Bursts and the Efficacy of Selective Serotonin Reuptake Inhibitors

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Abstract

We present a new hypothesis for the efficacy of selective serotonin reuptake inhibitors (SSRIs). We propose that SSRIs bring the response to the phasic firing of raphe nucleus cells back to normal, even though the average extracellular 5HT concentration remains low. We discuss burst firing in the raphe nuclei and use mathematical models to argue that tonic firing and phasic firing may be decoupled and may come from different mechanisms. We use a mathematical model for serotonin synthesis, release, and reuptake in terminals to illustrate the responses in terminal regions to bursts in a normal individual and in an individual with low vesicular serotonin. We then show that acute doses of SSRIs do not bring the response to bursts back to normal, but that chronic doses do return the response to normal. These model results need to be confirmed by new electrophysiological and pharmacological experiments.

I Introduction

Despite more than 50 years of research, the etiology of depressive illness remains unknown. A hypothesis that has been central to much work in pharmacology and electrophysiology is that depression is caused by a deficiency of serotonin [18,54]. This hypothesis led to the development of monoamine oxidase inhibitors (MAOIs), tricyclic anti-depressants, and the selective serotonin reuptake inhibitors (SSRIs). The idea of the MAOIs is that inhibiting the degradation of serotonin (5HT) should make more 5HT available for packaging into synaptic vesicles. The idea of the tricyclics and the SSRIs is that blocking the 5HT reuptake transporters (SERTs) should leave more serotonin in the extracellular space. All of these drugs have shown some efficacy in the treatment of depression, but the causal chain of events and the reasons why they benefit some patients and not others remain elusive.

Early evidence [18,54] showed that reserpine, which inhibits the monoamine transporter and thereby limits the packaging of 5HT into vesicles, lowers tissue 5HT and causes depression. More recent evidence [6,65] shows that the depletion of tryptophan (the precursor of 5HT) from the diet is followed by mood lowering in humans and decreases 5HT release in rat hippocampus [57]. Both of these lines of evidence suggest strongly that low vesicular 5HT is a major cause of depression. We accept this as a working hypothesis and assume in our mathematical modeling that depressed patients differ from normal patients by having considerably less 5HT in the vesicles of their 5HT neurons. This leaves open the question of what is the mechanism of efficacy of the SSRIs, especially since there is evidence [5,31,64,66] that the presence of an SSRI lowers vesicular 5HT and this is confirmed by our model. Any theory of the mechanism of efficacy of the SSRIs must also account for the fact that pharmacological changes happen on a very short time scale but patients typically show improvements on a time scale of 3–6 weeks.

In this paper we develop a new hypothesis about the efficacy of SSRIs – that they enable the response (in projection regions) to burst firing of cells in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) to return to normal. We use our mathematical model for a 5HT terminal, developed in [8], as a platform for describing our ideas. Confirmation or rejection must come, of course, from pharmacological and electrophysiological experiments. In Section II we discuss 2 of the traditional hypotheses about the efficacy of SSRIs and the experimental evidence against them. In Section III we discuss burst firing in the DRN and MRN and our hypothesis. In Section IV we use a mathematical model to show that it is reasonable that SSRIs could

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lower the rate of tonic firing without affecting the response to bursts. Finally, in Section V we use our neuron terminal model to compute the responses in projection regions to DRN and MRN bursts in normal patients, depressed patients, depressed patients with an acute dose of an SSRI, and depressed patients with chronic dosing with SSRIs. The chronic SSRI patients differ from the acute SSRI patients in that their SERT numbers on terminal membranes are lower as found in many studies [7,23,38,46].

II Traditional Hypotheses About the Efficacy of SSRIs

The question of how SSRIs "increase serotonergic signaling" has 2 parts. First, how do SSRIs change the pharmacology (and possibly the cell biology) of the serotonin system? Second, how do the pharmacological changes affect the electrophysiology of the serotonin system? The second question is particularly difficult, because the details of the electrophysiology of the 5HT system have not been worked out. Do 5HT neurons transfer information through one-on-one signaling, by modifying the signaling of neurons using other neurotransmitters by synapsing on cell bodies or terminals, or by simply keeping a basal level of 5HT in tissues via volume transmission? Probably all of the above in different projection regions. Thus it was natural for hypotheses to focus on the pharmacological changes.

The basic hypothesis was that SSRIs would raise the level of 5HT in serotonergic synapses by blocking reuptake by the SERTs on 5HT terminals. This simple hypothesis was thrown into doubt by the discovery that the cell bodies of DRN and MRN neurons also release 5HT and have SERTs. Furthermore, increased extracellular 5HT (e5HT) in the DRN and MRN decreases the tonic firing rate of those cells via the $5HT_{1A}$ autoreceptors [21]. Thus, there are 2 conflicting effects. Blocking the SERTs in the terminal region would tend to raise e5HT there, and blocking the SERTs in the DRN or MRN would tend to decrease e5HT in the terminal region. The balance between the 2 effects will depend on the densities of $5HT_{1A}$ autoreceptors on different 5HT populations in the DRN and MRN and on the densities of SERTs in different projections, both quite variable, and perhaps on other factors as well. Thus one would expect that the amount of increase (or decrease) of e5HT would depend on dose and on the projection region being studied. This was indeed the case, as experimentalists found variable increases and some decreases [1,4,28,42]. The net result of these experiments was that it was not clear how much e5HT could be expected to rise (or even decrease) in projection regions with acute doses of SSRIs.

