# Model of Calcium Channel Inactivation: A Qualitative Analysis

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**Abstract**. A simple model of calcium channel inactivation has been developed, based on the accumulation of calcium ions at the inner mouth of the channel and on their binding to a receptor which inactivates the channel. A qualitative analysis has shown that upon an appropriate choice of parameters corresponding to the cell structure and to kinetic properties of its components, the calcium dependent inactivation and that assumed to be voltage dependent can both be emulated. The model suggests that the supposed variety of calcium channels might be explained by quantitative differences in nonlinear interactions of the channels with other cell components.

**Key words**: Calcium channel inactivation — Mathematical model — Qualitative analysis

## Introduction

The mechanism of excitability is based on mutual, membrane potential mediated coordination of ionic permeabilities of integral membrane proteins, called channels.

Since calcium is the universal intracellular messenger which regulates numerous cell functions, calcium channels are among the most commonly occurring ionic channels.

Experimentally, different types of the membrane calcium permeability behaviour have been observed. Usually data have been interpreted as linear composition of several processes, i.e. as contributions of two or more types of calcium channels.

Significant differences in calcium channel characteristics have been reported to exist between various preparations; these may be related to some specific role of this channel type in the particular type of cells, or with possible artefacts of the methods used (Hagiwara 1983; Tsien 1983). A number of these problems

have been overcome recently with the development of single-channel recording (Hagiwara and Byerly 1981).

In their nature, biological processes are highly nonlinear due to interactions of a large number of components and strong thermodynamic forces.

The aim of the present paper has been to analyse the types of behaviour of calcium channels, in particular inactivation, that may be explained by nonlinearities of their mechanism and/or by differences in experimental conditions.

Our model has been based on the hypothesis of Brehm and Eckert (1978). These authors studied inactivation dependent on calcium concentration at the inner mouth of the calcium channel. This effect was modelled also by Standen and Stanfield (1982). The latter authors used kinetics of the $m^3h$ type, where $h$ was a function of the calcium concentration inside the cell.

The present analysis is only qualitative; this means that the model components have been chosen as simple as possible to allow general conclusions. For a satisfactory approximation of experimental data, more sophisticated model components should be chosen.

The theoretical considerations of the problem of various channel types presented herein cannot be used to prove or disprove the existing hypotheses. They merely stress which effects should be considered when interpreting experimental data.

## The Model and Results

Let us assume the following simple model of calcium channel permeability. A depolarizing voltage step opens Q channels in surface unit of a membrane. Calcium can bind at the inner side of the membrane to the channel and block it. When X stands for open channel and X* for the blocked one, then

$$\mathrm{X} + \mathrm{Ca} \underset{k_r}{\overset{k_f}{\leftrightarrow}} \mathrm{X}^* \text{ and } [\mathrm{X}] + [\mathrm{X}^*] = \mathrm{Q}$$

where $k_f$ is the rate constant of Ca binding to the channel, and $k_r$ is the dissociation rate constant. Then, the number of open channels meets the relationship

$$\mathrm{d}X/\mathrm{d}t = k_r . (\mathrm{Q} - X) - k_f . X . c_i \tag{1}$$

where $c_i$ is the calcium concentration in the vicinity of the inner side of the membrane.

In the simplest case the flow of calcium ions through the channel is proportional to the electrochemical gradient across the membrane (thermodynamical force)

$$J = \mathrm{k} \,.\, X \,.\, (c_\mathrm{o} \,.\, \exp(\mathrm{zFV}/\mathrm{R}T) - c_\mathrm{i}) \tag{2}$$

where potential inside the cell is zero. The calcium concentration at the inner surface of the membrane depends on the diffusion flow from the membrane towards cell inside

$$\mathrm{d}c_\mathrm{i}/\mathrm{d}t = J - J_\mathrm{d} \tag{3}$$

where

$$J_\mathrm{d} = \varkappa c_\mathrm{i} \tag{4}$$

when the internal calcium concentration is negligibly low.

Denoting $c = c_\mathrm{o} \,.\, \exp(\mathrm{zFV}/\mathrm{R}T)$, then from (2), (3) and (4), we get

$$\mathrm{d}c_\mathrm{i}/\mathrm{d}t = \mathrm{k} \,.\, X \,.\, (c - c_\mathrm{i}) - \varkappa c_\mathrm{i} \tag{5}$$

We shall analyse the system of nonlinear differential equations (1,5). The initial state is $X = 0$, $X^* = 0$ and $c_\mathrm{i} = 0$. At $t = 0$, Q channels open, i.e. $X = \mathrm{Q}$ ($X$ and $X^*$ are identical with their surface concentrations).

