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Abstract

To understand how the cerebellum adaptively times the classically conditioned nictitating membrane response (NMR), a model of the metabotropic glutamate receptor (mGluR) second messenger system in cerebellar Purkinje cells is constructed. In the model slow responses, generated postsynaptically by mGluR-mediated phosphoinositide hydrolysis, and calcium release from intracellular stores, bridge the interstimulus interval (ISI) between the onset of parallel fiber activity associated with the conditioned stimulus (CS) and climbing fiber activity associated with unconditioned stimulus (US) onset. Temporal correlation of metabotropic responses and climbing fiber signals produces persistent phosphorylation of both AMPA receptors and Ca^{2+}-dependent K^{+} channels. This is responsible for long-term depression (LTD) of AMPA receptors. The phosphorylation of Ca^{2+}-dependent K^{+} channels leads to a reduction in baseline membrane potential and a reduction of Purkinje cell population firing during the CS-US interval. The Purkinje cell firing decrease disinhibits cerebellar nuclear cells which then produce an excitatory response corresponding to the learned movement. Purkinje cell learning times the response, while nuclear cell learning can calibrate it. The model reproduces key features of the conditioned rabbit NMR: Purkinje cell population response is properly timed, delay conditioning occurs for ISIs of up to four seconds while trace conditioning occurs only at shorter ISIs, mixed training at two different ISIs produces a double-peaked response, and ISIs of 200-400ms produce maximal responding. Biochemical similarities between timed cerebellar learning and photoreceptor transduction, and circuit similarities between the timed cerebellar circuit and a timed dentate-CA3 hippocampal circuit, are noted.

Keywords: Classical conditioning, nictitating membrane response, cerebellum, long-term depression, metabotropic glutamate receptors, AMPA receptors, neural network

The cerebellum is involved in the learned timing of classically conditioned eye blinks. Maladaptively timed conditioned responses (CRs) occur following cerebellar cortical lesions (McCormick & Thompson, 1984; Perrett et al., 1993). Neural activity patterns in cerebellar Purkinje cells and interpositus nuclear cells precede and model the CR (McCormick et al., 1982; Thompson & Krupa, 1994). Direct stimulation of mossy fiber inputs to the cerebellum can substitute for external conditioned stimulus (CS) presentation, while direct stimulation of the source of climbing fibers can serve as the unconditioned stimulus (US) (Steinmetz, Lavond & Thompson, 1989). Classical conditioning with direct brain stimulation results in an adaptively timed CR, and a correspondingly timed increase in interpositus activity (Steinmetz, 1990b).

A number of mechanisms have been proposed to explain timing of eye blinks, including delay lines (Moore et al, 1989; Zipser, 1986), slow responses in neurons (Bartha et al, 1991; Bullock et al, 1994; Grossberg & Merrill, 1992, 1995; Grossberg & Schmajuk, 1989; Jaffe, 1992), and temporal evolution of the network activity pattern (Buonomano & Mauk, 1994; Chapeau-Blondeau & Chauvet, 1991). Given that eye blinks may be delayed for up to four seconds following onset of the CS (Gormezano, 1966), there do not appear to be delay lines of sufficient length in cerebellar cortex (Freeman, 1969). Noise in network activity pattern models seem to preclude their operation over these long intervals as well (Bounomano & Mauk, 1994). The most likely candidate mechanism is a slow neuron response. Given the above evidence that timing occurs in cerebellar cortex and the fact that granule cells appear to have only short latency responses (Thompson & Bower, 1993), the simplest explanation is that slow responses in Purkinje cells are the operative mechanism in adaptive timing. We hypothesize that Purkinje cell slow responses are produced by activation of metabotropic glutamate receptors (mGluRs) and that the latency of the mGluR response spans the range of conditionable eye blink interstimulus intervals (ISIs).

Experimental study of metabotropic responses in Purkinje cells is difficult. Slow excitatory postsynaptic potentials mediated by mGluRs have been observed in some preparations (Batchelor & Garthwaite, 1993; Batchelor, Madge & Garthwaite, 1994), but not others (Eilers et al, 1995; Midtgaard et al, 1993; Miyakawa et al, 1992). This difficulty may be related to the fact that the endoplasmic reticulum is rapidly reorganized in Purkinje cells following perfusion with artificial media such that normal release of calcium from intracellular stores is blocked (Takei et al, 1994). In the present study, the basic hypothesis was tested by constructing a mathematical model of the mGluR response in Purkinje cells. Simulations of the model demonstrate how adaptive mechanisms within Purkinje cells can produce a temporal regulation of the firing rate of these cells which times the disinihibition of interpositus nuclear cells and thereby "opens a timed gate" which enables gains learned at the nuclear stage to modulate ongoing movements (Figure 1). The model suggests new experiments that can be used to test which metabotropic pathways influence the learned timed response.

— Figure 1 —

Methods

The Biochemistry of Adaptively Timed Cerebellar Learning

The present work develops a model that links behavioral properties of adaptively timed classical conditioning to the biochemistry and biophysics of metabotropic glutamate responses in the cerebellum. A key paradigm for studying cerebellar classical conditioning is the rabbit nictitating

membrane response (NMR). The NMRcan be delay or trace conditioned to an auditory, vibrotactual or light conditioned stimulus (CS) (Gormezano, 1966). The CS is usually paired with a periorbital shock or air puff unconditioned stimulus (US). To reach the same level of performance, trace conditioning, in which the CS terminates before US onset, requires five times as many learning trials as delay conditioning, in which the CS and US overlap in time. Asymptotic performance levels of 95 to 98 percent conditioned responses (CRs) can be obtained within three or four delay conditioning sessions, each consisting of 50-60 CS-US pairings with an interstimulus interval (ISI) of 250ms and an intertrial interval of 1 minute. The amplitude of individual CRs is positively correlated with the frequency of CRs. The more generic term *strength of CR* is used to refer to either amplitude of individual CRs or CR frequency.

An individual CR has a distinctive topography with a number of timing related properties. The CR is adaptively timed such that the peak amplitude occurs near the expected onset of the US. CR onset is smooth, such that the CR onset typically occurs much before expected onset of the US. The CS must precede the US by more than 50ms for successful conditioning (Smith et al., 1969). The onset of the CS can precede the US by up to 4s in delay conditioning (Gormezano, 1966), while trace conditioning cannot be obtained when the CS precedes the US by more than 2s (Solomon et al., 1986). The strength of the CR depends on the interstimulus interval (ISI), the time between onset of CS and onset of US, in a characteristic way. CR strength is maximal at ISIs of 200-400ms and is reduced at shorter or longer ISIs (Smith et al., 1969). This property is traditionally referred as the *inverted-U* property of the CR. The strength of CRs diminishes more quickly as a function of long ISIs for trace versus delay conditioning.

— Figure 2 —

When the NMR is conditioned to a particular ISI_1, such that the peak response occurs at that time, continued conditioning to a different ISI_2 will produce a discrete peak shift in which the response peak at ISI_1 diminishes and a new response peak grows at ISI_2 (Coleman & Gormezano, 1971). Alternating between two different ISIs during training with a single CS will produce a double-peaked CR (Figure 2), each peak coincident with one of the ISIs (Hoehler & Leonard, 1976; Millenson, Kehoe & Gormezano, 1977).

The cerebellum has been posited as a locus of conditioned NMR timing (Perrett et al., 1993). A properly timed neural expression of the CR occurs in the interpositus nucleus of the cerebellum as revealed by electrophysiological recordings (McCormick et al., 1982; Steinmetz, 1990b). Interpositus neurons exhibit a firing pattern which resembles the conditioned NMR topography. However, interpositus responses precede the NMR by 50ms or more (Thompson & Krupa, 1994). Such a temporal precedent is required if the interpositus is the originator of the response and the slow muscle-plant system is to produce eyelid closure which peaks at US onset (Bartha & Thompson, 1992a, 1992b).

Direct stimulation of inputs to cerebellum can substitute for external CS presentation during conditioning of an adaptively timed response in the cerebellum. An auditory CS (tone) normally activates the cerebellum via mossy fibers that originate in the pontine nuclei (Steinmetz et al., 1987). Conditioning with direct stimulation of mossy fibers as the CS results in properly timed CRs (Steinmetz et al., 1986). The ISI may be as long as 2000ms or as short as 100ms, and CRs peak near the time of the expected US (Steinmetz, 1990a). Interpositus expression of the CR has the same timing properties in conditioning with direct brain stimulation as when peripheral stimulation is used (Steinmetz, 1990b). This evidence strongly suggests that a delayed response to the

CS can be generated by the cerebellar network.

Earlier work has developed lumped neural models of how adaptive timing of neural responses may occur. These models have successfully simulated key properties of timed behavior and neural spiking, and have given credence to the hypothesis that adaptive timing is produced by selective enhancement of certain responses from an entire *spectrum* of responses distributed through time (Grossberg & Merrill, 1992; Grossberg & Schmajuk, 1989). The present work has used these model properties as a point of departure for interpreting and modeling biochemical properties of Purkinje cells in the cerebellar cortex that are consistent with the behavioral and neural data. In the model, the CS activates metabotropic glutamate receptor responses that are used for spectral timing in eye blink conditioning. The CS does this by activating mossy fibers, which in turn activate granule cells whose parallel fibers synapse on Purkinje cells (Figure 1). The metabotropic glutamate receptors are located just outside of the synaptic junction with parallel fiber terminals (Nusser et al, 1994).

Metabotropic Response Model

Purkinje cells of the cerebellar cortex express metabotropic glutamate receptors of the subtype mGluR1 (Figure 3). Releases of glutamate from parallel fiber terminals activate mGluR1 receptors by binding to the receptor (Blackstone et al, 1989). The activated receptor binds the G-protein/GDP complex, which promotes the exchange of GTP for GDP and cleavage of the G-protein into α and $\beta\gamma$ components (Berstein et al, 1992). The G_α component of the G-protein diffuses to PLC in the cell membrane and enables its enzymatic activity. Activated PLC ($PLC \cdot G_\alpha \cdot GTP$) catalyzes the production of the second-messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatydylinositol 4,5-bisphosphate (PIP_2) (Blackstone et al, 1989). IP_3 diffuses through the cytoplasm to the membrane of the endoplasmic reticulum (ER). IP_3 binds to receptors in this membrane (IP_3R), opening calcium channels and allowing Ca^{2+} to flow into the cytoplasm. The rapid increase of the cytosolic calcium concentration activates a Ca^{2+}-dependent K^+ conductance, which leads to hyperpolarization and a decrease in excitability (Khodakhah & Ogden, 1993).

— Figure 3 —

The binding of glutamate to mGluR exhibits first-order kinetics with a Hill coefficient of 1 and a dissociation constant of 0.296 μM (Thomsen et al, 1993). With the inclusion of an inactivated state, the time course of mGluR activation is modeled by

$$\frac{dB}{dt} = k_1(B_{max} - A - B)[glu] - 0.296k_1B - k_2BC \tag{1}$$

and

$$\frac{dA}{dt} = k_2BC - k_3A, \tag{2}$$

where B is the concentration of bound, activated receptor, B_{max} is the concentration of available receptors, and A is the concentration of inactivated receptors; [glu] is the concentration of glutamate to which the receptors are exposed as a result of CS input. The calcium- and DAG-

dependent protein kinase, protein kinase C, regulates the inactivation of receptors and the G-protein-mediated response (Catania et al., 1991; Herrero et al., 1994; Nakanishi, 1988; Yarfitz & Hurley, 1994). Protein kinase C (PKC) activity in the model is given by the variable C (below).

