Models of Dopaminergic and Serotonergic Signaling

Authors J. Best[1], M. Reed[2], H. F. Nijhout[3]

Affiliations [1] Department of Mathematics, Ohio State University, Columbus, OH, USA
[2] Department of Mathematics, Duke University, Durham, NC, USA
[3] Department of Biology, Duke University, Durham, NC, USA

Abstract

Mathematical models of dopaminergic and serotonergic synapses have enabled the authors to study quantitative aspects of the synthesis, release and reuptake of dopamine and serotonin, to investigate the effects of autoreceptors, and to explore the influence of the neurochemistry on the firing patterns of cells known to be involved in the behavioral responses to dopaminergic and serotonergic signaling.

The models consist of coupled ordinary differential equations. Parameters are determined from biochemical and physiological measurements.

Three results from recent *in silico* experiments with the dopaminergic and serotonergic synapse models are described: (1) influence of substrate inhibition on the stability of dopamine and serotonin synthesis; (2) a predicted connection between serotonin reuptake transporter (SERT) density on terminals and tonic firing rates; (3) an explanation of data from autoreceptor knock-out experiments.

Mathematical models are useful for studying the biology of dopaminergic and serotonergic signaling because these systems are complex and involve interactions between neurochemistry and neurobiology.

Introduction

The task of understanding the central nervous system is exceptionally difficult for several reasons. The chemistry of cells forms the basis for electrical activity, so that synthesis of neurotransmitters, cell regulatory mechanisms, oxidative stress, and inter-cell communication between neurons and glia all influence neuronal network behavior. However, the firing patterns of neurons and networks of neurons also influence the chemistry of cells and tissues. Experimentation, both clinical and biological, is difficult, and brain systems are plastic and influenced by environment and past history. It is a daunting scientific task to go from neurons to networks to behavior.

Mathematical models of very different kinds have been used to organize data, to test hypotheses about how systems might work, and to aid in the biological investigation of specific systems. We are interested in understanding how brain chemistry and brain electrophysiology influence each other in serotonin (5HT) and dopamine (DA) signaling and what the consequences are for behavior. To that end, we have constructed a mathematical model of a dopaminergic synapse [1] and a mathematical model of a serotonergic synapse [2]. Our purpose is not simply to make models that summarize what is known, but to use the models for *in silico* biological experimentation that sheds light on the complex, intricate behavior of these systems.

We present here three kinds of results that show how experimentation with dopamine and serotonin models can give biological information. First we discuss the physiological consequences of substrate inhibition of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH). Second, we discuss the relationship between tonic firing rates and the density of reuptake transporters on terminals. Finally, we use the model to briefly discuss and interpret experimental data from 5-HT autoreceptor knockout mice.

Methods

Our models consist of systems of ordinary differential equations that we solve using MatLab. The models are based on real physiology and biochemistry in the sense that the kinetic forms of enzymatic reactions and transporters and associ-

Bibliography
DOI http://dx.doi.org/10.1055/s-0030-1252024
Pharmacopsychiatry 2010; 43 (Suppl. 1): S61–S66

ISSN 0176-3679

Correspondence
Dr. J. Best
Department of Mathematics
Ohio State University
Columbus, OH
43210 USA
jbest@math.ohio-state.edu

ated constants are chosen from the literature or from discussions with experimentalists. This is not an easy task since parameters vary from cell to cell, from tissue to tissue and from species to species. A schematic description of the model is shown in **Fig. 1** and the differential equations are given in the Appendix. A full description of our approach is not possible in this short note, but interested readers can consult the lengthy methods sections in [1] and [2]. The serotonin synapse model includes: transport of tryptophan into the terminal, synthesis of 5-hydroxytryptophan by TPH and of 5HT by aromatic amino acid decarboxylase, the transport of 5HT to vesicles by the monoamine transporter, the release of 5HT from vesicles into the synaptic cleft depending on firing rate, reuptake by serotonin reuptake transporters (SERTs), the effects of the autoreceptors, and degradation of 5HT to 5-hydroxyindoleacetic acid in the terminal and in the extracellular space. The model allows us to conduct experiments on the millesecond time scale (*e.g.*, response to individual action potentials) up to the scale of hours and days (*e.g.* reactions to single or repeated doses of fluoxetine).

