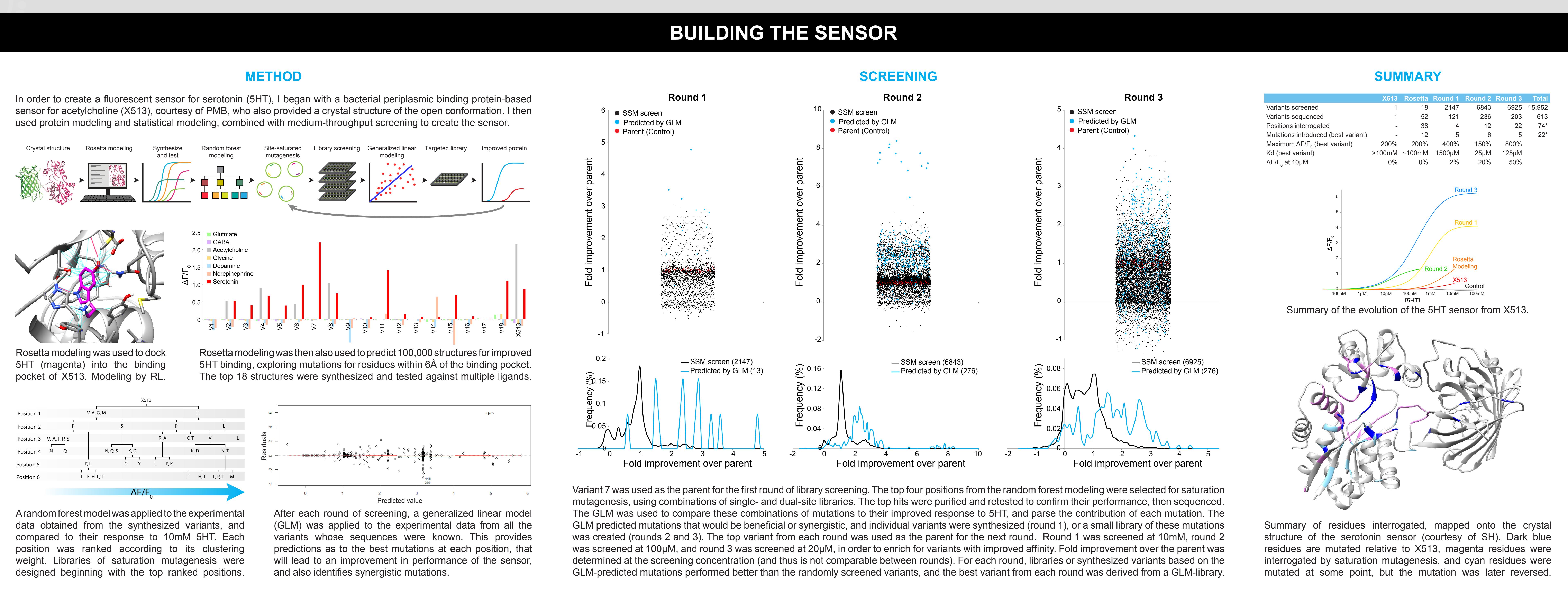


Engineering a fluorescent serotonin sensor using machine learning

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ABSTRACT

The goal of our BRAIN Initiative funded project (U01NS013522) is to generate genetically encoded fluorescent sensors for serotonin, dopamine and norepinephrine, in order to enable high spatial and temporal resolution optical interrogation of neuromodulatory circuits. I am working to produce a fluorescent serotonin sensor. To do this, I chose to start from an existing sensor built from a bacterial periplasmic binding protein, which has three major benefits: it is amenable to bacterial screening methods, it has the potential for a large dynamic range, and no known endogenous activity in eukaryotes. However, there are no bacterial proteins that bind serotonin, thus it was necessary to redesign the binding pocket of an existing binding protein to recognize serotonin. In addition to using traditional methods for protein engineering, I developed a machine learning and probability theory-based approach to directed evolution that has the potential to produce very large improvements in performance with only low to medium throughput screening burden. My method first employs a random forest model to identify which positions will be most effective to mutate, then an absorbing markov chain monte carlo simulation to calculate the minimum library size to be screened, and finally, once screening is complete, a generalized linear model to determine the contribution of individual mutations to the overall improvement in performance of the sensor, which also predicts the best amino acid combinations. This method is straightforward, easy to implement (requiring only rudimentary knowledge of statistics and coding), and can be used in conjunction with other methods, thus adding a powerful new tool to the protein engineering toolbox. Using this method I was able to redesign the binding pocket to generate a fluorescent serotonin sensor whose affinity for serotonin is four orders of magnitude greater than the parent sensor, and has three times the dynamic range. I am now applying this technique to a similar sensor for dopamine.