Activated mGluRs stimulate the activation of G-proteins. The rate of G-protein activation increases linearly with the concentration, B, of activated receptors (Berstein et al, 1992). The concentration of activated G-proteins is given by the variable G.

$$\frac{dG}{dt} = k_4(G_{max} - G)B - k_5G - k_6GC \quad (3)$$

The final term represents G-protein inactivation by PKC (Nestler & Duman, 1994; Yarfitz & Hurley, 1994).

In addition to PLC activity dependent on the presence of activated G-protein, cerebellar membranes contain a form of PLC activated by cytoplasmic calcium (Mignery et al, 1992). The production of IP_3 by PLC is thus modeled by

$$\frac{dI}{dt} = (I_{max} - I)(k_7G + k_8PLC([Ca^{2+}]_{cyt})) - k_9I \quad , \quad (4)$$

where I is the IP_3 concentration. Calcium-dependent PLC activity exhibits a steep dependence on calcium with half maximal activation in the range of 1-20 μM (Homma et al., 1988; Mignery et al, 1992). Therefore we choose a Hill coefficient of 2 and K_D of 20 μM for this PLC activation.

$$PLC([Ca^{2+}]_{cyt}) = \frac{[Ca^{2+}]_{cyt}^2}{[Ca^{2+}]_{cyt}^2 + 20.0} . \quad (5)$$

Diacylglycerol is produced in conjunction with IP_3 by PIP_2 hydrolysis. Therefore the amount, D, of DAG is given by

$$\frac{dD}{dt} = (D_{max} - D)(k_7G + k_8PLC([Ca^{2+}]_{cyt})) - k_9D \quad . \quad (6)$$

PKC is activated by binding a calcium ion and DAG (Schwartz & Kandel, 1991), as in

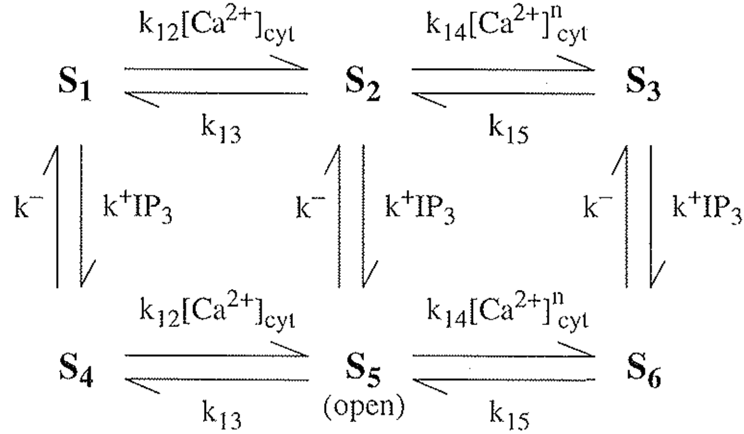
$$\frac{dC}{dt} = k_{10}(C_{max} - C)D[Ca^{2+}]_{cyt} - k_{11}C . \quad (7)$$

IP_3 binding to IP_3R in ER membrane is one-to-one with half maximal binding at 100-300 nM (Joseph et al, 1989; Missiaen et al, 1994; Watras et al, 1991). Assuming this reaction is fast compared to other reactions in the model yields as a formula for the fraction of bound receptors,

$$R = \frac{I}{I + 0.2}. \tag{8}$$

IP_3 binding is required to open the IP_3R calcium channels and release calcium into the cytosol, but channel opening also demonstrates a biphasic dependence on the cytosolic concentration of calcium (Bezprozvanny et al, 1991; Iino, 1990; Joseph et al, 1989). A similar biphasic calcium dependence is seen in the ryanodine-sensitive calcium channels of endoplasmic and sarcoplasmic reticulum (Bezprozvanny et al, 1991; Meissner et al, 1986). A fragmentary sequence homology between the ryanodine receptor (RyR) and IP_3R underlies the functional similarities of these channels (Furuichi & Mikoshiba, 1995). RyR and IP_3R contain at least two types of binding sites for Ca^{2+} or Ca^{2+}-activated proteins, one stimulating channel opening and another inhibiting it (Chen & MacLennan, 1994; Chen et al, 1992).

We model IP_3R kinetics with six states:



Here, $\{S_2,S_5\}$ represent the receptor with Ca^{2+} bound to the stimulating site and $\{S_3,S_6\}$ with Ca^{2+} bound to the inhibitory site. The cooperativity of the later binding produces a Hill coefficient of n=1.65 (Meissner et al, 1986). By considering IP_3 binding to be at equilibrium with respect to the Ca^{2+} binding reactions (5), the channel kinetics are described by two differential equations:

$$\frac{dR_o}{dt} = k_{12}(R_{max} - R_o - R_i)[Ca^{2+}]_{\mathrm{cyt}} + k_{15}R_i - k_{13}R_o - k_{14}R_o[Ca^{2+}]^n_{\mathrm{cyt}} \tag{9}$$

$$\frac{dR_i}{dt} = k_{14}R_o[Ca^{2+}]^n_{\mathrm{cyt}} - k_{15}R_i \tag{10}$$

The channel open state is R_o, and R_i is the channel inhibited (closed) by calcium binding. At steady state, the open probability is

$$P_o = R_{max}\left(\frac{I}{I+0.2}\right)\left(\frac{\frac{k_{15}}{k_{14}}[Ca^{2+}]_{\mathrm{cyt}}}{[Ca^{2+}]^{n+1}_{\mathrm{cyt}} + \frac{k_{15}}{k_{14}}[Ca^{2+}]_{\mathrm{cyt}} + \frac{k_{13}k_{15}}{k_{12}k_{14}}}\right) \tag{11}$$

With proper choice of parameters this model is in agreement with the open probability data of Bezprozvanny et al (1991), as shown in Figure 4.

— Figure 4 —

As depicted in Figure 3, the cytoplasmic calcium concentration in Purkinje cells is regulated by two transport systems, a Na/Ca exchanger in the plasmalemma (Staub et al, 1992) and a Ca^{2+}-ATPase pump in the reticular membrane (Takei et al, 1992; Villa et al, 1991). The exchanger has a low affinity for cytosolic Ca^{2+} (1-10μM) but a high capacity, while the ATPase pump has a smaller capacity and much higher affinity (0.2μM) (Yamada et al, 1989). The ATPase pump in reticular membrane exhibits a cooperativity of two calcium ions per transfer (De Meis & Inesi, 1982), and the calcium flux can thus be characterized by a Hill equation of the form

$$\frac{[Ca^{2+}]_{cyt}^2}{[Ca^{2+}]_{cyt}^2 + 0.2}. \tag{12}$$

The exchanger is at equilibrium when (Carafoli, 1987):

$$[Ca^{2+}]_{cyt} = c_0 = [Ca^{2+}]_{ext}\left(\frac{[Na^+]_{cyt}}{[Na^+]_{ext}}\right)^3 \exp\left(\frac{VF}{RT}\right), \tag{13}$$

where "ext" denotes extracellular ion concentrations, V is plasma membrane potential, F is the Faraday constant, R is the gas constant, and T is thermodynamic temperature. Assuming the other ionic concentrations are relatively constant, the Ca^{2+} flux produced by the exchanger is proportional to (Hodgkin & Nunn, 1987)

$$\frac{[Ca^{2+}]_{cyt} - c_0}{2 + [Ca^{2+}]_{cyt} - c_0}, \tag{14}$$

where c_0 is the equilibrium concentration, above, and 2μM is the half activation point.

Therefore, by equations (9)-(10) and (12)-(14), the cytoplasmic calcium concentration can be described by

$$\begin{aligned} \frac{d[Ca^{2+}]_{cyt}}{dt} &= k_{16}R_o\left(\frac{I}{I+0.2}\right)([Ca^{2+}]_{ER} - [Ca^{2+}]_{cyt}) \\ &\quad - k_{17}\left(\frac{[Ca^{2+}]_{cyt}^2}{[Ca^{2+}]_{cyt}^2 + 0.2}\right) - k_{18}\left(\frac{[Ca^{2+}]_{cyt} - c_0}{2 + [Ca^{2+}]_{cyt} - c_0}\right) \end{aligned} \tag{15}$$

We assume that calcium flux into the cytoplasm from the ER does not produce a significant change in the concentration of free Ca^{2+} in the lumen. Although the concentration of free Ca^{2+} in ER is not known, total calcium is in the range of 4-10 mM (Baumann et al., 1991).

The intracellular calcium response affects the Purkinje cell membrane potential. Two conductances are principally involved, a depolarizing conductance due to the electrogenic nature of the Na/Ca exchanger (Glaum et al, 1992; Staub et al, 1992), and a hyperpolarizing Ca^{2+}-dependent

K^+-conductance (Fagni et al, 1991; Khodakhah & Ogden, 1993). Since adequate data on the Ca^{2+}-dependent K^+-conductance in Purkinje cells are not available (DeSchutter & Bower, 1994a), our model is derived from data on the mGluR activated Ca^{2+}-dependent K^+-conductance in cultured cerebellar granule cells (Fagni et al, 1991).

— Figure 5 —

As shown in Figure 5A, the dependency of open probability on voltage follows the Boltzmann relation with an e-fold change in P_o per 22.5 mV. The half-activation values (V_0) of this equation vary linearly with the log of the cytoplasmic calcium concentration. Fitting a straight line to the data of Figure 5A gives

$$V_0 = 11 - 134\log[Ca^{2+}]_{cyt} \tag{16}$$

where voltage is given in mV and calcium concentration in μM. (A similar relation holds for Ca^{2+}-dependent K^+ channels in other preparations; e.g., Bielefeldt & Jackson, 1994.) Combining this result with the Boltzmann relation gives an expression for channel opening in terms of calcium concentration and membrane potential (Figure 5B).

$$g_K([Ca^{2+}]_{cyt}, V) = \frac{[Ca^{2+}]_{cyt}^{2.6}}{[Ca^{2+}]_{cyt}^{2.6} + \exp\left(\frac{11 - V}{22.5}\right)} \tag{17}$$

The reversal potential of Purkinje cell potassium channels is about -85 mV (De Schutter & Bower, 1994a). Thus, incorporating the calcium-driven plasma membrane currents due to (14) and (17), membrane potential is

$$\frac{dV}{dt} = k_{19}\left(\frac{[Ca^{2+}]_{cyt} - c_0}{2 + [Ca^{2+}]_{cyt} - c_0}\right) - \bar{g}g_K([Ca^{2+}]_{cyt}, V)(85 + V) + k_{20}(V_b - V) \quad , \tag{18}$$

where $\bar{g}$ is the peak conductance of the Ca^{2+}-dependent K^+ channel. This conductance is modulated through conditioning as described in the next section.

The baseline membrane potential, V_b, is used to set an approximate level of tonic activity. It has been observed (DeSchutter & Bower, 1994b) that a resting potential of around -68 mV as commonly seen in Purkinje cells *in vitro* gives rise to a quiescent state in which there is little or no tonic simple spike activity. Purkinje cells *in vivo*, on the other hand, have a continual background level of parallel fiber activity, which gives them a more depolarized resting potential of about -50 mV. This elevated baseline potential gives rise to a state of tonic simple spike firing. We assume that V_b=-50mV, such that when the mGluR activation raises intracellular calcium levels, a net increase or decrease in simple spike firing rate is produced based on whether the Na/Ca exchange- and Ca^{2+}-dependent K^+-currents depolarize or hyperpolarize the cell.

Learning Model

The processes hypothesized to mediate learning in the present model are depicted in Figure 6

which shows how climbing fiber and parallel fiber signals (*top*) can affect receptors (*bottom*) that control transmembrane current. The hypothesis is based primarily on evidence for long-term depression (LTD) of AMPA receptors at the parallel fiber-Purkinje cell synapses (Ito, 1991; Ito & Karachot, 1992). LTD is a result of phosphorylation of AMPA receptors, but there are additional substrates for phosphorylation which also effect the Purkinje cell response, especially the metabotropic response component, as described below.