The system is simplified by substituting

$$s = k_\mathrm{f}/k_\mathrm{r} \,.\, c_\mathrm{i} \tag{6}$$

$$Y = \mathrm{k} \,.\, X \tag{7}$$

and then

$$\mathrm{d}Y/\mathrm{d}t = k_\mathrm{r} \,.\, (\mathrm{R} - Y \,.\, (1 + s)) \tag{8}$$

$$\mathrm{d}s/\mathrm{d}t = s_\mathrm{o} \,.\, Y - Y \,.\, s - \varkappa s \tag{9}$$

where $\mathrm{R} = \mathrm{k} \,.\, \mathrm{Q}$ and $s_\mathrm{o} = k_\mathrm{f}/k_\mathrm{r} \,.\, c$.

To be able to infer solution properties to the system (8, 9), we must know the course of trajectories in the phase space. The right sides of equations (8) and (9) supply, in each point of the plane ($Y$, $s$), a tangent to the trajectory. I.e., starting from a point defined as initial condition, the course of the trajectory is determined by a tangent in each successive point of the phase space reached. Usually, it is sufficient to draw curves, for which $\mathrm{d}Y/\mathrm{d}t$ and/or $\mathrm{d}s/\mathrm{d}t$ are zero, and directions of tangents in their neighbourhood. Therefore,

$$\mathrm{d}Y/\mathrm{d}t = 0 \Rightarrow s = \mathrm{R}/Y - 1 \tag{10}$$

$$\mathrm{d}s/\mathrm{d}t = 0 \Rightarrow s = s_\mathrm{o} \,.\, Y/(Y + \varkappa) \tag{11}$$

The intercept of these curves (nullclines) is a critical point, which represents a steady solution to the system (8,9). Elimination of the variable $s$ gives a quadratic equation

$$Y^2 \,.\, (s_\mathrm{o} + 1) + Y \,.\, (\varkappa - \mathrm{R}) - \varkappa \mathrm{R} = 0 \tag{12}$$

which has roots

$$Y_{1,2} = [R - \varkappa \pm ((\varkappa - R)^2 + 4R\varkappa(s_o + 1))^{0.5}]/(2.(s_o + 1))$$

The answer to the problem is only the solution in the first quadrant, and

$$\bar{Y} = [R - \varkappa + ((\varkappa + R)^2 + 4R.\varkappa.s_o)^{0.5}]/(2.(s_o + 1)) \tag{13}$$

and from (10)

$$\bar{s} = R/\bar{Y} - 1 \tag{14}$$

The critical point $(\bar{Y}, \bar{s})$ can be specified by the criteria shown in Appendix.

The matrix of a linear approximation of the system (8,9) in the neighbourhood of the critical point has elements

$$\begin{aligned} a_{11} &= -k_r.(1 + \bar{s}),\ a_{12} = -k_r.\bar{Y} \\ a_{21} &= s_o - \bar{s},\ a_{22} = -(\bar{Y} + \varkappa) \end{aligned} \tag{15}$$

Therefore, coefficients of a characteristic equation are

$$b = -k_r.(1 + \bar{s}) - (\bar{Y} + \varkappa),\ c = k_r.(1 + \bar{s}).(\bar{Y} + \varkappa) + k_r.\bar{Y}.(s_o - \bar{s})$$

For $s_o - \bar{s} > 0$ obviously $c > 0$; i.e. the critical point cannot be a saddle point. The sign of

$$b^2 - 4c = [k_r.(1 + \bar{s}) + (\bar{Y} + \varkappa)]^2 - 4k_r.\bar{Y}.(s_o - \bar{s})$$

has to be positive, since in an opposite case the critical point would be either a centre or a spiral, and neither periodical nor dumped oscillations have been observed experimentally. Hence, the critical point is a node, and

$$[k_r.(1 + \bar{s}) - (\bar{Y} + \varkappa)]^2 > 4k_r.\bar{Y}.(s_o - \bar{s}) \tag{16}$$

Based on these calculations we can draw the phase portrait (Fig. 1) of the system (8,9), particularly its nullclines, (10) and (11). In these points tangents of trajectories intersecting curve (10) are vertical and those intersecting curve (11) are horizontal. Curve (10) is a hyperbola, intersecting the axes $Y$ at point (R,0) and asymptotically approaching the axis $s$. It divides the first quadrant in two parts. According to Eq. (8), $dY/dt > 0$ on the left, and $dY/dt < 0$ on the right. This shows the directions of tangents of trajectories at points of their intersection with curve (10) (see Fig. 1). Analogically, we draw curve (11), which starts from point (0,0) and asymptotically approaches the line $s = s_o$. With the known signs of time derivatives in any part of the phase space, it is easy to draw tangents anywhere.

