



Serotonergic paradoxes of autism replicated in a simple mathematical model

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Summary The biological causes of autism are unknown. Since the early 1960s, the most consistent pathophysiological finding in autistic individuals has been their statistically elevated blood 5-hydroxytryptamine (5-HT, serotonin) levels. However, many autistic individuals have normal blood 5-HT levels, so this finding has been difficult to interpret. The serotonin transporter (SERT) controls 5-HT uptake by blood platelets and has been implicated in autism, but recent studies have found no correlation between SERT polymorphisms and autism. Finally, autism is considered a brain disorder, but studies have so far failed to find consistent serotonergic abnormalities in autistic brains. A simple mathematical model may account for these paradoxes, if one assumes that autism is associated with the failure of a molecular mechanism that both regulates 5-HT release from gut enterochromaffin cells and mediates 5-HT signaling in the brain. Some 5-HT receptors may play such a dual role. While the failure of such a mechanism may lead to consistent abnormalities of synaptic transmission with no alteration of brain 5-HT levels, its effects on blood 5-HT levels may appear paradoxical.

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Introduction

Autism is a pervasive developmental disorder whose biological causes are poorly understood despite decades of research [1]. Since the early 1960s, the most consistent pathophysiological finding in autistic individuals has been their statistically higher serotonin (5-hydroxytryptamine, 5-HT) levels in whole blood and blood platelets [2–7]. This finding has been difficult to interpret. Blood serotonin (released by enterochromaffin

cells) and brain serotonin (released by serotonergic neurons) are synthesized by two entirely different tryptophan hydroxylases [8], and very little 5-HT crosses the blood–brain barrier. While tryptophan, the main 5-HT precursor, does cross the blood–brain barrier, its levels are reported to be unaltered in autistic individuals [9]. In light of these data, it is not surprising that brain 5-HT synthesis (as reflected in CSF 5-hydroxyindoleacetic acid (5-HIAA) levels) may not be altered in autism [10]. However, it is generally agreed that autism is a disorder of the brain, not blood.

Two other related paradoxes have been well documented in the literature:

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1. Any autistic group always has individuals whose blood 5-HT levels are normal [3,5,11] irrespective of their level of mental retardation [5,11]. Despite this substantial overlap between autistic and control groups, the statistical mean of the 5-HT levels in an autistic group is usually significantly higher than the mean of a control (normal) group. However, the distribution of the 5-HT levels in an autistic group is not simply the control distribution shifted toward higher 5-HT values; instead, it has a broader "tail" extended toward higher 5-HT values [12] or even appears bimodal [11].
2. The serotonin transporter (SERT) gene has been recently suspected as a major player in the pathogenesis of autism [13]. A naturally occurring SERT promoter polymorphism is associated with 5-HT uptake rates in human blood platelets [14]. Therefore, it is possible that some SERT gene variants may lead to higher 5-HT levels in blood platelets and may occur more frequently in autism. While statistical analyses of the platelet 5-HT levels in SERT polymorphic variants have yielded somewhat inconsistent results [12,14–16], these same studies found no correlation between SERT polymorphisms and autism [12,14,16].

Here I present a simple and biologically realistic model that replicates these paradoxes. The model assumes that autism is associated with the failure of a molecular mechanism that plays a dual functional role by both regulating 5-HT release from enterochromaffin cells in the gastrointestinal system and mediating 5-HT signaling in the brain.

Model description

As arterial blood flows through the gastrointestinal system, it receives new 5-HT released from enterochromaffin cells. Most of this free 5-HT is taken up by cells expressing SERT in the liver, kidney, lungs and other organs [17–19] and is destroyed by monoamine oxidases (MAOs) [20,21]. The regulation of 5-HT release from enterochromaffin cells relies, at least in part, on 5-HT receptors and G-protein signaling cascades [22–24], which also are present in the brain [25–27].

A very simple model of this regulation is shown in Fig. 1. Let γ ($0 < \gamma < 1$) be the proportion of free 5-HT that is removed from the flow after the flow leaves the 5-HT synthesis system (gastrointestinal system) and before it re-enters this system. Let F be the 5-HT flow that exits the synthesis system.