— Figure 6 —

Stimulation of the mGluR1 receptor activates PKC by the production of DAG and the release of Ca^{2+} intracellularly (Nishizuka, 1986). PKC activation is necessary for induction of LTD of AMPA receptors (Linden & Conner, 1991). The mGluR1 receptor must be present (Aiba et al., 1994; Shigemoto et al., 1994) and activated (Daniel et al., 1992; Hartell, 1994; Linden et al., 1991) for LTD to occur. However, Kasono and Hirano (1995) recently reported that an intracellular IP_3 increase in conjunction with AMPA receptor activation and depolarization is sufficient for LTD. Blockage of IP_3 binding to IP_3R (Kasono & Hirano, 1995) or blockage of the intracellular Ca^{2+} rise (Sakurai, 1990; Konnerth et al., 1992) prevents LTD. Therefore, it appears that the metabotropic second messenger responses in the parallel fiber-activated pathway are important for LTD induction.

Climbing fiber stimulation is the second pathway involved in LTD. Climbing fiber activation strongly depolarizes the Purkinje cell and produces Ca^{2+} spiking and plateau potentials in the dendrites (Llinás & Sugimori, 1992). It has been proposed that the influx of Ca^{2+} into the Purkinje cell from the extracellular media following climbing fiber activation was directly responsible for LTD (Ito, 1984; Linden et al., 1991), but an alternative explanation is that the Ca^{2+} spiking provides a means to ensure sufficient depolarization to activate NO synthase in the terminals of basket and stellate interneurons (Ito & Karachot, 1992; Linden & Conner, 1993). The axons of basket and stellate cells climb along the dendrites of Purkinje cells, often in close proximity with climbing fibers (Palay & Chan-Palay, 1974). Activation of NO synthase produces a rise in NO, which permeates the membranes of Purkinje cells. NO elevation is essential for the production of LTD and for concommitant motor learning (Crepel & Jaillard, 1990; Ito, 1991; Shibuki & Okada, 1991).

Cerebellar Purkinje cells are replete with components of the cGMP system. Purkinje cells possess abundant guanylyl cyclase (Bredt et al., 1990). Elevated NO levels stimulate guanylyl cyclase to produce cGMP from GTP (Nestler & Duman, 1994). Purkinje dendrites also contain high levels of a Ca^{2+}/calmodulin-dependent phosphodiesterase which hydrolyzes cGMP (Nestler & Duman, 1994). High levels of cGMP-dependent protein kinase (PKG) in the brain are found only in cerebellar Purkinje cells (Nestler & Greengard, 1984, p.37). The principle substrate for PKG is G-substrate, a protein found only in Purkinje cells of the cerebellum (Nestler & Greengard, 1984, p.169).

Climbing fiber stimulation may be replaced by cGMP application in the induction of LTD (Ito & Karachot, 1992; Shibuki & Okada, 1992). Elevated cGMP levels activate PKG which phosphorylates G-substrate. Phosphorylated G-substrate inhibits protein phosphatase-1 (Ito & Karachot, 1992). Application of an exogenous protein phosphatase inhibitor, such as calyculin or microcystin-LR, can also substitute for climbing fiber activation in LTD induction (Ajima & Ito, 1995). Protein phosphatase-1 (PP-1) dephosphorylates two target proteins, the AMPA receptor (Ito & Karachot, 1992), and the Ca^{2+}-dependent potassium conductance, g_K (Reinhart & Levitan, 1995).

Dephosphorylation of G-substrate is produced by calcineurin, a Ca^{2+}-activated protein phosphatase (King et al., 1984). Phosphorylation of AMPA receptors underlies the LTD of AMPA-mediated EPSPs (Ito & Karachot, 1992). An increase in the open probability of Ca^{2+}-dependent K^+ channels in plasma membrane is produced by phosphorylation of these proteins by PKC (Baraban et al., 1985; Reinhart & Levitan, 1995). Thus, *simultaneous activation of PKG and PKC will result in a persistent phosphorylation of the target proteins*. Interestingly, behavioral learning in *Drosophila* (Griffith et al., 1994) and *Hermissenda* (Alkon, 1984; Nishizuka, 1986) is also dependent on phosphorylation of Ca^{2+}-dependent K^+ channels.

In summary, learning in the present model is based on the hypothesis that a robust and maintained level of phosphorylation of specific target proteins is obtained by an increase in the mGluR1-mediated Ca^{2+} and DAG signals, coincident with an increase in cGMP through the climbing fiber pathway. Note that this assumes that substantial increases in cytoplasmic free Ca^{2+} in spines are not induced by climbing fiber activation under normal conditions. It is likely that spine heads are insulated from these Ca^{2+} increases by the activity of inhibitory interneurons (Callaway et al., 1995). Extinction of the learned response results from dephosphorylation produced by activation of the mGluR pathway alone, without a coincident climbing fiber signal. We model this learning process as follows, with a system that avoids unnecessary details of the processes depicted in Figure 6.

A climbing fiber burst produces a rapid increase in [cGMP] followed by an exponential decay. We describe this signal by a dual exponential function,

$$[cGMP] = \exp\left(-\frac{max(0, t-s)}{\tau_1}\right) - \exp\left(-\frac{max(0, t-s)}{\tau_2}\right) \qquad (19)$$

where τ_1 and τ_2 are the decay and rise time constants, respectively. Each conditioning trial starts with CS onset at t=0. The onset of the US occurs at t=s.

The Ca^{2+}-dependent activation of calcineurin involves multiple calcium/calmodulin binding sites on the regulatory subunit of the enzyme and exhibits a Hill coefficient of 3 (Burroughs et al., 1994; Stemmer & Klee, 1994). Thus, the level of activated calcineurin, N, is described by

$$\frac{dN}{dt} = k_{21}(N_{max} - N)[Ca^{2+}]^3_{cyt} - k_{22}N \qquad (20)$$

Learning can now be expressed as change in $\bar{g}$, the peak conductance for the Ca^{2+}-dependent K^+ channel.

$$\frac{d}{dt}\bar{g} = k_{23}(g_{max} - \bar{g})C[cGMP] - k_{24}N\bar{g} \qquad (21)$$

Because our model proposes that the learned Purkinje response topography arises primarily from the interaction between the metabotropic pathway and the Ca^{2+}-dependent K^+ channel, the phosphorylation of AMPA receptors and their individual responses were omitted from the present simulations (see discussion). However, we note that if an equation analogous to (20) governs AMPA

de/phosphorylation, then climbing fiber-parallel fiber coincidence would produce AMPA receptor LTD and parallel fiber activity alone would produce AMPA receptor LTP, consistent with data of Sakurai (1988) and Hirano (1990).

Modeling and Parameter Assumptions

To simplify numerical simulation of the model, only three compartments are considered with respect to chemical concentrations at a given site: extracellular, cytosol, and lumen of the endoplasmic reticulum. Extracellular and reticular Ca^{2+} concentrations are assumed constant and uniform, relative to cytoplasmic concentrations. Within the cytoplasmic compartment, all points are considered to have the same concentration; that is, the temporal delays produced by diffusion are ignored. This is reasonable for the present simulations because diffusional delays in this second messenger pathway are only 10-20ms (Lamb & Pugh, 1992), a tiny fraction of the expected overall response time.

The CR expressed at the interpositus is influenced by a number of Purkinje cells distributed in the cortex. Thus, there are multiple mGluR response sites which contribute to the behavioral response. For simulation purposes, we consider 10-60 such sites, as described below. Each site was simulated by an identical set of equations (1)-(21) with identical parameters except for B_{max}, which was varied over a range. This range of values was selected to produce responses in the observed behavioral response range of approximately 0.1-4 seconds. Although the variation in the number of mGluR receptors in cerebellar response pathways is not known, this assumption appears reasonable since this kind of variation is seen in other cell preparations. For example, neuroblastoma cells exhibit different individual $[Ca^{2+}]_{cyt}$ response latencies (range: 0.4-20s) following carbachol application (Wang et al, 1995). Since the carbachol concentration was always the same in these experiments, latency variation is produced by variation in the number of metabotropic receptors.

Simulations were performed on a 486-based computer, using a fourth-order Runga-Kutta algorithm with a step size in the range of 0.0005-0.002 seconds. Simulations used the parameters given in Table 1. Hill coefficient, ion concentration, and dissociation constant values were taken from the literature, where possible. Other parameters were fit to published data in cases where such data were available, such as with IP_3R kinetics. The results are not particularly sensitive to any given parameter value. Values near the given values produce the same qualitative results.

— Table 1 —

Results

Response to mGluR Activation

As shown in Figure 7A, activation of mGluR by glutamate results in a rise in intracellular calcium due to release from endoplasmic reticulum. The calcium response builds slowly as IP_3 accumulates, until the threshold for activation of positive feedback is reached. The positive feedback of calcium on IP_3 production and on the IP_3R channel opening, results in a rapid rise in $[Ca^{2+}]_{cyt}$. As the calcium level increases further, the biphasic nature of the IP_3R calcium dependency (Figure 4) switches the feedback from excitatory to inhibitory. Calcium release is thereby terminated. The intracellular calcium level is quickly returned to the resting level by the action of the calcium

pump in ER membrane and the Na/Ca exchanger in the plasma membrane. Since mGluR is inactivated during the calcium transient, the IP_3 levels also return to baseline following the calcium transient. If the receptor continued to activate G-proteins after the initial response, intracellular calcium oscillations would develop, as observed in many preparations (Berridge et al., 1988; Devor et al., 1991).

— Figure 7 —

Since the Na/Ca exchanger is electrogenic, it will produce a depolarizing membrane current from resting potential (Figure 7B). The membrane depolarization produced by this current may be responsible for the slow EPSPs observed in Purkinje cells following activation of mGluR at the parallel fiber-Purkinje cell synapse, as discussed below (Batchelor & Garthwaite, 1993; Batchelor, Madge & Garthwaite, 1994). When the Ca^{2+}-dependent K^+ current is also activated by the intracellular calcium transient, the net effect on the membrane potential can be hyperpolarization rather than depolarization (Figure 7C). Thus, if the input to the Purkinje cell elevates its membrane potential and establishes a certain rate of simple spike firing, the firing rate can be decreased from this level during the calcium transient by the activation of the Ca^{2+}-dependent K^+ conductance. This "pause" in Purkinje cell firing will allow an increase in activity in the interpositus cells which govern the eye blink response (Bullock et al., 1994). If the Purkinje cell pause is made adaptive, then a mechanism for eye blink conditioning is realized.

Population Response

Large quantities of glutamate are released presynaptically following activation. It has been estimated that the postsynaptic concentration of glutamate in the center of the synapse reaches levels much greater than 1 mM (Clements et al., 1992). Metabotropic receptors, however, are located at the periphery of the synapse (Nusser et al., 1994). This means that a much lower level of glutamate will reach these receptors. This concentration at the periphery will exhibit a slower decay than that in the synaptic cleft. Therefore, we assume that in response to maintained parallel fiber firing of sufficient frequency, the population of mGluR receptors at the synapse will be exposed to a 10μM level of glutamate.

Many of the G protein-coupled receptor types utilize the second messenger system involving PLC and IP_3-mediated calcium release (McGonigle & Molinoff, 1994). Responses mediated by these receptors can exhibit a wide range of temporal latencies. Serotonergic and muscarinic receptor responses can exhibit latencies of a few seconds to more than 30 seconds (Berridge et al, 1988; Devor et al, 1991). The photoresponse due to activation of rhodopsin in the invertebrate photoreceptor has latencies on the order of tens of milliseconds (Fuortes & Hodgkin, 1964). The rapidity of the response for a given receptor type is dependent on the level of G protein activation. This, in turn, is dependent on the level of activation of receptors. Assuming a relatively constant glutamate level of 10μM, latency of the response will be dependent on the number of available receptors in the vicinity of the parallel fiber synapse.

