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A Model for the Cellular Mechanisms of Morphine Tolerance and Dependence

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Abstract—The *Locus Coeruleus* (LC) is the major noradrenergic nucleus, with approximately 30,000 neurons located on the floor of the fourth ventricle in the rostral pons. LC neurons submitted to acute exposure to morphine develop addiction ranging from acute to chronic, because of the alteration in concentration of intracellular messengers and in genetic expression of some proteins. Although the main electrophysiological effects of opiates in the LC are well established, some of the cellular mechanisms of morphine tolerance and dependence have required further research. This work contains results related to both a mathematical model that simulates the effects of morphine in a LC neuron, and the hypothesis that only the increase in genetic expression of a protein is sufficient to allow the neuron to develop tolerance and range from acute to chronic addiction. Simulations based on the model show interesting results that allow us to infer that the hypothesis proposed is sufficient for neurons to attain chronic dependence.

Keywords—Morphine, Addiction, Locus Coeruleus, Molecular psychiatry.

1. INTRODUCTION

Many types of neuronal cells and brain nuclei have the property of changing, acutely or chronically, their regular behavior by the action of pharmacological agents, such as psychoactive drugs. Acute changes, those that cease in a short time, would not be important to the chronic altered behavior if the cell recovered its original drug-free state, but it is observed that some adaptation occurs that impairs such a recovery. In fact, the disturbed cell under the influence of a drug tries to compensate for its acute effects by promoting changes in the opposite direction, transiently restoring its homeostasis. However, when the acute action of the drug is finished, the cell is imbalanced by its own reactive response [1]. As a consequence, the phenomenon of tolerance develops, that is, the need for an increased dose of the drug to produce the same effect. After tolerance is established, the withdrawal of the drug may produce physical or psychological symptoms opposed to the acute pharmacological actions of the drug itself. Dependence is defined as the need for constant exposure to the drug in order to avoid the withdrawal syndrome.

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Addiction, a commonly used term classically associated with the withdrawal syndrome, cannot be characterized by either tolerance or dependence, since some nonaddictive drugs may cause se-

a general and imprecise definition of addiction would be the use of a drug despite its noxious consequences [3].

The oldest known drug of abuse is opium, whose name derives from a Greek word that means "juice". A white liquid is extracted from immature fruits of a specific poppy family, and after air-dried becomes the brown or black putty of opium. Raw opium has been used as a pain reliever to control dysentery, and in the production of a sort of tea since 4000 B.C. by the Sumerians [4]. The main active ingredient of opium is the alkaloid morphine, named after Morpheus, the mythological god of dreams. The isolation and purification of morphine from the raw opium and the invention of the hypodermic needle greatly contributed to the increase of the number of addicts in the nineteenth century. Although tolerance and dependence do not develop when the access to the drug is limited, chronic unconstrained use leads to profound tolerance and serious withdrawal syndrome with muscle and gastrointestinal pain followed by diarrhea [5].

In the seventies, a sequence of binding experiments in certain tissues established the existence of specific opioid receptors which were originally classified as μ, for those that had a great morphine-like affinity, κ, for those whose affinity was to ketazocine-like compounds, and σ, for those that preferentially associated to N-Allylnormetazocine-like substances [6]. Later, it was observed that the σ receptor was not solely related to opiates, and a new receptor type called δ was discovered [7]. The existence of opioid receptors in mammalian nervous cells hinted at the presence of an internal opioid system, which was indeed confirmed when opioid activity was detected in pig brain preparations. The two opioid peptides discovered were named Leu-enkephalin and Met-enkephalin. Later, any endogenous opioid substance was collectively referred to as an endorphin [8]. Recently, evidence for the existence of anti-opioid peptides that attenuate the action of endorphins brought additional complexity to the endogenous opioid system [9].

After the discovery of the endogenous opioid system, a great research effort was directed to detect changes in receptor density or affinity under conditions of opioid addiction [10]. The failure in detecting these changes shifted the attention to intracellular mechanisms of opioid dependence. The role of G-proteins [11]—proteins that couple receptors to effector systems inside the cell—was studied, and differences in their concentrations were observed under chronic and acute opioid administration. A direct consequence was that intracellular messengers like adenylate cyclase, cyclic adenosine monophosphate (cAMP), protein kinases, and protein phosphatases were also regulated by opioids [12]. Furthermore, since many characteristics of opioid addiction develop gradually and persist for a long time after drug withdrawal, it is likely that opioids control gene expression in some way [13].

Due to a high density of opiate receptors and the presence of endorphins, the Locus Coeruleus (LC) is a brain nucleus well suited for the study of opiate dependence [14]. This pontine homogeneous set of noradrenergic neurons projects to many brain regions and has been electrophysiologically and anatomically characterized [15]. Injection of opiate antagonists in the LC of dependent rats produces a severe withdrawal syndrome, suggesting that this nucleus mediates opiate dependence [16]. Acutely, opiates inhibit LC neurons by the action of G-proteins and cAMP-dependent kinases in ionic channels. As tolerance develops, neuronal firing rates are restored and the cAMP system becomes up-regulated, in a clear compensatory reaction. As a consequence, alterations in the levels of certain nuclear proteins, called transcription factors, lead to changes in the rate of gene transcription, establishing the genetic expression of the addicted state [17]. It is worth noting that as LC projects to several regions and receives axons from many brain nuclei, changes in its behavior may be the synergetic sum of afferent signals, including the feedback, through some pathways, of its own action. Addiction would then be the result of a complex interaction between brain sites imbalanced by excessive opiate exposure.

This work proposes a mathematical model for the cellular alterations produced in the Locus Coeruleus by short- and long-term treatment with morphine. For this purpose, a model of an LC neuron is defined that includes its membrane ionic channels, the receptor-drug interaction, the transmembrane signaling by G-proteins, the biochemical pathways between adenylate cyclase and nuclear proteins, and the feedback of the genetic expression over the whole cell. The model is based on recent physiological and molecular studies, and incorporates few plausible working hypotheses when the experimental data are inconclusive. The mathematical equations are numerically solved and some simulation results show the acute and chronic effect of morphine over an LC neuron from the drug-free to the addicted state.

To make the exposition easier, the remainder of this text is divided into four additional sections. Section 2 explains the molecular mechanisms of opiate tolerance and dependence known up to now, while Section 3 states a mathematical model of the LC neuron. Section 4 shows the computer simulations and their results, and Section 5 concludes the text.

2. THE MOLECULAR MECHANISMS OF OPIATE DEPENDENCE

2.1. Drug-Receptor Interaction

Morphine is absorbed readily from the gastrointestinal tract, nasal mucous membrane, and lungs, when it is smoked. Parenteral administration promotes passive diffusion of the drug in the tissue until reaching the blood stream, which distributes it in high doses to the kidney, liver, thyroid, and adrenals. Although morphine concentration at brain sites are low due to the blood-brain barrier, its effects of analgesia, respiratory depression, and sedation are marked and complex. The limbic system, the brainstem respiratory nuclei, the cerebral cortex, and the Locus Coeruleus are some of the centers where the drug has clear influence. At these CNS centers, opioid receptors can be found attached to cell membranes, mainly in the synaptic region.

In the Locus Coeruleus, opiates act through μ receptors and acutely hyperpolarize the cells by opening K^+ channels [18] and blocking an Na^+-dependent current that seems to be responsible for pacemaker activity [19]. Rapid loss of response to morphine occurs while the receptors are exposed to the drug, reducing the mentioned acute hyperpolarization. This receptor desensitization represents a first process of compensation in which the cell tries to recover its equilibrium [10]. Although experiments about μ receptor desensitization are not conclusive [20], studies of the β-adrenergic receptor suggest that desensitization may happen either via phosphorylation of the receptors by cAMP-dependent protein kinases (heterologous desensitization), or via their phosphorylation by proteins that depend on the level of receptor in ligand-bound state (homologous desensitization) [21].

Homologous desensitization is a two-step process that begins with the "sequestration" of the receptor and ends with the receptor "down-regulation". The observation that the number of receptors decreases during desensitization suggests that they are internalized and stored in an isolated compartment. This sequestration action is possibly induced by the phosphorylation of the receptors in ligand-bound state by a β-adrenergic receptor protein kinase (βARK). The sequestration is fast and allows the return of the receptors to the surface of the cellular membrane by a reverse process of resensitization through protein phosphatases. Following a longer time scale, down-regulation seems to be a consequence of the actual disappearance of the receptors by their proteolytic degradation. Clearly, it would be an irreversible procedure, and the return of the number of receptors to its basal level would require new receptor synthesis [22].

