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Bistability in Purkinje neurons: Ups and downs in cerebellar research

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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.

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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.
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 in 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 (Ullinas & 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; Ullinas & 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 \(-60\) 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 \(-20\) 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
FIG. 1. Transitions between up- or down-states can be triggered by synaptic inputs. (A), Extracellular recordings of guinea pig Purkinje cell spike SS spike output in vivo in relation to climbing fiber input (asterisk). SS firing can either end or be activated in relation to the occurrence of climbing fiber input. At right are plots of the cross-correlation of complex spikes with the end or beginning of SS spike firing, as shown in the representative traces at left. (B), Whole-cell recordings of SS from a rat Purkinje cell in vitro demonstrates the ability for climbing fiber input (arrows) to cause transitions between rest and firing states. (C), Parallel fiber EPSP are capable of generating transitions to up-states from rest, while input from molecular layer interneurons (MLIs) can return the cell to the down-state. (D), Dual whole-cell recordings from rat MLIs and Purkinje cells in vitro demonstrate the ability for MLI activity to generate both up- and down-state transitions.

Source: Modified from Loewenstein et al. (2005), McKay et al. (2007), Rokni et al. (2009), and Oldfield et al. (2010).

consistent with coordinated and sustained activity of voltage-gated conductances in Purkinje cells instead of a cell with compromised health.

A transition from SS firing to a trimodal-like oscillatory pattern emerges in vivo when CF input is silenced (Cerminara & Rawson, 2004) (Fig. 4(A), (B)), suggesting that trimodal activity is normally
Fig. 2. Bifurcation analysis of bistability in Purkinje cells. (A), (B), Single-parameter bifurcation diagrams for somatic membrane voltage. Driving current (I_e) was used as the bifurcation parameter and was varied from −650 to 200 µA/cm². Stable fixed points (node point) are denoted by a thin gray line, unstable fixed points (saddle points) by a black line, and the unstable limit cycle by a thick gray line. Upper and lower limits of the spike (limit cycle) amplitude are denoted by a thick black line. Note that the limit cycle and stable fixed point coexist between −0.05 and 0.21 µA/cm² of driving current (between dashed lines in (B)). While the stable fixed point and limit cycle also coexist between ∼100 and 200 µA/cm², the level of current injection required to enter this region is outside the physiological range. An asterisk indicates a point where a saddle-node (or fold) bifurcation of fixed points occurs. (C), A Purkinje cell response to a 4 s ramp protocol (2 s on each side) with a slope of 100 pA/s. Strong hysteresis is evident in the ability to fire for lower current levels on the downstroke of the current-ramp protocol. (D), Plots of the F–I relationships for the cell in (C) calculated during the upstroke and downstroke of the ramp. Grey lines illustrate gain change in the low frequency range. (E), Frequency–current (F–I) relationship for the model cell for different levels of driving current. Note that the F–I relationship is discontinuous if the model is taken from rest to firing but continuous if taken from firing to rest. An upward arrow indicates the discontinuity in the F–I relationship when moving from rest to firing and a gray shaded area the bistable region. Also note that the discontinuity indicates a bifurcation and loss of bistability, and delineates the bistable region when moving from firing to rest.

Source: Modified from Fernandez et al. (2007).