In any case, it was clear that raising e5HT in projection regions was, at best, only part of the story since patient improvement did not occur immediately but on a time scale of 3–6 weeks. So, attention focused next on the $5HT_{1A}$ autoreceptors on the DRN and MRN cell bodies. It was shown in numerous studies (for example [15]) that giving $5HT_{1A}$ antagonists potentiates the SSRI-induced increase of e5HT in projection regions. Similarly, $5HT_{1A}$ knockouts show increased release in projection regions [35]. Both types of studies confirm the role of the $5HT_{1A}$ autoreceptors in decreasing tonic firing of 5HT neurons in the DRN and MRN in the presence of SSRIs. Furthermore, a number of studies [10,15,29,32] showed that chronic treatment with SSRIs desensitizes the $5HT_{1A}$ autoreceptors in the DRN and MRN. And thus, one could explain the improvements of patients on the time scale of 3–6 weeks by the slow desensitization of autoreceptors. Consistent with this hypothesis are several studies that showed that e5HT levels in projection regions are higher after chronic treatment as compared to acute treatment [37,53,60]. These studies did not measure e5HT in projections regions during the full course of chronic SSRI treatment. Unfortunately, when this was done, Smith et al. [56] found that e5HT concentrations in neocortex, caudate, and hippocampus of awake monkeys went up initially and then declined somewhat over the course of treatment. Similar findings were found by Anderson et al. [2] who saw an initial quick rise in e5HT in the cerebral spinal fluid of rhesus monkeys but then a plateau during chronic treatment. Thus the autoreceptor desensitization hypothesis seems unlikely to explain the delay of beneficial effects of SSRI treatments.

III A Hypothesis About Burst Firing and the SSRIs

The 5HT neurons are only a miniscule fraction of the neurons in the brain, and of these about 80% are in the DRN and MRN [18]. The DRN receives a complicated pattern of afferents from the lateral habenula, hypothalamus, pontine reticular formation, the locus coeruleus, and other raphe nuclei [39,49], as well as direct innervation from the retina [19] and descending projections from the medial prefrontal cortex [14]. The efferent projections from the DRN and MRN project to myriad brain regions including striatum, hippocampus, thalamic nuclei, the olfactory system, and all regions of the neocortex [18]. Thus it is not surprising that the serotonergic system has been associated with so many different types of behavior or that the connections between the electrophysiology of the system and behavior have been difficult to work out.

In a series of pioneering studies [20,30,34,61], Jacobs, Fornal and coworkers studied the relationship between the electrophysiology of the 5HT system and various behaviors in non-anaesthetized cats. They showed that the firing rate and pattern of some DRN 5HT neurons differ in active waking, quiet waking, slow-wave sleep, and REM sleep [34]. They also showed that while most serotonergic DRN neurons show a strong relationship between tonic motor activity and firing rate, a subset of these cells is strongly activated during feeding and grooming [61]. Already in the early paper [30], it was noted that some of the spikes of 5HT neurons (in that case in the nucleus raphe pallidus) are, in fact, doublets or triplets. This phasic activity is the bursting that we are discussing here. It is not known whether DRN and MRN cells fire bursts spontaneously or only in response to afferent stimulation, but they do respond phasically to diverse and specific sensory, motor and reward events [50,55]. For our hypothesis, the origin of the bursts does not matter.

In a landmark paper, Hajos et al. [25] studied the firing patterns of DRN and MRN neurons in anaesthetized rats and classified them into 2 groups. The first group fired single action potentials in a regular rhythm (approximately 1 Hz), while the second group also fired rhythmically, but doublets or triplets of action potentials sometimes replaced single spikes. These "bursts" had short duration (usually <12 msec) with spikes approximately 7 msec apart. The spikes in a burst had decreasing amplitude but the first was always identical to single spikes. These were extracellular recordings at the cell bodies, but in a later paper [26] it was shown that the spikes in bursts actually create action potentials in the axon that can, therefore, release 5HT in terminal regions.

Our hypothesis is that chronic treatment with SSRIs of depressed patients returns to normal the response to bursts arriving in terminal regions. The sequence of ideas that support this hypothesis, as well as mathematical model simulations, are given in Section V.

