From reversible to irreversible bistable switches via bifurcations in a gene regulatory network

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Abstract

The interplay of small, noncoding microRNAs (miRNAs), mRNAs and proteins plays crucial roles in almost all cellular processes. MiR-124, widely known as a memory-related miRNA, can regulate LTM by binding to the mRNA of CREB1 stimulated with 5-HT. In this paper, we establish a regulatory network model of CREB1 and miR-124 stimulated by 5-HT, in which miR-124 inhibits CREB1, which in turn enhances miR-124. Our model validates three protocols based on 5-HT in experiments on the induction of LTM in Aplysia. A steady-state analysis and numerical bifurcations of the abstracted system beyond memory formation, when the fast reaction has been in the equilibrium, can facilitate more abundant dynamical behaviors such as bistability and oscillation. The original system also exhibits bistability under appropriate feedback strengths, which is relevant to the mechanism of LTM formation. Furthermore, we specifically show a change in the transition from a reversible switch to an irreversible switch via bifurcations of the negative regulation of miR-124 on CREB1, which eventually maintains a high phosphorylated CREB1 level after initially elevated by 5-HT. These findings indicate that miR-124 provides an inhibitory constraint on long-term synaptic plasticity through the regulation of CREB1.

1. Introduction

Induction of long-term memory (LTM), as well as consolidation of newly acquired memory, requires both RNA and protein synthesis [1, 2] by activation of transcription factors (TFs). The gill-withdrawal reflexes of Aplysia after long-term sensitization (LTS) is a simple form of LTM that is used extensively to study cellular and molecular mechanisms of LTM and are possibly connected with serotonin (5-HT)-induced long-term facilitation (LTF) of synaptic connections between sensory neurons and motor neurons. Cyclic AMP (cAMP)-response element-binding protein (CREB) TFs are crucial for the regulation of the gene expression required for LTF and LTM [3–5]. Moreover, treatment of ganglia with five pulses of 5-HT, a protocol commonly used to induce LTF [6, 7], led to enhanced recruitment of the transcription activator CREB1 to its promoter [8]. These observations suggest that the presence of a positive feedback loop in which CREB1 regulates its own expression, results in a prolonged increase in CREB1 expression and a consequent increase in CREB1-mediated transcription that could extend beyond LTF induction [3].

At the molecular level, LTM formation requires the recruitment of multiple regulators, among which are microRNAs (miRNAs), small non-coding RNAs that are vital post-transcriptional regulators of gene expression that control key components of memory formation [9]. The most abundant neuron-specific miRNA, miR-124, as a suppressor of CREB-dependent transcription has a role in a simple form of memory in invertebrates [10–12]. A previous study has also demonstrated that miR-124 recognizes specific binding sites in the 3'UTR (untranslated region) of the CREB1 mRNA in the neurons of Aplysia [11, 13, 14]. Additionally, miR-124 is downregulated by 5-HT, which in turn enhances various components with synaptic plasticity by regulating the expression of CREB1 [6, 11].

These observations imply the existence of considerable cross-talk between the miRNA-mediated post-transcription layer and the transcriptional regulation
layer, which dominates the production of protein-coding mRNAs as TFs. These precedents led us to discuss how miR-124 mediates the CREB1 cascade or directly modulates the expression of CREB1 that is stimulated by 5-HT. As a result of our inquiry, mathematical models integrating large amounts of experimental data were essential for discovering the miRNA regulation pathway.

In this paper, using mathematical modeling of the simple architecture abstracted from the complex network between miR-124 and CREB1, we characterized the regulation between CREB1 and miR-124 stimulated by 5-HT, in which miR-124 inhibits CREB1, which in turn enhances miR-124. We validated the model by three stimulation protocols that were used to simulate experiments of the induction of LTM in Aplysia using 5-HT. The bifurcation analysis beyond memory formation indicated that the abstracted system displays diverse dynamics, including bistability and oscillation. However, bifurcations of the original system only repeated bistability under proper feedback strength, which is relevant to LTM formation. Moreover, we altered the levels of miR-124 to perform bifurcation analyses and found that the decrease in miR-124 can trigger a transition from a reversible bistable switch to an irreversible bistable switch and further maintain the high pCREB1 levels increased by 5-HT.