blocked by synaptic inputs. Under these conditions, a Purkinje cell in vivo exhibits oscillatory output consisting of a continual increase in SS firing frequency during the tonic firing periods followed by a rapid drop and finally cessation of SS firing (Fig. 4(A)). This pattern of SS output is consistent with trimodal activity (Fig. 4(C), (E)) rather than toggling behavior resulting from synaptic inputs (Fig. 4(D), (E), right). In fact, the slow oscillatory shifts and high frequency SS firing are blocked upon restoring CF input in vivo (Cerminara & Rawson, 2004), consistent with the finding that as few as 10 CF stimuli (1 Hz) reintroduced in the in vitro slice preparation rapidly blocks trimodal activity (Mckay et al., 2007) (Fig. 4(C)–(E)). This net inhibitory or stabilizing influence on sodium spike output may incorporate GABAergic inhibitory interneurons during CF input (Barmack & Yakhnitsa, 2011). Yet, in the slice preparation, repetitive activation by just the postsynaptic depolarization associated with a complex spike is sufficient to block trimodal output (Mckay et al., 2007), revealing that postsynaptic conductances triggered by the complex spike can fully replicate the inhibitory effects of CF input on trimodal activity. These studies were important in establishing that trimodal activity is not associated with periodic CF input, but rather arises when CF input is lost. We thus consider trimodal activity to represent the intrinsic activity of healthy cells in the absence of CF input, as is the case when the inferior olive is lost during slice preparation. If the inferior olive is compromised in vivo, we would expect the same activity to become apparent in a slow oscillatory output of Purkinje cells.

The study of Mckay et al. (2007) further showed that once CF input stabilizes the rate of Purkinje cell firing, a CF-evoked toggling between up- and down-states can become apparent, demonstrating true bistable behavior (Fig. 4(D)) (Mckay et al., 2007). Although trimodal activity may incorporate bistable properties in its generation, these data again indicate that a trimodal pattern of discharge is distinct from a CF-evoked toggling of Purkinje cells between up- and down-states (Section 2.3). These two patterns then arise through separate mechanisms: trimodal activity through a loss of activity in inferior olivary neurons that drive CF input, and toggling (up- and down-states) through an expression of bistable properties of Purkinje cells. However, there are indications in published records that both trimodal activity and...
Fig. 3. Purkinje cells in vitro can exhibit a spontaneous trimodal pattern of activity. (A), Expanded view of the different components of a trimodal pattern consisting of initial tonic Na+ spike discharge (Tonic), Ca–Na bursts (Burst), and a quiescent phase (Silent). Tonic firing is further subdivided into tonic-early (TE) and tonic-late (TL) phases for sake of comparison. (B), Plots of the average proportion of spontaneously active Purkinje cells expressing the trimodal pattern at the indicated days of postnatal development. The incidence of recording a trimodal pattern in vitro increases from zero prior to P12 to >0.8 by P24. (C), Superimposing the Boltzmann fits for the development of the trimodal pattern (B) and mean dendritic area calculated for Purkinje cells over the same time period reveals that dendritic growth precedes and then parallels the incidence of trimodal pattern expression. Sample values in (B), n = 9–25. Source: Modified from McKay and Turner (2005) and McKay et al. (2007).

The term toggling will be used when referring to synaptically-evoked transitions between up- and down-states, which is an expression of bistability.

As emphasized earlier, it is important to note that toggling is distinct from other forms of pauses or patterns of SS firing. These can include the long duration pauses in SS firing associated with trimodal activity (typically a period of seconds) or the shorter duration pauses (20–100 ms) recorded following PF or CF input, the latter of which arise from feed-forward inhibition or postsynaptic ion channels (Davie, Clark, & Haussser, 2008; De Schutter & Steuber, 2009; Engbers et al., 2012; Mittmann & Haussser, 2007; Mittmann, Koch, & Haussser, 2005; Rancz & Haussser, 2010; Schmolesky, Weber, De Zeeuw, & Hansel, 2002; Steuber et al., 2007; Williams et al., 2002). Distinguishing between these different sources of pauses in Purkinje cell unit discharge in vivo is necessary to identify occurrences of toggling behavior. Indeed, the incidence of toggling encountered in vivo has been debated, often on the grounds of patterns of Purkinje cell extracellular unit discharge showing bimodal interspike interval (ISI) distributions (Schonewille et al., 2006; Yartsev et al., 2009) (Figs. 5–7). Regular oscillatory patterns in extracellular unit discharge have also been interpreted as representing toggling behavior in anesthetized animals (Fig. 6(D)). However, while bimodal ISI distributions and rapid and extended alterations in SS output are consistent with bistability, they are not in themselves conclusive as they do not differentiate between bistable state transitions, trimodal patterns, or long periods of inhibition (Schonewille et al., 2006; Yartsev et al., 2009) (Figs. 1(A), 5(A), 6(D) and 7). Studies that successfully demonstrated toggling in vivo used a combination of cell-attached and whole-cell recordings in anesthetized preparations, providing direct evidence that extended pauses in spike output were associated with a transition to a hyperpolarized resting state (Fig. 5(B), (C)) (Kitamura & Haussser, 2011; Loewenstein et al., 2005). While such advanced techniques are not possible for all studies, they will be necessary to conclusively confirm toggling behavior in awake in vivo preparations.