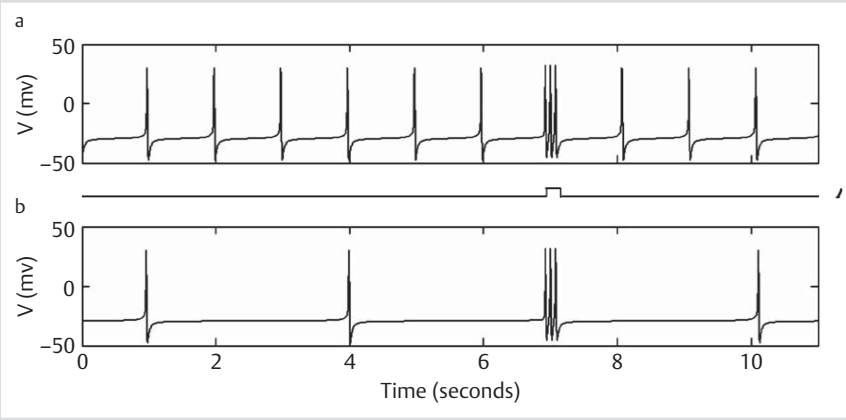

Fig. 1 Burst firing of a model cell is independent of tonic firing frequency. A 10 μA/cm^2 current pulse, indicated between Panels **a** and **b**, applied from $t = 6.9$ s to $t = 7.2$ s causes the cell to fire a triplet of spikes. Panel **a**: A background current of 39.996 μA/cm^2 sets the firing frequency at approximately 1 Hz. Panel **b**: A lower applied current of $I = 39.9666$ μA/cm^2 results in a tonic frequency of approximately 0.33 Hz.

Our physiological point of view is that the 5HT neurons in the DRN and MRN have 2 functions. By rhythmic tonic firing they maintain (or change if they change their frequency) the 5HT tone in target tissues by volume transmission. By burst firing they convey specific information to one-on-one synapses that are known to exist [43,48]. These DRN and MRN bursts are very short. In a sense they have to be, because as Conley et al. [16] have pointed out, a longer signal would raise e5HT in the DRN and MRN and dampen the response via the autoreceptors. An important part of our argument is that pharmacological agents can reduce the rate of firing of DRN or MRN cells without reducing the amplitude of individual spikes or bursts. In the next section we present modeling results that show that this hypothesis is reasonable.

IV Tonic Firing and Burst Firing

SSRI administration reduces the rate of tonic firing of serotonergic DRN and MRN cells [22,26,54]. Yet in the simulations in Section V (shown in **Fig. 3, 4**), we assume that the spike frequency within a burst remains essentially unchanged. In this section we show, using common mathematical modeling techniques, 2 different mechanisms by which a neuron retains its ability to fire a burst of spikes despite a reduced frequency of tonic spiking. Thus we illustrate the reasonableness of this possibility. In the first example, the burst results from a temporary increase in membrane excitability, modeled as a current pulse. In the second example, the burst occurs because the cell is driven by a bursting afferent. Since we have not yet developed a mathematical model for the spike generation of a serotonergic neuron, we use here the relatively simple, 2-dimensional Morris-Lecar model for neuronal activity. The 2 state variables correspond to membrane potential and to a slower recovery variable associated with spiking, the gating of a delayed-rectifier potassium current. Although the Morris-Lecar model was originally developed for a barnacle muscle fiber [47], it is widely used to study a variety of neuronal behaviors. The spirit in which we use it here is the following: that behaviors readily found in a Morris-Lecar model can be expected to be common in many neuronal models. The model includes voltage-gated calcium and delayed-rectifier potassium channels. The state variable w represents the fraction of open potassium channels. The calcium channels are assumed to very rapidly reach steady state values, so this process is modeled as instantaneous. The equations we use are

$$C\frac{dV}{dt} = g_{Ca}m_{\infty}(V)(V - V_{Ca}) + g_K w(V - V_K) + g_L(V - V_L) + I$$

$$\frac{dw}{dt} = \varphi\frac{w_{\infty}(V) - w}{\tau_w(V)},$$

where g_{Ca}, g_K, and g_L are the maximal conductances of the calcium, potassium, and leak currents, respectively. V_{Ca}, V_K, and V_L are the reversal potentials for calcium, potassium, and for the leak current. A nice discussion of this model and its analysis can be found in [51]; aside from varying the applied current, I, we use the parameter values specified there.

Panel **a** of **Fig. 1** shows the behavior of the Morris-Lecar model where the excitability has been adjusted (via the applied current, I) so that the model cell fires at approximately 1 Hz. A current pulse of 10 μA/cm^2 temporarily increases the membrane excitability, causing the 7th spike to be replaced by a burst of 3 spikes. This figure should be compared to Figure 1, Panel c of [25] which shows a mix of tonic spiking and short bursts in a serotonergic cell of the DRN; Figure 2 of that paper shows doublets and triplets of spikes.

In Panel **b** of **Fig. 1**, the current I has been reduced in order to lower the frequency of tonic firing, as occurs under SSRI administration. The model cell now fires at a frequency of approximately 0.33 Hz. The model cell still responds to a 10 μA/cm^2 current pulse with a triplet burst (replacing the 3rd spike) similar to the case in Panel **a**. In this simple example, the increased membrane excitability underlying the burst has been modeled as a current pulse; other variations of the example also work, for instance one can inject, instead, a conductance-based current.

