Neural Networks ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at SciVerse ScienceDirect

Neural Networks

journal homepage: www.elsevier.com/locate/neunet

2012 Special Issue

Bistability in Purkinje neurons: Ups and downs in cerebellar research

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ARTICLE INFO

Keywords:
Bistability
Toggling
Cerebellum
Purkinje cells

ABSTRACT

The output of cerebellar Purkinje cells has been characterized extensively and theories regarding the role of simple spike (SS) and complex spike (CS) patterns have evolved through many different studies. A bistable pattern of SS output can be observed *in vitro*; however, differing views exist regarding the occurrence of bistable SS output *in vivo*. Bistability in Purkinje cell output is characterized by abrupt transitions between tonic firing and quiescence, usually evoked by synaptic inputs to the neuron. This is in contrast to the trimodal pattern of activity which has been found *in vitro* and *in vivo* when climbing fiber input to Purkinje cells is removed. The mechanisms underlying bistable membrane properties in Purkinje cells have been determined through *in vitro* studies and computational analysis. *In vitro* studies have further established that Purkinje cells possess the ability to toggle between firing states, but *in vivo* studies in both awake and anesthetized animals have found conflicting results as to the presence of toggling in the intact circuit. Here, we provide an overview of the current state of research on bistability, examining the mechanisms underlying bistability and current findings from *in vivo* studies. We also suggest possible reasons for discrepancies between *in vivo* studies and propose future studies which would aid in clarifying the role of bistability in the cerebellar circuit.

1. Introduction

Since the first *in vivo* recordings of cerebellar Purkinje cells, their complex patterns of output have been well-characterized. However, the nature of their output under physiological conditions remains an area of controversy. Early unit recordings of Purkinje cells *in vivo* revealed the presence of both simple spikes (SS) and complex spikes (CS). The advent of the *in vitro* slice preparation and intracellular recordings revealed new dimensions of Purkinje cell output in the form of intrinsic conductances that could drive calcium spikes and plateau depolarizations, along with slow oscillatory output of sodium spike discharge (Llinas & Sugimori, 1980a, 1980b; Tank, Sugimori, Connor, & Llinas, 1988). Slow oscillations were eventually dubbed a "trimodal" pattern consisting of an initial period of tonic spike discharge that proceeds into a repetitive series of calcium spike-mediated bursts of sodium spike discharge and then finally a long period of quiescence (Womack & Khodakhah, 2002). However, trimodal activity in Purkinje cells slowly became accepted by most as a pattern not representative of the tonic activity of Purkinje cells *in vivo* (Cerminara & Rawson, 2004; McKay et al., 2007). More recently, SS activity of Purkinje cells *in vivo* were reported to exhibit patterns of regular spike output separated by intermittent periods of quiescence (Loewenstein et al., 2005; Yartsev, Givon-Mayo, Maller, & Donchin, 2009), with additional "pauses" of firing evoked following either parallel fiber (PF) or climbing fiber (CF) inputs (De Schutter & Steuber, 2009; Shin et al., 2007; Steuber et al., 2007). The short- and long-term rates of synaptically-evoked output of Purkinje cells are further modified by the specific pattern and interaction between PF and CF inputs (Schmolesky, De Zeeuw, & Hansel, 2005; Shin et al., 2007; Steuber et al., 2007). Furthermore, CF-evoked responses were reported to trigger transitions between up- and down-states of sodium spike discharge *in vivo* (Kitamura & Hausser, 2011; Loewenstein et al., 2005; Yartsev et al., 2009) or *in vitro* (Fernandez, Engbers, & Turner, 2007; McKay et al., 2007; Oldfield, Marty, & Stell, 2010; Rokni, Tal, Byk, & Yarom, 2009), a result consistent with bistable membrane properties. However, bistability *in vivo* was subsequently reported to be influenced by the state of anesthesia (Schonewille et al., 2006).

Therefore, the field has progressed through multiple stages of discussion around the natural intrinsic patterns of Purkinje cells and their response to PF or CF input. Recent issues center on the extent to which Purkinje cells exhibit slow oscillations, bistable membrane properties, CF-evoked toggling between up- and down-states, or regular patterns of sodium spike discharge.

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doi:10.1016/j.neunet.2012.09.006

This review considers the patterns of spike output indicative of bistable membrane responses and their relation to trimodal activity vs. CF-evoked toggling between up- and down-states. We discuss these issues in the context of conventional terms used in physiology and those in the field of non-linear dynamics, which prove to be instrumental to identifying the underlying basis of Purkinje cell output. Finally, we summarize some of the factors that computational analyses identify as potentially key to regulating the prevalence of bistability and toggling *in vivo*, and thus potential targets for future research. Given the long history of research in this area, we do not intend to provide an exhaustive documentation of first reports of different factors, but rather focus on studies that define some of the most recent discussions in the field.

2. Defining the terms

Purkinje cells exhibit several forms of output, but only some reflect bistable membrane properties. For the purpose of focusing this review, we will define some key output patterns and the underlying cellular mechanisms in order to help identify responses that represent bistable behavior.