This phase portrait of the system (8,9) can be interpreted as follows. Let us assume that activation is much faster than inactivation, as has been generally concluded for calcium channels (Tsien 1983). At rest, the system is in point (0,0). A depolarizing pulse at time $t = 0$ opens Q channels in a surface unit of

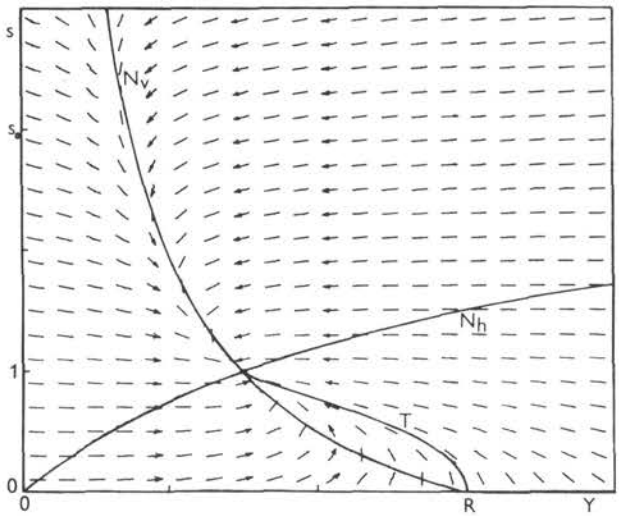


**Fig. 1.** The phase portrait of system (8,9) for parameter values $s_o = 3$, $k_r = 10$, $\varkappa = R$. Short segments are tangents to trajectories at corresponding points of plane $(Y, s)$. Nullclines (10) and (11) are drawn, where tangents are vertical ($N_v$) or horizontal ($N_h$). The arrows are drawn to give an idea about the direction of the tangents in the neighbourhood of the critical point, where nullclines (10) and (11) intersect. The trajectory (T) of the solution for the initial condition (R,0) is shown.

membrane. Hence the trajectory moves in the phase space towards point (R,0). Calcium ions, entering the cell, accumulate in the near-membrane space and block a part of the channels. The trajectory of this process continues from point (R,0) along the right side of curve (10) into the critical point $(\bar{Y}, \bar{s})$ denoting the steady state, which the process will reach asymptotically at time $t = +\infty$. This is qualitatively illustrated in Fig. 2.

The flux of calcium ions into a cell is given by Eq. (2); it can be rewritten for a steady flux as

$$\bar{J} = \bar{Y} \,.\, k_r/k_f \,.\, (s_o - \bar{s})$$

while the maximal flux is

$$J_m = R \,.\, k_r/k_f \,.\, s_o$$

The ratio maximal to steady flux expresses inactivation related to the inactivation determined by the two pulse method. Considering equations (13) and (14), the inactivation can be expressed as

$$\begin{aligned} \bar{J}/J_m &= \bar{Y}/R \,.\, (1 - \bar{s}/s_o) \\ &= [-(R + \varkappa) + ((R + \varkappa)^2 + 4R \,.\, \varkappa \,.\, s_o)^{0.5}]/(2R \,.\, s_o) \end{aligned} \tag{17}$$