Results

Substrate inhibition

Both tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) show substrate inhibition. What this means it that the reaction velocity does not follow Michaelis-Menten kinetics but, instead, after a particular substrate concentration, S_m, the velocity of the reaction declines as substrate concentration goes up; see **Fig. 2**. The value of S_m and the rate of decline depend on the particular enzyme and substrate and the mechanism that causes substrate inhibition.

The dark red and blue bars on the x-axis in **Fig. 2** show typical cellular concentrations of tyrosine and tryptophan in CNS neurons under fasting conditions. However, before and after meals blood concentrations of amino acids vary dramatically. Typical serum concentrations of tyrosine are in the range 40–180 micromolar [7,14], and typical brain concentrations are in the range 100–150 [3,9]. Furthermore, it is known ([10,11]) that the brain concentrations of tyrosine and tryptophan can vary by as much as a factor of two (indicated by the pink and light blue bars in **Fig. 2**). In the case of DA, we used our model for the DA synapse [1] to show that these large changes in tyrosine have almost no effect on the synthesis rate of DA or the vesicular stores of DA and this corresponds to what is seen experimentally [5,10]. The reason for this homeostasis is that the normal concentration of tyrosine is near the point where the velocity curve has its maximum, that is, well above the K_m of TH. Thus even quite large increases in tyrosine concentration do not change the synthesis rate of DA very much.

The case is very different for tryptophan. The normal fasting concentrations of tryptophan lie below the K_m for TPH and therefore large fluctuations in tryptophan cause large changes in the velocity of synthesis of 5HT. This is what we see in experiments with the model, and these large changes in synthesis affect the vesicular stores of 5HT. Experiments on rats [11] confirm that brain serotonin is, indeed, sensitive to the levels of tryptophan in the diet. This is, of course, consistent with the fact that serotonin is known to be an appetite suppressant [8]. If elevated 5HT is a signal to stop eating, then the synthesis of 5HT