In heterologous desensitization, receptors' phosphorylation through cAMP-dependent protein kinases can be the result of many events, like the isolated or compound action of neurotransmitters, hormones, and also other drugs. This process of desensitization may need the uncoupling of the receptor from G-proteins or, in other words, only receptors in ligand-bound state uncoupled from G-proteins would have their phosphorylation sites available. This would explain why

cAMP-dependent proteins' kinases do not phosphorylate free receptors [17]. Since opiates reduce the level of activity of the cAMP system, this mechanism of desensitization would not be so important to acute exposures to the drug as it seems to be in the chronic treatment, when an up-regulated cAMP system develops.

2.2. Transmembrane Signaling

Acute opiate action is mediated by G-proteins that are membrane-bound GTP (guanosine triphosphate) binding proteins that couple receptors to intracellular effectors. These proteins function as signal transducers, which receive receptor-drug pair information from the cell's outer surface and transform it into an internal code that regulates the adenylate cyclase catalytic unit [23].

The G-proteins are heterotrimers, with subunits called α, β, and γ. The α subunit characterizes the oligomer, since the β and γ subunits are indistinguishably present in all forms of G-proteins. Toxins from bacteria like *Vibrio cholera* and *Bordetella pertussis* can modify the G-proteins by adding a ribose to the α subunit. In this sense, the G-proteins can be classified into four classes, depending on the susceptibility of ribosylation, i.e., ribosylation by cholera toxin only, pertussis toxin only, both toxins, or neither toxin. The adenylate cyclase catalytic unit is stimulated by the cholera toxin substrate and inhibited by the pertussis toxin substrate. As a consequence, their α subunits are named α_s and α_i, respectively [24,25]. There is another α subunit, called α_0, which is also pertussis-toxin sensitive and acts on ionic channels across the cellular membrane [26].

The activation of the G-protein occurs when the ligand-bound receptor transforms the guanosine diphosphate (GDP) linked to the α subunit to GTP, resulting in the dissociation of the α-GTP complex from the $\beta\gamma$ heterodimer. The free α-GTP complex interacts with second messengers, ionic channels, and enzymes until its GTP is hydrolyzed to GDP [27]. The α subunit may then reassociate with the $\beta\gamma$ complex.

The μ receptors in ligand-bound state activate two forms of G-proteins, liberating the subunits α_i and α_0. The opening of K^+ channels, which is in part responsible for the acute inhibition of LC cells, is mediated by the subunit α_0, maybe by the phosphorylation of the channel itself. The role of the α_{it} subunit is to inhibit the action of the adenylate cyclase catalytic unit, reducing cAMP formation and, consequently, protein phosphorylation [17].

Many experiments have shown that chronic exposure to morphine leads to an increase in the levels of the subunits α_i and α_0 and, inconsistently, a proportional increase in the adenylate cyclase activity. This apparent paradox can be explained if we consider that in the basal state, the α_i and the α_s subunits are associated with the common $\beta\gamma$ complex in an equilibrated form, where the free subunits have a net effect of stimulating the activity of the adenylate cyclase. When the drug induces a chronic increase in the α_i subunit, the association between the $\beta\gamma$ complex and this subunit is also increased, and thereby the association between the complex and the α_s subunit is decreased. A higher level of free α_s subunit and a lower level of free α_i subunit lead the system to a new equilibrium state where the adenylate cyclase catalytic unit is marked stimulated. In the acute effect of the drug, dissociation of the α_i subunit from the $\beta\gamma$ complex facilitates the association of the complex with the free α_s subunit, reducing its action. Higher levels of free α_i and lower levels of free α_s subunits would produce the inhibition of the adenylate cyclase, as observed. The competition between the various α subunits for the constant amount of $\beta\gamma$ heterodimer may be the control mechanism of the adenylate cyclase activity by the G-protein network [28].

2.3. Protein Phosphorylation

Protein phosphorylation is the final common process to which many molecular pathways converge. Indeed, neurotransmitters, hormones, and some drugs can alter the rate of phosphorylation of specific proteins, indirectly regulating neuronal functions such as neurotransmitter synthesis

and release, ionic channels conductance, genetic expression, and plasticity. The substrate protein is converted from the dephospho form to the phospho form by a protein kinase, and converted back by a protein phosphatase. Protein kinases transport a phosphate group from the ATP (adenosine triphosphate) molecule to the substrate protein, while protein phosphatases remove the phosphate group by hydrolysis. The control of kinasis and phosphatasis activation by external signals is an important mechanism for regulation of protein phosphorylation [29].

Generally, protein kinases are activated by second messengers such as cAMP, and up to now, only two types of cAMP-dependent protein kinases are known to exist. The four types of observed protein phosphatases may be controlled either by second messengers, like cAMP, or indirectly, by phosphatase inhibitors which are themselves activated by cAMP. The intracellular level of cAMP is regulated by the enzyme adenylate cyclase, which produces cAMP from ATP, and some cyclic nucleotide phosphodiesterases, which destroy this second messenger [30].

As the acute exposure to morphine decreases the activity of adenylate cyclase, and consequently the levels of cAMP, the process of cAMP-dependent protein phosphorylation is inhibited. Such regulation of protein phosphorylation is the way opiates act to promote changes in many aspects of neuronal function, as the closing of the nonspecific Na^+-dependent ionic channel by the reduction of the phosphorylation rate and the triggering of processes associated to chronic adaptation [17].

The chronic administration of morphine produces an up-regulated cAMP system, leading to increased levels or to higher phosphorylation states, of cAMP-dependent protein kinases and morphine- and cAMP-regulated phosphoproteins (MARPPs). The two processes of phosphoprotein activation, i.e., increasing their amounts or changing their phosphorylation state, may subserve different forms of addiction and withdrawal syndromes [31]. One important identified MARPP is the enzyme tyrosine hydroxylase, which regulates the rate of cathecolamine production. Recalling that the LC spreads its noradrenergic projections throughout the brain, an upper level of tyrosine hydroxylase may lead to disturbances in norepinephrine release and, consequently, marked changes in brain function. Certainly, there are other unknown MARPPs that participate in basic neuronal processes related to addiction, and their identification is a topic of current research [32].

2.4. Genetic Expression

When long exposed to morphine, LC neurons have their spiking frequency disturbed due to changes in protein synthesis, as a consequence of a new state of genetic expression [17,33]. Synaptic activity produces alterations in genetic expression through the immediate early genes (IEGs), that are quickly induced in response to morphine stimulation [34–36]. Two families of such transcription factors are well defined: the cAMP response element binding protein (CREB) and the Fos/Jun proteins [37–39].

It seems that morphine regulates genetic expression in two ways. First, the total amount of transcription factors is changed through feedback with its own transcription, like the Fos/Jun family; second, a phosphorylation process takes place to control the activity of the transcription factors [40]. The CREB proteins are regulated by phosphorylation through cAMP- and calcium-dependent protein kinases [41–43], and their levels, reduced in acute administration of morphine, recover their basal values in the chronic contact with the drug [40].

In a short time scale, CREB phosphorylation could happen because morphine inhibits adenylate cyclase [18,44]. This is consistent with the known effects of acute chronic morphine administration to the LC neurons [45]. In a longer time scale, due to the need of protein synthesis, the levels of Fos/Jun transcription factors would be changed. Acute morphine administration decreases the levels of mRNA and Fos in LC neurons, while higher levels of Fos are observed in the withdrawal syndrome [13].

In an apparent contradiction, an increase in the concentration of the α_i subunit is associated with a proportional increase in the adenylate cyclase activity [17,28,44]. This can be explained

noting that higher levels of α_i would promote, by competition, the dissociation of G_s, liberating free α_s that could stimulate adenylate cyclase.

3. THE MODEL

Several facts concerning the electrophysiology of the LC neuron are important to our model. Typically, LC cells display long-duration action potentials (more than 1 ms) and a basal slow spontaneous activity of about two spikes per second. The evoked response has a biphasic burst-pause character in which a short latency spike train—about ten spikes per second—is followed by a long-lasting inhibition of, approximately, three seconds [17]. The resting membrane potential ranges from −65 mV to −55 mV, values above which spontaneous spiking arises. Two components produce the 75 mV rising phase of the action potential: first, a slow depolarization caused by an inward voltage-dependent calcium current, here named I_{Ca}, and, second, the action potential proper which is due to a fast and also voltage-dependent sodium current, denoted by I_{Na}.

The potential repolarization is obtained through a voltage-dependent outward potassium current, I_K, which functions as a delayed rectifier. After a train of spikes, another outward potassium current, $I_{K,Ca}$, which depends on the intracellular calcium concentration, is triggered and hyperpolarises the cell to about 75 mV. At this potential, a voltage-dependent outward potassium current I_A slows the return of the membrane potential to its resting value, thus controlling the interspike frequency [18].

The spontaneous activity observed in LC neurons is possibly due to a cAMP-dependent sodium persistent current, $I_{Na,p}$, which slowly depolarizes the cell from its membrane resting potential to the threshold potential of the I_{Ca} current [19]. This pacemaker activity would then be regulated by drugs, neuromodulators, or neurotransmitters, acting directly or indirectly on the cAMP system. It is still questionable whether this mechanism really takes place. For example, other authors [18,20] have proposed that the important current to the LC neurons' pacemaker activity is calcium-dependent instead of cAMP-dependent. In this paper, Alreja's results [19] are assumed for simplicity, but other mechanisms can be adopted without changing the main results of the model.