Thus, variation in the number of mGluR1 receptors, B_{max}, at different synapses produces intracellular calcium responses with different latencies. Figure 8 demonstrates the effect of variation of B_{max}. A *spectrum* of calcium responses spanning the behaviorally relevant interval for eye blinks of about four seconds is created in Purkinje cells by choosing B_{max} in the range of 0.1 to 500. The particular values used in generating a given spectrum are given in the associated figure caption. No other parameters are varied in producing these responses. The present model is thus a

biochemically-derived variant of a *spectral timing* model (Bullock et al., 1994; Grossberg & Merrill, 1992; Grossberg & Schmajuk, 1989). The spectrum of response times can be used to learn an adaptively timed eye blink, as discussed below.

— Figure 8 —

Although for purposes of simulation we assumed that the latency variations are wholly due to a natural spectrum of B_{max} values, it is possible that variance in other mGluR pathway components may contribute to generation of different latencies. For example, variations in IP_3R density or in luminal calcium stores, which effect the rate of mGluR-mediated Ca^{2+} release, will effect response latency.

The model exhibits different calcium response properties to transient versus maintained agonist concentrations. Although maintained parallel fiber inputs produce a spectrum which spans four seconds, a transient parallel fiber activation of 50ms duration only admits a spectrum spanning about two seconds. This is because the dynamics engendered by a 50ms stimulus fail to generate a $[Ca^{2+}]$ spike in mGluR pathways whose B_{max} values are associated with longer latencies. Figure 9 shows the spectral response properties of the model to 50ms parallel fiber activations. This difference in the spectral properties of transient versus maintained inputs is analogous to the differences in the maximal ISIs for trace versus delay eye blink conditioning (Smith et al., 1969; Solomon et al., 1986).

— Figure 9 —

Measurement of the potential of a population of Purkinje cells in slice reveals a slow response following brief activation of parallel fibers (Batchelor & Garthwaite, 1993; Batchelor, Madge & Garthwaite, 1994). The response has a slow rise-time, with a peak at 300-700ms and a slow decay over several seconds (Figure 10A). The response is observable in a bath of ionotropic glutamate and GABA antagonists, which suggests that the response is mediated by metabotropic glutamate receptors at the parallel fiber-Purkinje cell synapses. This type of response is the result of the summation of the individual signals in a spectrum, such as that of Figure 8 or 9. Figure 10B shows the summation of the potential changes produced by Na/Ca exchange current in a heterogeneous population of mGluR response pathways. This population signal, P(t), is computed by

$$P(t) = \alpha \sum_{i=1}^{N} \Delta V_i + V_b \tag{22}$$

where N is number of response pathways, $\Delta V_i = (V_i - V_b)$ is the mGluR induced potential change in a given pathway, α is a constant scaling factor, and V_b is the baseline resting potential of the population.

As shown in Figure 10, even though the individual responses are localized in time, the population signal is broad and smooth, due to distribution of the localized signals throughout a long interval. This population response phenomenon is also seen in other IP_3-mediated response systems, such as histamine receptors of HeLa cells (Bootman, 1994).

— Figure 10 —

Conditioning

Given a CS-activated spectrum of responses distributed among a population of response pathways, a US input can select spectral components which will produce the desired behavioral response (Grossberg & Schmajuk, 1989). In the present model, the CS is parallel fiber activation of metabotropic glutamate receptors, while the climbing fibers produce the [cGMP] increase at US onset. Figure 11 shows the population response during 36 pairings of a 600ms CS and a 100ms US. The CS and US coterminate, such that the ISI is 500ms. Note the rate of learning is accelerated from that observed in experiments to decrease simulation time. Also, learning is asymptotic due to the balance between CS-driven dephosphorylation and CS- and US-driven phosphorylation. CS-driven dephosphorylation alone causes extinction of the learned response.

Those mGluR response pathways which have PKC activity at the time of climbing fiber activation correlated with US onset exhibit a persistent phosphorylation of Ca^{2+}-dependent K^+ channels. This increases the peak conductance of these channels in response to the intracellular calcium transient, such that these pathways produce a more hyperpolarizing response after repeated pairings. Thus, those Purkinje cells whose CS-activated mGluR1 pathway has a latency that approximates the ISI will exhibit a progressive decrease in simple spike firing during the CS-US interval. Other Purkinje cells will exhibit increases in simple spike firing in the CS-US interval due to the depolarizing Na/CA exchanger as well as the AMPA receptor input. Those cells exhibiting a decrease in firing will realize a minimum firing rate near the expected time of US onset. These characteristics are in agreement with *in vivo* recordings of Purkinje cell activity during eye blink conditioning (Berthier & Moore, 1986; Thompson, 1990).

— Figure 11 —

Interpositus nuclear cells receive input from a population of Purkinje cells. Therefore, the population response shown in Figure 11 is responsible for the observed CR related activity in interpositus. In agreement with recordings from interpositus (Figure 12), the population response peak occurs prior to the time of the expected US. Both the latency of the response peak and the response onset latency decrease during learning.

— Figure 12 —

The strength of the CR depends on ISI in a characteristic way. CR strength is maximal at ISIs of 200-400ms and is reduced at shorter or longer ISIs (Smith et al., 1969; Steinmetz, 1990a). By taking the depth of the population response as a measure of CR strength, it is possible to reconstruct the CR strength-ISI dependency curve produced by the model. Figure 13 shows the curve for the model in comparison with the experimental data obtained by Steinmetz (1990a). Strength of CR in the experiment is calculated as percent CRs over test trials. For the model, CR strength is calculated by the magnitude of hyperpolarization below the baseline value of -50 mV. As shown in the figure, the model reproduces the characteristic ISI dependency as measured behaviorally.

— Figure 13 —

A spectral timing model is able to produce double responding following conditioning with two different ISIs in alternation. Figure 14 depicts the effect of conditioning with alternating ISIs of 350ms and 1000ms. A double-peaked CR is produced with peaks near the expected times of the US, and with the Weber law property (cf. Figure 2) whereby the earlier peak is narrower and the later peak broader. The figure also demonstrates extinction of a learned response with repeated presentation of the CS alone.

— Figure 14 —

Two sites of learning need to be considered in eye blink conditioning, cerebellar cortex and interpositus. Although the present model focuses on learning in cortex, the result is compatible with an additional learning site in the interpositus (Figure 1). Learning at mossy fiber synapses on nuclear cells can provide a learned gain that can be expressed through the interpositus when Purkinje cell activity pauses. In this way, learning at Purkinje cells opens a timed gate which enables learned gains at the intracerebellar nuclei to control a movement at the appropriate time. We have previously demonstrated that the existence of this type of interpositus learning in conjunction with cortical learning can explain the maladaptively-timed CRs which can occur following cortical lesions (Bullock et al, 1994).

Discussion

As described in the introduction, the most parsimonious explanation for direct mossy fiber stimulation producing a timed response in interpositus is that a timing function is present in the cerebellum. The present model demonstrates that the mGluR1-phosphoinositide hydrolysis second messenger system in cerebellar Purkinje cells can perform a timing function, both in maintaining a CS trace for association with a temporally remote US, and in the delayed onset of the CR.

The basic scheme of Figure 5 for control of phosphorylation has been recognized for many years (e.g. Nestler & Greengard, 1984, Figure 9.3). It is important to realize, however, that the cGMP signal which increases levels of phosphorylation is antagonized by the parallel fiber-mediated intracellular calcium signal which decreases phosphorylation. The fact that the cGMP signal corresponds to a US signal, while the mGluR activation corresponds to a CS signal, makes it clear that conditioning is obtainable only when activation of these pathways occurs in temporal conjunction. Activation of the mGluR pathway alone gradually reverses the effects of any previous conjunctive activation.

Long-Term Depression of AMPA Receptors

Although the phosphorylation of AMPA receptors is not crucial to behavioral learning in the model, it certainly has some bearing *in vivo*. The exact role played by the AMPA receptor in eye blink conditioning remains an unresolved issue. It is not even clear whether AMPA receptor activation is necessary for AMPA receptor LTD (Linden, Smeyne, & Connor, 1993). Nonetheless, it appears that the mechanisms inducing AMPA receptor LTD are also responsible for behavioral learning, possibly through the phosphorylation of Ca^{2+}-dependent K^{+} channels. The fact that mGluR1 is critical for induction of AMPA receptor LTD (Aiba et al., 1994; Shigemoto et al., 1994) motivates our hypothesis that it is temporal correlation of the mGluR1-mediated second messengers and the climbing fiber evoked cGMP signal which produces behavioral learning.

Aiba et al. (1994) report loss of AMPA receptor LTD, and diminished but extant eye blink conditioning in mice lacking mGluR1. Further, the eye blink appears to be correctly timed, although a detailed study over various ISIs was not conducted. This would seem to argue that mGluR1 is not involved in timing. However, another possible explanation for this finding is that mGluR5, which also couples to phosphoinositide hydrolysis, is able to partially replace mGluR1 functionally in the mutant mice. The mGluR5 subtype is present in Purkinje cells of immature rat brain (Abe et al., 1992), but during development is normally supplanted by a proliferation of

mGluR1 (Shigemoto et al., 1992). It order to completely rule out a role for mGluR-mediated phosphoinositide hydrolysis in timing, both subtypes would need to be eliminated.

A recent report by Linden et al. (1995) demonstrates that activation of the NO/cGMP pathway is not required for LTD in culture. It suffices to significantly depolarize Purkinje cells (3 seconds of +10mV) in conjunction with application of glutamate. Kasono and Hirano (1994) found that the depolarization can be replaced by an artificial elevation of intracellular calcium to 6μM in LTD induction. Linden et al. report that the LTD they observe is blocked by PKC inhibitors, but not inhibitors of PKG. According to the model of Figure 5, a large enough $[Ca^{2+}]_{cyt}$ rise can evoke LTD in the absence of cGMP. This is due to the fact that calcium activates PLC which produces DAG. The combination of high levels of calcium and DAG could drive PKC phosphorylation beyond that recoverable by baseline protein phosphatase activity. This can be realized in equation (21) by assuming non-zero resting levels of cGMP. However, our hypothesis is that this situation is not occurring *in vivo*. Both mGluR1 activated PKC and climbing fiber activated PKG must be present for LTD.

A possible role for AMPA receptor LTD could be to unblock the mGluR-mediated response, which has been reported to be inhibited by AMPA receptor stimulation (Lonart et al., 1993). The mechanism studied by Lonart et al. appears to involve AMPA activation of voltage-dependent calcium channels and subsequent activation of a calcium-dependent protein kinase. In the present model, significant calcium influx would result in activation of calcium-dependent PLC, and thus would invariably stimulate, rather than inhibit, formation of IP_3. Therefore, LTD does not appear to unblock mGluR responses in the Purkinje cell. The manner of interaction between mGluR and AMPA receptors in cerebellar Purkinje cells awaits further investigation.

Purkinje Cell and Invertebrate Photoreceptor

The biochemistry of the invertebrate photoresponse is very similar to the biochemistry of the Purkinje cell mGluR response. In the invertebrate photoreceptor light activates rhodopsin. Activated rhodopsin stimulates phospholipase C through a G-protein (Yarfitz & Hurley, 1994), as described above for mGluR. In both invertebrate photoreceptors and Purkinje cells, activated PLC catalyzes the production of the second-messengers IP_3 and DAG from PIP_2, and IP_3 subsequently releases calcium from intracellular stores. The rapid increase of the cytosolic calcium concentration in the invertebrate photoreceptor activates a plasma membrane Na^+ conductance which produces a depolarizing photoresponse (Shin, Richard & Lisman, 1994). The specific mechanisms which activate this conductance in the invertebrate photoreceptor are not well understood, but it may involve Ca^{2+}-stimulated increases in cGMP (Bacigalupo et al., 1991; Richard et al., 1995).