3. Ionic mechanisms of bistability and toggling

Purkinje cells have been reported to toggle between up- and down-states spontaneously, in response to synaptic input, or with current pulse injections (Fernandez et al., 2007; Jacobson et al., 2008; Loewenstein et al., 2005; McKay et al., 2007; Rokni et al., 2009; Williams et al., 2002). The ionic basis for bistability in Purkinje cells has been well-characterized (Fernandez et al., 2007; Oldfield et al., 2010; Williams et al., 2002), providing insights into in vivo recordings where bistable behavior may underlie a toggling of spike output. Analyses of the non-linear dynamics of Purkinje cell models have also been used to examine these behaviors and provide several testable predictions about factors that may influence the probability of detecting toggling in Purkinje cells (Fernandez et al., 2007; Loewenstein et al., 2005).

The potential role for $I_h$ in generating bistable responses in Purkinje cells has been examined in several studies (Fernandez et al., 2007; Loewenstein et al., 2005; Oldfield et al., 2010; Williams et al., 2002). It was established that the ability for short current pulse injections to elicit toggling is not affected by blocking hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Figs. 8(A), 9(A)). Nonetheless, experimental and modeling studies show that $I_h$ narrows the current input range over which bistability exists (Fernandez et al., 2007; Williams et al., 2002). Examination of the non-linear dynamics of a 5-equation Purkinje cell model using bifurcation analyses showed that the size of the bistable range is strongly and inversely related to the conductance of HCN channels ($g_{HCN}$) (Fig. 8(B), (C)). In fact, increasing $g_{HCN}$ by a factor of 10 nearly eliminated bistability.
Fig. 4. Loss of climbing fiber input uncovers slow oscillations of Purkinje cell SS firing in vivo and trimodal activity in vitro. (A), Frequency plots of Purkinje cell SS output recorded in vivo before and after blocking climbing fiber (CF) input by injecting 10% lignocaine in the inferior olive. Over time Purkinje cell firing transitions from a stable tonic mode to a slow oscillatory output, with lower records obtained 2 h after blocking CF input. Note the long period of oscillatory output. (B), Lesioning the inferior olive to produce long-term block of CF input raises the frequency of Purkinje cell SS output. Subsequent 1 Hz CF stimulation (bar) lowers SS frequency closer to pre-inactivation levels. The lower plot in (B) shows an expanded time scale of the onset of CF stimulation. Bin width 1 s. (C), Expanded view of the different components of a trimodal pattern consisting of initial tonic Na\(^+\) spike discharge (Tonic), Ca–Na bursts (Burst), and a quiescent phase (Silent). Tonic firing is further subdivided into tonic-early (TE) and tonic-late (TL) phases for sake of comparison. (D), Climbing fiber stimulation at 1 Hz (CF, arrows) in the same cell as in (C) blocks the trimodal pattern, leaving tonic Na\(^+\) spike firing between CF stimuli. A slight hyperpolarization of the membrane then uncovers the ability for CF input to toggle a cell between bistable up- and down-states. (E), Plot of the average spike frequency during trimodal activity and after initiating CF stimulation. Sample numbers in (E) n = 10.