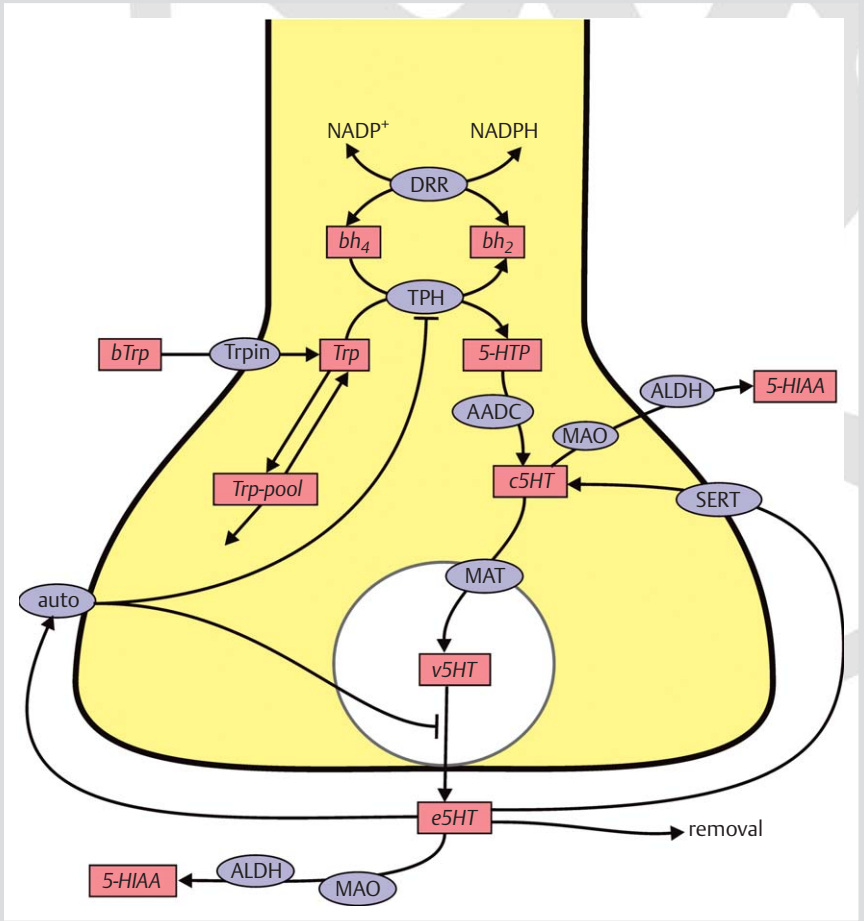

Fig. 1 Serotonin synthesis, release, and reuptake. The figure shows the reactions in the model. Rectangular boxes indicate substrates and blue ellipses contain the acronyms of enzymes or transporters. Acronyms: bh_2, dihydrobiopterin; bh_4, tetrahydrobiopterin; bTrp, serum tryptophan; Trp, intracellular tryptophan; 5-HTP, 5-hydroxytryptamine; c5HT, v5HT, e5HT, cytosolic, vesicular, and extracellular serotonin, respectively; 5-HIAA, 5-hydroxyindoleacetic acid; Trpin, neutral amino acid transporter; DRR, dihydrobiopterin reductase; TPH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; MAT, vesicular monoamine transporter; SERT, 5HT reuptake transporter; auto, 5HT autoreceptors; MAO monoamine oxidase; ALDH, aldehyde dehydrogenase. Removal means uptake by capillaries or glial cells or diffusion out of the system.

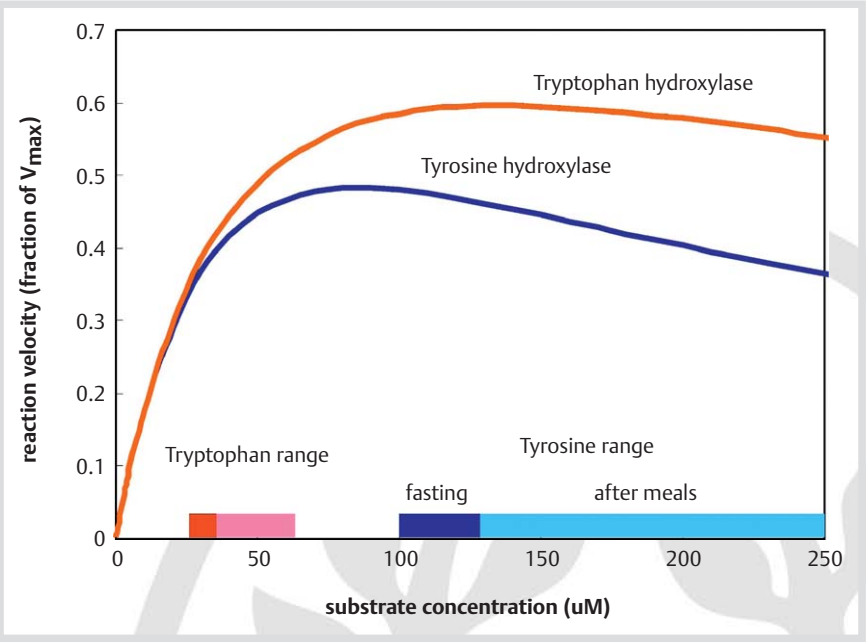

Fig. 2 Velocity Curves for TH and TPH. The velocity curves for tyrosine hydroxylase and tryptophan hydroxylase are shown in blue and red, respectively. The tyrosine curve was obtained by fitting experimental data in [16] and the tryptophan curve uses the parameters determined in [15]. The range of normal fasting values for brain tyrosine and tryptophan concentrations are shown by the dark blue and dark red bars while the light blue and pink bars show the range of concentrations after meals. The light blue range is on the flat part of the TH velocity curve but the pink range is in the sharply rising part of the TPH curve.