2. Model

Gene regulatory networks have been investigated in mathematical modeling for memory formation in Aplysia but regulations related to miRNAs have not been considered in previous models. Here, we established a regulatory motif of CREB1 and miR-124 stimulated by 5-HT for the induction of LTM. Since there are several putative CREB binding sites in the presumed promoter region upstream of the Aplysia miR-124 gene, biologists have suggested that CREB may be able to regulate miR-124 expression levels. Thus, we assumed that the transcriptional activator CREB1 promotes miR-124 gene transcription. We focused on a particular network motif (see figure 1): a single miRNA-mediated negative feedback loop in which CREB1 (TF) activates the miR-124 (miRNA) gene but CREB1 is negatively regulated by miR-124. Interval applications of five pulses of 5-HT to the sensory neurons reduced the expression of several miRNAs, especially mature miR-124 [11]. Additionally, injections of 5-HT increased CREB1 protein levels and contributed to its phosphorylation [3, 6]. We considered serotonin by defining a function for the 5-HT promotion of CREB1 phosphorylation through $k_5$ and $K_5$ and a function of 5-HT inhibition of miR-124 through $K_{5-HT}$ and $\lambda$.

We denote the levels of CREB1 mRNA and miR-124 mRNA in the cell as $m_{CREB1}$ with a translation rate $\gamma$ and miR-124, respectively. The roles of phosphorylated CREB1 (pCREB1) in the transcription of the CREB1 and miR-124 genes can be described as $g_{a_1} = \frac{V_1 [pCREB1]^2}{[pCREB1]^2 + K_1}$ and $g_{s_1} = \frac{V_2 [pCREB1]^2}{[pCREB1]^2 + K_2}$, respectively, where $V_1$ and $V_2$ are the feedback strengths between mRNA and miR-124 genes with sufficient pCREB1, and $K_1$ and $K_2$ are two dissociation constants of two complexes of pCREB1 from the promoter regions of the CREB1 and miR-124 genes, respectively. $g_{a_0}$ and $g_{s_0}$ illustrate the basic transcription rates of the CREB1 and miR-124 genes; therefore, the total transcription rates of the CREB1 and miR-124 genes, $g_a$ and $g_s$, can be described as $g_a = g_{a_0} + g_{a_1}$ and $g_s = g_{s_0} + g_{s_1}$. The degradation rates of miR-124, $d_{m_{CREB1}}$, and CREB1 are defined by $d_a$, $d_m$, and $d_s$, respectively. The rate of CREB1 phosphorylation promoted by 5-HT
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3. Results

3.1. Verification of the three experimental protocols under 5-HT stimulation

A large body of experimental evidence suggests that the sustained high level of pCREB1 is often associated with the formation of LTM and is dependent on the input from 5-HT signaling after only five, not one or three, short pulses [3, 18]. Moreover, with exposure to five spaced pulses of 5-HT, the level of miR-124 begins to decrease, then slowly reaccumulates and finally returns to baseline for a long time [11]. The three stimulus protocols for one (a), three (b) and five (c) short pulses, as presented in figure 2, were applied in our model to verify the experimental results. 5-HT induces a transient decrease in [miR-124], which then returns to its original level after stimulation. As shown in figure 2(c), the five pulses of 5-HT induced low levels of [miR-124] for a much longer time than the pulses depicted in figures 2(a) and (b). Indeed, only five short pulses induced high levels [pCREB1], while one or three short pulses failed to induce it, as shown in figure 2.

According to the one-parameter bifurcation analysis shown in figure 3, a one-way irreversible switch is induced by a transition from bistability to monostability with increasing 5-HT. We can see from figure 3(a) that the concentrations of miR-124 are quite high and at similar levels for both stable steady states at [5-HT] = 0 μM, but they are very low for the monostable state at [5-HT] = 10 μM. That makes the miR-124 level return to the baseline within 12 h after 5-HT exposure, which is consistent with the experimental findings in [11]. However, the pCREB1 levels were considerably different for the two stable steady states at [5-HT] = 0 μM, and the [pCREB1] is always maintained at the high steady state, even after stimulation, as shown in figure 3(b).