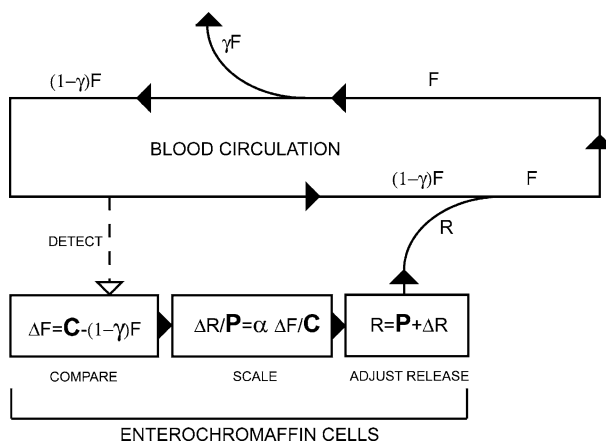


Figure 1 Diagram of the model showing the circulation of free 5-HT in blood (see the text for details).

Then $(1 - \gamma)F$ is the 5-HT flow that enters the synthesis system; let C be its normal ("expected") value. Assume that the 5-HT release rate (R) has a normal value of P , but it gets adjusted if $\Delta F = C - (1 - \gamma)F \neq 0$. A simple rule for this adjustment can be given by $R = P + \Delta R$, where $\Delta R/P = \alpha \Delta F/C$ and $\alpha > 0$.¹ Note that α (gain) reflects the strength of the feedback mechanisms. This feedback may be mediated by 5-HT_{1A}, 5-HT₂, 5-HT₃ and 5-HT₄ autoreceptors that can alter 5-HT release from enterochromaffin cells [22,24]. Also, indirect regulation mechanisms may be available for enterochromaffin cells [28]. The equilibrium state (steady state) of the system is then described by the following system of equations:²

¹ Another way to obtain R would be to calculate $x = \beta(C - (1 - \gamma)F)/C$ and plug it into a simple sigmoid function, such as $R(x) = 2P/(1 + e^{-kx})$, to obtain R . $R(x)$ is always positive and has a threshold but otherwise is almost linear. This linear part can be approximated by $R(x) = xP(k/2) + P$. By using this approximation, one again arrives at Eq. (1) if $\alpha = \beta k/2$. It is guaranteed that even with this linearization $R \geq 0$ and $R \leq P(\alpha + 1)$ at equilibrium (see Table 1).

² More precisely,

$$R_{n+1} = P + \alpha P(C - (1 - \gamma)F_n)/C \quad \text{and} \quad F_{n+1} = (1 - \gamma)F_n + R_{n+1}$$

and we demand that, at equilibrium, $R_{n+1} = R_n = R$ and $F_{n+1} = F_n = F$ for any n . This system of difference equations can also be written as a system of linear differential equations:

$$\tau \frac{dR}{dt} = -R + P + \alpha P(C - (1 - \gamma)F)/C$$

and

$$\tau \frac{dF}{dt} = -F + (1 - \gamma)F + P + \alpha P(C - (1 - \gamma)F)/C.$$

The equilibrium point of this system is an attracting node, since both eigenvalues of the system are negative ($\lambda_1 = -1/\tau$ and $\lambda_2 = -(\alpha P(1 - \gamma) + \gamma C)/\tau C$).

$$R = P + \alpha P(C - (1 - \gamma)F)/C \quad \text{and} \quad (1)$$

$$F = (1 - \gamma)F + R.$$

Solving the system yields

$$F = \frac{CP(\alpha + 1)}{P\alpha(1 - \gamma) + C\gamma} \quad (2)$$

and

$$R = \gamma F. \quad (3)$$

Note that $F, R \geq 0$ with any $\alpha > 0$.