The mechanism underlying the phenomenon in this simple model is easy to understand. Panel **a** of **Fig. 2** shows a bifurcation diagram for this model: for each value of injected current, I, the diagram indicates the corresponding steady state values of membrane potential; spiking solutions are also indicated for a range of values of I between approximately $I_L = 40$ μA/cm^2 and $I_R = 116$ μA/cm^2. Panel **b** shows the frequency-current (f/I) diagram for the spiking solutions. The transition from quiescence to spiking in this example, at I_L occurs at a bifurcation that is a saddle node on an invariant cycle. This type of transition, called "type I excitability" [33] is often used in models of neurons that can fire at frequencies of 1 Hz or less since, as the f/I curve indicates, this bifurcation produces spikes at arbitrarily low frequencies. Generically for such a bifurcation, the frequency scales as $O(\sqrt{\mu})$ for μ small, where μ is an appropriate measure of distance from the bifurcation point [59]. Thus tonic firing frequencies of 1 Hz and 0.33 Hz are expected to correspond to similar values of I, and therefore a current pulse of a fixed size is expected to result in similar levels of membrane excitability and similar frequencies within the burst.

In the above example, the increase in membrane excitability occurs due to mechanisms intrinsic to the cell. There are, however, a variety of afferents to the raphe nucleus. Using retrograde

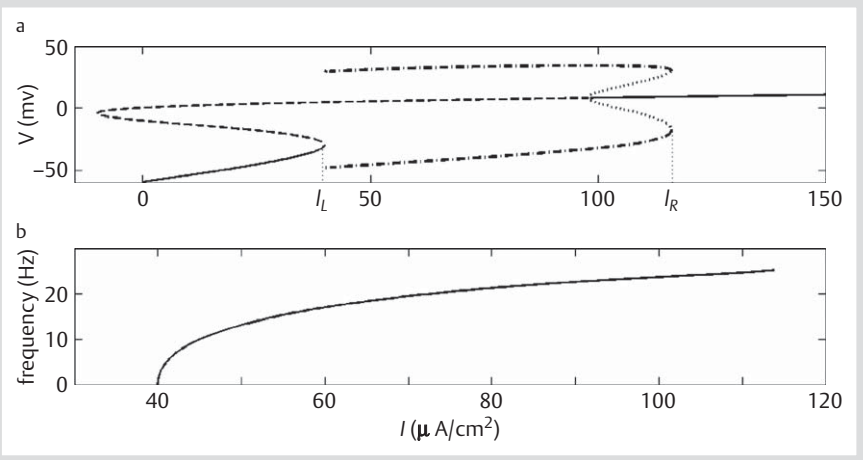

Fig. 2 Bifurcation diagram and f/I curve. Panel **a**: Bifurcation diagram for the Morris-Lecar model cell. The S-shaped curve depicts equilibrium membrane potential values for the corresponding level of applied current, *I*. These equilibria are stable where the line is solid and unstable where dashed. Each value of *I* between I_L and I_R also corresponds to stable tonic firing; the maximum and minimum values of *V* during a spike are indicated by the dot-dashed curves that parallel the S-shaped curve between these values of *I*. The background values of *I* resulting in the 1 Hz and 0.33 Hz spiking of **Fig. 1** are near I_L; the 10 μA/cm² current pulse moves *I* to the right. Panel **b**: f/I curve for the Morris-Lecar model cell. For *I* values between I_L and I_R (for which the cell spikes), the diagram shows the frequency of spiking. Near $I = I_L$, the frequency is proportional to $\sqrt{(I - I_L)}$.

and anterograde tracing methods, Peyron et al. [49] have shown that each DRN subdivision receives a differential distribution of afferents from numerous forebrain structures. The DRN is innervated by descending projections from the medial prefrontal cortex [14]. Such data suggest an alternative mechanism in which the properties of the burst are passed to the serotonergic cell from its afferent. We experimented with this mechanism by having a bursting model cell synaptically project to the Morris-Lecar model described above. We found that when the afferent cell fires a triplet burst it stimulates a triplet burst in the Morris-Lecar cell (simulations not shown). And, the response of the Morris-Lecar cell to the afferent burst is unchanged, even when the tonic firing rate of the Morris-Lecar cell is reduced.

In the 2 examples above, we utilized previously published sets of parameters; we have not tuned the model or parameters (other than *I*) to reflect specific properties of serotonergic neurons. We are not proposing that either of these examples is necessarily the actual mechanism causing bursts in serotonergic cells. However, these 2 simple examples illustrate that it is reasonable to suppose that the frequency of firing within a burst may remain essentially unchanged even while the tonic firing slows.