### 3.2. Bifurcation analysis of the regulatory mechanism between pCREB1 and miR-124 without 5-HT

#### 3.2.1. A steady-state analysis of the abstracted system

MiR-124, as a suppressor of CREB-dependent transcription, is important for LTM. Here, we first aimed to find a regulatory mechanism for CREB1 and miR-124 in our model by setting [5-HT] = 0 μM. The phosphorylation of the TF was rapid relative to other timescales in the system [19]. Therefore, we presumed that the fast reaction indicated a prior state of equilibrium, that is, $\frac{dx}{dt} = 0$. Furthermore, we substituted $x$, $y$, $z$ and $w$ for levels of [CREB1], [miCREB1], [miR-124] and [pCREB1] in a cell, respectively, to obtain the abstracted system described herein.
Figure 2. Time courses of [miR − 124] (the second column) and [pCREB1] (the third column) under different stimulus protocols using 5-HT (the first column): one pulse (a), three pulses (b), and five pulses (c) of 10 µM 5-HT for 5 min and the inter-pulse interval (from the end of one pulse to the onset of the next) of 15 min.

Figure 3. Bifurcation diagrams of [pCREB1] (a) and [miR − 124] (b) versus [5-HT]. The stable and unstable states are represented by red solid lines and black dashed lines, respectively. Saddle-node bifurcation points are marked as SN.

After a nondimensionalizing process, equations (5)–(7) can be rewritten as follows,

\[
\frac{dx}{dt} = \gamma y - d_0 x, \tag{8}
\]

\[
\frac{dy}{dt} = g_0 \frac{m + \beta x^2}{m + x^2} - d_m y - \delta yz, \tag{9}
\]

\[
\frac{dz}{dt} = g_0 \frac{n + \alpha x^2}{n + x^2} - d_s z - \delta yz, \tag{10}
\]

where \(\alpha = 1 + \frac{V_2}{V_1}, \beta = 1 + \frac{V_1}{V_2}, K = \frac{k_1}{k_2}, m = \frac{k_1^2}{k_2}, n = \frac{k_2^2}{k_1}.

We computed the overall steady state response of the abstracted system (8)–(10) to reveal the regulation of CREB1 and miR-124. Specifically, we set the right-hand sides of equations (8)–(10) equal to zero and solved for the roots in the algebraic equations.

Then, a relation of \(x^*, y^*\) and \(z^*\) was obtained:

\[
y^* = \frac{d_0}{\gamma} x^*, \tag{11}
\]

\[
z^* = \frac{g_0 \frac{m + \alpha x^2}{m + x^2} - d_m y^*}{d_s + \delta y^*} = \frac{g_0 \frac{m + \alpha x^2}{m + x^2} - d_m \frac{d_0}{\gamma} x^*}{d_s + \delta \frac{d_0}{\gamma} x^*}, \tag{12}
\]

\[
z^* = \frac{g_0 \frac{n + \alpha x^2}{n + x^2}}{d_s + \delta \frac{d_0}{\gamma} x^*} \tag{13}
\]

where \(x^*, y^*\) and \(z^*\) represent the steady states of \(x, y\) and \(z\), respectively. \(\alpha, \beta > 1\).

According to equation (13) and \(\alpha \geq 1\), for all \(x^* \geq 0\),

\[
z^* = \frac{g_0 \frac{n + \alpha x^2}{n + x^2}}{d_s + \delta \frac{d_0}{\gamma} x^*} > \frac{g_0}{d_s + \delta \frac{d_0}{\gamma} x^*}.
\]
Thus, \( z^* \) monotonically decreases with respect to \( x^* \); i.e., the steady states of CREB1 and miR-124 change in the opposite direction. This confirms that miR-124 has a negative regulation on CREB1, in accordance with observations in Aplysia, for which increasing (or decreasing) the levels of miR-124 in sensory neurons can cause a significant reduction (or increase) in LTF [11].