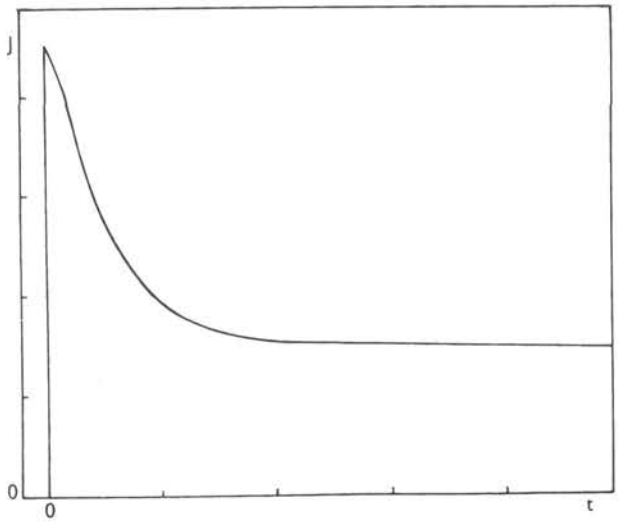


**Fig. 2.** The time course of calcium flux, corresponding to the trajectory shown in Fig. 1. It represents the numerical solution of the system (8,9) for the initial condition (R,0). The Heun method with step $10^{-3}$ was used, and the relative error was less than $10^{-5}$.

In this term, the potential dependent variables are R and $s_o$. The dependence of the inactivation on the individual parameters is illustrated in Fig. 3. We see that upon an appropriate choice of parameters all the experimentally observable results can be obtained. The potential dependence of R is S-shaped, it increases with larger depolarizations. For a low depolarization the number of activated channels, and hence R, are small. The voltage dependence of $s_o$ is opposite, it exponentially decreases with the depolarization. Hence, for $\varkappa \gg k \,.\, Q$, there is no inactivation. For $\varkappa \ll k \,.\, Q$, the inactivation is total; moreover for $k_f \gg k_r$ the voltage dependence of inactivation is negligible. For $s_o$ approaching zero, the ratio $\bar{J}/J_m$ converges to $\varkappa/(R + \varkappa)$, which happens at large depolarizations or when the receptor affinity to calcium is low ($k_f \ll k_r$). Mutual relations of these parameters can therefore yield also a nonmonotonic voltage dependence of inactivation.

A more realistic approach to describe a current through the calcium channel is to use a saturable transport instead of using linear dependence on the driving force. Also, rectifying properties of the channel should be considered. This modification does not introduce qualitative changes in conclusions drawn from the model. The phase portrait remains topologically the same, with the time course of the current being better adjusted to the experimental data.

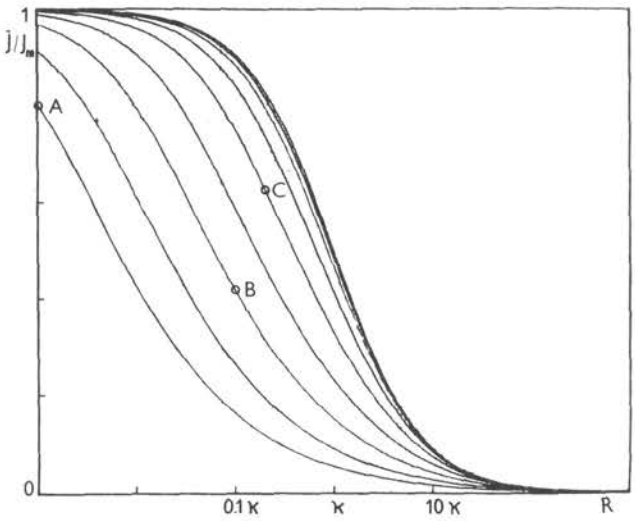


**Fig. 3.** Inactivation curves for parameters $s_0 = 300, 100, 30, 10, 3, 1, 0.3, 0.1$, i.e. an approx. 15 mV step; the limit curve on the right is $\varkappa/(R + \varkappa)$. The independent variable R is expressed in $\varkappa$ units on a logarithimc scale. Filled points represent the U-shaped voltage-steady current relation, where point *A* corresponds to $-58$ mV, $R = 0.001\,\varkappa$, $s_0 = 300$; *B* to $-29$ mV, $R = 0.1\varkappa$, $s_0 = 30$; and *C* to 0 mV, $R = 0.2\varkappa$, $s_0 = 3$. These points represent the S-shape voltage dependence of R and the exponential dependence of $s_0$.

## Discussion

The calcium channel has been supposed to have a number of modifications and to present different characteristics in different preparations (Tsien 1983; Hagiwara and Byerly 1981; Hagiwara 1983). The aim of this paper was to show, on a simple model, that the channel variability can be explained by its nonlinear interactions with other membrane and/or cell components.