2.1. Bistable membrane properties

Some of the confusion surrounding the existence of bistability in Purkinje cells results from the ambiguity surrounding the term "bistable". In this review, we use the general definition provided by the field of non-linear dynamics: a system is *bistable* when two attractors coexist for a given set of parameters. This could be the coexistence of stable depolarized and hyperpolarized states (fixed points), or, in the case of Purkinje neurons, a hyperpolarized stable membrane potential (fixed point) and a tonic firing state (limit cycle) (Fernandez et al., 2007). *Bistability* is evident when a transitory input (e.g. current pulse or synaptic event) is capable of moving the system between either stable state without changing the topology of the system. This is in contrast to a *bifurcation*, which occurs when the topology of the system changes via the creation or elimination of attractors due to a steady-state change in a system parameter (for a review of these concepts, see Izhikevich (2007)). To describe these concepts in physiological terms, bistability can be seen when a unitary event, such as a CF input, can evoke a transition from a hyperpolarized state to tonic firing ("up-state"), as well as from a tonic firing state to a hyperpolarized state ("down-state") (Fig. 1) (Fernandez et al., 2007; Kitamura & Hausser, 2011; Loewenstein et al., 2005; McKay et al., 2007; Oldfield et al., 2010; Rokni et al., 2009). A bifurcation occurs when increasing levels of depolarizing current are applied to a neuron until the cell enters a tonic firing state (as for a current-frequency plot). In this case, the fixed point representing the stable membrane potential is eliminated by the increase in applied current, the *bifurcation parameter*. A common method of examining bistability and bifurcations in a model is through the use of *bifurcation diagrams* (Fig. 2(A), (B)), which plot all attractors (stable and unstable) that exist for a given value of the bifurcation parameter. A *bistable range* is identified as the range of bifurcation parameter values for which two stable attractors coexist (Fig. 2(B), *dashed lines*). A bifurcation is identified by the elimination or creation of an attractor as the bifurcation parameter is changed (Fig. 2(B), *asterisk*).

Input from CFs, PFs, and molecular layer interneurons have all been shown to produce a "toggling" between up- and down-states *in vitro* (in the absence of trimodal activity—see below) (Fernandez et al., 2007; Jacobson, Rokni, & Yarom, 2008; McKay et al., 2007; Oldfield et al., 2010; Rokni et al., 2009; Williams, Christensen, Stuart, & Hausser, 2002) (Fig. 1). Synaptic input can thus shift a cell from a depolarized state of ~ -50 mV that supports tonic spike firing to a hyperpolarized quiescent state until perturbed by subsequent synaptic inputs that can shift the cell back to a state of firing (Fig. 1) (Fernandez et al., 2007; Loewenstein et al., 2005; McKay et al., 2007). Bistability in neurons is further identified by the generation of a plateau depolarization and the presence of hysteresis in the firing rate, both of which have been reported in Purkinje cells. Plateau depolarizations driven by voltage-dependent calcium currents and a non-inactivating sodium current were reported in some of the first Purkinje cell recordings *in vitro* (Llinas & Sugimori, 1980a, 1980b). Hysteresis can be observed upon injection of triangular current waveforms to progressively ramp membrane voltage up and down (Fernandez et al., 2007; Williams et al., 2002; Yuen, Hockberger, & Houk, 1995), revealing spike firing during lower levels of current injection on the down slope of the ramp as compared to the rising phase (Fig. 2(C), (D)). The discontinuity of the F–I relationship and minimum discharge frequency observed upon transition from rest to firing is consistent with the bistability associated with a saddle–homoclinic bifurcation (Fig. 2(E)) (Fernandez et al., 2007). These dynamics are also associated with a high gain in the F–I relationship near the minimum spike discharge frequency, which is also observed in experimental recordings of Purkinje cells (Fig. 2(D), (E)) (Fernandez et al., 2007; McKay et al., 2007). Thus, the existence of bistable membrane properties in Purkinje cells is firmly established as an intrinsic membrane property of these cells under isolated conditions *in vitro*. The question is the extent to which this activity is incorporated in the generation of Purkinje cell output *in vivo* under physiological conditions.

2.2. Trimodal activity

Purkinje cells can exhibit a form of slow membrane potential oscillation that drives a trimodal pattern of output (Fig. 3(A)). The soma–dendritic interactions that contribute to trimodal activity have been extensively examined in the *in vitro* slice preparation (Brenowitz, Best, & Regehr, 2006; Llinas & Sugimori, 1980a, 1980b; McKay et al., 2007; McKay & Turner, 2005; Swensen & Bean, 2003, 2005; Tank et al., 1988; Womack & Khodakhah, 2002, 2004). During a trimodal pattern of firing, the cell regularly transitions from tonic firing to repetitive calcium spikes to a quiescent, hyperpolarized state with a period of $\sim$60 s. This continual switching between firing states could be due to one or more slow, modulatory currents pushing the neuron through different equilibria and bifurcations (Izhikevich, 2007). This type of bursting pattern would then be the result of interactions between oscillators of different time scales: a fast oscillator to produce the observed simple spikes and bursts, and an intrinsic slow oscillator to predictably drive the system between states (this is in contrast to a bistable system that will remain in one of two states indefinitely in the absence of noise or input and only transitions between states due to extrinsic input). Therefore, the trimodal system is not unstable, but exhibits stability over a longer time scale.

Importantly, the trimodal pattern is not a result of network activity or a sign of unhealthy cells (McKay et al., 2007; McKay & Turner, 2005; Womack & Khodakhah, 2002). Indeed, the trimodal pattern can be prevalent *in vitro* at physiological temperatures and proceed for hours without interruption in the presence of excitatory and inhibitory synaptic blockers. The incidence of recording trimodal activity also changes over the first $\sim$20 days of postnatal development (McKay & Turner, 2005; Womack & Khodakhah, 2002) in a manner that correlates with expansion of the dendritic tree and associated conductances (McKay & Turner, 2005) (Fig. 3(B), (C)). Trimodal activity can also be reversibly blocked by modest levels of bias current injection to hyperpolarize the cell or by shifting between physiological (34 °C) and room temperature (McKay & Turner, 2005). All of these properties are

Please cite this article in press as: Engbers, J. D. T., et al. Bistability in Purkinje neurons: Ups and downs in cerebellar research. Neural Networks (2012), doi:10.1016/j.neunet.2012.09.006