We can obtain a “typical” value of γ (denoted γ^*) from the model itself. We assumed that, if the flow entering the synthesis system is normal (i.e., $(1 - \gamma)F = C$), the release rate needs no adjustment. But then, from Eq. (1), $R = P$ and $F = (1 - \gamma)F + P$. This yields

$$\gamma^* = P/(C + P). \quad (4)$$

It is natural to assume that the actual γ should be close to γ^* ; however, γ need not be equal γ^* for the system to reach an equilibrium. The distribution of γ around γ^* will be important in the following analysis.

Model parameters

In normal humans, the concentration of free 5-HT in distal venous blood has been theoretically and experimentally estimated to be around 0.153 ng/ml [20] or 0.77 nM [29]. Considerably higher values have been reported in some other studies; however, they are likely to overestimate the actual free 5-HT levels, as discussed in detail elsewhere [20,29–31]. Here, free 5-HT levels in venous blood were assumed to be 0.140 ng/ml, and free 5-HT levels in arterial blood were calculated to be around 0.175 ng/ml based on the model by Anderson et al. [20]. This concentration of 5-HT is similar to extracellular 5-HT levels in the brain [27,32] and is sufficiently high to activate 5-HT autoreceptors based on their reported affinities [33–35]. The arterial blood flow to the gastrointestinal tract var-

ies with food intake; here, the average flow was considered to be 1200 ml/min [20]. These estimates yield $C = (0.175)(1200) = 210$ ng/min. The production of 5-HT in the human gastrointestinal tract (P) has been estimated to be around 3000 ng/min [20]. From Eq. (4) we now obtain $\gamma^* = 0.93$. It should be pointed out that the obtained value of γ^* does not contradict the evidence that more than 99% of blood 5-HT is sequestered in platelets [5]. In fact, γ^* is the proportion of the free 5-HT that is removed from the flow in one circulation cycle (minutes), whereas platelets accumulate 5-HT over a long time (days).

In the present model, α is the only parameter whose value is chosen somewhat arbitrarily. We set the normal $\alpha > 1$ and assume that in autism the regulation of 5-HT release is consistently failing ($\alpha \leq 0.1$). It should be noted that the essential features of the model remain the same, as long as the autistic α is smaller than the normal (see Table 1). It is feasible, but difficult, to obtain experimental values of α , for which pharmacological manipulation of blood 5-HT levels may be of considerable value [36]. In any case, assuming the normal $\alpha > 1$ appears realistic. If, for instance, $\alpha = 5$, 5-HT release rate should double if the detected 5-HT levels are 20% below their pre-set value. In fact, enterochromaffin cells can triple their 5-HT synthesis in vitro [37] and they can double 5-HT release or reduce it by 50% in response to 5-HT receptor agonists [22].

Individual variability of γ

Recent studies suggest that γ varies from individual to individual. Individual 5-HT uptake rates are highly variable in human blood platelets (standard deviations of 50–80% of the mean have been reported), which may be at least partly due to the presence of several variants of the SERT gene in the population [12,14,16]. However, blood platelets remove only a small fraction of the 5-HT released by enterochromaffin cells [20,38] and,

Table 1 Properties of $(1 - \gamma)F$, R , and F as the functions of γ and α

	Direction when $\gamma \uparrow$	$\gamma = 0$	$\gamma = 1$	Direction when $\alpha \downarrow$
$(1 - \gamma)F$	↓	$C(\alpha + 1)/\alpha$	0	↑ if $\gamma < \gamma^*$ ↓ if $\gamma > \gamma^*$
R	↑	0	$P(\alpha + 1)$	↑ if $\gamma < \gamma^*$ ↓ if $\gamma > \gamma^*$
F	↓ if $C > P\alpha$ ↑ if $C < P\alpha$	$C(\alpha + 1)/\alpha$	$P(\alpha + 1)$	↑ if $\gamma < \gamma^*$ ↓ if $\gamma > \gamma^*$

therefore, their contribution to γ may be negligible. Nevertheless, the same SERT gene is also expressed in the lungs [39] and may also be expressed in the liver, kidneys and other organs [17,18], which together remove most of the 5-HT released by enterochromaffin cells. It can be expected, therefore, that their 5-HT uptake rates should also show considerable variability in the population. The importance of this peripheral variability for understanding mental disorders has been underappreciated [40]. The human gene encoding monoamine oxidase A also has several variants [41,42]. The actual statistical distribution of γ is unknown and depends on how these and other mechanisms interact to remove free 5-HT from blood circulation.