CHARACTERIZING THE SENSOR

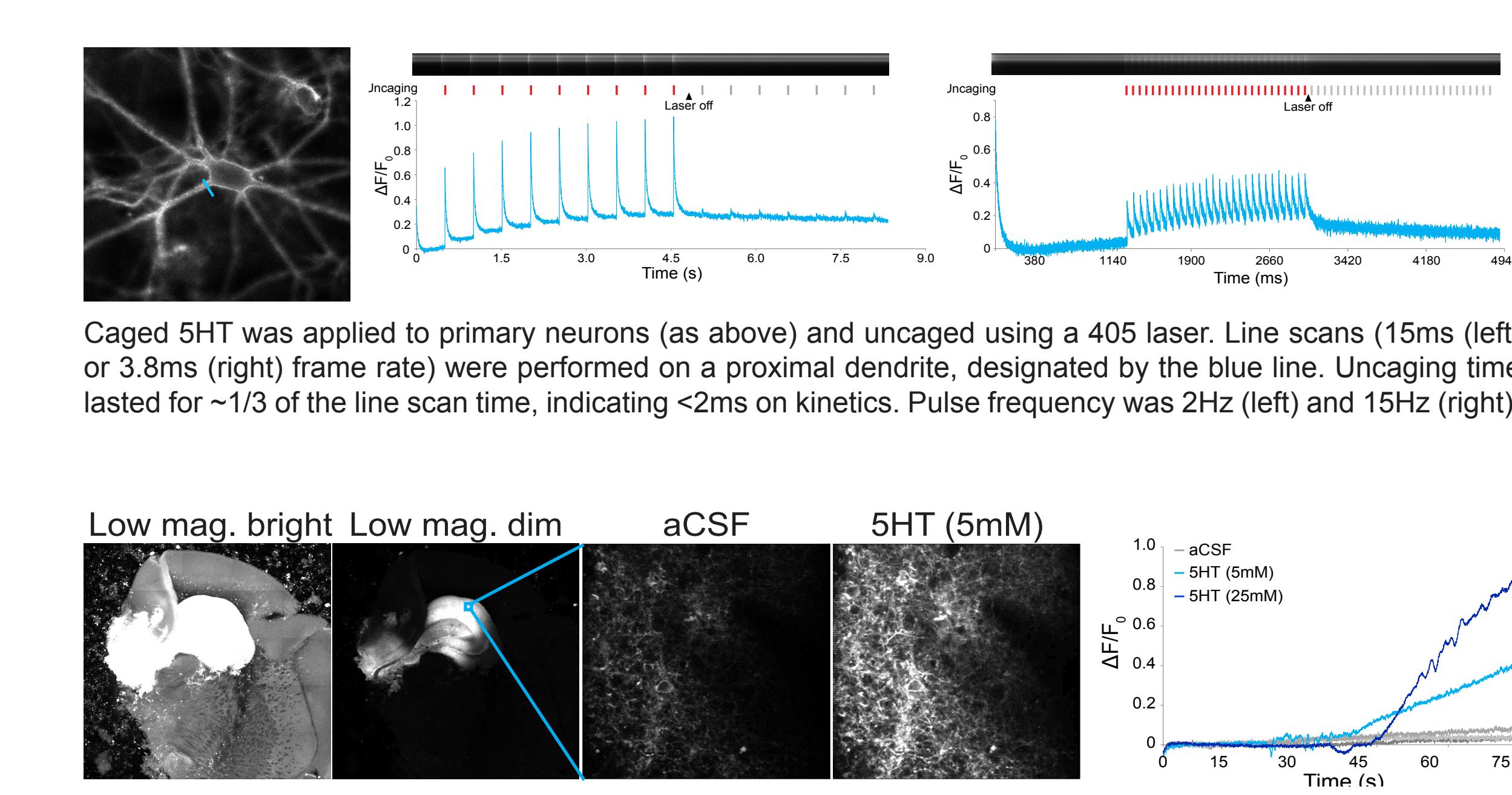
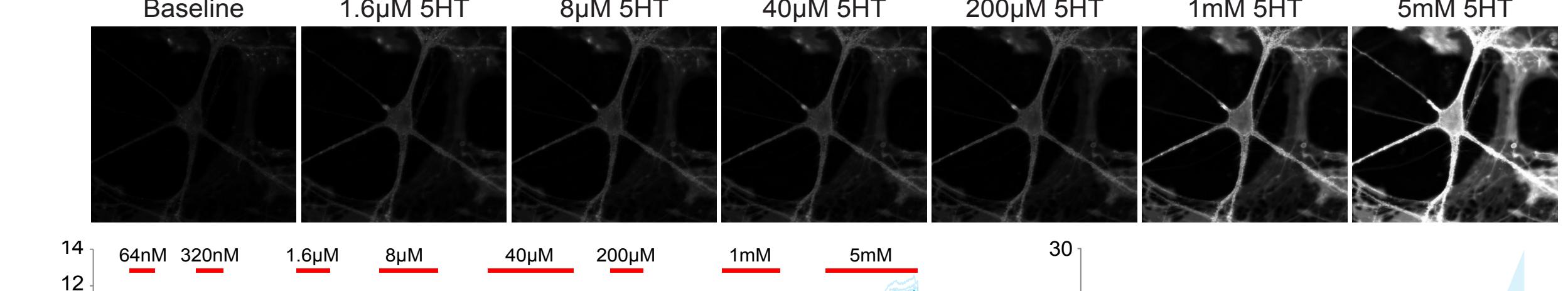
CHARACTERIZATION

The sensor was purified and tested for its response to several compounds related to 5HT, including other indoles, and other neurotransmitters. 5-HTP: 5-hydroxy-tryptophan, 5-HIAA: 5-hydroxy-indole-acetic acid, DMT: dimethyl tryptamine, TMT: trimethyl tryptamine, (DMT, TMT, and bufotenin courtesy of LPC.)