V Simulations of burst firing in the presence of SSRIs

We will use our mathematical model for serotonin synthesis, release, and reuptake in terminals [8] to illustrate our ideas about burst firing and SSRIs. The model is exactly as described in [8]; no parameters have been changed except as indicated below. We begin by briefly describing some aspects of the model that are relevant for the simulations that follow. The rate of release of 5HT at time *t* (in μM/hr) is given by the function:

$$RATE = release(e5HT(t)) \times fire(t) \times v5HT(t).$$

v5HT(t) is the current concentration of 5HT in the vesicular compartment. *fire(t)* is a function of time that represents our assumptions about the rate of firing of action potentials at the cell body of the (DRN or MRN) neuron. For example, we model action potentials as causing constant release over a 3 millisecond time interval. The amount released is set so that if the neuron is firing tonically at 1 Hz (typical for DRN neurons [18]) then the average extracellular 5HT concentration is 0.768 nM, which is the steady state concentration that one obtains by setting *fire(t)* = 1. This choice means that the vesicular pool turns over once an hour at steady state. The factor *release(e5HT(t))* describes the effect of the extracellular 5HT concentration on release via the terminal autoreceptors. At steady state in the model, the concentration of vesicular 5HT is 21.5 μM. Much more detailed discussions can be found in [8].

Panels **a**, **b**, and **c** of **Fig. 3** show the "normal" response of the model to tonic firing at 1 Hz where the 7th spike has been replaced by a burst of 3 spikes 7 msec apart as described in [24,25]. Panel **a** shows the individual spikes, each 3 msec long, and the burst of 3. Panel **b** shows the concentration of e5HT as a function of time in response to the spikes. Each spike causes the e5HT concentration to rise to 6 μM and the burst causes e5HT to rise to 16 μM. In Panel **c** we graph the area under the e5HT curve for the previous millisecond. For tonic firing this is flat at 0.768 nM since the frequency of the tonic spikes is 1 Hz, but at the burst the area goes up considerably to approximately 2.3 (μM × sec). This area is a good measure of the effect of the burst on the post-synaptic membrane since it includes not only the peak height of the e5HT curve but also how long the e5HT remains available to the post-synaptic membrane.

It is known from some of the early studies on humans and animals that depression is correlated with lower tissue levels of 5HT [18,54]. And, a number of studies have shown ([18,54,57]) that tryptophan depletion in the diet can lead to depression. Thus it is our working hypothesis that "depressed" patients have low levels of tissue 5HT. Since the cytosolic and extracellular 5HT concentrations are very low, most 5HT is in the vesicles, so if tissue 5HT is low, vesicular 5HT (v5HT) is low. To dramatically reduce v5HT in the model, we reduced the V_{max} of the tryptophan hydroxylase (TPH) reaction to 5% of normal (possibly corresponding to a polymorphism in the TPH gene). At steady state, this change reduces v5HT from 21.5 to 4.57 μM (see **Table 1**), or about 20–25% of normal. Note that v5HT has

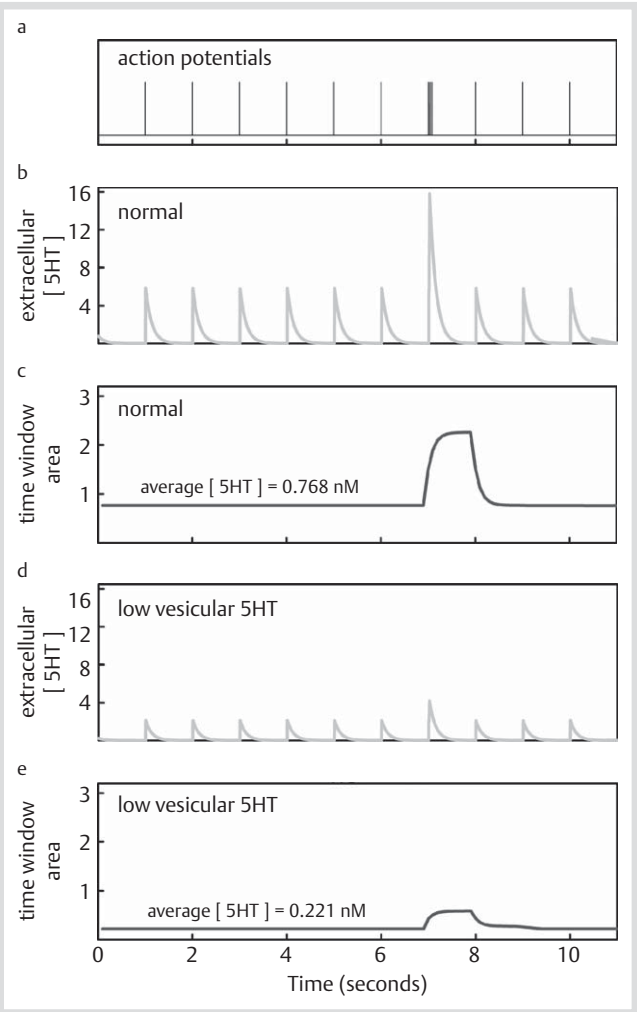

Fig. 3 Responses to spikes and bursts for normal and low vesicular 5HT. Panel **a** shows the timing of spikes; in the model 5HT is released into the extracellular space for 3 msec for each spike. The seventh spike is replaced by a burst of three, 7 msec apart (drawn slightly farther apart). Panel **b** shows the "normal" time course of e5HT in the extracellular space at the terminal in response to this pattern of firing. Panel **c** shows the area under the e5HT curve in Panel **b** over the previous 1 msec; this is a measure of impact of release on the post-synaptic membrane. Panels **d** and **e** show what the response curves in Panels **b** and **c** would look like for an individual with low vesicular 5HT (4.57 µM instead of 21.5 µM) caused by tryptophan depletion or a defect in tryptophan hydroxylase.