Model replicates autistic paradoxes in periphery

Blood platelets accumulate 5-HT over a relatively long time (days), and it has been suggested that they take up very little 5-HT released from enterochromaffin cells, before most of it is cleared by the liver, lungs and other organs [20]. It has also been suggested that, at equilibrium, platelet 5-HT levels depend almost linearly on the levels of free plasma 5-HT [20]. Therefore, platelet 5-HT levels can be approximated by $K[(1 - \gamma)F]$, where K is a constant.

In Fig. 2, the values of $(1 - \gamma)F$, R and F are plotted as functions of γ . It can be shown analytically that the relevant properties of these functions remain the same with any $\alpha > 0$ (Table 1).

As shown in Fig. 3(a), a decrease in α may elevate platelet 5-HT levels, but only in those individuals whose $\gamma < \gamma^*$. Individuals whose $\gamma = \gamma^*$ will have normal 5-HT levels irrespective of α . Individuals whose $\gamma > \gamma^*$ will have their blood 5-HT levels in the same range as normal individuals whose $\gamma > \gamma^*$. The model predicts, therefore, that the distribution of platelet 5-HT levels in autistic individuals (who have a lower than normal α) will have a longer “tail” extended toward higher 5-HT values, or that it may even appear bimodal. The model produces such distributions even if the “expected” values themselves (C and P) are allowed to vary across individuals (Fig. 3(b) and (c)). Such distributions have been consistently reported in human studies [3,5,11,12]. Since $\gamma = \gamma^*$ may be considered “typical”, more autistic individuals should have normal 5-HT levels than not, which is consistent with the evidence that only some 11–36% of autistic children are hyperserotonemic

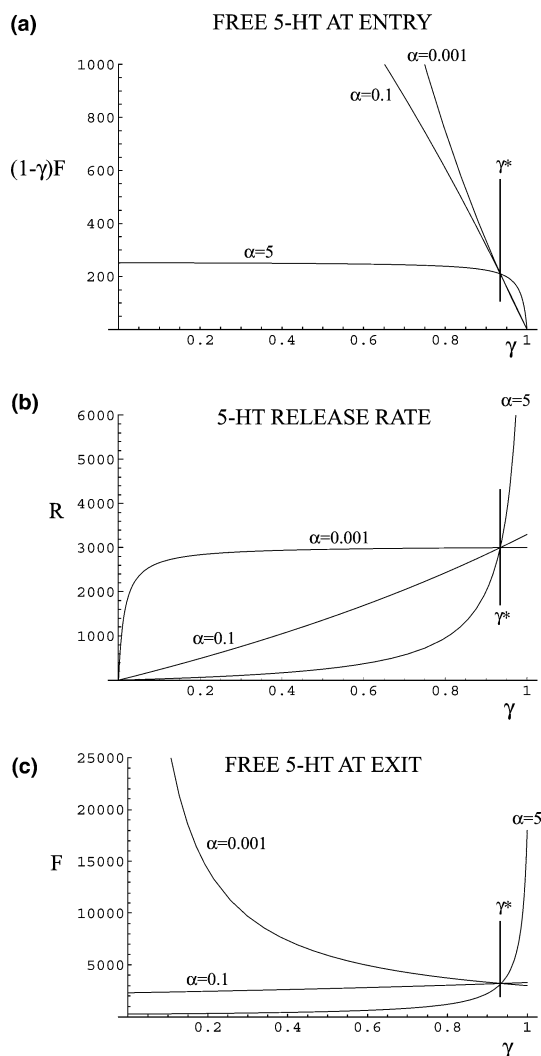


Figure 2 (a) The equilibrium 5-HT flow that enters the gastrointestinal system $[(1 - \gamma)F]$; (b) the 5-HT release rate in the gastrointestinal system $[R]$; (c) the 5-HT flow that exits the gastrointestinal system $[F]$, all plotted as functions of the 5-HT removal rate $[\gamma]$. The ordinate axis values are in ng/min. The functions are plotted with three α values: $\alpha = 5$ represents normal feedback, whereas $\alpha = 0.1$ and $\alpha = 0.001$ represent failing feedback in autism.