The photoreceptor is a site of associative conditioning in marine mollusks such as *Hermissenda* (Alkon, 1984; Crow, 1988). Repeated pairings of light and rotation with a forward ISI results in a persistent suppression of photokinesis in these animals (Matzel et al., 1990). This behavioral change is effected by a modification of voltage-dependent and Ca^{2+}-dependent K^+ conductances within the photoreceptor (Alkon, 1986). Similarly, the Purkinje cell appears to play an essential role in certain forms of classical conditioning. Our theory proposes that, like the invertebrate photoreceptor, behavioral learning in the cerebellum can be produced by persistent modification of a Ca^{2+}-dependent K^+ conductance.

The biochemical cascade producing the invertebrate photoresponse is designed to remain sensitive to light over a wide range of stimulus intensities and durations. Weak signals are amplified and prolonged by the positive feedback in the biochemical cascade. This amplification results

in a single absorbed photon opening 1000 plasma membrane channels and eliciting a current transient of several nanoamps in *Limulus* ventral photoreceptors (Nagy, 1991). The photocurrent in response to a maintained stimulus is reduced through negative feedback in the second messenger pathway, ensuring that a transient photoresponse can be produced even at high background intensities (Fuortes & Hodgkin, 1964). A similar mechanism appears to occur in turtle cones (Baylor & Hodgkin, 1974), and has been modeled by a Ca^{2+}-mediated gating function (Carpenter & Grossberg, 1981).

Our theory suggests that the Purkinje cell utilizes something very similar to the robust signal transduction mechanism of photoreception for the specialized purpose of forming associations between temporally separated stimuli. In both cases, there is a functional need to respond reliably to signals whose intensity and duration may vary over a wide range. In the photoreceptor, this variation is due to changes in photon density. In the cerebellar cortex, it is due to variations in the number of convergent CS-activated cells. The mechanisms in question may have evolved to improve the signal-to-noise ratio in response to weak signals by amplifying and prolonging them without losing sensitivity or temporal resolution to more intense signals. Whether this relationship between Purkinje cell and invertebrate photoreceptor represents convergent evolution or a true homology is an open question. Homology is possible because associative learning arises in the invertebrates and probably postdates the evolution of photoreceptors, whereas cerebella and Purkinje cells are not found until the vertebrates, for which the cerebellum is virtually a defining feature. Data on protochordates might be able to shed light on this question.

Another link warranting exploration is with the dentate-CA3 circuit in hippocampus, which exhibits adaptive timing (Berger et al., 1986; Hoehler & Thompson, 1980), and seems to employ mechanisms on the circuit level that are similar in many respects to those utilized here. Grossberg and Merrill (1995) have discussed how the hippocampal circuit may fit into a larger model neural architecture for timed reinforcement learning, attention, and movement control through interactions between the hippocampal system and cerebellum, among other brain regions. Taken together, these functional similarities suggest that learned timing in the cerebellum uses a specialized version of neural mechanisms that are of much broader occurrence and functional significance.

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Figure Captions

Figure 1. Basic neuronal circuitry of the cerebellum which forms the basis for the present model of adaptive timing of eye blinks. Inhibitory neurons, *dark*; Excitatory neurons, *white*. PC, Purkinje cell; BA, basket cell; ST, stellate cell; GR, granule cell; PF, parallel fiber; MF, mossy fiber; CF, climbing fiber; N, cerebellar nuclear cell; PN, precerebellar neuron that issues mossy fibers; IO, inferior olive; CS, conditioned stimulus; CR, conditioned response; US, unconditioned stimulus.

Figure 2. Nictitating membrane responses following mixed ISI delay conditioning. Group 200F received all 200ms ISI trials. Group P n/8 received mixed trials in a ratio of n 200ms to 8-n 700ms ISI trials. Group 700F received all 700ms ISI trials. As shown in the right-hand column, 700ms CS test trials result in double responding. (Reprinted with permission from Millenson et al., 1977.)

Figure 3. Components of the metabolic transmission pathway within a Purkinje cell dendrite. DAG, diacylglycerol; G, guanine nucleotide-binding protein; glu, glutamate; mGluR1, metabotropic glutamate receptor subtype 1; PKC, protein kinase C; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate.

Figure 4. Open probability of IP_3R in model (*dashed line*) and in experiments with IP_3R reconstituted into planar lipid bilayers (Bezprozvanny et al, 1991) in the presence of 2 μM IP_3. Normalized from maximum open probability of 15%. Model parameter values: $R_{max}=1$, $k_{13}/k_{12}=0.81$, $k_{15}/k_{14}=0.0556$, $n=1.65$.

Figure 5. *A*: Voltage and calcium dependencies of the mGluR-activated Ca^{2+}-dependent K^+ channels of cultured cerebellar granule cells (Reprinted with permission from Fagni et al, 1991). *B*: Equation (17) plotted as a function of voltage for various cytoplasmic calcium concentrations.

Figure 6. Processes mediating learning of a timed response in cerebellar Purkinje cells. AMPA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-sensitive glutamate receptor; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; glu, glutamate; GC, guanylyl cyclase; g_K, Ca^{2+}-dependent K^+ channel protein; GTP, guanosine triphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; mGluR1, metabotropic glutamate receptor subtype 1; NO, nitric oxide; NOS, nitric oxide synthase; P, phosphate; PLC, phospholipase C; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PP-1, protein phosphatase-1. NOS is probably not localized in Purkinje cell, as discussed in text.

Figure 7. Model responses to mGluR activation. Parameters as described in text, with [glu]=10μM, $B_{max}=1.5$. *A*: Rise in cytoplasmic calcium concentration following release from endoplasmic reticulum. *B*: The plasma membrane potential driven by the Na/Ca exchange current in the absence of Ca^{2+}-dependent K^+ current ($\bar{g} = 0$). *C*: The plasma membrane potential change when both Na/Ca exchange current and Ca^{2+}-dependent K^+ current are present ($\bar{g} = 100$).

Figure 8. Spectrum produced by variation in B_{max} in response to sustained [glu] concentration. B_{max}={360, 21, 4.7, 1.73, 0.97, 0.625, 0.458, 0.368, 0.315, 0.283, 0.261, 0.245, 0.236, 0.23, 0.226}; these receptor concentration values were chosen to give approximately equally spaced responses spanning 4s. With a sustained [glu] input, $[Ca^{2+}]$ spike response can be observed out to about 5s if the B_{max} distribution is allowed to range down to 0.

Figure 9. Spectrum produced by variation in B_{max} in response to a 50ms [glu] application. B_{max}={360, 18, 6.5, 3.9, 3.18, 2.93, 2.87, 2.859, 2.858, 2.8579}; values within the indicated range were chosen to give equally spaced responses. The value 2.585 is the smallest B_{max} for which the 50ms [glu] stimulus was sufficient to induce a [Ca^{2+}] spike in the mGluR pathway..

Figure 10. *A*: Metabotropic glutamate response in a slice population of Purkinje cells recorded using the three-chamber grease-gap method. (Reprinted with permission from Batchelor and Garthwaite, 1993.) *B*: Model population response produced by summation of spectral components in response to a 150ms agonist application at the arrow, with α=0.1, N=60, B_{max}={360, 170, 100, 65, 42, 29, 21, 15.7, 12, 9.2, 7.2, 5.8, 4.7, 3.8, 3.15, 2.65, 2.25, 1.96, 1.73, 1.55, 1.4, 1.27, 1.15, 1.06, 0.97, 0.89, 0.82, 0.763, 0.706, 0.66, 0.625, 0.59, 0.555, 0.525, 0.5, 0.478, 0.458, 0.44, 0.422, 0.407, 0.393, 0.38, 0.368, 0.357, 0.347, 0.338, 0.33, 0.322, 0.315, 0.309, 0.303, 0.298, 0.293, 0.288, 0.283, 0.279, 0.275, 0.271, 0.267, 0.264}; values within the indicated range were chosen to give a smooth population response. This distribution was used for all results except those reported in Figures 8 and 9.

Figure 11. Progress of model population response during 30 pairings of CS and US at an ISI of 500ms. Initially, mGluR activation produces a depolarizing response, but as learning progresses a timed hyperpolarization is realized. Spectral components are the same as for Figure 10B.

Figure 12. Average nictitating membrane movement (*top trace*) and peristimulus histogram of interpositus nucleus neural activity (*bottom*) during classical conditioning of a rabbit with a 25ms pontine stimulation as the CS and an air puff delivered 225ms later as the US. (Reprinted with permission from Steinmetz, 1990b.)

Figure 13. Comparison of CR strength-ISI dependency curves for the model and the behavioral data. Data of Steinmetz (1990a) is normalized to 86% CRs. Model data is the magnitude of the learned hyperbolarization below -50mV, normalized to the amount of hyperpolarization obtained at asymptote during training with an ISI of 250ms.

Figure 14. Progress of population response during first 10 extinction trials following 30 CS-US pairings with alternating ISIs of 350ms and 1000ms. After conditioning, the 1100ms CS2 is used to elict a double-peaked CR.

Table 5.1. mGluR timing model simulation parameters.

Parameter	Value	Reason for Choice
B_{max}	variable	see discussion in text
k_1	50	steady-state from Thomsen et al (1993); assume fast reaction
k_2	80	
k_3	0	assume slow enough to ignore
k_4	0.1	assume forward reaction is slow (c.f. Berstein et al, 1992; Casey et al, 1990); total PKC inact.
k_5	1	
k_6	20	
G_{max}	1	arbitrary
k_7	4	chosen to produce latencies in observed CR responce range (c.f. Wang et al, 1995)
k_8	40	
k_9	8	
I_{max}	1	arbitrary
D_{max}	1	arbitrary
k_{10}	5	K_D~6μM (Nishizuka, 1988); rate set for proper inactivation
k_{11}	30	
C_{max}	6	more PKC than calcineurin
k_{12}	60	steady-state fit to Bezprozvanny et al (1991); rate of reactions fit to fast desensitization data of Levitan et al (1993)
k_{13}	48.6	
k_{14}	7.55	
k_{15}	0.42	
n	1.65	
k_{16}	2	chosen to produce a $[Ca^{2+}]_{cyt}$ transient of appropriate duration and amplitude
k_{17}	8	
k_{18}	25	
T	293°K	Eilers et al., 1995
$[Ca^{2+}]_{ER}$	1 mM	Baumann et al., 1991
$[Na^+]_{cyt}$	8 mM	Eilers et al., 1995
$[Na^+]_{ext}$	125 mM	Eilers et al., 1995
$[Ca^{2+}]_{ext}$	2 mM	Eilers et al., 1995
k_{19}	100	set amplitude of depolarizations
k_{20}	10	set rate of decay of potential
τ_1	0.025	assume [cGMP] signal has rapid onset and decay
τ_2	0.005	
k_{21}	1	set amplitude & duration of N
k_{22}	12	set amplitude & duration of N
N_{max}	2	less calcineurin than PKC
k_{23}	2	set rate of learning
k_{24}	0.4	set rate of extinction
g_{max}	600	need large $\bar{g}$ to hyperpolarize