The membrane of an excitable cell can be modeled as an equivalent electric circuit in which the membrane capacitance C is in parallel with ionic channels [46,47]. In this model, the membrane is concentrated in one point and has an electric potential V.

The membrane equation for the LC neuron is

$$C\frac{dV}{dt} = I_{Na} + I_K + I_{Na,p} + I_{Ca} + I_{K,Ca} + I_A. \tag{1}$$

Assuming a linear relation between the instantaneous voltage across the membrane and the instantaneous current flowing through the ionic channels, Ohm's law yields

$$I_{Na} = -g_{Na}(V - E_{Na}), \tag{2}$$

$$I_K = -g_K(V - E_K), \tag{3}$$

$$I_{Na,p} = -g_{Na,p}(V - E_{Na}), \tag{4}$$

$$I_{Ca} = -g_{Ca}(V - E_{Ca}), \tag{5}$$

$$I_{K,Ca} = -g_{K,Ca}(V - E_K), \tag{6}$$

$$I_A = -g_A(V - E_K), \tag{7}$$

where E_{Na}, E_K, and E_{Ca} are the Nernst potential of the ionic channels of Na^+, K^+, and Ca^{2+}, respectively. The terms g_{Na}, g_K, $g_{Na,p}$, g_{Ca}, $g_{K,Ca}$, and g_A correspond, respectively, to the conductance of the currents I_{Na}, I_K, $I_{Na,p}$, I_{Ca}, $I_{K,Ca}$, and I_A.

The dynamical behavior of the g_{Na} conductance in (2) is expressed by the product of an activation gating variable $d \in (0,1)$ and inactivation gating variable $f \in (0,1)$ representing, respectively, the opening and closing of the Na_+ channel, and a constant maximal conductance $\bar{g}_{Na}$

$$g_{Na} = \bar{g}_{Na} df. \tag{8}$$

The inactivation variable f follows a steady-state value $f_\infty \in (0,1)$ with a relaxation time τ_f, according to

$$\frac{df}{dt} = \frac{f_\infty - f}{\tau_f}, \tag{9}$$

where f_∞ expresses the voltage-dependency of the I_{Na} current inactivation,

$$f_\infty = \frac{1}{1 + e^{(V - V_f)/S_f}}, \tag{10}$$

V_f being the half-maximal potential for f, and S_f the slope of the sigmoidal function. The relaxation time τ_f is also a function of the membrane potential and usually has a bell-shaped form [48] like

$$\tau_f = \frac{\bar{\tau}_f}{e^{(V - V_{\tau_f})/S_{\tau_f}} + e^{-(V - V_{\tau_f})/S_{\tau_f}}}, \tag{11}$$

where S_{τ_f} is the slope, V_{τ_f} is the potential for maximum, and $\bar{\tau}_f$ is the maximal relaxation time constant. As the activation gate d has a fast dynamics, it always assumes the voltage-dependent steady-state value d_∞ given by

$$d_\infty = d = \frac{1}{1 + e^{-(V - V_d)/S_d}}, \tag{12}$$

where V_d is the half-maximal potential for d and S_d is a constant to regulate the curve slope. Analogously, the conductance g_K of the current I_K in (3) is modeled as

$$g_K = \bar{g}_K l, \tag{13}$$

where g_K is the constant maximal conductance, and the activation gating variable $l \in (0,1)$ follows the relaxation equation

$$\frac{dl}{dt} = \frac{l_\infty - l}{\tau_l}, \tag{14}$$

with

$$l_\infty = \frac{1}{1 + e^{-(V - V_l)/S_l}} \tag{15}$$

and

$$\tau_l = \frac{\bar{\tau}_l}{e^{(V - V_{\tau_l})/S^{\tau}_{\tau_l}} + e^{-(V - V_{\tau_l})/S^{l}_{\tau_l}}}. \tag{16}$$

The steady-state value l_∞ has a half-maximal potential V_l and a slope S_l, while the relaxation, bell-shaped time τ_l has a maximal value of $\bar{\tau}_l$ with slopes $S^{\tau}_{\tau_l}$ and $S^{l}_{\tau_l}$, at the right and left sides, respectively, and potential for maximum V_{τ_l}.

The Na^+ channels of the $I_{Na,p}$ current in (4) are phosphorylated by cAMP [19] from the basal $\bar{g}_{Na,p}$ to a maximum value $g^*_{Na,p}$. Assuming a sigmoidal relation between the intracellular levels of cAMP, [cAMP], and the conductance $g_{Na,p}$ of the channel, we have

$$g_{Na,p} = \frac{g^*_{Na,p}}{1 + e^{-([cAMP] - [cAMP_h])/S_{Na,p}}} + \bar{g}_{Na,p}, \tag{17}$$

where $[cAMP_h]$ is the level of cAMP to produce a half-maximal channel phosphorylation. The dynamics of the g_{Ca} conductance in (5) is represented as the product

$$g_{Ca} = \bar{g}_{Ca} mh \tag{18}$$

of the maximal conductance $\bar{g}_{Ca}$ by the activation and inactivation $m \in (0,1)$ and $h \in (0,1)$. The inactivation gating variable h follows its steady-state h_∞ with a relaxation time τ_h, according to

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h}, \tag{19}$$

where

$$h_\infty = \frac{1}{1 + e^{(V - V_h)/S_h}} \tag{20}$$

and

$$\tau_h = \frac{\bar{\tau}_h}{e^{(V - V_{\tau_h})/S^\tau_{\tau_h}} + e^{-(V - V_{\tau_h})/S^l_{\tau_h}}}. \tag{21}$$

The voltage dependency of h is established by the half-maximal potential V_h, in the maximal relaxation time $\bar{\tau}_h$, in the slopes S_h, $S^\tau_{\tau_h}$, and $S^l_{\tau_h}$, and by the potential for maximum V_{τ_h}. The activation variable m equals its steady-state value m_∞, given by

$$m_\infty = \frac{1}{1 + e^{-(V - V_m)/S_m}}, \tag{22}$$

where V_m and S_m stand for the half-maximal potential and the constant slope for m, respectively. For the current $I_{K,Ca}$, the conductance $g_{K,Ca}$ in equation (6) is represented as

$$g_{K,Ca} = \bar{g}_{K,Ca} opq, \tag{23}$$

where $\bar{g}_{K,Ca}$ is the maximal conductance for this current and $o \in (0,1)$ is the activation gating variable that depends on the intracellular calcium concentration [Ca]. The voltage-dependent activation and inactivation gating variables are, respectively, $p \in (0,1)$ and $q \in (0,1)$. The behavior of the gating variable o is simply a function of the calcium concentration as

$$o = \frac{1}{1 + e^{-([Ca] - [Ca]_o)/S_o}}, \tag{24}$$

$[Ca]_o$ being the lower limit for the opening of the gate. The gating variable p has a fast dynamics and controls the voltage dependency of the channel as

$$p = \frac{1}{1 + e^{(V - V_p)/S_p}}, \tag{25}$$

where V_p is the half-maximal potential and S_p is the slope. Finally, q has a slow dynamics that obeys a relaxation equation like

$$\frac{dq}{dt} = \frac{q_\infty - q}{\tau_q}, \tag{26}$$

where the steady-state value q_∞ has a slope S_q and a half-maximal potential V_q as

$$q_\infty = \frac{1}{1 + e^{-(V - V_q)/S_q}}, \tag{27}$$

and the relaxation time τ_q is a bell-shaped function of the membrane potential V like

$$\tau_q = \frac{\bar{\tau}_q}{e^{(V - V_{\tau_q})/S^\tau_{\tau_q}} + e^{-(V - V_{\tau_q})/S_{\tau_q}}}. \tag{28}$$

The term $\bar{\tau}_q$ is the maximal relaxation time constant, while S_{τ_q} is a slope and V_{τ_q} is the potential for maximum.