Source: Modified from Cerminara and Rawson (2004) and McKay et al. (2007).
Fig. 5. Purkinje cells in vivo display membrane bistability in spike output pattern. (A), In vivo guinea pig Purkinje cell recording shows periods of tonic, high frequency SS firing separated by long periods of quiescence. At right is a histogram illustrating a bimodal distribution of the instantaneous frequency over 10 min (bin width 5 Hz). (B), On-cell, in vivo patch clamp recording from a rat Purkinje cell shows a bimodal firing pattern. (C), Whole-cell recording from the same Purkinje cell shown in (B) demonstrates that the bimodal firing pattern detected in on-cell mode corresponds to membrane potential bistability. Source: Modified from Loewenstein et al. (2005). (Fernandez et al., 2007; Kitamura & Hausser, 2011). In contrast, an intermediate amplitude CF input has a higher probability of causing a down-to-up state transition as it does not recruit enough slow potassium current to overcome the positive feedback following an action potential (detailed below). By comparison, a large amplitude CF input recruits sufficient slow potassium current to generate a hyperpolarization that pulls the neuron back to a resting state, preventing transition to a firing state. As variable amplitudes of CF-induced complex spikes and inward currents have been documented (Davie et al., 2008; Kitamura & Hausser, 2011), the relative magnitude of depolarization provided by a CF input is another variable in evoking bistable activity in vivo (see below).

Importantly, recent imaging of calcium influx in Purkinje cells in vivo also shows an increased calcium influx during a complex spike associated with the transition from an up-state to down-state (Kitamura & Hausser, 2011; Rokni & Yarom, 2009). A transition to a down-state may then involve an increase in the activation of KCa channels when a CF input is presented during a state of tonic SS firing. The identity of the putative KCa current has not yet been identified. It is known that blocking SK or BK channels prevents CF regulation of SS output in vitro (Mckay et al., 2007), but the intense bursting that accompanies a blockade of these channels prevents a determination of their role in bistability. While the oscillatory bursting pattern that follows blockade of SK or BK channels has been interpreted as bistability in some studies (Williams et al., 2002), it represents an activity pattern more closely resembling a trimodal or astable pattern, since the cell does not display any long-term stability associated with a bistable system. Since transitions to a down-state can still occur in the presence of Cd2+ that blocks HVA calcium channels (Fig. 9(A)), a second potential source to activate KCa channels may be low voltage-activated (LVA) calcium channels, which are less sensitive to Cd2+. In this regard, it was recently shown that Cav3.2 T-type calcium channels form a complex with the intermediate conductance KCa channel (KCa3.1) in Purkinje cells (Engbers et al., 2012). The low voltage for activation of Cav3.2 calcium channels allows even PF EPSPs to activate sufficient calcium influx to generate a KCa3.1-mediated AHP. This complex may then play an important role in regulating bistability and the ability for CF inputs to control the expression of trimodal activity, but this idea has not been tested yet.