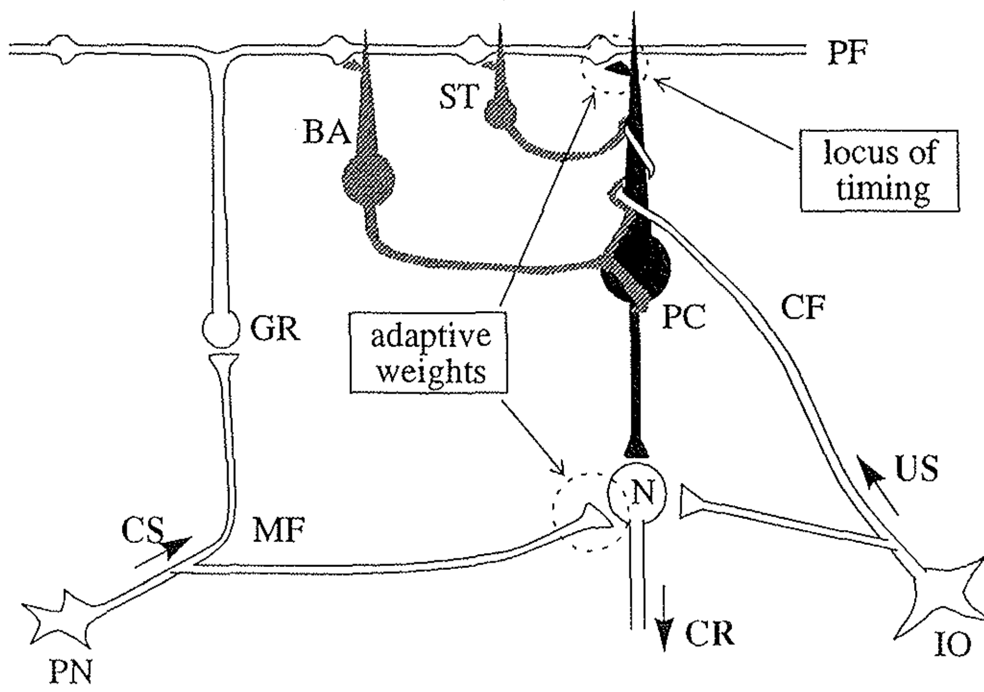


Figure 1

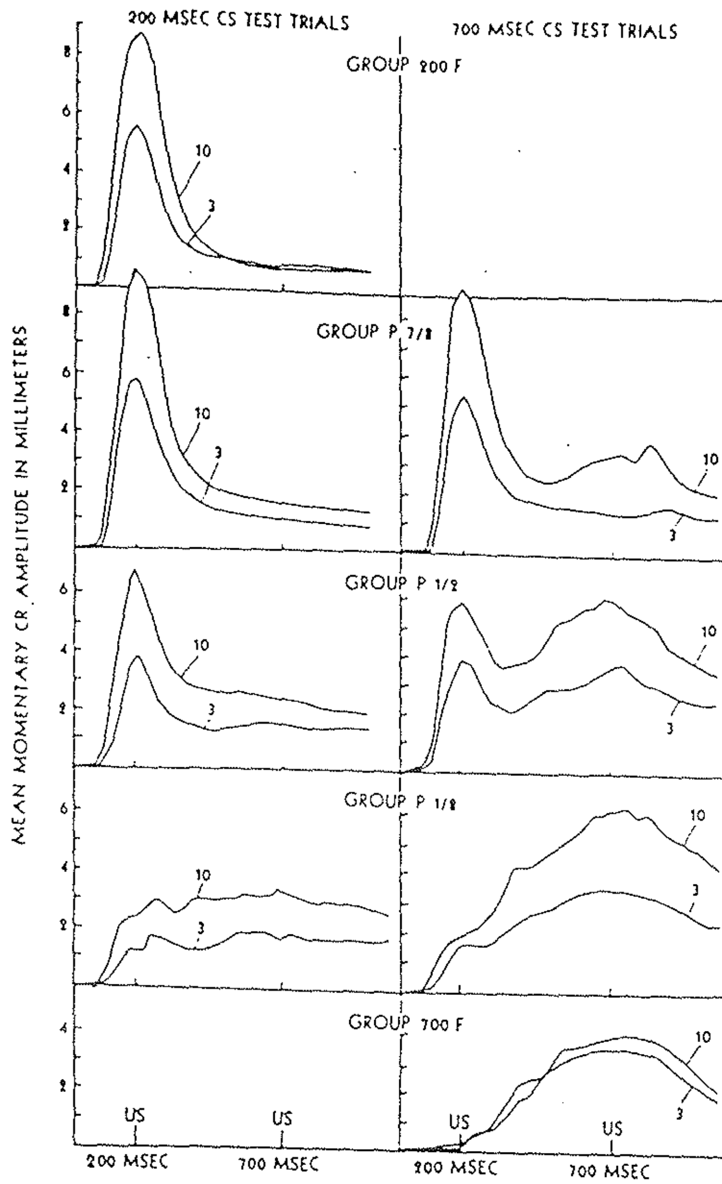


Figure 2

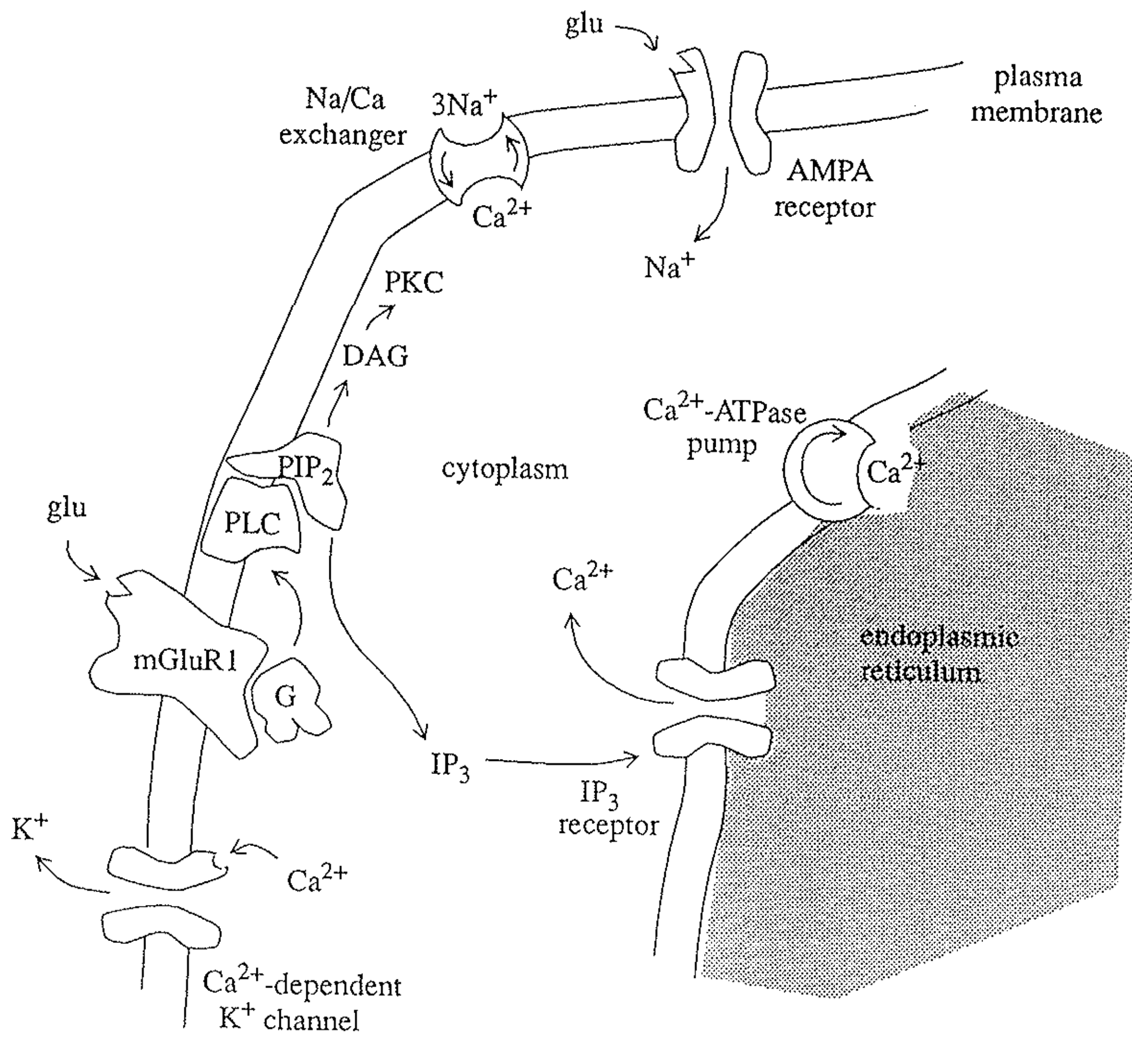


Figure 3

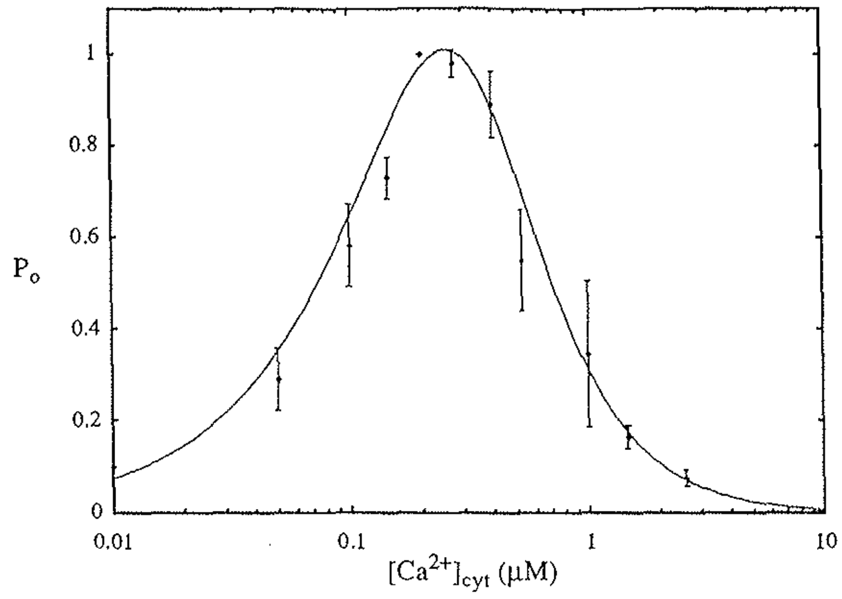


Figure 4

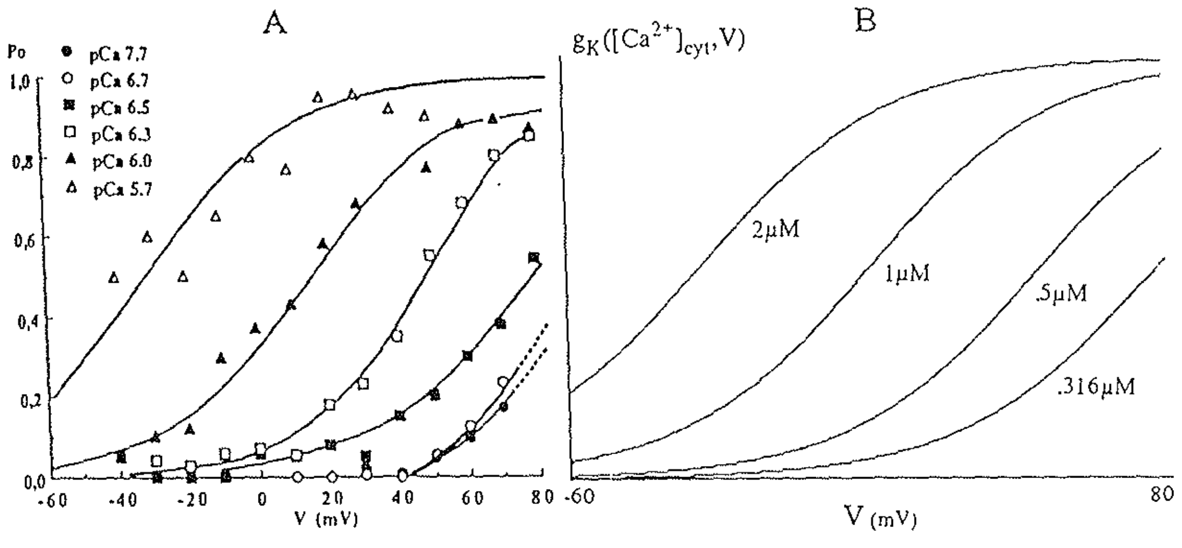


Figure 5