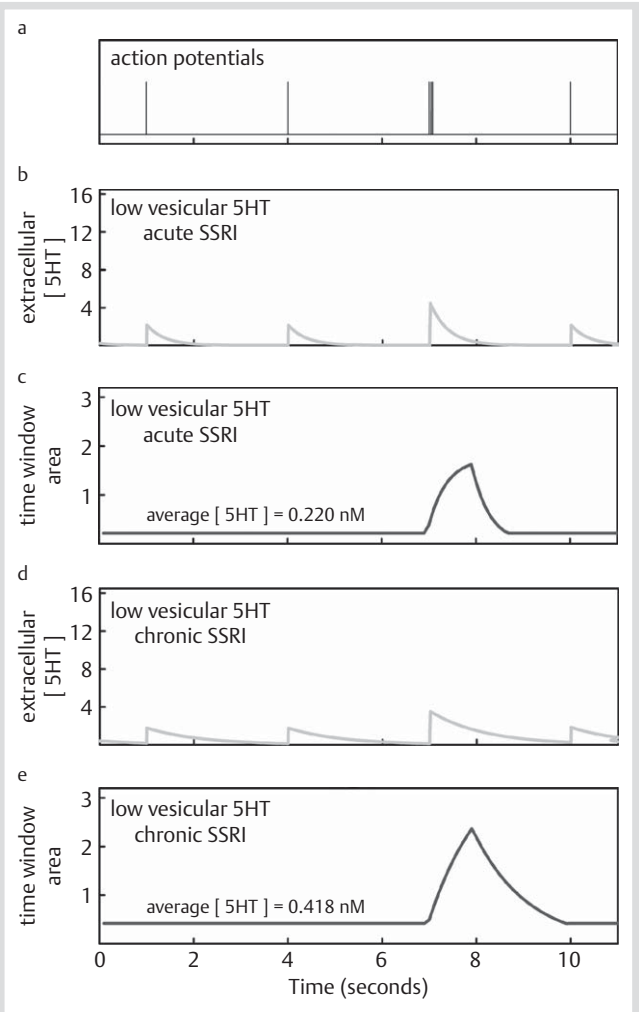

Fig. 4 Responses to spikes and bursts of the low vesicular 5HT patient after acute and chronic treatment with SSRIs. Panel **a** shows the timing of spikes; the DRN or MRN neuron is firing at 0.33 Hz in the model because of inhibition via the $5HT_{1A}$ autoreceptors. 5HT is released into the extracellular space for 3 msec for each spike. The third spike is replaced by a burst of three, 7 msec apart (drawn slightly farther apart). Panel **b** shows the time course of e5HT in the extracellular space at the terminal in response to this pattern of firing for the low vesicular 5HT patient who has been given an acute dose of an SSRI. Panel **c** shows the area under the e5HT curve in Panel **b** over the previous 1 msec during the burst; before and after the burst we graph the average e5HT due to tonic firing. Panels **d** and **e** show what the response curves in Panels **b** and **c** would look like for the same individual given chronic doses of SSRIs that further deplete the number of SERTs on the terminal membrane. The peak response to the burst is now back to normal (Panel **e**).

not declined to 5% of its normal value. This is because of the homeostatic effects of the terminal autoreceptors that increase synthesis if e5HT decreases (see [8]). We could also have lowered v5HT by lowering the amount of tryptophan available in the blood, or by a combination of tryptophan reduction and V_{max} reduction. For our discussions of the effects of the SSRIs below all that matters is that in depression there are lower than normal vesicular stores. The result of the lower level of v5HT is obvious and is evident in Panels **d** and **e** of ◉ **Fig. 3**. The response of e5HT to individual spikes and bursts is much smaller (Panel **d**), the steady state e5HT concentration is now 0.220 nM, and the curve representing the one second time integral of the e5HT curve now peaks at 0.6 µM × s during the burst.

We want to determine the acute effects of an SSRI dose on our model of a depressed patient. The SSRIs block a percentage of the SERTs depending on dose. We'll assume that the dose blocks 2/3 of the SERTs. In the model, this is equivalent to reducing the Vmax of the SERTs to 33% of normal (◉ **Table 1**). The SERTs on the DRN and MRN serotonergic cells are also blocked and this raises the e5HT concentration in the DRN and MRN which reduces the rate of tonic firing in the DRN and MRN [1, 12, 21]. We will assume that the rate of tonic firing is reduced from 1 Hz to 0.33 Hz. With these parameter changes (row 3 in ◉ **Table 1**), we obtain the results shown in Panels **b** and **c** of ◉ **Fig. 4**. The average extracellular concentration of e5HT at the terminal hardly changes at all. This is because the 2 acute effects of the SSRIs influence e5HT in the terminal region in opposite directions as discussed in Section II. The blocked SERTs on the terminal tend to raise e5HT, while the blocked SERTs in the DRN and MRN raise e5HT there, stimulating the $5HT_{1A}$ autoreceptors and

Table 1 Steady states values and parameters for simulations.

