HT), 6.63 (1H, dd, J=8.5 Hz  
24 and J=2 Hz, 5HT), 6.21 (1H, d, J=2 Hz, 5HT), 3.57 (1H, t, 5HT), 2.65 (1H, t, 5HT), 2.61-2.47 (2H, m,  
25 5HT), 2.42 (1H, m, 5HT), 1.76 (1H, m, 5HT), 0.92 (9H, d, PMe<sub>3</sub>).

### 50 Spectroscopic Measurements and Photolysis

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52 The optical bench used for UV-Vis measurements consists, briefly, of a set of collinear lasers directed  
53 towards a four-faceted cuvette, stirred and thermostated at 25 °C. The solution's absorbance is  
54 monitored perpendicularly to the laser path by an OceanOptics PC2000 diode-array spectrophotometer  
55 run by OOIChem software.  
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1 Quantum yield measurements of photouncaging were performed recording full absorption spectra  
2 while the photoreaction occurs. Then, the quantum yield of photolysis ( $\phi_{PD}$ ) was adjusted as a parameter  
3 in order to fit the equation 1 to the measured spectra.  
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7 NMR spectra were obtained with a 500 MHz Bruker AM-500. The compound was photolyzed inside  
8 the unopened NMR test tube with an own-designed illuminator (see supporting information, Figure S2)  
9 using an array of high power LEDs centered at  $450 \pm 20$  nm FWHM.  
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### 15 16 17 **Voltammetry**

18 Cyclic voltammetry was recorded at 100 mV/s scan rate in acetonitrile solution and  
19 tetrabutylammonium salt as supporting electrolyte, using a three-electrode potentiostat based on an  
20 operational amplifier TL071 in current-voltage configuration. A Pt wire with a diameter of 500  $\mu$ m was  
21 used as a working electrode and acquisition software was written in QuickBasic 4.5. Ferrocene was used  
22 as internal reference.  
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### 33 **Electrophysiology. Slice Preparation.**

34 Coronal brain slices (250  $\mu$ m) containing medial prefrontal cortex were made from a P45 C57BL/6  
35 male mouse. Slicing solution was chilled to 4°C and contained (in mM): 234 sucrose, 26 NaHCO<sub>3</sub>, 11  
36 glucose, 10 MgSO<sub>4</sub>, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 0.5 CaCl<sub>2</sub>, bubbled with 5% CO<sub>2</sub>/ 95% O<sub>2</sub>. Slices were  
37 incubated in artificial cerebrospinal fluid (aCSF) at 32°C for 30 minutes and then at room temperature  
38 until recording. ACSF contained (in mM): 123 NaCl, 26 NaHCO<sub>3</sub>, 11 glucose, 3 KCl, 2 CaCl<sub>2</sub>, 1.25  
39 NaH<sub>2</sub>PO<sub>4</sub>, 1 MgCl<sub>2</sub>, also bubbled with 5% CO<sub>2</sub>/ 95% O<sub>2</sub>.  
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### 52 **Serotonin Uncaging & Patch Clamp Electrophysiology**

53 [Ru(bpy)<sub>2</sub>(PMe<sub>3</sub>)(5HT)]Cl was applied as a powder to the aCSF bath solution (100  $\mu$ M) and was  
54 photolysed by 1 photon excitation with a single 5 ms light flash of a 470 nm LED (~3.6 mW).  
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1 Neurons were visualized using DODT contrast microscopy on an upright microscope (Bruker).  
2 Recordings were made using a Multiclamp 700B (Molecular Devices) amplifier and acquired with  
3 IgorPro. Patch pipettes (2-5 M $\Omega$  tip resistance, Schott 8250) were filled with the following (in mM): 130  
4 K<sub>2</sub>Gluconate, 10 KCl, 10 HEPES, 10mM Tris-phosphocreatine, 1.1 EGTA, 2 MgCl<sub>2</sub>, 2 MgATP, 0.3  
5 Na<sub>3</sub>GTP, and 0.1 Alexa-594. All recordings were made at 32-34°C. Series resistance ranged from 10-15  
6 M $\Omega$  and pipette capacitance was compensated.  
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17 Current pulses were applied through the somatic patch pipette for electrophysiological  
18 characterization (-300 and +150 pA in 250 ms duration). The neuron that displayed voltage sag at step  
19 onset and rebound at step offset when hyperpolarized (indicative of high expression of HCN channels)  
20 was identified as a putative corticopontine (CPn) projecting neuron [41, 42, 46]. The neuron lacking  
21 significant sag and rebound was identified as a putative commissural (COM) projecting neuron [41, 42,  
22 47]. A 9 s positive current step was then applied to elicit spiking during uncaging trials (100 and 75 pA  
23 in CPn and COM cells, respectively). 1  $\mu$ M MDL100907 (stock in DMSO), a potent 5HT<sub>2A</sub> receptor  
24 antagonist, was applied before uncaging for some experiments.  
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## TOC Graphic