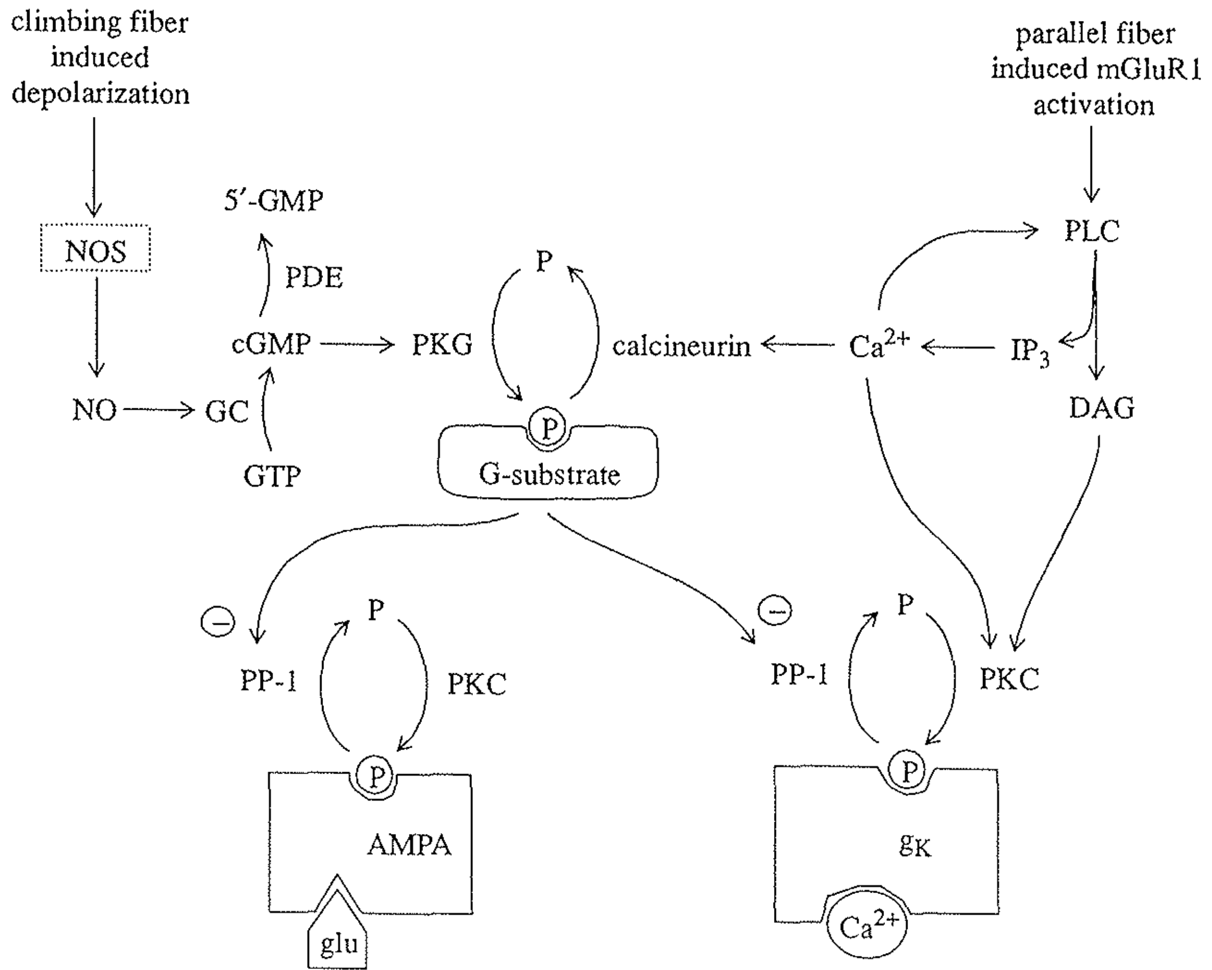


Figure 6

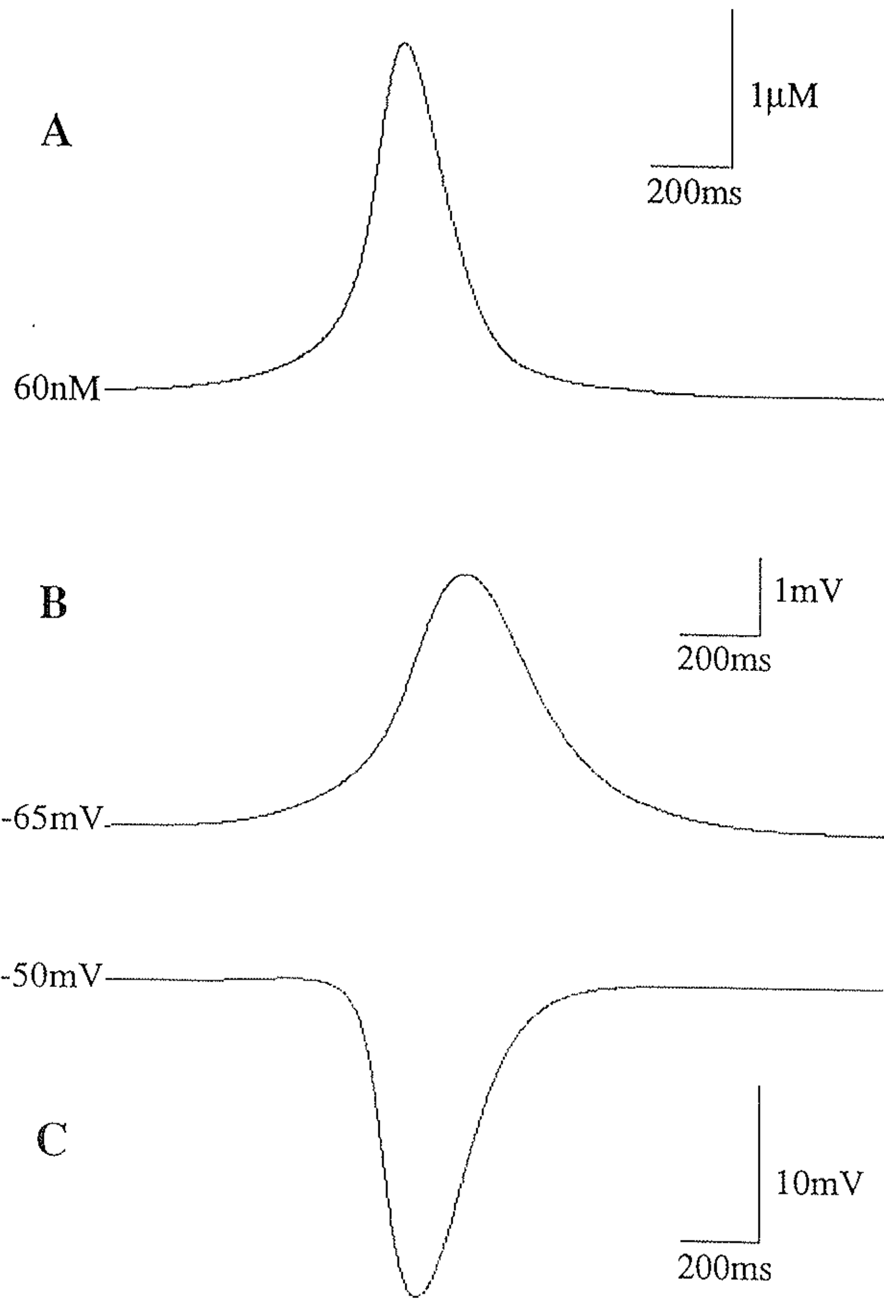


Figure 7

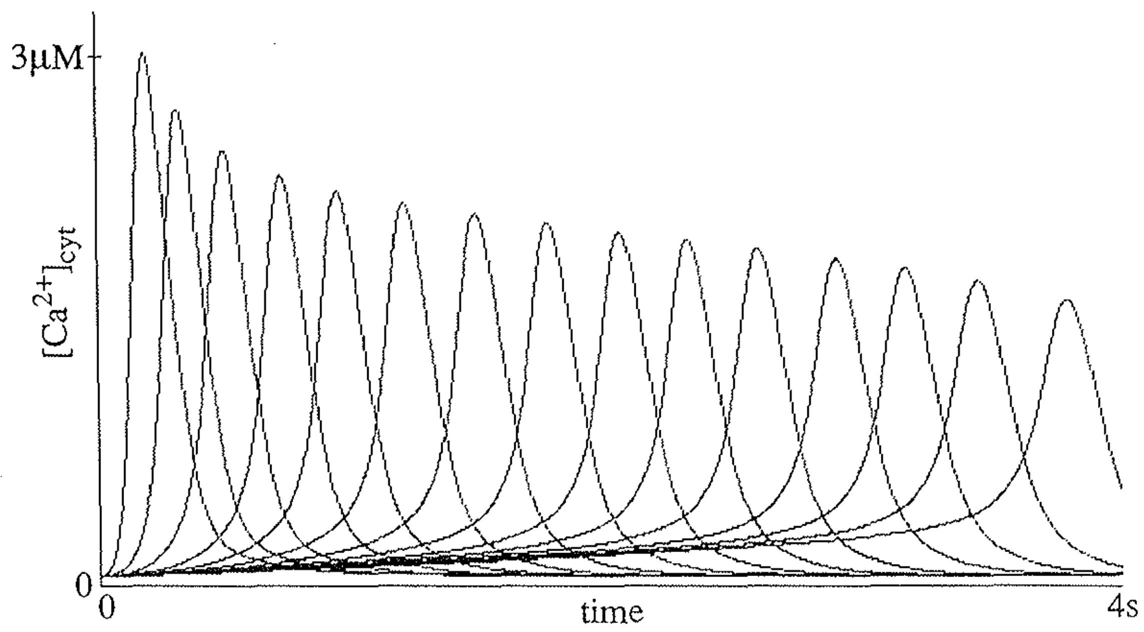


Figure 8

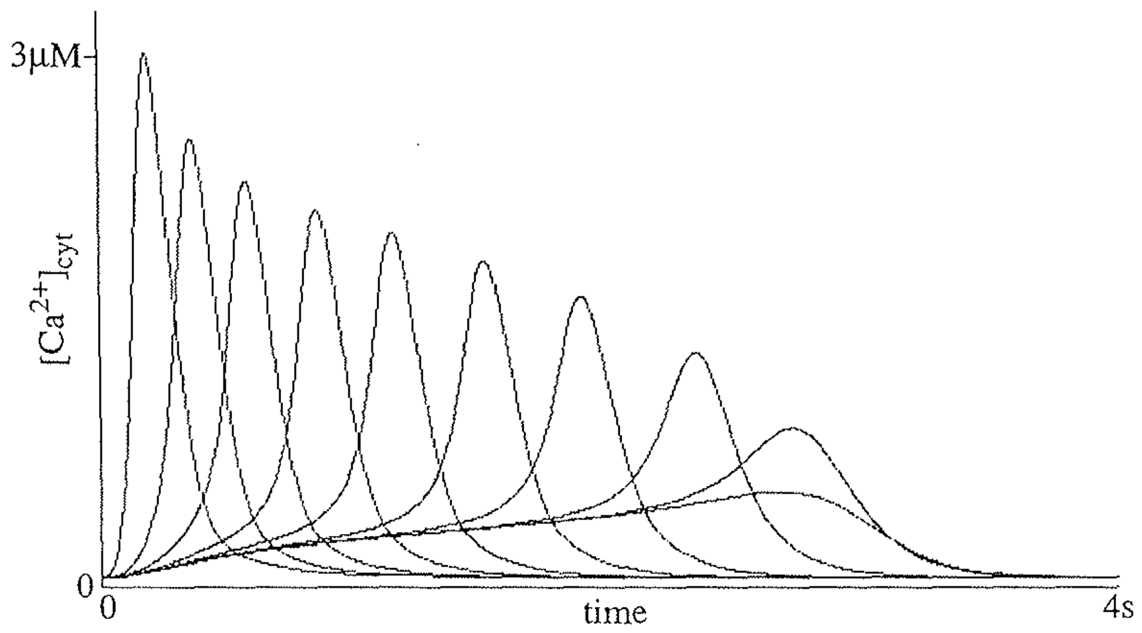
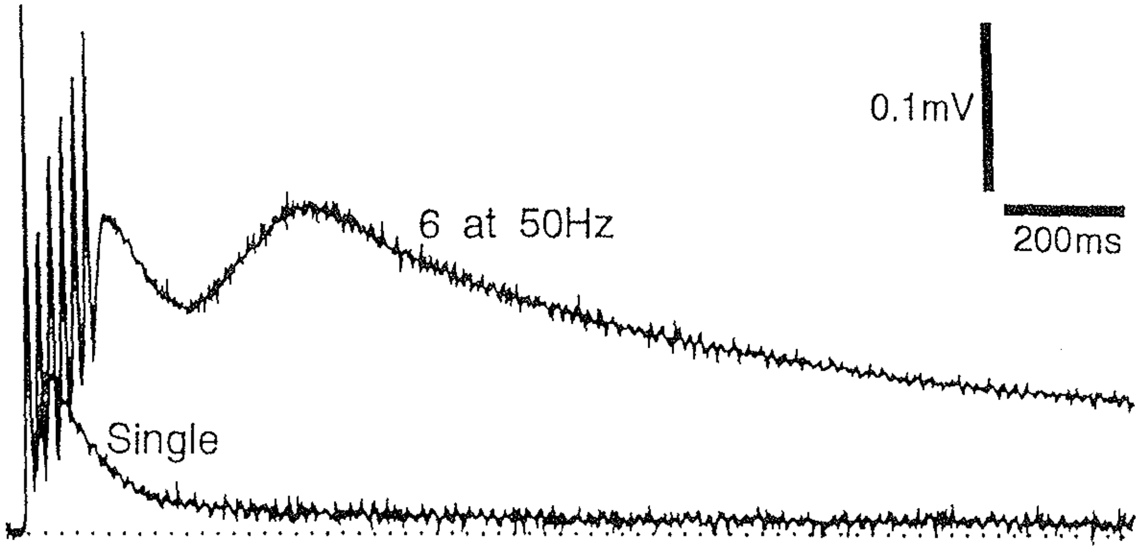
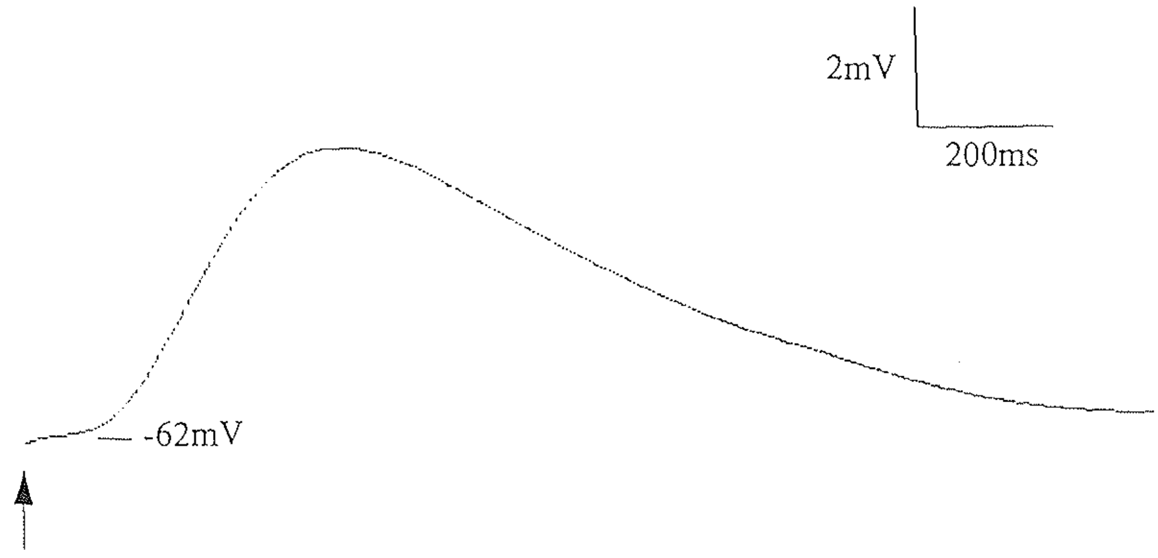