[2,3,11,12]. Nevertheless, the difference between the mean autistic and normal 5-HT levels is usually statistically significant, which is replicated in the model (Fig. 3). Furthermore, the model predicts that normal individuals with $\gamma < \gamma^*$ should have slightly elevated platelet 5-HT levels, but that these levels should be lower than those of autistic individuals (Fig. 3(a)). In fact, one study has reported that some SERT gene variants (one component of γ) elevate the blood 5-HT levels in normal and autistic individuals, but only autistic individuals become hyperserotonemic [12]. The model also

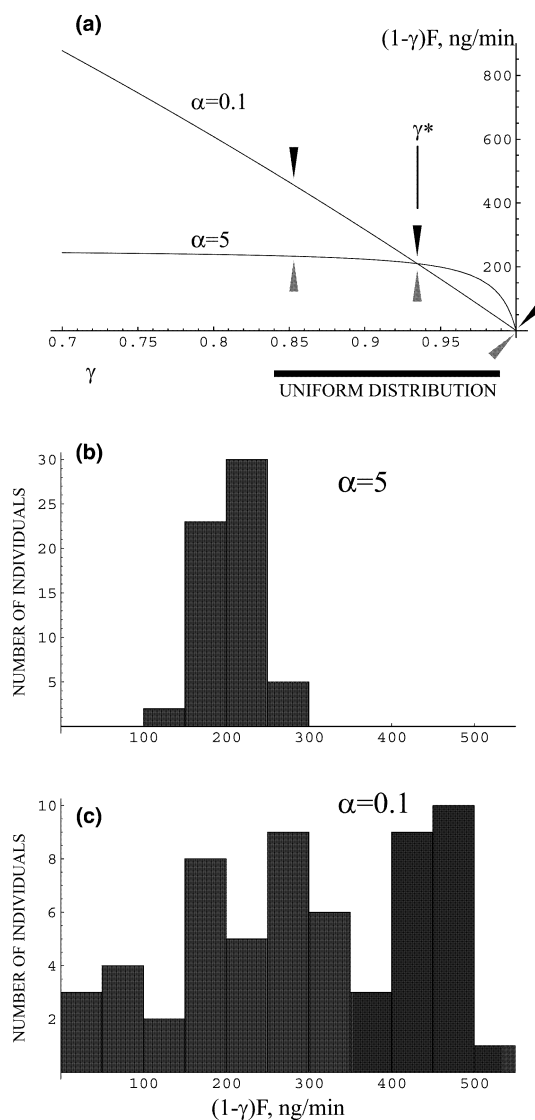


Figure 3 Simulated sampling of blood 5-HT levels in control and autistic individuals. (a) The levels of free 5-HT after most of the 5-HT released by enterochromaffin cells has been cleared by the liver, lungs and other organs. As discussed in the text, platelet 5-HT levels can be approximated by $K[(1-\gamma)F]$, where K is a constant. The control (normal) condition is represented by $\alpha=5$ and the autistic condition by $\alpha=0.1$. In both groups, γ was assumed to have a continuous uniform distribution in the interval $[0.84; 0.99]$. A uniform distribution was assumed for γ , because different SERT and MAO activities may have similar population frequencies, as suggested by the reported frequencies of some SERT polymorphic variants [54]. (b, c) Simulated sampling of 60 control ($\alpha=5$) and 60 autistic ($\alpha=0.1$) individuals (the simulation was run in Mathematica 5 (Wolfram Research, Inc.)). The parameters C and P were assumed to vary across individuals with Gaussian distributions [mean, standard deviation] that were $[210, 20 \text{ ng/min}]$ and $[3000, 100 \text{ ng/min}]$, respectively. The variables γ , C and P were assumed to be statistically independent. Note that the autistic distribution overlaps with the control distribution, but that the autistic distribution extends toward higher 5-HT values and appears to have a few (false) peaks. A bona fide multimodal distribution can be generated by the model if the distribution of γ is assumed to be discrete (not shown). Such an assumption may be biologically valid, given the discrete polymorphic variants of the SERT and MAO genes. In the present simulation, the mean autistic levels (299 ng/min) were 46% higher than the mean control levels (205 ng/min), and this difference was highly significant ($P < 0.001$, the unpaired t -test and the Kruskal–Wallis test). This increase is in good agreement with experimental data, where the median increase is about 50% [5]. Note that due to the shape of the curves to the right of γ^* (a), an autistic group may have occasional individuals whose blood 5-HT levels will be lower than the 5-HT levels in a control group. Such cases have been reported in some studies [2,43]. The distributions in (b) and (c) are in good agreement with the actual control and autistic distributions reported in Fig. 1 of [11].