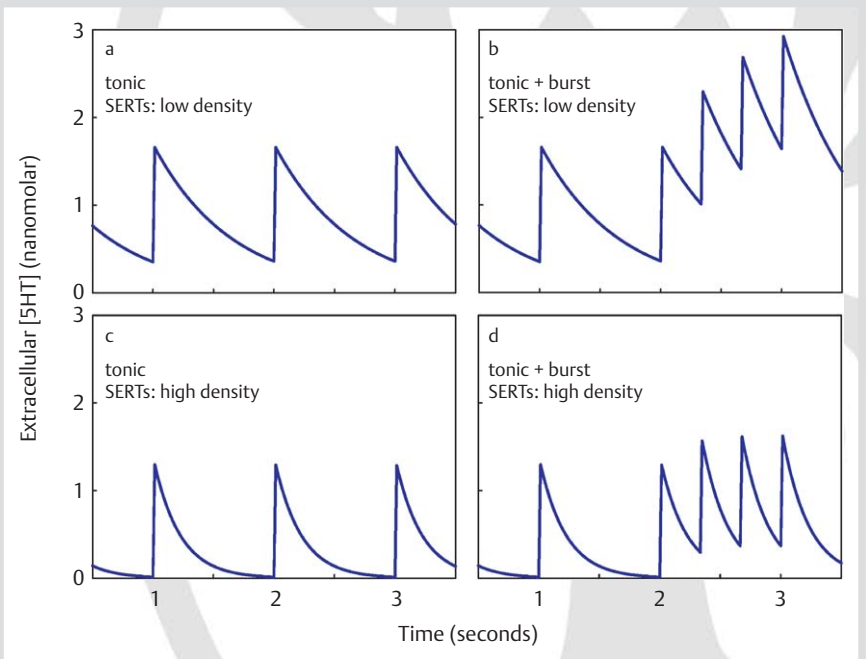

Fig. 3 Extracellular 5HT for tonic firing and bursts. Panel **a** shows model simulations of the extracellular 5HT concentration for a serotonergic neuron firing at 1 Hz. In Panel **b**, the second spike has been replaced by a burst of three spikes and the extracellular 5HT concentration rises significantly because of the burst. Panel **c** shows the extracellular 5HT concentration under tonic firing for the same neuron except that the density of SERTs has been tripled; 5HT is cleared much more rapidly from the cleft. When this neuron receives a burst, its response is very small (Panel **d**).

should be sensitive to the levels of tryptophan in the blood. Substrate inhibition is a common phenomenon that often has functional biological significance [17].

Tonic firing rates and the density of SERTs in terminals

During the last ten to fifteen years dramatic improvements in electrochemistry (reviewed in [19]) have permitted the measurement of the time courses of extracellular DA and 5HT in different brain regions and under different experimental protocols. These experiments and theoretical calculations have enabled the determination of the V_{max} values of the dopamine reuptake transporters (DATs) and the SERTs in different brain regions [4,6]. These V_{max} values correspond roughly to the density of DATs or SERTs on the terminal: higher density, higher V_{max}, faster reuptake.

Interestingly, these studies [4,6] found big differences in SERT densities in different projection regions. What could be the function of these different densities? A terminal with a higher density of SERTs clears the 5HT in the extracellular space from a single action potential faster than a terminal with lower density. We propose that the density of SERTs in a terminal region of a projection neuron is tuned to the tonic firing rate so that the reuptake time corresponds to the interspike interval during tonic firing of the neuron. We begin by explaining why this proposal makes sense physiologically. It is known that 5HT neurons in the dorsal Raphe nucleus (DRN) fire tonically with regular spikes at rates 0.4–2.5 spikes/second [8] and that they also fire bursts at higher frequencies [12] that convey sensory or motor information. In ◉ **Fig. 3**, we show model simulations of extracellular 5HT concentrations. In Panel **a** we see regular spiking and that the time to clear the synaptic cleft is approximately the