As equation (12) is equivalent to equation (13), we obtain

\[
g_0 \frac{\frac{m + 2x^2}{m + x}}{\delta x} - d_m \frac{d}{\gamma} x^* = g_0 \frac{\frac{n + x^2}{m + x}}{\delta x} - d_l + \frac{\delta}{\gamma} x^*. 
\]

For simplification, we let \( M = \frac{d_m}{d_{sa}}, S = \frac{d_s}{d_{sa}}, D = \frac{d_l}{\delta}, K = \frac{d}{\gamma}. \) Then equations (11)–(13) were simplified into a single quartic equation of the form

\[
x^4 + a_3 x^3 + a_2 x^2 + a_1 x + a_0 = 0, \tag{14}
\]

where \( a_0 = \frac{DM}{K}, a_1 = \frac{D+SM}{K}, a_2 = 1 - \frac{DM+S}{K}, a_3 = \frac{-DM}{K}. \)

Notably, the number of solutions for equation (14) changes with the different values of the parameters on which the stability of system (8)–(10) depends. Here we focus on the changes to stability in the abstracted system by adjusting the values of the parameters \( \alpha \) and \( \beta \) (see figure 4). The red and the blue lines indicate the saddle-node bifurcation points and Hopf bifurcation points, respectively, in the two-parameter plane \( (\alpha, \beta) \) in figure 4(a). The monostable region largely covers the top and bottom parts, while the bistable region located in the middle part becomes narrower and narrower before it switches to the oscillatory region via a Hopf bifurcation with the increasing \( \alpha \) and \( \beta \).

Bifurcation diagrams of \( x \) versus \( \alpha \) at different values of \( \beta \) are shown in figure 4(b). When \( \beta = 54 \) (figure 4(b1)), the system is bistable at \( \alpha = 0 \). As \( \alpha \) is increased, a saddle and a high stable steady state collide and disappear via a saddle-node bifurcation (SN2). When \( \beta = 68 \) (figure 4(b2)), an unstable limit cycle appears through a subcritical Hopf bifurcation at sub-\( H \) on the upper branch, which grows gradually with decreasing \( \alpha \) and then collides with a saddle at a homoclinic bifurcation (HC) point on the middle branch. For the case of \( \beta = 75 \) (figure 4(b3)), the system is monostable at \( \alpha = 0 \), and a high steady state

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**Figure 4.** Bifurcation diagrams of the abstracted system (8)–(10). (a) Bifurcation diagram in the \((\alpha, \beta)\) parameter plane. Red and blue lines depict the loci of saddle-node bifurcation points and Hopf bifurcation points, respectively. (b) Bifurcation diagrams of \( x \) versus \( \alpha \) at six values of \( \beta \). Stable and unstable steady states are represented by red solid lines and black dashed lines, respectively. For the stable and unstable limit cycles, the maximum and minimum values of \( x \) are denoted by blue solid circles and green open circles, respectively. Bifurcation points are marked as SN, saddle-node bifurcation point; sup-H, supercritical bifurcation point; sub-H, subcritical bifurcation point; HC, homoclinic bifurcation point; LPC, fold limit cycle bifurcation point; and SNIC, saddle-node-invariant-circle bifurcation point. (b1) \( \beta = 54 \), (b2) \( \beta = 68 \), (b3) \( \beta = 75 \). (b4) \( \beta = 105 \), (b5) \( \beta = 122 \). (b6) \( \beta = 132 \). Other parameter values \( \gamma = 0.02, d_s = 0.06, g_{sa} = 0.06, g_{sh} = 0.4, m = 0.5, n = 0.62, d_m = 0.02, d_l = 0.02 \), and \( \delta = 0.03 \).
transits to three steady states via a saddle-node bifurcation (SN1) and further to a low stable steady state via another saddle-node bifurcation (SN2). An unstable limit cycle generated by a subcritical Hopf bifurcation (sub-H) grows increasingly and then vanishes at HC. At $\beta = 105$ (figure 4(b4)), a saddle and a low stable steady state collide, and a stable limit cycle appears at the same time through a saddle-node invariant circle bifurcation (SNIC). The stable limit cycle grows with decreasing $\alpha$ and then collapses with an unstable limit cycle through a fold limit cycle bifurcation (LPC). At $\beta = 122$ (figure 4(b5)), a stable and an unstable limit cycle appear pairwise at an LPC. Then the unstable limit cycle shrinks with increasing $\alpha$ and the stable steady state loses stability through a subcritical Hopf bifurcation (sub-H), while the stable limit cycle persists. Furthermore, the unstable steady state becomes stable again and another unstable limit cycle emerges at another subcritical Hopf bifurcation (sub-H). Eventually, the unstable limit cycle collides with the stable limit cycle via another LPC. Similarly, a stable and an unstable limit cycle appear pairwise at an LPC when $\beta = 132$ (figure 4(b6)). However, in contrast to figure 4(b5), the stable limit cycle shrinks to a supercritical Hopf bifurcation (sup-H) point with increasing $\alpha$, and the unstable steady state becomes stable.
Figure 7. Bifurcation diagram of the original system in the 
\((V_1, V_2)\) parameter plane. Red lines depict the loci of the 
saddle-node bifurcation points.