The intracellular free calcium concentration increases as a function of the inward I_{Ca} current and decreases due to membrane-bound ionic pumps and calcium binding proteins (buffers) as

$$\frac{d[\mathrm{Ca}]}{dt} = \alpha I_{\mathrm{Ca}} - \beta[\mathrm{Ca}]^n, \tag{29}$$

where α, β, and n are constants. The conductance g_A of the voltage-dependent I_A current in (7) is modeled as

$$g_A = \bar{g}_A r z, \tag{30}$$

where $\bar{g}_A$ is the maximal conductance for this current and the fast activation gating variable $r \in (0,1)$ takes its steady-state value from

$$r = \frac{1}{1 + e^{(V-V_r)/S_r}}, \tag{31}$$

with a slope S_r and a half-maximal potential V_r. The gating variable z has a slow dynamics that obeys a relaxation equation like

$$\frac{dz}{dt} = \frac{z_\infty - z}{\tau_z}, \tag{32}$$

where the steady-state value z_∞ has a slope S_z and a half-maximal potential V_z as in

$$z_\infty = \frac{1}{1 + e^{-(V-V_z)/S_z}}, \tag{33}$$

and the relaxation time τ_z is a bell-shaped function of the membrane potential V like

$$\tau_z = \frac{\bar{\tau}_z}{e^{-(V-V_{\tau_z})/S_{\tau_z}} + e^{-(V-V_{\tau_z})/S_{\tau_z}}}. \tag{34}$$

The term $\bar{\tau}_z$ is the maximal relaxation time constant, while S_{τ_z} is a slope and V_{τ_z} is the potential for maximum.

The synthesis of activated receptors μ^* is done through the association of the morphine molecule m with the free receptor μ, driven by the affinity constant k_l. The activated receptors bind to the G_i and G_o proteins, breaking them into the subunits α_i, α_s, and $\beta\gamma$. At this phase, the morphine molecule frees itself from the activated receptor, possibly due to a change in its tertiary structure. Finally, the activated receptors are phosphorylated by a protein kinase, P_μ, then loosing their capacity of breaking G-proteins. These phosphorylated receptors, μ_p, are internalized in vesicles and, after resensitization, are delivered to the cellular membrane as free receptors μ.

The phosphorylation process is a way through which the neuron develops tolerance in a short time scale, because it reduces the amount of receptors in the membrane. Similarly, the hindering of the resensitization process also reduces the amount of free receptors in the membrane, leading to the down-regulation in a longer time scale. Indeed, for our purposes, the processes of internalization, phosphorylation, and resensitization can be considered different phases of the same phenomenon of transmembrane signalling homeostasis. Then, the synthesis of activated receptors can be modeled as

$$\frac{d[\mu^*]}{dt} = k_1[\mathrm{morf}][\mu] - k_2[\mu^*][G_i] - k_3[\mu^*][G_o] - k_4[\mu^*][P_\mu], \tag{35}$$

where k_1, k_2, k_3, and k_4 are association constants.

The process of phosphorylation and resensitization of activated receptors μ^* depends on the protein kinase concentration, P_μ, and on the constant k_6 of resensitization, as follows:

$$\frac{d[\mu_p]}{dt} = k_5[\mu^*][P_\mu] - k_6[\mu_p]. \tag{36}$$

Similarly, the process of down-regulation of the μ receptors can be represented as an irreversible reduction in the concentrations of the μ_p receptors, with a constant rate k_{60}

$$\frac{d[\mu_d]}{dt} = k_{60}[\mu_p]. \tag{37}$$

We hypothesize, without jeopardizing the generality of the model, that the total amount of μ receptors is a constant $\overline{\overline{\mu}}$, and so the conservation of mass allows us to write

$$\overline{\overline{[\mu]}} = [\mu] + [\mu^*] + [\mu_p] + [\mu_d]. \tag{38}$$

The pharmacokinetics of the morphine can be represented by

$$\frac{d[\text{morf}]}{dt} = -k_7[\text{morf}], \tag{39}$$

where k_7 stands for a constant rate of metabolisation.

The association of the dimmer $\beta\gamma$ with the monomers α_i, α_s, and α_o generates the proteins G_i, G_s, and G_o, respectively. These proteins are dissociated through the action of the opiate receptor μ^*. The formation of a G-protein may be formulated as the compound action of the dissociation of other forms of G-protein, the competition between the $\beta\gamma$ dimmer and the monomers α_i, α_s, and α_o, and the analytic process of interaction with the activated opioid receptor

$$\frac{d[G_i]}{dt} = k_8[\alpha_i][\beta\gamma] - k_9[\mu^*][G_i] - k_{10}[G_i][\alpha_s] - k_{11}[G_i][\alpha_o], \tag{40}$$

$$\frac{d[G_o]}{dt} = k_{12}[\alpha_o][\beta\gamma] - k_{13}[\mu^*][G_o] - k_{14}[G_o][\alpha_s] - k_{15}[G_o][\alpha_i], \tag{41}$$

$$\frac{d[G_s]}{dt} = k_{16}[\alpha_s][\beta\gamma] - k_{17}[G_s][\alpha_i] - k_{18}[G_s][\alpha_o], \tag{42}$$

where k_8 through k_{18} are constants of the model. The conservation of mass allows us to write

$$\overline{\overline{[\beta\gamma]}} = [G_i] + [G_s] + [G_o] + [\beta\gamma], \tag{43}$$

$$\overline{\overline{[\alpha_i]}} = [G_i] + [\alpha_i], \tag{44}$$

$$\overline{\overline{[\alpha_s]}} = [G_s] + [\alpha_s], \tag{45}$$

$$\overline{\overline{[\alpha_o]}} = [G_o] + [\alpha_o], \tag{46}$$

where $\overline{\overline{[\beta\gamma]}}$, $\overline{\overline{[\alpha_i]}}$, $\overline{\overline{[\alpha_s]}}$, and $\overline{\overline{[\alpha_o]}}$ are the maximal basal concentrations of the proteins $\beta\gamma$, α_i, α_s, and α_o, respectively.

The activation of the protein adenylate cyclase [AC*] may be represented by the competition between the activation and inhibition proteins, α_s and α_i, that alters the basal drug-free level, $\overline{\overline{[AC^*]}}$, of activated adenylate cyclase

$$\frac{d[AC^*]}{dt} = \overline{\overline{[AC^*]}} - [AC^*] + k_{19}[\alpha_s] - k_{20}[\alpha_i], \tag{47}$$

where k_{19} and k_{20} are constants that control the competition between activation and inhibition.

The activated adenylate cyclase is responsible for the production of cAMP from ATP at a constant rate k_{21}. Part of the cAMP concentration is degraded to AMP as the cAMP system activates protein kinases at a constant rate k_{22}

$$\frac{d[\text{cAMP}]}{dt} = k_{21}[AC^*] - k_{22}[\text{cAMP}]. \tag{48}$$

On the other hand, the protein kinases, PQ, are activated by cAMP and part of them are used in the formation of phosphoproteins as

$$\frac{d[\mathrm{PQ}^*]}{dt} = k_{24}[\mathrm{PQ}][\mathrm{cAMP}] - k_{25}[\mathrm{PQ}^*], \tag{49}$$

where k_{24} is the rate of activation and k_{25} the rate of utilization by the phosphoproteins. The amount of protein kinase is constant in the cell for the purposes of our model,

$$\overline{\overline{[\mathrm{PQ}]}} = [\mathrm{PQ}] + [\mathrm{PQ}^*], \tag{50}$$

where $\overline{\overline{[\mathrm{PQ}]}}$ is the maximal amount present.

The transcription factors, CREB, are phosphorylated through the action of the protein kinases and at a constant rate k_{26}. The phosphorylated CREBf acts as a transcription factor at a rate k_{27}. Then, we can write

$$\frac{d[\mathrm{CREB}_f]}{dt} = k_{26}[\mathrm{PQ}^*][\mathrm{CREB}] - k_{27}[\mathrm{CREB}_f]. \tag{51}$$

The maximum amount of transcription factor $\overline{\overline{[\mathrm{CREB}]}}$ in the cell is divided into inactive and phosphorylated

$$\overline{\overline{\mathrm{CREB}}} = \mathrm{CREB} + \mathrm{CREB}_f. \tag{52}$$

The genetic activation, denoted *gene*, obtained through the transcription factors, is closely related to the amount of *fos* in the cell and can be represented by a sigmoidal function of the type

$$\mathrm{gene} = \frac{1}{1 + e^{-\gamma_g([\mathrm{CREB}_f] - [\mathrm{fos}] - T_{h_g})}}, \tag{53}$$

where γ_g controls the sensitivity of the genetic activation and T_{h_g} determines the amount of fos necessary to activation.

The genetic activation leads to changes in the production of the α_i protein. This can be represented by an increase in the basal levels $\overline{\overline{[\alpha_i]}}$, proportional to the level of genetic activation, as

$$\overline{\overline{[\alpha_i]}}_{(t)} = (k_{\mathrm{gene}} * \mathrm{gene} + 1)\,\overline{\overline{[\alpha_i]}}\Big|_{t=0}, \tag{54}$$

where k_{gene} is a constant of the model.

Finally, the production of fos is dependent upon the gene activation and upon the amount of phosphorylated transcription factors present at the cell as

$$\frac{d[\mathrm{fos}]}{dt} = k_{28}\left\{\frac{2}{1 + e^{-\gamma_r\{([\mathrm{CREB}_f] - [\mathrm{CREB}_{fb}]) - (T_{h_r} * \mathrm{gene})\}}} - 1\right\}, \tag{55}$$

where T_{h_r} controls the amount of CREB_f necessary for triggering the fos production, and CREB_{fb} is a constant basal value of CREB_f.