Status	s. s. v5HT	s. s. e5HT	TPH	tonic rate	SERTs
normal	21.5 µM	0.768 nM	100%	100%	100%
depressed	4.57 µM	0.221 nM	5%	100%	100%
SSRI acute	4.68 µM	0.220 nM	5%	33%	33%
SSRI chronic	3.26 µM	0.418 nM	5%	33%	10%

decreasing the tonic firing rate, which tends to lower e5HT in terminal regions. The balance between these competing effects depends on how many SERTs are blocked in the terminal region and how much the tonic firing rate is depressed in the DRN and MRN (and other factors, see the Discussion). Since the number of SERTs varies widely from terminal region to terminal region ([11,17]) and the number of $5HT_{1A}$ autoreceptors are thought to differ between the DRN and MRN [62], one would expect that the acute effects of SSRIs would raise e5HT in some terminal regions and decrease it in others. There is clear evidence for such differential effects of SSRIs on e5HT in different terminal regions including decreases ([13,35,41]).

Though the SSRI dose does not raise the average concentration of e5HT, it does raise the response to bursts considerably. Panel **c** of ▶ **Fig. 4** shows that the area under the e5HT curve now rises to 1.6 µM × s. This is much higher than 0.6 µM × s in Panel **e** of ▶ **Fig. 3** for the depressed patient without the SSRI, but not as high as 2.3 µM × s for the normal patient in Panel **c** of ▶ **Fig. 3**.

Several studies have found that chronic treatment with SSRIs reduces the number of SERTs in terminal regions. For example, Benmansour et al. [7] found that chronic treatment of rats with the SSRI sertraline caused an 80% decrease in SERTs on the membrane of cells in the CA3 region of the hippocampus. Mizra et al. [46] found a 40% decrease in the SERT density in mouse cortex after 28 day treatment with various SSRIs. Gould et al. [23] reported a 75–80% decrease in the SERT binding sites in the lateral nucleus of the amygdala, dentate gyrus, and dorsal raphe after 6 weeks of paroxetine administration to rats. Lau et al. [38] showed that exposure of 5HT neurons in culture to citalopram caused an internalization of SERT proteins from the cell surface but also induced a redistribution of SERT from neurite extensions into the soma.

To simulate the effect of the internalization of SERTs due to chronic treatment with an SSRI we kept the parameters the same as for acute SSRI treatment (row 3 of ▶ **Table 1**) except that we lowered the fraction of SERTs available for transport from 33% to 10%; see row 4 of ▶ **Table 1**. The results can be seen in Panels **d** and **e** of ▶ **Fig. 4**. The steady state level of e5HT rises considerably compared to acute treatment (Panel **c**) and compared to depressed patients with no treatment (Panel **e** of ▶ **Fig. 3**), but is still not back to the "normal" level (Panel **c** of ▶ **Fig. 3**). By contrast, the response to a burst has recovered completely (peak at 2.3 in Panel **e**) and is equal to the response to a burst of the normal individual (peak at 2.3 in Panel **c** in ▶ **Fig. 3**). Thus it is the chronic reduction of SERTs on the membrane in the terminal region that brings the response to bursts back to normal.

VI Discussion

There is much controversy about the mechanisms by which SSRIs have their therapeutic effect. In this paper we present a novel hypothesis about the efficacy of SSRIs. We propose that SSRIs bring the response to the burst firing of raphe nucleus cells back to normal, even though the average extracellular 5HT concentration remains low, and we present a realistic physiological mechanism by which this can occur. The thrust of our argument is that chronic doses of SSRIs can bring the response in terminal regions to DRN or MRN bursts back to normal for individuals with low vesicular 5HT. We explain why this can be true even though the chronic dosing has not returned the average extracellular 5HT concentration in terminal regions to normal. We used mathematical models for spike generation (Section IV) and our model for serotonergic terminals (Section V) to illustrate the steps in the argument. The arguments are suggestive but not conclusive, of course. Proof can come only from more electrophysiological and pharmacological studies. There are many other hypotheses for the efficacy of SSRIs including the norepinephrine reuptake hypothesis, the cytokine hypothesis, the hypothalamic-pituitary-thyroid hypothesis, and the brain derived neurotrophic factor hypothesis; for a review see [63].

The mathematical model [8] used for the simulations in Section V is based on physiological measurements. Some of the parameters of the model are likely to vary considerably in different projection regions. For example, it is known that the SERT density varies by about a factor of 5 in different brain regions [11,17]. For our simulations in this paper we use the parameters from [8]. The model should be regarded as a platform on which to try out ideas and hypotheses and to investigate how the parts of the system contribute to its overall regulation.