Figure 9



A



B

Figure 10

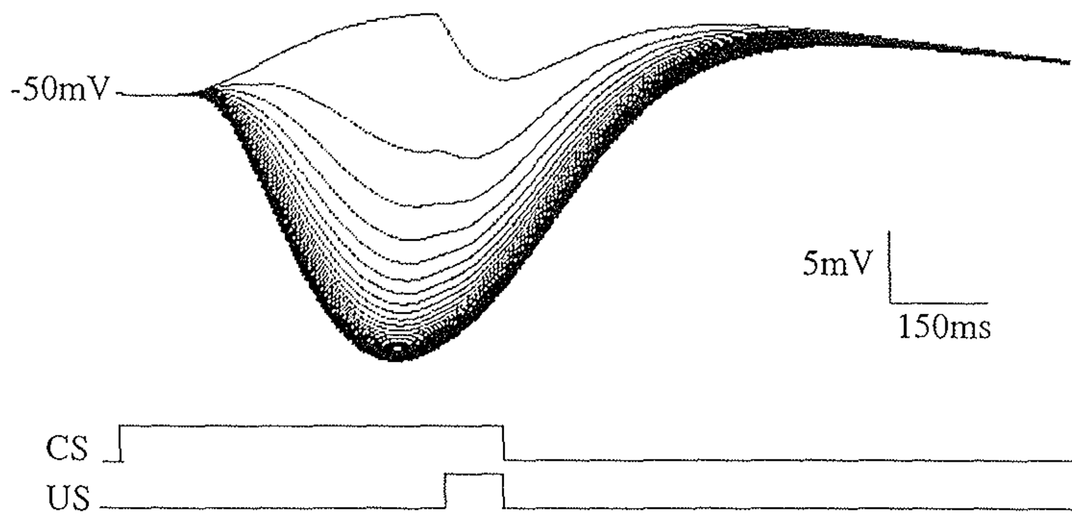


Figure 11

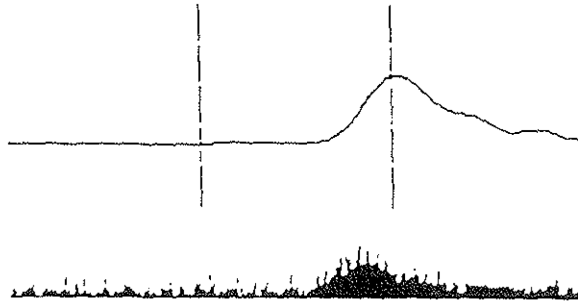


Figure 12

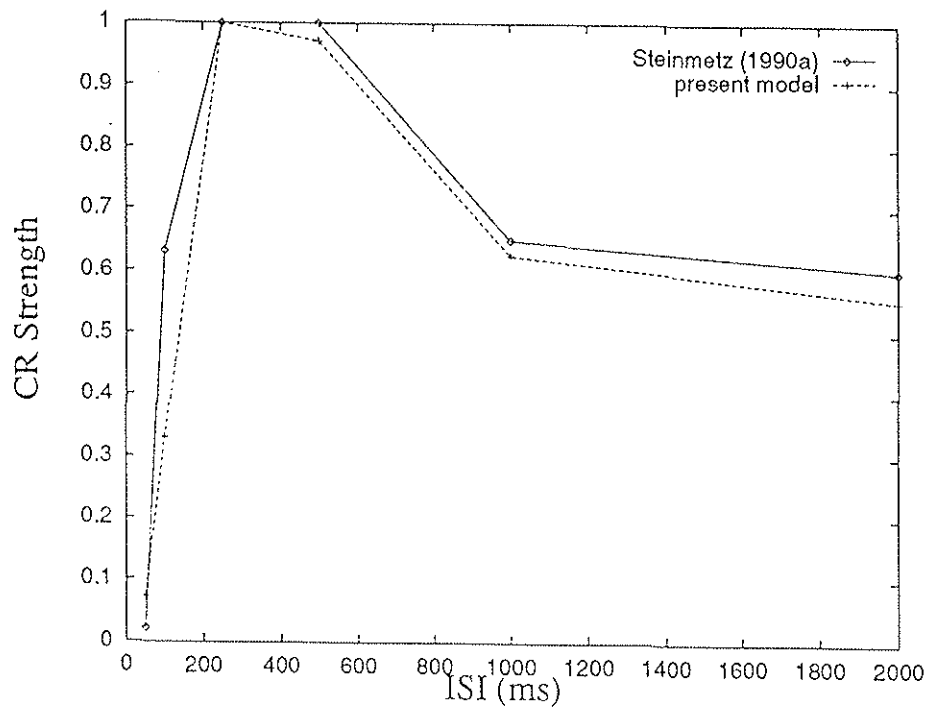


Figure 13

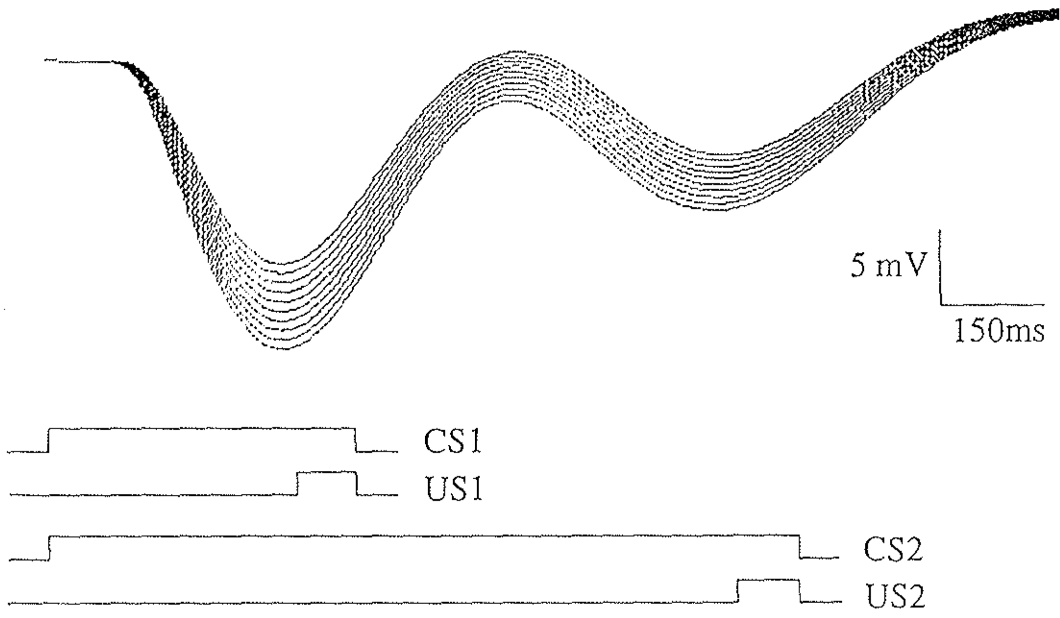


Figure 14