Modelling Phasic Firing in Vasopressin Neurones

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Abstract

Hypothalamic neurons secreting the hormone vasopressin (VP cells) exhibit asynchronous phasic bursting activity which is crucial for hormone release. A computational model of a VP cell is presented that reproduces phasic behaviour observed *in vivo*. The model includes various ionic currents and a stochastic term to represent the synaptic input. A dynamic for the cytosolic and the endoplasmatic reticulum Ca^{2+} concentrations is introduced, that incorporates a process of Ca^{2+}-induced Ca^{2+} release (CICR) from internal stores. Simulated response of the model cell to a range of applied stimuli show a consistent match with experimental recordings, suggesting that an interplay between a calcium-dependent potassium current and an amplified Ca^{2+} signaling system via CICR could play a key role in shaping the observed spiking pattern.

1 Introduction

The release of the peptide hormones vasopressin (VP) and oxytocin (OT) strongly depends on the pattern of neuronal activity of magnocellular neurosecretory neurones located in the supraoptic and paraventricular nuclei of the hypothalamus [11]. VP is primarily concerned with body fluid homeostasis and is released in response to increased plasma osmotic pressure and reduced plasma volume. At normal plasma osmolarity, VP cells are generally silent or fire slowly and irregularly with no apparent patterning. When the plasma concentration of Na^+ rises, VP cells increase their firing rate, and eventually adopt an asynchronous phasic bursting pattern characterized by alternating periods of activity (7-15 Hz) and silence lasting tens of seconds each [5]. This pattern optimises the efficiency of hormone secretion from nerve terminals through several facilitatory mechanisms [16]. During chronically maintained stimulation, the bursts become shorter and more intense, and their timing becomes more regular [15].

The mechanism underlying the emergence of the phasic firing pattern in the VP cells has been the subject of intense investigations. It is now widely recognized that phasic firing is neither a passive response to a phasically patterned input, nor does it reflect spontaneous oscillation of membrane potential, but instead results from the interaction between synaptic activity and intrinsic ionic mechanisms [9].

The excitability and the discharge pattern of magnocellular neurones strongly depend on hyperpolarising (AHP) and depolarising after-potentials (DAP). In particular, VP cells display long DAPs which arise slowly following action potentials thereby increasing the likelihood of successive spikes [2]. In phasically active cells, DAPs following consecutive action potentials summate temporally into a depolarising plateau. Spike activity induced by the synaptic input is believed to trigger and maintain the plateau potential, thus providing a feedback mechanism by which a burst could be sustained [10].

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Bursts evoked by current injection or by antidromic stimulation are followed by an activity-dependent after-hyperpolarisation (AHP) resulting mainly from a slow, apamin-sensitive Ca^{2+} - dependent K^+-current ($I_{K(Ca)}$) which functions as a feedback inhibitor of spike activity [13, 8]. Ca^{2+} entry through voltage-gated channels during each spike causes Ca^{2+} to accumulate in the cell body and progressively activate $I_{K(Ca)}$. As a result, a hyperpolarizing shift is developed during the course of the burst that may eventually lead to abolition of the plateau potential and shut the cell into silence. Growing evidence [14, 1] has made it possible to propose the hypothesis that the subcellular process of Ca^{2+}-induced Ca^{2+} release (CICR) from the intracellular stores, could be involved in the process of excitation-secretion coupling of VP cells by recruiting Ca^{2+}-dependent K^+ channels.

In this paper we show how the inclusion of a Ca^{2+}-dependent K^+ current in a single-compartment neuronal model, together with a suitable description of the intracellular calcium dynamics, can account for the production of phasic bursting. In particular the *inactivation* of $I_{K(Ca)}$ is suggested to be responsible for the graded depolarisation at the start of the burst, whereas burst termination and prolonged inter-burst intervals would be due to the strong activation of $I_{K(Ca)}$ following large intracellular Ca^{2+} oscillations mediated by CICR.

2 Methods

A model of a single compartment is considered. The dynamic of the membrane potential V is described as follows:

$$C_m \frac{dV}{dt} = -I_{Na} - I_K - I_{K(Ca)} - I_{HVA} - I_{LVA} - I_L - I_{syn} - I_{osm} \tag{1}$$

where C_m is the membrane capacitance. Six ionic currents have been included in the model: a fast inward Na^+ current (I_{Na}); a transient outward K^+ current (I_K), mainly responsible for membrane repolarisation after the action potential, which also contributes to hyperpolarisation after-potential (HAPs) [6]; a Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$) which produced adaptation during repetitive firing, and is responsible for the prolonged AHP following burst termination [4]; a low-voltage activated Ca^{2+} current (I_{LVA}) which is de-inactivated by hyperpolarizing the cell below its resting potential and contributes to DAP; a high-voltage activated Ca^{2+} current (I_{HVA}), which is activated during action potentials; a linear leakage term (I_L).