predicts that 5-HT levels depend on γ and that γ may contribute to hyperserotonemia if α is low; however, the model assumes that autism is caused by a low α and not by abnormal values of γ . In the model, normal and autistic individuals have identical γ distributions and, therefore, no causal relationship between autism and γ exists. This is consistent with actual observations. Even though earlier reports have implicated the SERT gene in autism [13], recent studies have failed to find correlation between SERT gene variants and autism [12,14,16]. Similarly, no correlation has been found between polymorphisms of MAO-A and autism [41,42]. Also, no correlation has been found between the activities of MAO-B (which may reflect its different polymorphic variants) and autism [43].

The model makes other, more subtle predictions. If γ is distributed around γ^* , autistic platelet 5-HT levels should show more population variability than normal platelet 5-HT levels, as already discussed (Fig. 2(a)). In contrast, autistic 5-HT release from enterochromaffin cells should be less variable than normal 5-HT release (Fig. 2(b)). Also, if γ is spread on both sides of γ^* , autistic 5-HT release rates will fall within the normal range, but will be skewed toward higher values for those individuals whose $\gamma < \gamma^*$ (Fig. 2(b)). This subtle difference between autistic and normal groups may escape statistical detection. Since $R = \gamma F$, 5-HT release rate can be assessed by measuring urinary levels of 5-HIAA, a major 5-HT metabolite. A number of studies have failed to find significant differences between autistic and normal urinary 5-HIAA levels when the autistic group was studied as a whole [2,9,43]. However, if we chose only those autistic individuals whose $\gamma < \gamma^*$, their 5-HT release rate would be always higher than the normal 5-HT release rate (Fig. 2(b)). These are the same autistic individuals whose platelet 5-HT levels are higher than normal (Fig. 2(a)). In agreement with this prediction, higher urinary 5-HIAA levels have been reported in the hyperserotonemic subgroup of autistic individuals compared with normal controls (no difference has been found when the autistic group was treated as a whole). Interestingly, this same study has reported lower standard deviations of the urinary 5-HIAA levels in the autistic group compared with the control group, but higher standard deviations of the whole blood 5-HT levels in the same autistic group compared with the control group [9]. While this is consistent with the model's prediction, no published reports have rigorously examined differences between the standard deviations (as opposed to the means) of the 5-HT and 5-HIAA levels in normal and autistic groups. Finally, the relationship between $(1 - \gamma)F$ and $R = \gamma F$ is

non-linear in the model, so it is not surprising that no significant correlation has been found between blood 5-HT levels and urinary 5-HIAA levels [9].

Model suggests the nature of an abnormality in the autistic brain

As discussed in the previous section, the distributions of peripheral 5-HT appear rather complex and, when observed in an experimental study, may even suggest that the autistic group is heterogeneous. Contrary to this intuition, these distributions can be obtained from the model by assuming that all normal individuals have one high value of α and all autistic individuals have one low value of α , i.e., that normal and autistic groups are perfectly homogeneous with respect to α . This suggests that autism may be associated with the low α value, itself, as opposed to how it alters the peripheral 5-HT system. What does it mean biologically?