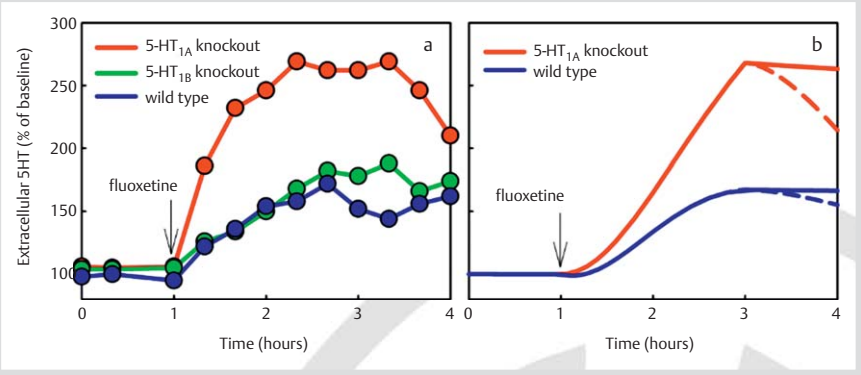

Fig. 4 Experimental data and model simulations for a mouse autoreceptor knockout. Panel **a** shows experimental data redrawn from [13]. Panel **b** shows corresponding model simulations in the WT and 5-HT_{1A} knockout cases (see discussion in the text).

same as the interspike interval. Panel **b** shows the same model but the second spike has been replaced by a burst of 3 spikes. The extracellular 5HT concentration rises dramatically with the burst. In Panel **c** we show the extracellular 5HT concentration under tonic firing where the density of SERTs has been increased by a factor of 3 so that clearance of 5HT from the extracellular space is much more rapid. Panel **d** shows the result of burst firing in this case. Although the burst raises the mean extracellular 5HT it does not raise the maximum amount of 5HT in the cleft very much. Thus, matching the clearance time to the interspike interval greatly enhances the serotonergic signal in bursts.

There is a small amount experimental evidence for our proposal. For example, the V_{max} for the DATs in DA terminals in the caudate putamen was found to be 3.9 nM/sec [20] and the V_{max} for the SERTs in the substantia nigra reticulata was found to be 0.78 nM/sec [4]. Since DA neurons fire tonically at about 5 Hz and 5HT neurons at about 1 Hz this ratio of V_{max} values is what one would expect from our proposal. Also the range of tonic firing rates in the DRN varies by about a factor of 5 or 6 [8] and the range of SERT V_{max} values found in four projection regions in [6] also varies by about 5–6. These pieces of evidence are consistent with our proposal but are by no means conclusive. Definitive proof (or rejection) of our proposal must come from correlating the tonic firing rates of the subpopulations of DRN cells that project to different brain regions with the SERT densities in those regions.

Extracting information from time-course data

Finally, we give one example of how models can be used to extract information from time-course data on extracellular 5HT concentrations. Panel **a** of ○ **Fig. 4** shows data redrawn from experiments that compare extracellular 5HT concentrations in the striatum for wild type (WT) and autoreceptor knockout mice [13] after a dose of fluoxetine. The extracellular 5HT for the 5-HT_{1A} knockout rises to 269% of baseline, presumably because the firing rate has not decreased. This is because there are no 5-HT_{1A} receptors on the cell body in the DRN that would normally inhibit firing when extracellular 5HT near the cell body rises. The extracellular 5HT for the 5-HT_{1B} knockout (terminal receptors) and the WT curve are very similar rising to 167% of baseline.

We can immediately extract some information from the graphs in Panel **a**. Since presumably the firing has not decreased for the 5-HT_{1A} knockout after the dose of fluoxetine, we can assume that the release of 5HT from the terminal remains the same. Since the extracellular concentration rises by a factor of 2.69, the fraction of SERTs on the terminal not blocked by fluoxetine must be 1/2.69 = 0.37. This assumes that reuptake is a linear process but this is reasonable because at these low concentrations we are well below the K_m of the SERTs. The WT and 5-HT_{1B} curves rise much less presumably because the 5-HT_{1A} receptors have decreased the firing rate. How much? Well, if we assume that the same amount of 5HT is released per action potential, then since these curves rise only a factor of 1.67, the firing rate must have decreased to 0.62 = 1.67/2.6 of normal. The fact that the WT and 5-HT_{1B} curves are similar shows that at these modest increases in extracellular 5HT the terminal autoreceptors play little role in the WT case.