The osmotic stimulus has been represented by a voltage-independent inward current (I_{osm}) which accounts for the effect of stretch-sensitive cation channels [3]. A stochastic term (I_{syn}) has also been included in the model to account for the synaptic input. This is given by the sum of two independent Poissonian processes that represent the effect of randomly occurring EPSPs and IPSPs.

The dynamics for the free intracellular Ca^{2+} concentration $[Ca^{2+}]_i$, and the Ca^{2+} concentration in the endoplasmatic reticulum $[Ca^{2+}]_{ER}$, are given by the following balance equations (see [7]),

$$\frac{d[Ca^{2+}]_i}{dt} = F_0 + F_1 I_{HVA} + F_2 I_{LVA} + F_{ER,leak} - F_{SL,pump} + F_{ER,rel} - F_{ER,pump} \tag{2}$$

$$\frac{d[Ca^{2+}]_{ER}}{dt} = F_{ER,pump} - F_{ER,rel} - F_{ER,leak} \tag{3}$$

where F_0 represents a constant entry rate of Ca^{2+} into the cell body; the constants F_1, F_2 convert the calcium currents into flux rates; $F_{ER,leak}$ is a linear leakage from the internal store, and $F_{SL,pump}$ is a linear process of pumping into the extra-cellular space. The terms $F_{ER,rel}$ and $F_{ER,pump}$ represent the calcium-induced release rates from the stores, and the pumping rate into the stores, respectively, and are given by the expressions,

$$F_{ER,rel} = F_5 \frac{[Ca^{2+}]_i^3}{K_4^3 + [Ca^{2+}]_i^3} \frac{[Ca^{2+}]_{ER}^2}{K_5^2 + [Ca^{2+}]_{ER}^2}, \qquad F_{ER,pump} = F_6 \frac{[Ca^{2+}]_i^2}{K_6^2 + [Ca^{2+}]_i^2} \tag{4}$$

All model parameters, including current conductances and kinetics, and calcium dynamics, have been adjusted to fit observed behaviour of VP cells (see [12]).

3 Simulation results

The model neurone has a resting potential of about -63 mV and a threshold of about -55 mV. Action potentials are followed by a brief HAP, of about 6 mV amplitude, and a DAP of 2 mV amplitude, lasting for about 300 ms. During the DAP, the cell excitability is increased, resulting in a more sensible response to synaptic input. During an evoked burst, the progressively activated $I_{K(Ca)}$ results in the enhancement of the HAPs and the attenuation of the DAP. At the end of the burst, a prolonged after-hyperpolarisation (AHP) is apparent.

Phasic bursting behaviour is observed during simulated activity, in response to synaptic inputs and steady depolarisation (Fig. 1). At low levels of input, the model cell fires irregularly, without apparent patterning. As the rate of the synaptic input is increased, clear bursting activity emerges. At higher levels of synaptic input, or in the presence of increased osmotic stimulation, the VP cell tends to fire continuously and the patterning of spikes is progressively lost.