By definition, α reflects the strength of the feedback mechanisms that regulate 5-HT release from the gastrointestinal system. These feedback mechanisms may be represented, at least in part, by 5-HT_{1A}, 5-HT₂, 5-HT₃, 5-HT₄, and perhaps other 5-HT receptors [22]. These same receptors are expressed in the brain, where they play important roles in brain development [44–46] and in synaptic transmission. Therefore, their abnormal expression in the gastrointestinal systems is likely to be accompanied by their abnormal expression in the brain, which may lead to consistent cognitive defects. This abnormal expression in the brain may not alter brain 5-HT levels, because all of these 5-HT receptors can be expressed postsynaptically, where they do not directly regulate 5-HT release from serotonergic neurons. Even those 5-HT receptors that have been thought to function as autoreceptors in the brain (5-HT_{1A}, 5-HT_{1B}) may not inhibit 5-HT release unless extracellular 5-HT levels become excessive [27,47]. This is consistent with the observation that the tissue 5-HT levels are not altered in a 5-HT_{1A} knockout mouse [48]. The 5-HT_{1A} receptor has been suggested to be important in autism [5,49], but its actual role remains unclear.

In light of these arguments, the 5-HT receptors that act as autoreceptors in the gastrointestinal system but are expressed exclusively postsynaptically in the brain may deserve special attention. One such example is 5-HT₂ receptors that may regulate 5-HT release from enterochromaffin cells [22], but have no known autoreceptor function in

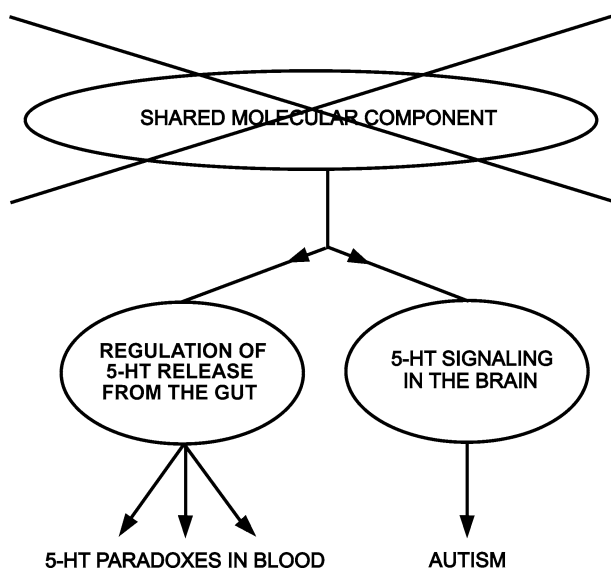


Figure 4 Autism may be associated with the failure of a molecular mechanism that participates both in the regulation of 5-HT release from enterochromaffin cells in the gastrointestinal system, and in 5-HT neurotransmission in the brain.

the brain [27]. Therefore, their abnormal expression is unlikely to directly alter brain 5-HT levels; nevertheless, it may lead to severe cognitive defects, as evidenced by research into schizophrenia [50]. Remarkably, several lines of evidence do strongly implicate 5-HT₂ receptors in autism [51–53].

In conclusion, a number of autistic serotonergic “paradoxes” observed experimentally may be explained if one assumes a failure of a molecular mechanism that both regulates 5-HT release from the gastrointestinal system and participates in 5-HT signaling in the brain (Fig. 4). While 5-HT receptors may be the most obvious candidates, other molecular mechanisms can satisfy these criteria, as well. It is important to consider these possibilities, given our very incomplete knowledge about the regulation of 5-HT release from enterochromaffin cells and serotonergic neurons [27,28]. Nevertheless, the model imposes rather strict constraints on the mechanisms that may be involved in autism, and, therefore, may be helpful in guiding future experimental and theoretical studies.

Conclusion

The model demonstrates that some apparent serotonergic paradoxes in autism research can be accounted for if one assumes that autism is associ-

ated with a failure of a molecular component that both (1) regulates 5-HT release from enterochromaffin cells in the gastrointestinal system and (2) participates in 5-HT signaling in the brain. Genes that code for proteins that have such dual functions are especially likely to play a role in autism.

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