Panel **b** shows the results of simulations with our full terminal model using these numbers. Between hours 1 and 3 we decrease the percentage of available SERTs from 100–37% and we obtain the extracellular 5HT curve labelled 5-HT_{1A}. If we ramp down the firing rate from 100–62% between hours 1–3, then we obtain the curve labelled WT. Both curves are quite similar to the corresponding experimental curves. We remark that our model treats the intra-synaptic and extra-synaptic spaces as part of one, well-mixed compartment, yet provides a reasonably good approximation to the extracellular dynamics of experimentally measured 5HT concentrations.

But what about the interesting substantial decrease in the experimental 5-HT_{1A} curve after hour 3? This is unlikely to be clearance of fluoxetine since fluoxetine has a long half-life and in any case the WT curve only declines slightly. To test whether this decrease could be due to decreased synthesis (for example, caused by the terminal 5-HT_{1B} receptors), we decreased the synthesis rate to 50% of normal for the model 5-HT_{1A} curve and to 25% of normal for the WT curve between hours 3 and 4. Computations gave us the dashed curves in Panel **b**. The rate of decline of the curves depends on the balance between rates of synthesis and catabolism and the size of the vesicular stores, none of which we changed except as indicated. This shows that a decline in synthesis rate may be the underlying reason for the decreases seen in the experimental curves after hour 3.

Discussion

We have given three brief examples of how our mathematical models of dopaminergic and serotonergic synapses can be used to shed light on the difficult systems-level issues in neurobiology. The first example shows that in some circumstances the details of the cellular biochemistry really matter. In the second example, we propose a new connection between tonic firing rates and the density of SERTs on serotonergic terminals. The third example illustrates, very briefly, how one can use the model to explore and investigate the meaning of experimental data.

Mathematical models are not magic bullets that can easily solve biological problems. Good models, based on detailed physiological and biochemical information, are difficult and time-consuming to construct. However, if they represent well (a part of) physiological reality, they can be used for *in silico* biological experimentation to test ideas and hypotheses and to make predictions, as we did above in our discussion of tonic firing. Experiments with our DA synapse model enabled us to sort out the different factors that contribute to extracellular dopamine homeostasis in the striatum and why it is maintained until the late stages of Parkinson's disease despite massive loss of the projections from the substantia nigra [18].

Mathematical models are often questioned by pointing out that there surely are pieces, possibly important pieces, of the underlying biology that are not taken into account. This is always a legitimate question. It is worthwhile to point out, however, that this is also a serious issue for the interpretation of traditional biological experiments, though often unacknowledged. For example, in interpreting the data [13] discussed above, we tacitly assumed that the 5-HT_{1A} mouse is just like the WT mouse except that it is missing the 5-HT_{1A} receptors. But this mouse has lived its whole life without these receptors and we can't be certain that other aspects of its physiology have not been seriously affected.

We are in the process of constructing a mathematical model for the synthesis, release, and reuptake of 5HT in cell bodies of the DRN. When completed, we will combine that model with the model of the terminal that we have briefly discussed here. This will enable us to study the simultaneous effects of selective serotonin reuptake inhibitors on the cell body, the firing rate, and the release of 5HT in different terminal regions.

Acknowledgements

This research was partially supported by grant #061670 (MR, HFN) from the National Science Foundation (USA) and through NSF agreement 0112050 through the Mathematical Biosciences Institute (JB, MR).

Disclosure

The authors declare that they have no competing interests and no conflicts of interest regarding the paper.