The above analysis shows that the abstracted system possesses rich dynamic behaviors and various bifurcation phenomena with changing parameters, which is beyond memory formation as potential biological significance of oscillations in memory formation is unclear. Next, we need to continue with the original system to explore the dynamics and diverse bifurcations. As \(\alpha = 1 + \frac{V_1}{\kappa_0}\) and \(\beta = 1 + \frac{V_2}{\kappa_0}\), the parameters \(V_1\) and \(V_2\) of the original system (1)–(4) are considered in the bifurcation analysis below.

3.2.2. Bifurcation analysis of the original system

Here, we focus on the bifurcation analyses of the original system (1)–(4) for the two parameters \(V_1\) and \(V_2\) to measure the feedback strength of CREB1 self-induced activation and the strength of miR-124 activation by pCREB1, respectively. Saddle-node bifurcation points SN1 and SN2 of the parameter \(V_1\) enclose a bistable region of two stable equilibria with a low and a high value of pCREB1, as shown in figure 5(a1). Two branches of stable states monotonically increase with \(V_1\), which is consistent with the fact that increased production of the CREB1 protein is induced by the increase in the rate of CREB1 transcription. The above results confirm that the synthesis process of CREB1 with a positive feedback loop can create switching behaviors [3]. At the same time, miR-124 changed in an opposite manner to pCREB1 in terms of the parameter \(V_1\), as shown in figure 5(a2), which is evidence of the negative feedback regulation of miR-124 on CREB1.

In contrast to the states described above, the bistability of two stable steady states transits to a single state of monostability via saddle-node bifurcation (SN2) as the parameter \(V_2\) increases, as shown in figures 5(b1) and (b2). Bistability at \(V_2 = 0\), indicating that pCREB1 does not enable the promotion of the transcription of the miR-124 gene, is a self-promoting positive feedback loop. Furthermore, through the irreversible switching from the bistability to monostability via SN2, as shown in figures 5(b1) and (b2), pCREB1 must remain at the low steady state, but miR-124 at the high steady state is over the critical value at SN2. Actually, the promotion of pCREB1 to induce miR-124 gene transcription prompts an increasing amount of miR-124 to repress the transcription of CREB1 mRNA by forming the mCREB1–miR-124 complex and further reducing the CREB1 level.

Figure 5 shows two steady states with bistability in specific ranges of \(V_1\) and \(V_2\). However, in certain ranges of \(V_1\) and \(V_2\), the unstable states of miR-124 decrease below both the stable states but not between the two stable states. Therefore, it is of great biological significance that a stimulus can push miR-124 from one stable state through the stable manifold of the saddle to get into the attraction domain of the other stable state, regardless of the location of the saddle (the unstable state) between or below these two stable states.

The bistable regions, shown in figure 5, indicate that the system may switch from a low state to a high state after the application of a stimulus. However, the pulses of 5-HT required to generate a sustained high state of [pCREB1] depend on the parameters in the model, so we give the number of pulses needed at different values of parameters \(V_1\) and \(V_2\), as shown in figures 6(a1) and (b1). Figure 6(a1) illustrates that the number of pulses of 5-HT needed to maintain the high steady state of [pCREB1] gradually decreases with increasing \(V_1\); for example, six pulses are required for \(V_1 = 3.1\) but a single pulse is needed for \(4.7 \leq V_1 \leq 4.9\). In contrast, the pulse numbers increase with increasing \(V_2\), as shown in figure 6(b1). Furthermore, transient concentrations of pCREB1 at the termination point for the stimulations based on the different values of the parameters \(V_1\) and \(V_2\) in figures 6(a1) and (b1) are exhibited in figures 6(a2) and (b2) (blue stars), respectively. [pCREB1] tends to increase to the high state, where it persists for a long time after stimulation, as [pCREB1] has entered the attracting basin of the high steady state.