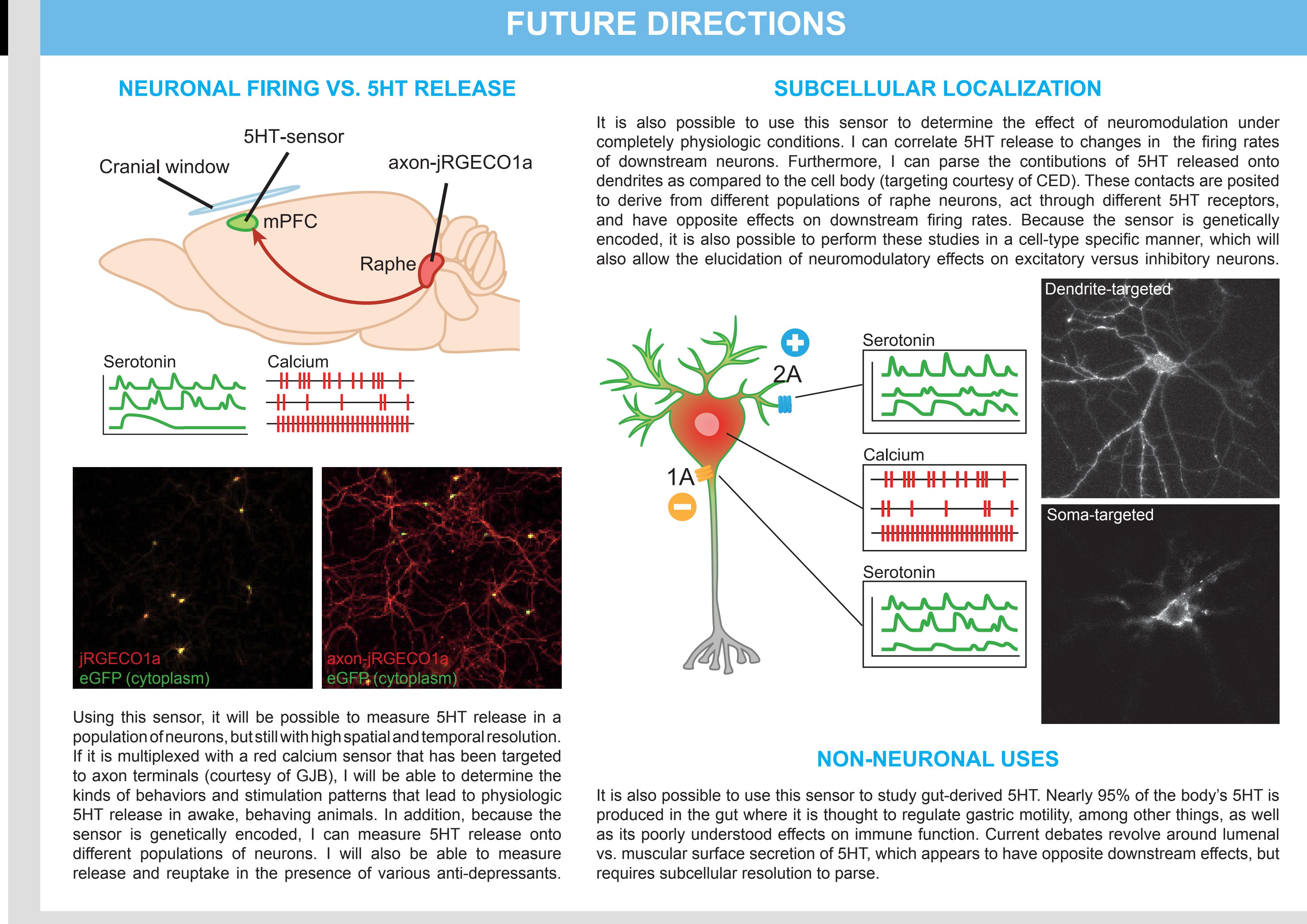
L-741,262, Chlorpromazine, Fluphenazine, Prochlorperazine, Clomipramine, Fluoxetine, Sertraline, L-741,262, Levomepromazine.

The sensor was also tested for its response to an array of clinically relevant compounds, including antidepressants known to interact with the serotonergic system. (Data courtesy of ALN.)

HEK293T cells were transfected with the sensor and imaged while being perfused with alternating HBSS with different concentrations of 5HT.



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