References

1 *Best J, Nijhout HF, Reed M.* Homeostatic mechanisms in dopamine synthesis and release: a mathematical model. Theor Biol Med Model 2009; 6: 21
2 *Best J, Nijhout HF, Reed M.* Control of serotonin synthesis, release, and reuptake in terminals: a mathematical model. In preparation
3 *Bongiovanni R, Young D, Newbould E et al.* Increased striatal dopamine synthesis is associated with decreased tissue levels of tyrosine. Brain Res 2006; 1115: 26–36
4 *Bunin MA, Prioleau C, Mailman RB et al.* Release and uptake rates of 5-Hydroxytryptamine in the dorsal Raphe and substantia nigra reticulata of the rat brain. J Neurochem 1998; 70: 1077–1087
5 *Cooper J, Bloom F, Roth R.* The Biochemical Basis of Neuropharmacology. New York, NY, Oxford U. Press; 2003
6 *Daws LC, Montanez S, Owens WA et al.* Transport mechanisms governing serotonin clearance in vivo revealed by high-speed chronoamperometry. J Neurosci Meth 2005; 143: 49–62
7 *Dickinson J, Rosenblum H, Hamilton P.* Ion exchange chromatography of the free amino acids in the plasma of the newborn infant. Pediatrics 1965; 36: 2–13
8 *Feldman RS, Meyer JS, Quenzer LF* Principles of Neuropsychopharmacology, Sinauer, Sunderland, MA; 1997
9 *Fernstrom JD.* Role of precursor availability in control of monoamine biosynthesis in brain. Physiol Rev 1983; 63: 484–546
10 *Fernstrom J, Fernstrom M.* Dietary effects on tyrosine availability and catacholamine synthesis in the central nervous system: possible relevance to the control of protein in-take. Proc Nutr Soc 1994; 53: 419–429
11 *Fernstrom J, Fernstrom M.* Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. Am J Clin Nutr 1995; 61: 312–319
12 *Hajos M, Allers KA, Jennings K et al.* Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. Eur J Neurosci 2007; 25: 119–126
13 *Knobelman DA, Hen R, Lucki I.* Genetic regulation of extracellular serotonin by 5-hydroxtryptamine-1A and 5-hydroxytryptamine-1B autoreceptors in different brain regions of the mouse. J Pharmacol Exper Therap 2001; 298: 1083–1091
14 *Long C* (Ed): Biochemist's Handbook, Princeton, NJ, Van Nostrand 1961
15 *McKinney J, Knappskog PM, Haavik J.* Different properties of the central and peripheral forms of tryptophan hydroxylase. J Neurochem 2005; 92: 311–320
16 *Nakashima A, Mori K, Suzuki T et al.* Dopamine inhibition of human tyrosine hydroxylase type I is controlled by the specific portion of the N-terminus of the enzyme. J Neurchem 1999; 72: 2145–2153
17 *Reed MC, Lieb A, Nijhout HF.* The biological significance of substrate inhibition: a mechanism with diverse functions. BioEssays, to appear 2010
18 *Reed M, Best J, Nijhout HF.* Passive and active stabilization of dopamine in the striatum. Bioscience Hypotheses 2009; 2: 240–244
19 *Robinson DL, Hermans A, Seipel AT et al.* Monitoring rapid chemical communication in the brain. Chem Rev 2008; 108: 2554–2584
20 *Venton BJ, Zhang H, Garris PA et al.* Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. J Neurochem 2003; 87: 1284–1295

Appendix

In this appendix we describe the differential equations that constitute the model. The time-dependent variables in the model are the concentrations of the substrates in the pink boxes in **Fig. 1**; full names are given in the legend. The velocities of reactions (or net velocities in case of reversible reactions) or velocities of transport are always indicated by a capital V with subscripts and superscripts indicating which enzyme, transporter, or other process is involved. In each case, the variables that the process depends on are indicated. On the left sides we include square brackets around the variables as a reminder that the units of the variables are concentration (μM); on the right sides of the equations we omit the square brackets because they make the equations harder to read.