4. SIMULATION RESULTS AND DISCUSSION

A computer program written in FORTRAN was used to solve the differential equations by the Euler method. The parameters of the model are described in the general glossary which appears in the Appendix.

In a first simulation, the neuron was submitted to a series of five equal doses of 100 μM of morphine, as in laboratory experiments [44]. The doses were administered with an interval of 100 minutes to give the necessary time of about 60 minutes for the total metabolization of the drug. In Figure 1, we show the graph of spikes per minute of the neuron during the experiment.

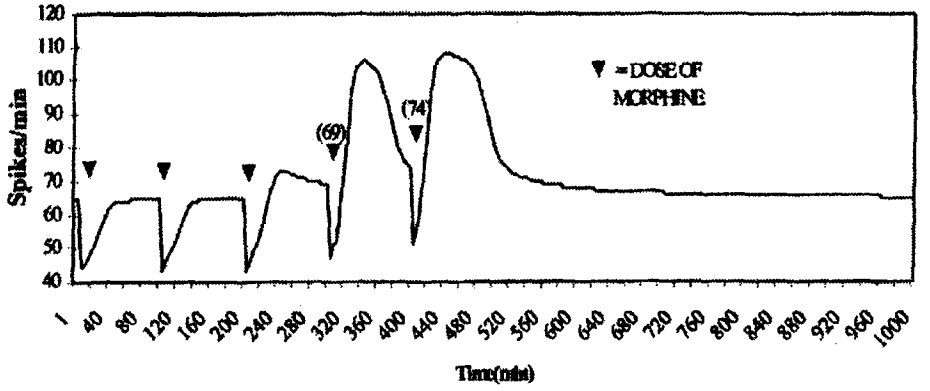

Figure 1. Number of spikes/min when the neuron is submitted to a series of five equal doses of 100 μM of morphine.

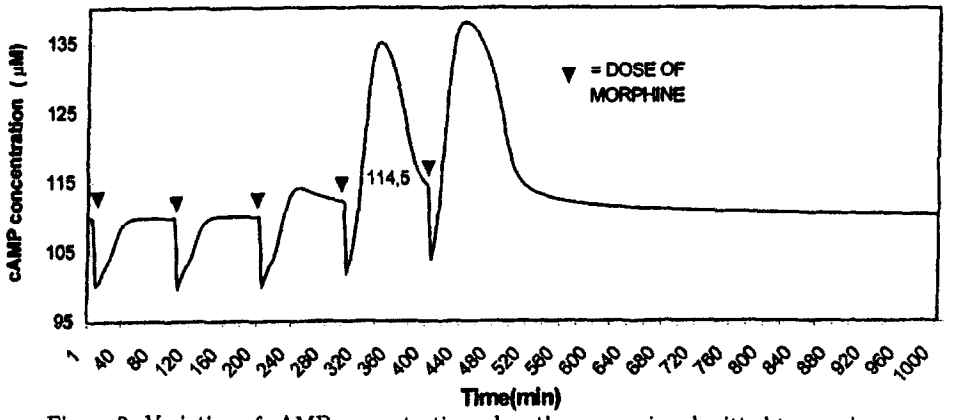

Figure 2. Variation of cAMP concentration when the neuron is submitted to a series of five equal doses of 100 μM of morphine.

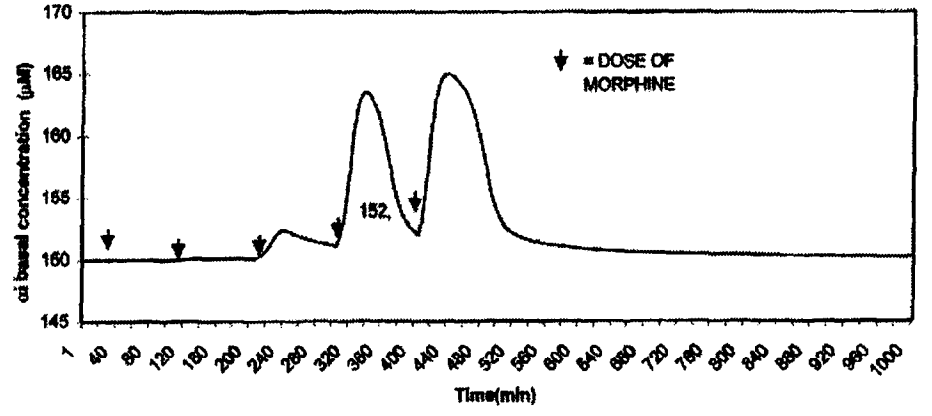

Figure 3. Variation of α_i concentration when the neuron is submitted to a series of five equal doses of 100 μM of morphine.

We can see that, after the third dose of morphine, the neuron begins to develop tolerance. The spiking rate grows from 65 spikes/min to 105 spikes/min after the last dose.

The neuron returns gradually to the basal spiking rate after the fifth dose. The cAMP and α_i concentrations have a similar behavior, as shown in Figures 2 and 3, respectively.

Until the third dose of morphine, the cAMP concentration decreases by about 10%, similar to what is observed experimentally [44]. After the next two doses, the cAMP concentration increases, having peaks in phase with the maximum expression of α_i. Both concentrations return to basal levels as long as no more morphine is applied. In Figure 4 it is shown that, as tolerance

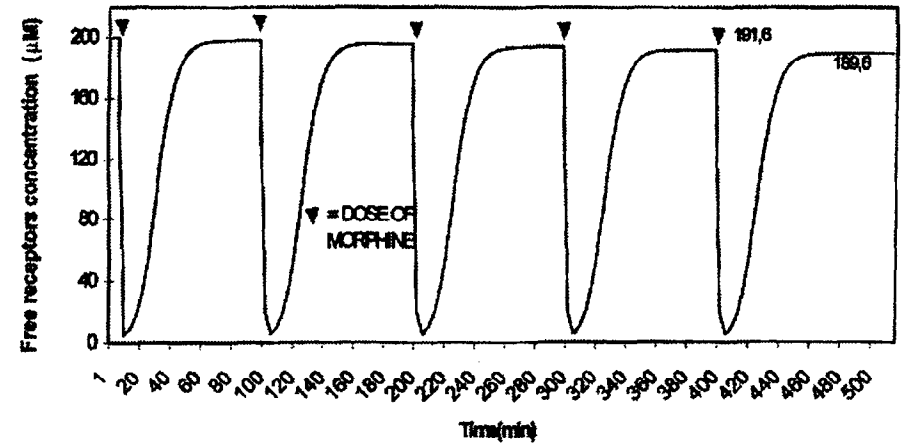

Figure 4. Variation of the concentration of free receptors μ when the neuron is submitted to a series of five equal doses of 100 μM of morphine.