The qualitative behaviour of the calcium channel was analysed on a time scale corresponding to the speed of inactivation. Two processes, which open and close the calcium channel, are considered. The first one is voltage dependent, and its mechanism is irrelevant for our analysis; it is only assumed that this process is faster than inactivation. The inactivation is represented as the blocking of the channel by calcium binding on a receptor at the inner mouth of the channel as was suggested by Brehm and Eckert (1978). The experimental confirmation of this hypothesis has been reviewed by Eckert and Chad (1984). The existence of these two mechanisms has been supported also by patch clamp experiments. The channel activity exhibits series of short openings which are

divided by longer intervals where the channel is closed (Fenwick et al. 1982). This could be interpreted as slow fluctuations of the voltage dependent mechanism with Boltzmann distribution. During an open state, the channel is blocked by the binding of calcium ions to the receptor. This explains the series of short closings of the channel. The distribution of these fluctuations is determined by the rate constants of calcium dissociation from and binding to, the receptor.

The patch clamp method has provided detailed information about the calcium channel kinetics. Still questions have remained which can be answered but by synthetising models. These questions concern the regulation of overall calcium flow into a cell; the role of channels with different kinetics and their mutual coordination; mechanisms preventing calcium overloading a cell etc.

The blocking of the calcium channel by ions entering the cell has been modelled also by Standen and Stanfield (1982), and Chad et al. (1984). They used the Hodgkin-Huxley kinetics to describe the channel. The modification consisted in the calcium concentration dependence of the inactivation variable $h$. It was possible to adjust parameters of the model to describe the experimentally observed time courses of calcium currents. Chad and Eckert (1984) showed that due to the discrete nature of the membrane ionic permeability, the calcium concentration in the layer at the inner membrane surface is not uniform, and that this nonuniformity can account for anomalous voltage relations of Ca-dependent processes.

Opposite to these detailed models, in quantitatively describing voltage dependences and time courses of particular calcium currents, the proposed model allows general conclusions to be drawn on the calcium feedback mechanism. The model is a general description of calcium flux into a cell and of its binding to the channel receptor. A qualitative analysis can infer properties of inactivation with a broad applicability, and it illustrates the range of variability offered by the supposed mechanism.

A strong argument in support of the hypothesis concerning the feedback inactivating effect of the influx of calcium ions is the fact that the cells keep internal calcium at very low concentrations. This is necessary for the messenger function of calcium. The uptake of calcium is an energy consuming process; the feedback inactivation is thus a very effective mechanism for the cell calcium homeostasis.

The results of this study can be applied to crayfish sarcolemma, which operates on the pure calcium electrogenesis principle. In an intact fibre, the calcium current inactivated almost completely (Henček and Zachar 1977). Morphological investigations (Uhrik et al. 1980) have shown a layer of mitochondria at the inner surface of the sarcolemma, which could form a diffusion barrier for calcium ions and contribute to their accumulation. On the other hand, almost no inactivation occurred in fibre segments dialysed with EGTA,

where calcium was chelated (Zahradník and Zachar 1982). Inactivation is the most important indicator of differences in calcium channels between different preparations. It is known that inactivation has a broad spectrum ranging from very slow, or absent, to complete, voltage or calcium dependent. Generally, compared with the sodium channel, the calcium channel inactivation is relatively slow with respect to activation (Tsien 1983).

Minimal inactivation was observed in synaptic terminals (Llinas et al. 1981), adrenaline chromaffin cells (Fenwick et al. 1982), and also in muscle fibres of Balanus (Keynes et al. 1973). Using Paramecium and neurons of Aplysia, Brehm and Eckert (1978) and Brehm et al. (1980) showed that the calcium channel inactivation is dependent on the flux of calcium ions entering the cell from extracellular space. Other cells have been supposed to operate on a similar voltage dependent mechanism of inactivation as does the sodium channel. The criteria for the nature of inactivation are usually the monotonous or U-shaped membrane potential dependence for voltage and/or calcium dependent inactivation. The proposed model shows that both dependences can by yielded by an appropriate choice of parameters $k_r/k_f$, $k$, and $\varkappa$ within an experimentally possible voltage range. These parameters can be influenced by the structure of the layer at the inner membrane surface and by aggregation of the calcium channel with specific membrane macromolecules through allosteric effects; this can in turn explain different sensitivities to calcium channel blockers.

A different type of inactivation was observed by Almers et al. (1981) on tubular membranes of frog fibres. There was a decrease of calcium current due to a decrease of calcium concentration in the confined space of the tubular system. This finding supports the principal idea of this discussion concerning the system approach to biological processes.