The two-parameter bifurcation diagram in the \((V_1, V_2)\)-parameter plane in figure 7 exhibits a bistable region, showing that the original system can switch between monostability and bistability by regulating the parameters \(V_1\) and \(V_2\). Biologically, the existence of bistable steady states is important for LTM formation, when a brief stimulus may induce persistent high level at the high steady state. However, compared with that in the abstracted system, no oscillatory region appears in the \((V_1, V_2)\)-parameter plane in the original system (1)–(4) in figure 7, as its
Figure 8. Stimulated by 5-HT with 10 μM 300 min, time courses of [pCREB1] for different levels of [miR-124] (12 μM, 14 μM, 26 μM and 30 μM) are plotted together.

Figure 9. (a) Bifurcation diagram of [pCREB1] versus [5-HT] for different [miR-124]. [5-HT] = 10 μM as the critical value. (b) Bifurcation diagram with [5-HT] and [miR-124] as control parameters. Red lines depict the loci of the saddle-node bifurcation points.

dynamical behavior is limited by the basal values of all the parameters in the physiological range to simulate experimental findings.

3.3. Switching from reversible to irreversible bistability is controlled by the bifurcation parameter 5-HT

In the experiments, injection of a duplex miR-124 mimic designed to elevate the levels of miR-124 in sensory neurons causes a significant reduction in LTF, while injection of a single-stranded antisense miR-124 inhibitor designed to reduce the levels of miR-124 leads to a significant increase in synaptic facilitation of the 5-HT-treated synapses [11]. Here, we attempt to alter the level of miR-124 in sensory neurons to investigate its ability to regulate pCREB1 stimulated by 5-HT.

Compared with the expression of TFs, miR-124 transcription can be regarded as a fast response. Therefore, we consider miR-124 as a parameter for use in a quasi-static analysis of the following equations.

\[
\frac{d[mCREB1]}{dt} = g_{m0} + \frac{V_1[pCREB1]^2}{[pCREB1]^2 + K_1^2} - \delta [miR-124][mCREB1],
\]

(15)

\[
\frac{d[CREB1]}{dt} = g_{a0} + \frac{V_1[pCREB1]^2}{[pCREB1]^2 + K_1^2} - \delta [miR-124][mCREB1],
\]

\[
- k_3 \left(1 + \frac{k_3[5-HT]}{[5-HT] + K_3}\right)[CREB1],
\]

(16)

\[
\frac{d[pCREB1]}{dt} = k_3 \left(1 + \frac{k_3[5-HT]}{[5-HT] + K_3}\right)[CREB1] - k_4[pCREB1].
\]

(17)

Indeed, even a single, long pulse of 5-HT leads to a significant change in the pCREB1 treated with miR-124 inhibitor [11]. Here, at different levels of [miR-124] (12 μM, 14 μM, 26 μM and 30 μM), we checked the change in pCREB1 stimulated by a long pulse of 5-HT (300 min, 10 μM) (see figure 8). When
Figure 10. Impact of changing each parameter with ±10% on the two-parameter bifurcation diagram in the \((V_1, V_2)\) plane. Red lines depict the loci of the saddle-node bifurcation points with basal values, and green and blue lines correspond to parameter values scaled down and up 10 percent, respectively.

As \([\text{miR-124}] = 12 \mu M\), the pCREB1 level was high both before and after stimulation (see figure 8(b)). As \([\text{miR-124}]\) is controlled at 14 \(\mu M\), stimulation of 5-HT can cause pCREB1 to increase significantly and even remain high after stimulation, as shown in figure 8(b). As \([\text{miR-124}] = 26 \mu M\) or 30 \(\mu M\), pCREB1 concentrations decrease to low baseline levels after being elevated by 5-HT, as shown in figure 8(b). Eventually, the lower concentration of miR-124 contributes to the higher level of pCREB1 while the higher miR-124 level leads to a lower pCREB1 level.