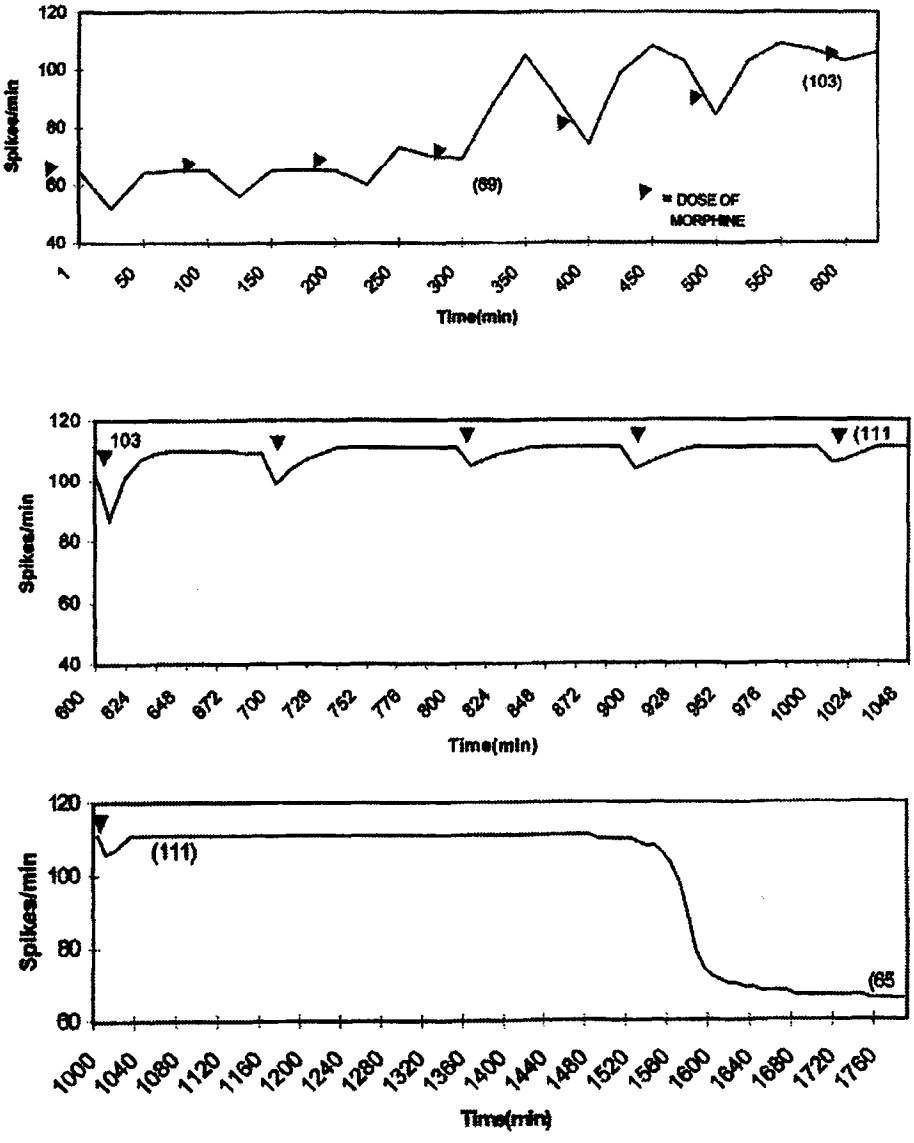

Figure 5. Variation of spikes/min when the neuron is submitted to 11 doses of morphine.

develops in the five contacts of the cell with the drug, the free receptors μ have their basal concentration level decreased from 200 μM to 189.6 μM.

This first experiment shows that the model captures the basic phenomena of tolerance development at both the molecular and electrophysiological levels.

The second simulation shows the behavior of the LC neuron when submitted to 11 successive doses of morphine with an interval of 100 minutes. In Figure 5 it can be seen that, immediately after the application of morphine, the spiking rate decreases to return to the basal level later. After the third contact with the drug, the spiking rates do not return to the basal level, which represents a process of tolerance. From the seventh dose on, the neuron remains highly excited, as is seen by its spiking rate that does not return to the basal level even without contact with the drug. A higher dose of morphine would be necessary to lead the LC neuron to its basal spiking rate. After the last contact with the drug, a long time is needed for the spiking rate to return to basal reference. A similar behavior is shown in Figures 6 and 7 when cAMP and α_i concentrations are observed.

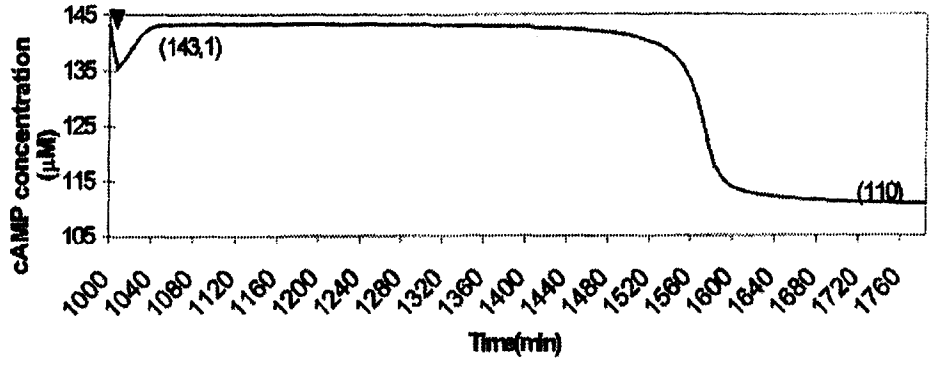

Figure 6. Variation of cAMP concentration when the neuron is submitted to 11 doses of morphine.

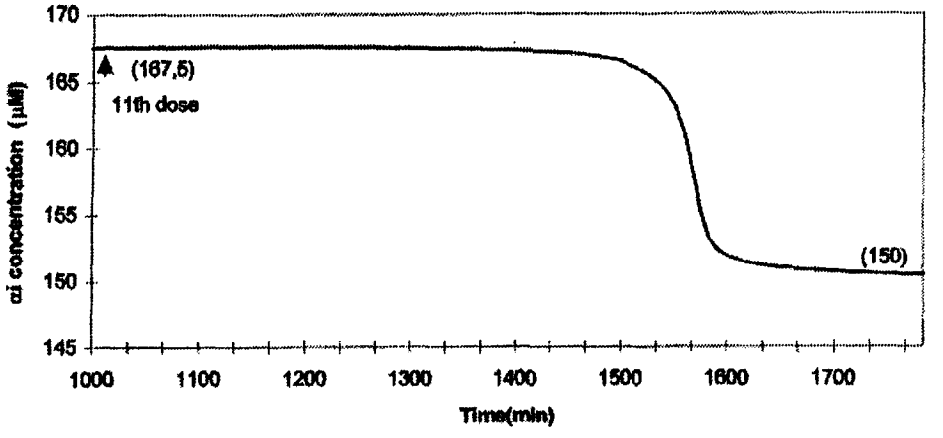

Figure 7. Variation of subunit α_i concentration when the neuron is submitted to 11 doses of morphine.

In another simulation, the number of doses was increased to 17. It can be seen in Figures 8–10 that, after the 11^{th} dose, the α_i concentration remains constant, indicating that due to the long exposure of the cell to the drug, genetic expression was disturbed. After the last dose, the LC neuron returns to its basal behavior in 1500 min, almost twice the time taken in the previous simulation. That is, if the contact with the drug is enough to promote genetic triggering, the time necessary for recovery increases substantially and, of course, a severe withdrawal syndrome takes place.

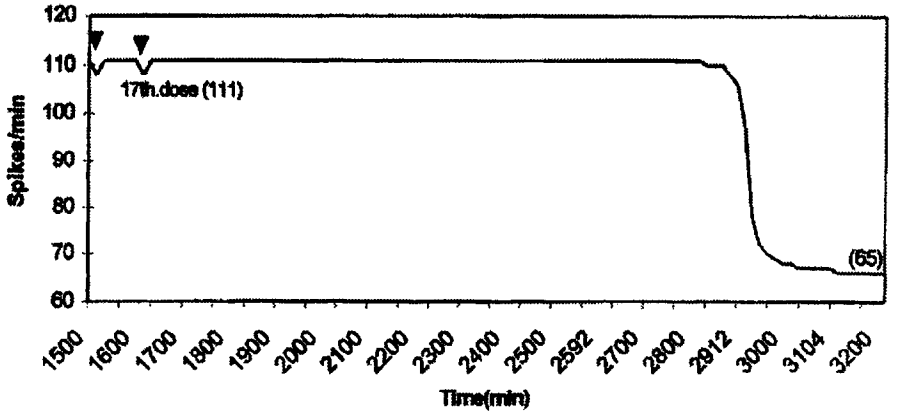

Figure 8. Variation of spikes/min when the neuron is submitted to 17 doses of morphine.

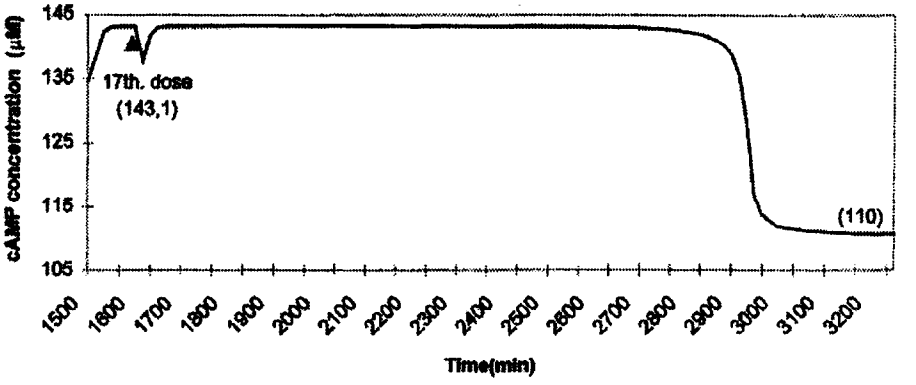

Figure 9. Variation of cAMP concentration when the neuron is submitted to 17 doses of morphine.

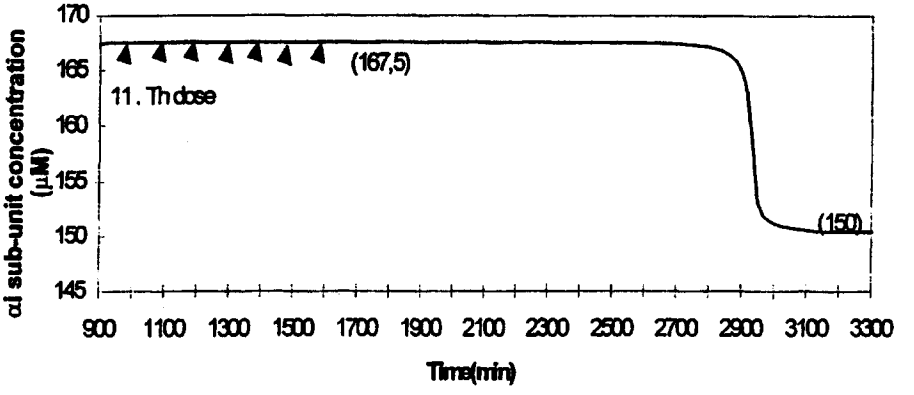

Figure 10. Variation of α_i subunit concentration when the neuron is submitted to 17 doses of morphine.