This paper has not been intended to provide a proof for the calcium channel inactivation being only a consequence of calcium accumulation in the space at the inner side of the membrane, as one possible interpretation arising from this simple model. Rather, we have attempted to show the variability a nonlinear system can yield in dependence on external conditions.

## Appendix

Very often, biological phenomena can be described by a system of ordinary differential equations. Such a system is always convertable into an autonomous system of ordinary first order differential equations in the form

$$\mathrm{d}y/\mathrm{d}t = f(y) \tag{A1}$$

A particular solution can be expressed as a trajectory in $n$-dimensional space, called phase space of the system (A1). At time $t$, any point of trajectory has coordinates $(y_1(t), y_2(t), \ldots, y_n(t))$. A vector $(f_1(y), f_2(y), \ldots, f_n(y))$ is tangent to to the trajectory at point $y$. It gives the direction and movement speed of a point along the trajectory. For the initial condition

$$\boldsymbol{y}(0) = \boldsymbol{c} \tag{A2}$$

the trajectory passing point $\boldsymbol{c}$ in the phase space is the solution to the initial problem (A1, A2).

All trajectories in the phase space contain characteristics of the system behaviour. An important role play points at which $\boldsymbol{f}(\boldsymbol{y}) = 0$. They are called critical, and represent steady states of the system.

If $\boldsymbol{f}(\boldsymbol{y})$ is continuously differentiable, then trajectories can intersect only at points $p$, where $\boldsymbol{f}(\boldsymbol{p}) = 0$. We can determine the qualitative behaviour of the system (A1) near a critical point by linearising the system (A1) in the vicinity of $\boldsymbol{p}$. The linearised system has the form

$$\mathrm{d}\boldsymbol{y}/\mathrm{d}\boldsymbol{t} = \boldsymbol{A}\boldsymbol{y} \tag{A3}$$

where $\boldsymbol{A}$ stands for the matrix of constant coefficients

$$a_{ij} = \partial f_i(\boldsymbol{p})/\partial y_j$$

The solution of the linear system of ordinary differential equations (A3) is

$$\boldsymbol{y}(t) = b_1\,{}^1\boldsymbol{e} \cdot \exp(\lambda_1 t) + \ldots + b_n\,{}^n\boldsymbol{e} \cdot \exp(\lambda_n t) \tag{A4}$$

where $\lambda_i$ is the eigenvalue and ${}^i\boldsymbol{e}$ the corresponding eigenvector of the system (A3), i.e.

$$\boldsymbol{A}\boldsymbol{e} = \lambda\boldsymbol{e}, \text{ resp. } (\boldsymbol{A} - \lambda\boldsymbol{I})\,\boldsymbol{e} = 0$$

where $\boldsymbol{e}$ is a nonzero vector only when

$$\det(\boldsymbol{A} - \lambda\boldsymbol{I}) = 0 \tag{A5}$$

$\det(\boldsymbol{A} - \lambda\boldsymbol{I})$ is a polynom of $n$-th order, and equation (A5) has $n$ roots.

Generally, it is the qualitative behaviour of the system (A1) rather than the exact solution to it that is the subject of our interests. Therefore, it is sufficient to determine only sings of the real parts of roots in Eq. (A5); e.g., if each $\lambda_i$ contains negative real parts, then (A4) implies that all solutions are stable and converge exponentially to a critical point for $t$ approaching $+\infty$.

With an autonomous system of two ordinary differential equations, the phase space is two-dimensional, representable in a plane; its analysis can thus be graphically represented. The system (A5) leads to the solution of quadratic equation

$$\lambda^2 - b\lambda + c = 0 \tag{A6}$$

where $b = a_{11} + a_{22}$ and $c = a_{11} \cdot a_{22} - a_{12} \cdot a_{21}$, and which has roots

$$\lambda_{1,2} = 1/2 \cdot (b \pm (b^2 - 4c)^{0.5}) \tag{A7}$$

Excluding multiple roots, we can classify critical points according to the signs of the real and the imaginary parts of the roots. The roots are either real or complex conjugates. If at least one root has a positive real part, the critical point is unstable. If imaginary parts are nonzero, the solutions near the critical point spiral about it. For the real roots with the same signs, the critical point is called a node. For the opposite sings, it is a saddle point.

More details can be found in every textbook of differential equations. For our purposes, this sketchy review is adequate.

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