The reversible and irreversible switches depending on the parameter \([\text{miR-124}]\) are shown in figure 9. For a reversible switch, the system is bistable between two saddle-node bifurcation points (for example, red and green curves in figure 9(a)). As the stimulus 5-HT increases, the pCREB1 level can switch from low to high at a saddle-node bifurcation point. If thereafter [5-HT] decreases, [pCREB1] will return to low level at another saddle-node bifurcation point. However, for an irreversible switch, only one saddle-node bifurcation point is located in the physiological range of parameter but another one falls in non-physiological range with negative value of the parameter (for example, blue and pink curves in figure 9(a)). Therefore, \([p\text{CREB1}]\) transits from low to high through the non-negative saddle-node bifurcation point as [5-HT] increases and then it has to remain high as [5-HT] afterward decreases to the basal level.

According to the bifurcation diagram of \([p\text{CREB1}]\) versus [5-HT], shown in figure 9(a), we found that the decrease of \([\text{miR-124}]\) led to a change from a reversible switch \([\text{miR-124}] = 26 \mu M\) or 30 \(\mu M\) to an irreversible switch \([\text{miR-124}] = 12 \mu M\) or 14 \(\mu M\). The reversible switch for \([\text{miR-124}] = 26 \mu M\) or 30 \(\mu M\) determines that pCREB1 must decrease rapidly to the low stable state after the stimulation of 5-HT, while the irreversible switch for \([\text{miR-124}] = 12 \mu M\) or 14 \(\mu M\) elevates pCREB1 levels by 5-HT pulse, and pCREB1 permanently remains at the high stable steady state, even after the stimulation is terminated. Physiologically, increased pCREB1 level correlates with LTF and LTM and the long-term high concentration of pCREB1 corresponds to the formation of LTM.

Additionally, both the reversible and the irreversible switches are better characterized by the transition between low and high monostable regions and the bistable region in the bifurcation diagram of miR-124 and 5-HT in figure 9(b). Notably, the system more easily reaches a high steady state, even at low levels of miR-124. Nevertheless, it barely achieves a high stable steady state at higher miR-124 levels, which greatly increases the threshold of 5-HT to the high stable steady state region. We can confirm that the inhibition of miR-124 in sensory neurons can lead to a high level of pCREB1, while overexpression of miR-124 is able to repress the expression of CREB1.
3.4. Bistability is robust to variations in parameters

Bistable dynamics is important in the system with physiological significance that underlies the mechanism of LTM formation. Therefore, it is necessary to investigate the robustness of bistability to variations in parameters, which could not be totally found in published literatures. Varying parameters in the system to survey changes in size and location of parameter regions where particular dynamics exist can investigate the robustness of system dynamics [16]. Figures 10 and 11 display the two-parameter bifurcation curves after changing each parameter value with ±10% in the model in the ($V_1$, $V_2$) plane and the ([5-HT], [miR-124]) plane, respectively. The results show that the bistability regions only have a little changes in both the size and the location, and so the bistability is robust to variations of all the parameters.

4. Discussion

MiRNAs are small noncoding RNAs that modulate gene expression at the post-transcriptional level, and miR-124 exclusively presented presynaptically in a sensory-motor synapse constrains 5-HT-induced LTF in Aplysia through regulation of transcriptional factor CREB1. In fact, the level of miR-124 reduced by an antagonist of miR-124 can elevate the level of CREB1 mRNA and protein; that is, miR-124 inhibits the synthesis of CREB1. Therefore, the precise regulation of CREB1 by miR-124 is indicative of the importance of miRNA during memory formation.