In the last simulation, the neuron is submitted to 17 doses of morphine and gradually returns to the basal state. When, the basal behavior is reached, more doses are applied. In Figure 11, it is shown that the spiking rate of the LC neuron increases, quickly reaching a maximum of tolerance development in only three doses.

This means that the path to tolerance development depends on the past history of the cell. Previous contact accelerates tolerance greatly. The same phenomena are observed in the cAMP and α_i concentrations, as depicted in Figures 12 and 13.

Finally, recalling that the proteins α_i and α_s compete for the association with the dimmer $\beta\gamma$, it is interesting to simulate a contact with morphine in which the concentration of α_s is deliberately

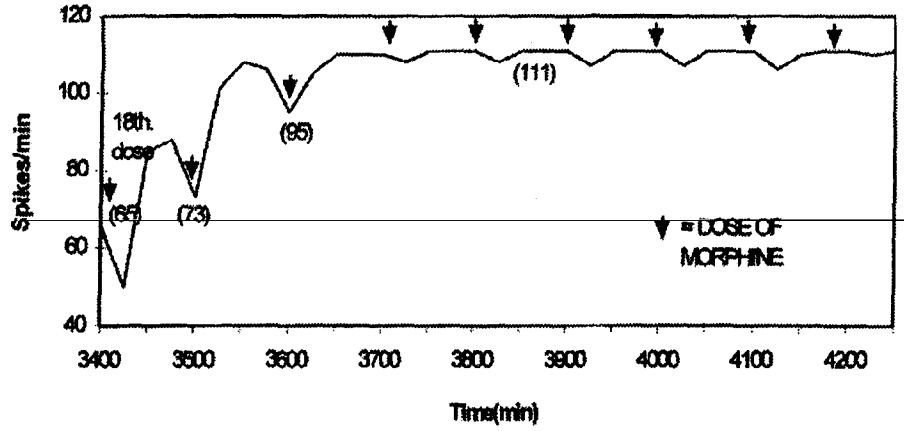

Figure 11. Variation of spikes/min when the neuron is submitted to 17 morphine doses, then comes back to normal and receives 11 more doses.

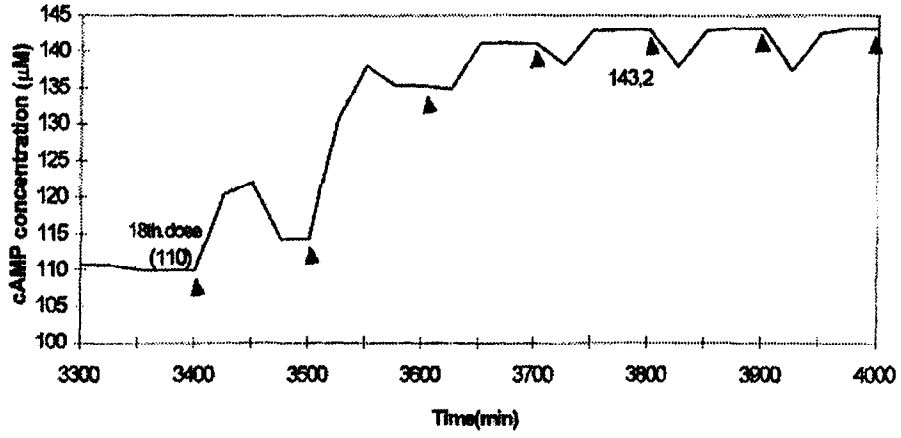

Figure 12. Variation of cAMP concentration when the neuron is submitted to 17 morphine doses, then returns to normal and receives 11 more doses.

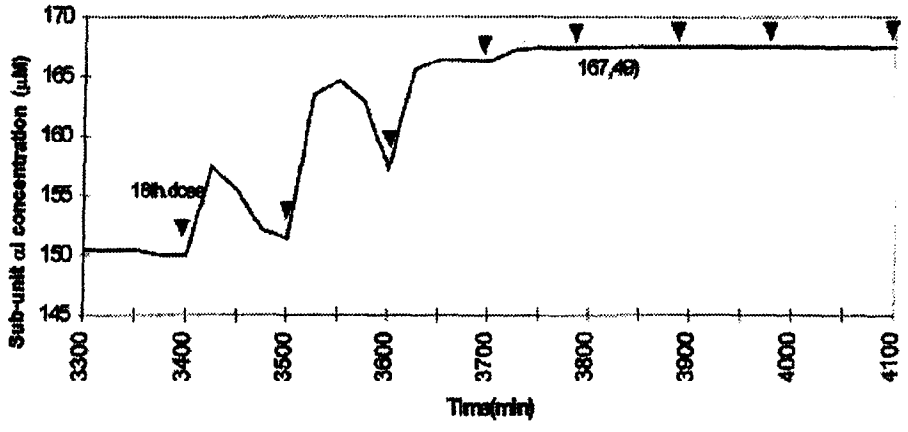

Figure 13. Variation of the concentration of the subunit α_i basal when the neuron is submitted to 17 morphine doses, then returns to normal and receives 11 more doses.

controlled. An experiment in which doses of morphine are applied every 100 min is shown in Figure 14. After the 11^{th} dose, the concentration of protein α_s is increased by 4% every five minutes. The result was that the LC neuron returned to its basal state in 200 min against the 760 min which would be necessary if α_s were constant. The increase of the α_s concentration seems to restore the cell's equilibrium, which hints at a possible treatment of the withdrawal syndrome of opiate addiction.

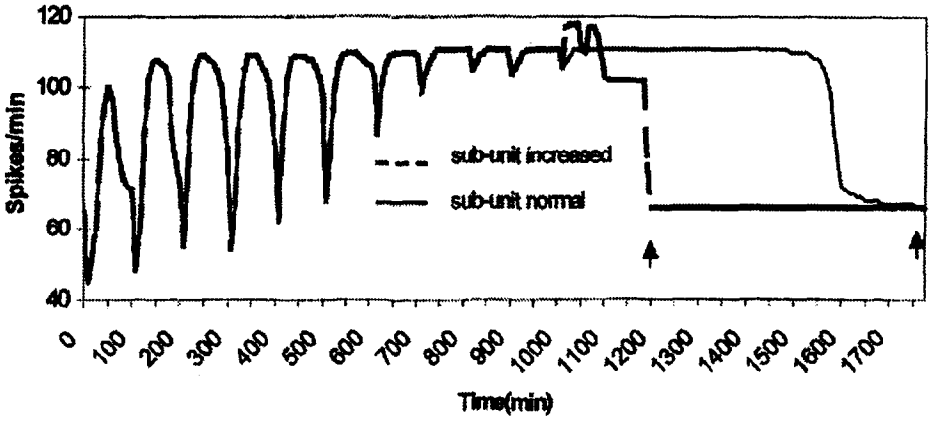

Figure 14. Comparison of the spikes/min under two different experimental conditions, with and without the increase in the concentration of the subunits α_s.

The model seems to capture the processes of tolerance and withdrawal syndrome of opiate intoxication with the reality of true experiments. The hypothesis that the disturbance of a single gene, responsible for the transcription of the α_i protein, is enough to promote opiate tolerance, seems to be plausible as shown by the simulations. The model also proposes that a possible treatment for opiate addiction would be the administration of the protein α_s, which could restore the original equilibrium in the G-protein system.