In order to indicate what is involved in the construction of such a model, we discuss a few of the terms. V_{TPH} (*Trp*, *bh4*, *e5HT*) is the velocity of the reaction catalyzed by tryptophan hydroxylase that depends on the concentrations of cytosolic tryptophan and dihydrobiopterin, as well as the concentration of extracellular 5HT via the autoreceptors. The term *release* (*e5HT*) · *fire* (*t*) · *v5HT* the rate of release of 5HT from the vesicles into the extracellular space per unit time at time *t*. *v5HT* is the concentration of 5HT in the vesicular compartment and *fire* (t) represents the firing rate of the neuron scaled so that it has value one in case of tonic firing. *release* (*e5HT*) represents the effect on release of vesicular 5HT by the extracellular 5HT concentration via the autoreceptors. The term *fluox* (t) · V_{SERT} (*e5HT*) represents the rate of reuptake of 5HT from the extracellular space into the cytosol by the SERT transporters. *fluox* (*t*) is the fraction of transporters that are unblocked by fluoxetine at time t, so it equals one in the absence of fluoxetine. The term V_{rem} (*e5HT*) represents the removal of 5HT from the extracellular space by uptake into capillaries and glial cells or diffusion out of the tissue. The most difficult part of the construction of the model is deciding on the functional form of the velocities (*i.e.*, Michaelis-Menten or other forms) and determining appropriate values for the constants involved. This is described in [2] to which we refer the reader for details.

It is worthwhile to point out that there is not a single "correct" model of a serotonergic synapse, nor are there single "correct" values for each parameter. As we remarked in the main body of the paper, the density of SERTs has been shown to vary by a factor of 5 in different terminal regions, which means the V_{max} of the SERT will vary by the same factor depending on which terminals one is discussing. Similarly, it is known that TPH synthesis rates vary from brain region to brain region as do the types and densities of autoreceptors. It is very likely that these variations in parameters are not random but have functional significance. The purpose of a model, such as the one we have described here, is that it enables one to study how the overall behavior of the system depends on the properties of each of the parts (SERTs, autoreceptors, TPH), and how the system behavior changes when the parts change or are influenced by pharmacological agents.

$$
\begin{aligned}
\frac{d[bh2]}{dt} &= V_{\mathrm{TPH}}(Trp, bh4, e5HT) - V_{\mathrm{DRR}}(bh2, \mathrm{NADPH}, bh4, \mathrm{NADP}) \\
\frac{d[bh4]}{dt} &= V_{\mathrm{DRR}}(bh2, \mathrm{NADPH}, bh4, \mathrm{NADP}) - V_{\mathrm{TPH}}(Trp, bh4, e5HT) \\
\frac{d[Trp]}{dt} &= V_{\mathrm{Trp}}(bTrp) - V_{\mathrm{TPH}}(Trp, bh4, e5HT) - V_{\text{Trp-pool}}(Trp, Trp{-}pool) - k_{Trp}^{catab} \cdot Trp \\
\frac{d[5{-}HTP]}{dt} &= V_{\mathrm{TPH}}(Trp, bh4, e5HT) - V_{\mathrm{AADC}}(5{-}HTP) \\
\frac{d[c5HT]}{dt} &= V_{\mathrm{AADC}}(5{-}HTP) - V_{\mathrm{MAT}}(c5HT, v5HT) + fluox(t) \cdot V_{\mathrm{SERT}}(e5HT) - V_{c5HT}^{catab}(c5HT) \\
\frac{d[v5HT]}{dt} &= V_{\mathrm{MAT}}(c5HT, v5HT) - release(e5HT) \cdot fire(t) \cdot v5HT \\
\frac{d[e5HT]}{dt} &= release(e5HT) \cdot fire(t) \cdot v5HT - fluox(t) \cdot V_{\mathrm{SERT}}(e5HT) - V_{e5HT}^{catab}(e5HT) - V_{rem}(e5HT) \\
\frac{d[5{-}HIAA]}{dt} &= V_{c5HT}^{catab}(c5HT) + V_{e5HT}^{catab}(e5HT) - k_{hva}^{catab} \cdot 5{-}HIAA \\
\frac{d[Trp{-}pool]}{dt} &= V_{\text{Trp-pool}}(Trp, Trp{-}pool) - k_{Trp\text{-}pool}^{catab} \cdot Trp{-}pool
\end{aligned}
$$