In this paper, we established a network model of the regulation between CREB1 and miR-124 stimulated by 5-HT, which is associated with LTF. Through three stimulation protocols of one, three and five short pulses, we performed numerical simulations of some experimental phenomena to validate the model. Moreover, miR-124 levels returned to baseline levels within 12 h after exposure to the five pulses of 5-HT, a finding that is in accordance with the experimental findings. Additionally, the regulatory mechanism of miR-124 as a suppressor of CREB-dependent transcription was confirmed by combining the steady-state analysis with bifurcations of feedback strength when setting [5-HT] = 0 µM in the model. The results from the bifurcation analysis beyond memory formation revealed that the abstracted system displays diverse dynamics, including monostability, bistability and oscillation. Varying the parameters in the abstracted system can generate various bifurcations, such as saddle-node bifurcation, Hopf bifurcation, HC, LPC and SNIC. Furthermore, the original system also exhibited bistability under appropriate feedback strength. More importantly, we altered the levels of miR-124 to survey the factors that lead to the high
pCREB1 levels increased by 5-HT and found that the decrease in miR-124 can trigger a transition from a reversible switch to an irreversible switch. Specifically, the irreversible switch at low miR-124 concentration permanently elevates 5-HT stimulated pCREB1 at the high stable steady state, while the reversible switch results in pCREB1 decreasing rapidly to the low stable state. As a persistent high level of pCREB1 is relevant to LTF and LTM, such an irreversible switch might correlate physiologically with the establishment of LTM.

The results from the bifurcation analysis of the abstracted model revealed diverse dynamics and multiple types of bifurcations. For LTM formation in *Aplysia*, bistability is a crucial dynamic property. However, in other biological systems, miRNAs can also induce some non-steady-state behaviors, in addition to bistability. For example, an miR-17–92 cluster can generate large-amplitude oscillations in a cancer network [20], and the accumulation of four *Arabidopsis* miRNAs (miR-171, miR-398, miR-168 and miR-167) oscillated during the diurnal cycle [21]. The insight gained from the abstracted model is expected to provide a basis for the investigation of other biological systems involving miRNA regulation.

In previous studies [16, 22], the positive feedback loop for generating bistability in CREB1 levels had been investigated without considering miR-124. According to experimental findings [11], miR-124 serves as a negative constraint on serotonin-induced LTF since miR-124 can tightly control CREB and CREB-mediated signaling during plasticity in synapses. In the absence of 5-HT, CREB1 protein can be controlled at a low level because miR-124 directly binds to CREB1 mRNA and downregulates the CREB1 mRNA level. Exposure to serotonin reduces the level of miR-124 and relieves negative constraints on CREB1 mRNA, enabling an increase in CREB1 protein levels. On the other hand, CREB1 can be activated by 5-HT and promotes its own gene expression to form a positive feedback loop for generating bistability. Then, the CREB1 protein reaches a high level, where it persists high after 5-HT stimulation, which leads to LTF.

However, long-term synaptic facilitation requires not only activation of memory-enhancer genes but also inactivation of memory-suppressor genes, through another TF (CREB2), the major inhibitory constraint of memory in *Aplysia*. Moreover, the Piwi/piRNA complex facilitates serotonin-dependent methylation of a conserved CpG island in the promoter of CREB2, leading to enhanced long-term synaptic facilitation [23]. These findings suggest another small RNA-mediated gene regulatory mechanism of CREB2 for establishing stable persistence of LTM, which will be further considered to more fully study the mechanism of miRNA on LTM. Finally, it should be noted that cellular processes at the molecular level are inherently stochastic. Noise may induce bistability not found in the deterministic model [24], or bifurcations on counterpart in the deterministic descriptions [25]. Future studies will emphasize stochastic dynamics in miR-124-mediated regulation of LTM.

miRNAs play essential roles in synaptic plasticity and memory in both *Aplysia* and mammals [26, 27]. There is a mammalian analog of miRNA-124 that contributes to a multitude of biological processes, such as neurogenesis, synapse morphology, and synaptosome transmission [27]. For example, the high expression of miRNA-124 and decreased levels of AMPAR are closely associated with hippocampal demyelination and memory impairment in mice, suggesting that intervention of potential miRNA-124/AMPA signaling may be a clue to improve memory performance. Therefore, the regulatory mechanisms of miRNA-124 in mammalian synaptic potentiation and memory formation are worth studying. Our model not only provides insights into the mechanisms of miRNA-124 modulation of CREB1 expression and LTF in *Aplysia*, but also provides a framework for understanding similar processes associated with synaptic plasticity in mammals. The mathematical modeling and dynamic analysis of miRNA-124 regulatory mechanisms in mammalian synaptic plasticity and memory will be researched in the future.

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