APPENDIX

GENERAL GLOSSARY

$\overline{\overline{[AC]}}$	Total adenylate cyclase concentration in the LC neuron	$80\,\mu$M
[AC]	Free adenylate cyclase concentration in the LC neuron	M
$\overline{[AC^*]}$	Basal activated adenylate cyclase concentration in the LC neuron	$80\,\mu$M
$[AC^*]$	Activated adenylate cyclase concentration in the LC neuron in time t	M
C	Capacitance of LC neuron membrane	$8\,\mu$F
[Ca]	Calcium concentration in the LC neuron	Mol
[cAMP]	cAMP concentration in the LC neuron	Mol
$[cAMP_h]$	cAMP of half-maximal phosphorylation	$40\,\mu$Mol
$\overline{\overline{[CREB]}}$	Basal CREB concentration	$400\,\mu$M
[CREB]	Free CREB concentration	M
$[CREB_f]$	Phosphoryled CREB concentration	M
d	Activation variable for I_{Na}	
d_∞	Steady-state value for d	
E_{Ca}	Nerst potential for ion Ca^{2+}	50 mV
E_{Na}	Nerst potential for ion Na^+	40 mV
f	Inactivation variable for I_{Na}	
f_∞	Steady-state value for f	
[fos]	Transcription factor c-FOS/JUN concentration	M
g_A	Conductance for the I_A current	.mhos
$\bar{g}_A$	Maximal conductance for I_A current	$0.6\,\mu$.mhos
g_{Ca}	Conductance for the I_{Ca} current	.mhos
$\bar{g}_{Ca}$	Maximal conductance for I_{Ca} current	$3.8\,\mu$.mhos
$[G_i]$	Protein G_i concentration	M
$[G_s]$	Protein G_s concentration	M
$[G_o]$	Protein G_o concentration	M
g_K	Conductance for the I_K current	.mhos

$\bar{g}_K$	Maximal conductance for I_K current	18 μ.mhos
$g_{K,Ca}$	Conductance for the $I_{K,Ca}$ current	.mhos
$\bar{g}_{K,Ca}$	Maximal conductance for $I_{K,Ca}$ current	1 μ.mhos
g_{Na}	Conductance for the I_{Na} current	.mhos
$\bar{g}_{Na}$	Maximal conductance for I_{Na} current	.mhos
$g_{Na,p}$	Conductance for the $I_{Na,p}$ current	.mhos
$g^*_{Na,p}$	Maximal conductance for $I_{Na,p}$ current	2.4 n.mhos
$\bar{g}_{Na,p}$	Basal conductance for the $I_{Na,p}$ current	0.6 n.mhos
h	Inactivation variable for I_{Ca}	
h_∞	Steady-state value for h	
I_A	LC "early" potassium current	nA
I_{Ca}	LC voltage-dependent calcium current	nA
I_K	LC delayed rectifier current	nA
$I_{K,Ca}$	LC calcium-dependent calcium current	nA
I_{Na}	LC fast voltage-dependent sodium current	nA
$I_{Na,p}$	LC persistent sodium current	nA
k_1	Affinity constant of the m for μ	1.8 (min.μM)$^{-1}$
k_2	Affinity constant of the μ^* for G_i	0.015 (min.μM)$^{-1}$
k_3	Affinity constant of the μ^* for G_o	0.05 (min.μM)$^{-1}$
k_4	Affinity constant of the μ^* for P_μ	0.05 (min.μM)$^{-1}$
k_5	Affinity constant of the μ^* for P_μ	0.05 (min.μM)$^{-1}$
k_6	Desensitization rate (Taxa de desensitização)	0.2 (min)$^{-1}$
k_7	Rate of morphine elimination (Taxa de metabolização da morfina)	0.2 (min)$^{-1}$
k_8	Affinity constant of the α_i for $\beta\gamma$	20/9 (min.μM)$^{-1}$
k_9	Affinity constant of the μ^* for G_i	0.015 (min.μM)$^{-1}$
k_{10}	Affinity constant of the α_s for G_i	0.1875 (min.μM)$^{-1}$
k_{11}	Affinity constant of the α_o for G_i	0.1875 (min.μM)$^{-1}$
k_{12}	Affinity constant of the α_o for $\beta\gamma$	0.6 (n.μM)$^{-1}$
k_{13}	Affinity constant of the μ^* for G_o	0.015 (min.μM)$^{-1}$
k_{14}	Affinity constant of the α_s for G_o	0.15 (min.μM)$^{-1}$
k_{15}	Affinity constant of the α_i for G_o	0.15 (min.μM)$^{-1}$
k_{16}	Affinity constant of the α_s for $\beta\gamma$	0.75 (min.μM)$^{-1}$
k_{17}	Affinity constant of the α_i for G_s	0.1875 (min.μM)$^{-1}$
k_{18}	Affinity constant of the α_o for G_s	0.1875 (min.μM)$^{-1}$
k_{19}	Part of α_s used in the formation of AC	5.5 (min)$^{-1}$
k_{20}	Part of α_i used in the formation of AC	5.5 (min)$^{-1}$
k_{21}	Part of AC used in the formation of cAMP	0.653125 (min)$^{-1}$
k_{22}	cAMP degration rate	0.475 (min)$^{-1}$
k_{24}	Affinity constant of the PQ for cAMP	0.3 (min.μM)$^{-1}$
k_{25}	PQ* degration rate	33 (min)$^{-1}$
k_{26}	Affinity constant of the PQ* for CREB	0.3 (min.μM)$^{-1}$
k_{27}	$CREB_f$ degration rate	30 (min)$^{-1}$
k_{28}	Part of $CREB_f$ in activation of fos	1.5
k_{60}	Part of μ_p that down-regulates	6.10^{-4}(min)$^{-1}$
ℓ	Activation variable for I_K	
ℓ_∞	Steady-state value for ℓ	
m	Activation variable for I_{Ca}	
[morf]	Morphine concentration	μM
m_∞	Steady-state value for m	
n	Constant for calcium buffering	0.33
o	Calcium activation variable for $I_{K,Ca}$	

p	Voltage activation variable for $I_{K,Ca}$	
[PQ]	Inactive protein kinase concentration	μM
[PQ*]	Active protein kinase concentration	100 μM
$\overline{[PQ]}$	Basal protein kinase concentration	200 μM
$[P_\mu]$	Protein kinase P_μ concentration	10 μM
q	Voltage inactivation variable for $I_{K,Ca}$	
q_∞	Steady-state value for q	
r	Activation variable for I_A	
S_d	Slope for d_∞	0.9 mV
S_f	Slope for f_∞	0.1 mV
S_{τ_f}	Slope for τ_f	2.5 mV
S_h	Slope for h_∞	1.5 mV
$S^r_{\tau_h}$	Right slope for τ_h	1 mV
$S^l_{\tau_h}$	Left slope for τ_h	1 mV
S_ℓ	Slope for ℓ_∞	0.1 mV
S_m	Slope for m_∞	0.48 mV
$S_{Na,p}$	Slope for $g_{Na,p}$	12.5 nMol
S_o	Slope for o	0.01 nMol
S_p	Slope for p	0.1 mV
S_q	Slope for q_∞	0.5 mV
S_{τ_q}	Slope for τ_q	1 mV
S_r	Slope for r	1 mV
$S^r_{\tau_l}$	Right slope for τ_ℓ	0.5 mV
$S^l_{\tau_l}$	Left slope for τ_ℓ	13.5 mV
S_z	Slope for z_∞	0.5 mV
S_{τ_z}	Slope for τ_z	0.3 mV
t	Time	minutes
T_{h_g}	Threshold for the gene sigmoidal function	40.0
T_h	Threshold for the fos sigmoidal function	12.13
V	Potential of the LC membrane	mV
V_d	Half-maximal potential for d_∞	−45 mV
V_f	Half-maximal potential for f_∞	0 mV
V_{τ_f}	Potential for maximum τ_f	0 mV
V_h	Half-maximal potential for h_∞	−50 mV
V_{τ_h}	Potential for maximum τ_h	−60 mV
V_ℓ	Half-maximal potential for ℓ_∞	−17.5 mV
V_{τ_l}	Potential for maximum τ_ℓ	−17.5 mV
V_m	Half-maximal potential for m_∞	−50 mV
V_p	Half-maximal potential for p	−65 mV
V_q	Half-maximal potential for q_∞	−68 mV
V_{τ_q}	Potential for maximum τ_q	−68 mV
V_r	Half-maximal potential for r	−74 mV
V_z	Half-maximal potential for z_∞	−73 mV
V_{τ_z}	Potential for maximum τ_z	−73 mV
z	Inactivation variable for I_A	
z_∞	Steady-state value for z	
α	Calcium accumulation constant rate	8 mMol/A
$[\alpha_i]$	α_i concentration	μM
$\overline{[\alpha_i]}$	Basal α_i concentration	μM
$[\alpha_s]$	α_s concentration	μM
$\overline{[\alpha_s]}$	Basal α_s concentration	150 μM

$[\alpha_o]$	α_o concentration	μM
$\overline{[\alpha_o]}$	Basal α_o concentration	150 μM
β	Calcium constant buffering rate	0.004
$[\beta\gamma]$	γ concentration	μM
$\overline{[\beta\gamma]}$	Basal $\beta\gamma$ concentration	350 μM
g	Slope for gene function	0.095
r	Slope for [fos] function	0.6
$[\mu]$	Free opioid receptor concentration	μM
$[\mu_d]$	"Down-regulation" receptor concentration	μM
$[\mu_p]$	Phosphorylated opioid receptor concentration	μM
$[\mu^*]$	Activated opioid receptor concentration	μM
$\overline{[\mu]}$	Basal opioid receptor concentration	200 μM
l	Relaxation time for l	ms
$\bar{\tau}_l$	Maximal relaxation time for l	150 ms

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