There is evidence [5,31,64,66] that the presence of an SSRI lowers vesicular 5HT. This fact is not discussed very much, perhaps because if depressed patients have low vesicular 5HT it seems unusual to give them a drug that lowers it still more. The reduction of vesicular 5HT in the presence of an SSRI also occurs in the model (see Table 4 in [8]) and the model can be used to understand this behavior. In the extracellular space, 5HT in the model has 3 fates. It can be returned to the cytosol by the SERTs, it can be metabolized by monoamine oxidase (MAO), and it can be removed from the system by uptake by glial cells and blood vessels or simply by diffusion out of the target tissue. Metabolism by MAO plays a very small role because the concentration of 5HT is so low in the extracellular space. The presence of the SERTs affects the balance between "reuptake" and "removal" because the longer the half-life of 5HT in the extracellular space, the more likely it is to be removed. If removal is higher and reuptake is lower, then at steady state there will be less v5HT because 5HT is being drained from the system more quickly. As the parameters are set in [8] and in this paper, removal takes away only about 2% of the released 5HT; almost all of the rest is returned to the cytosol. This corresponds to a terminal with a very high density of SERTs. This high density makes v5HT relatively insensitive to the blocking of SERTs. In Table 4 of [8] one sees that if 90% of the SERTs are blocked, then the steady state concentration of v5HT drops only from 21.5 µM to 17.05 µM. If a terminal has a lower density of SERTs, then removal could normally be much higher than 2%, perhaps as high as 10%–15%, in which case the blocking of SERTs would cause a much greater percentage decrease in the steady state value of v5HT. Since SERT density is known to vary between terminal regions by at least a factor of 5 [11,17,40], this may explain the differential effect of SSRIs on different terminal regions.

Given that SSRIs decrease the frequency of tonic spiking for serotonergic cells, one may ask whether bursts are not similarly affected. If bursts are intrinsically generated within DRN and MRN cells, then it is possible that there may be fewer bursts

when SSRIs are administered, although this has not been demonstrated experimentally. Even in the case of fewer bursts, we have provided in Section IV a simple illustration that the frequency of spikes within the burst may not be diminished; in Section V we have shown that, in that case, chronic dosing with SSRIs returns the efficacy of bursts to normal levels. On the other hand, it may be that bursts of serotonergic cells are driven by bursting afferents. SSRIs would not be expected to decrease either the frequency of bursts nor the frequency of spiking within the bursts of these non-serotonergic afferents.

Our examples in Section IV utilized previously published model cells and parameter values; we chose these models for their relative simplicity. Our purpose was to illustrate that slowed tonic firing need not imply slower firing within bursts. Indeed, Levine and Jacobs [39] showed that tonic firing and phasic responses may be decoupled for serotonergic neurons. The doublets and triplets in Figure 2 of [25] suggest more complex firing mechanisms than are captured in the 2 simple models we used. In future work, we will develop biophysical, conductance-based models of DRN and MRN cell electrophysiology. Such models will be useful in understanding the various firing patterns reported for serotonergic cells and how features of firing activity are affected by pharmacological manipulation.

We note that 30–50% of depressed patients do not respond to treatment by SSRIs [3,52]. There are several reasons why this may be the case. Such patients may have particularly low tissue serotonin, they may have other abnormalities in the serotonin system besides low tissue 5HT. Genetic polymorphisms, for example for SERT and TPH, are known to influence serotonergic signaling, and such patients may have particularly deleterious combinations of such polymorphisms.

Understanding the mechanisms of efficacy of the SSRIs is a daunting task. One has to understand mechanism and function on 4 different levels, genomic, biochemical, electrophysiological, and behavioral, but changes on each level affect function on the other 3 levels, and this makes the interpretation of experimental and clinical results very difficult. In addition, 5HT projections from the DRN and MRN go to myriad different brain regions [18]. 5HT influences dopaminergic signaling [22,45] and reward behavior [36] and may affect firing in the cerebral cortex by causing the release of glutamate [44]. 5HT may activate the hypothalamic-pituitary-adrenal axis by stimulating production of corticotropin-releasing hormone [27]. The endocrine system affects the 5HT system [9,58] and this may be the basis of gender differences in depression and response to SSRIs. And, finally, both gene expression and neuronal morphology are changing in time. In this circumstance, it is not surprising that many variables on all 4 levels are correlated with depression or to the efficacy of the SSRIs. All such correlations are candidates for causal mechanisms, so sorting out which mechanisms are causal is extremely difficult. The mechanism that we have proposed here suggests new approaches to the investigation of serotonergic signaling. This research may aid in the design of new and effective pharmacological agents.

Author's Contributions

All 3 authors (JB, MR, HFN) contributed equally to the formulation of the hypothesis, experimentation with the model, the biological interpretations and conclusions, and the writing and editing of the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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