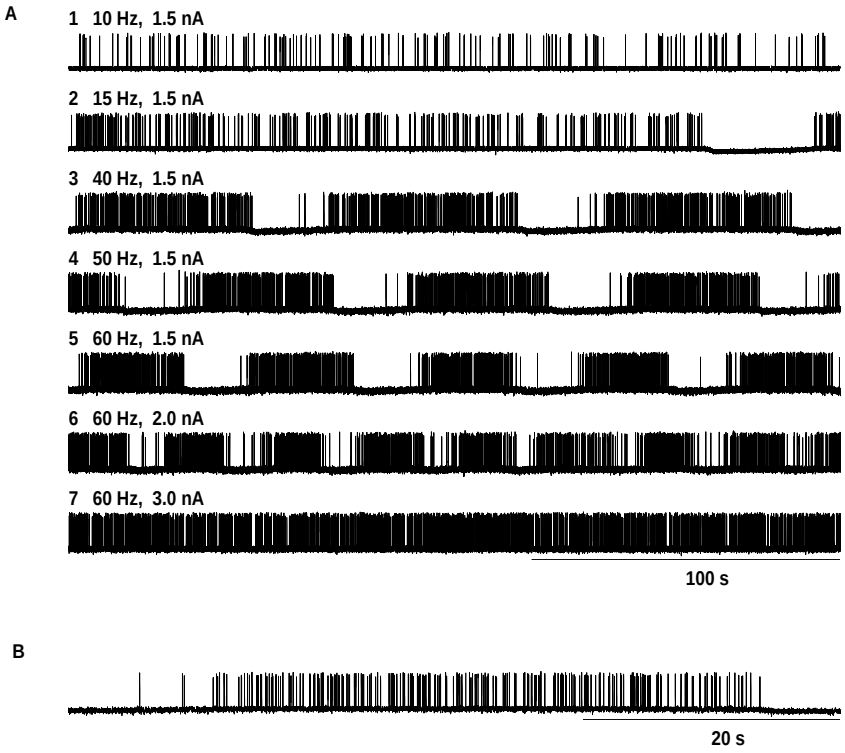

Figure 1: Phasic firing activity calculated from the mathematical model. The synaptic input rate (λ) and the osmotic stimulation (I_{osm}) are indicated in the legend of each panel. Panels A1-A5 show the effect of an increase in synaptic input rate; Panels A6-A7 show the effect of an increased osmotic stimulation. (B) Blow-up of the last burst in Panel A4.

Bursts of spikes last from tens of seconds to minutes and are separated by silent intervals from 10 s to about 1 min, depending on the rate of synaptic input and on the magnitude of the applied current. Mean duration of simulated burst is shortened as the rate of the synaptic input is increased. A moderate reduction in the inter-burst period is also observed with increasing λ. Firing frequency during simulated phasic activity compare well with *in vivo* recordings [12].

The intracellular and the store Ca^{2+} concentrations exhibit oscillations (Fig. 2) synchronized

with phasic bursts. During a burst, low-amplitude fluctuations in $[Ca]_i$ are seen, resulting from Ca^{2+} entry through voltage-gated channels. As soon as $[Ca]_i$ reaches a threshold concentration, it triggers a release of Ca^{2+} from the internal stores producing a rapid rise in $[Ca]_i$. The consequent large oscillation in $[Ca]_i$ fully activates $I_{K(Ca)}$, resulting in burst termination and a prolonged AHP. The excess in $[Ca]_i$ is then gradually cleared through ER-reuptake and pumping into the extra-cellular space. Finally, as $[Ca]_i$ returns to the baseline, $I_{K(Ca)}$ is inactivated, and the cell can start firing again in response to synaptic input.
The burst duration has been found to be dependent on spiking activity, this is consistent with previous observations. The higher the firing frequency, the sooner a Ca^{2+} oscillation is triggered, and the burst is terminated.

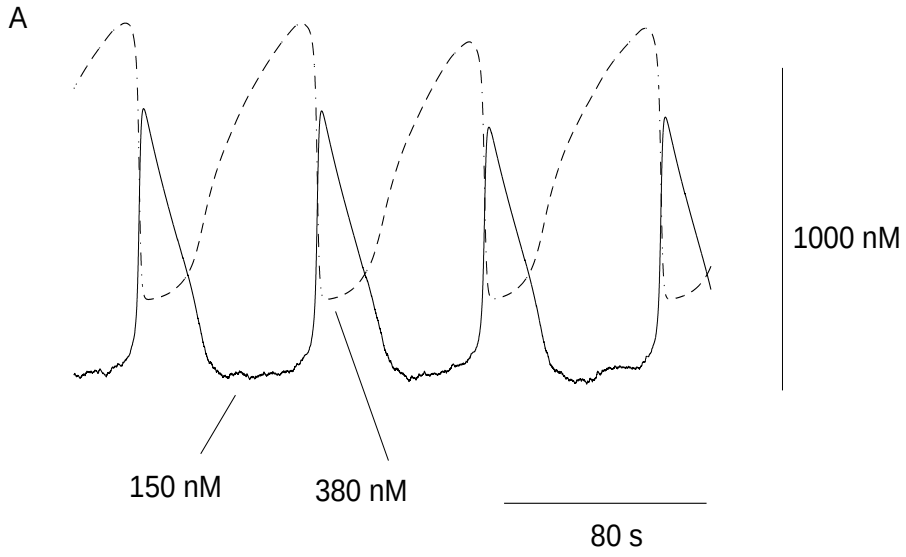

Figure 2: Intracellular Ca^{2+} dynamics. Time courses of free intracellular Ca^{2+} (solid line) and stored Ca^{2+} (dashed line), obtained by integration of the model; parameters are the same as in Fig. 1, Panel A2.

3.1 Response to afferent stimulation

Electrical stimuli applied to the neural stalk evoke spikes in the axons of magnocellular neurones, which are propagated antidromically to the cell bodies. Brief trains of antidromic spikes (or even single antidromic spike) can trigger full bursts of spikes in VP cells [10]. On the other hand, low-frequency antidromic spikes produce the interesting effect that cells appear to compensate for the additional evoked spikes by a matching reduction in spontaneous discharge. VP cells thus 'defend' their firing rate against perturbations, probably via the after-hyperpolarisation, which acts as a feedback inhibitor of spike activity.

Since antidromic activation mimics particular effects of synaptic excitation, one may infer that an increased frequency of excitatory input might trigger a burst if a VP cell is silent, might stop a burst if a cell is active, or might have no effect if it is week enough to allow the cell to defend its firing rate effectively. Paradoxically, a train of antidromic spikes can also stop established bursts, through exaggerating the activity-dependent inactivation of the plateau potential.

Obviously, an increased frequency of excitatory input might trigger a full burst if the cell is inactive and if it is not refractory following a burst. However, an increased frequency of excitatory input may also stop an ongoing burst in the model cell. This occurs in the model when, after an

increase in the firing rate, the consequent increase in the Ca^{2+} entry rate through voltage-gated is large enough to elicit CICR.

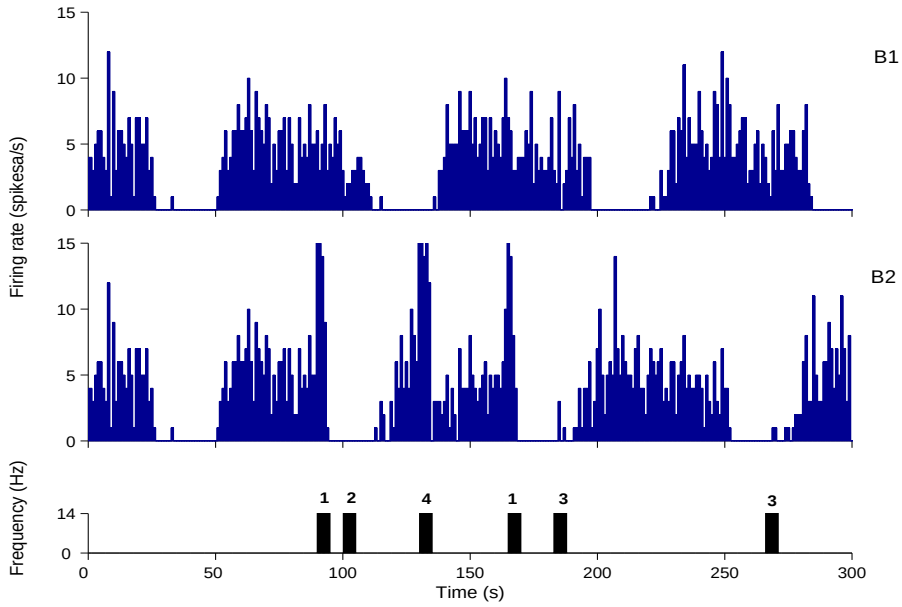

Figure 3: Responses of the model neurone to stimulation from afferent inputs. Panel (B1) show phasic bursting firing in a 'control condition', for a synaptic input rate of $\lambda = 40$ Hz. (B2) Phasic firing activity obtained for the same model neurone in the presence of afferent stimulation. Below a schematic of afferent stimulation and its frequency applied to the cell. Each afferent stimulation is represented as a train of current pulses at 14 Hz for 5 sec, and each pulse has of a duration of 3 ms and an amplitude of 5 nA.

Figure 3 shows how the simulated VP cell responds to discrete afferent stimulation. An increased frequency of excitatory input might either stop a burst (if the cell is active, stimulation 1), or trigger a burst (if the cell is inactive, stimulation 3). If a train of stimuli is applied at the earlier stage of phasic burst, it might not stop the burst (stimulation 4) while, if it arrives soon after the termination of the previous burst, it might fail triggering a new one.

4 Conclusion

We have presented a mathematical model that incorporates intrinsic and extrinsic properties of VP cells known to be important for phasic activity. The model successfully reproduces the behaviour of these cells under a variety of physiological circumstances. Consistently, the introduction in the model of a Ca^{2+} dynamics, allow us to delineate a mechanism through which a VP cell can *oscillates* between silence and activity. Indeed, Ca^{2+} entry through voltage-gated channels, Ca^{2+} release from internal stores, Ca^{2+} sequestration into the endoplasmatic reticulum and pumping into the extracellular space have found to combine to generate large oscillations in $[Ca^{2+}]_i$. These lead $I_{K(Ca)}$ to activate, so the cell is switched from the active to the silent state. On the other hand, the burst onset is given by $I_{K(Ca)}$ inactivation, after the decay of a Ca^{2+} oscillation.

The model also reproduces a number of subtleties of VP cells behaviour. In particular, it shows how bursts of activity can be both triggered or truncated by a transient flurry of EPSPs.

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