A depolarizing afterpotential (DAP) that follows an action potential plays an important role in contributing to the generation of each subsequent spike, providing positive feedback to support a stable up-state of firing (Fernandez et al., 2007; Fernandez, Mehaffey, & Turner, 2005). A study combining recordings in vitro and modeling identified several factors capable of reducing the amplitude of the DAP and thus the bistable region in Purkinje cells (Fernandez et al., 2007), as shown in Fig. 10. One of the mechanisms for generating a DAP is return current flow from dendritic regions (Fernandez et al., 2005; Lemon & Turner, 2000; Mainen & Sejnowski, 1996). The Purkinje cell has one of the most complex dendritic arbors in the CNS, with extensive branching that reduces the ability for sodium spike back-propagation (Kitamura & Hausser, 2011; Llinas & Sugimori, 1980a; McKay & Turner, 2005; Vetter, Roth, & Hausser, 2001). Nevertheless, the dendritic compartment remains sufficiently depolarized compared to the soma during an action potential to allow current flow from dendrites to contribute to the DAP after spike repolarization (Fernandez et al., 2007). As a result, removing the dendritic compartment in a model of Purkinje cells significantly reduces the DAP and the bistable region (Fig. 10). Sodium conductance can also increase the DAP (Fernandez et al., 2007) and interspike depolarization (Zagha, Lang, & Rudy, 2008), such that reducing sodium window current in the model decreases the DAP (Fig. 10). The presence of Ith reduces the DAP and positive feedback following...
The ability to detect Purkinje cell bistability in vivo has been examined in both anesthetized and awake preparations (Armstrong & Rawson, 1979; Cerminara & Rawson, 2004; Kitamura & Hauser, 2011; Loewenstein et al., 2005; Schonewille et al., 2006; Yartsev et al., 2009), but opposing conclusions have been drawn. In studies that examined the activity of Purkinje cells in anesthetized animals (Kitamura & Hauser, 2011; Loewenstein et al., 2005; Schonewille et al., 2006), toggling behavior is observable. A combination of whole-cell and extracellular recordings demonstrated conclusively that long pauses in Purkinje cell output were the result of full transitions to a hyperpolarized potential, rather than increases in inhibition or simply long pauses in output (Fig. 5) (Kitamura & Hauser, 2011; Loewenstein et al., 2005; Schonewille et al., 2006). Anesthetized animals (isofluorane or ketamine/xylazine) with CF input intact also show irregular patterns of state transitions rather than a regular oscillatory pattern, suggesting toggling between firing states (Kitamura & Hauser, 2011; Loewenstein et al., 2005). However, another study showed that anesthesia increases the propensity for observing pauses in spike output (Fig. 6), suggesting that toggling was only prominent in anesthetized animals (Schonewille et al., 2006). While no pauses were observed in awake animals in the study of Schonewille et al. (Fig. 6(A)), short pauses were observed when using ketamine/xylazine anesthetic and much longer pauses when isofluorane was used (Fig. 6(B), (C)). Some of the pauses under anesthetic may well reflect bistable behavior and toggling (i.e. Fig. 6(C)). However, the very long and repetitive pauses in SS firing reported for the case in Fig. 6(D) under isofluorane anesthesia may instead reflect anesthetic influence on the inferior olive that could produce trimodal activity reflecting a relative loss of CF input (Section 2.2). Indeed, oscillatory activity was rapidly blocked when anesthesia was removed (Schonewille et al., 2006) (Fig. 6), a change in activity resembling the near immediate effects of restoring CF input on trimodal activity (Fig. 4). Furthermore, the continual change in SS firing frequency during the tonic firing periods and increased mean frequency suggests a trimodal pattern of output (compare to Fig. 4(E)) rather than toggling. However, ketamine and isofluorane have also been shown to block sodium and potassium channels (Schneebeli et al., 2005; Zhou & Zhao, 2000), potentially affecting the DAP or plateau potentials in Purkinje cells that contribute to bistable behavior (Fernandez et al., 2007). Therefore, while anesthesia does affect the incidence of bistable activity, the cause of this regulation is not yet known and may depend on the behavioral state or direct effect on Purkinje cell ion channels.

4. In vivo studies of Purkinje cell bistability

It is possible that bistability may be present only in some Purkinje cells or a single Purkinje cell could exhibit both tonic and bistable firing patterns. Indeed, early recordings from Purkinje cells in awake cats showed periods that exhibited extended pauses in SS output, after which tonic SS activity was resumed (Armstrong & Rawson, 1979). Likewise, recent recordings in awake cats showed two distinct populations of Purkinje cells (Fig. 7) (Yartsev et al., 2009). Approximately 50% of recorded cells exhibited pauses separated by periods of tonic firing (Fig. 7(A)), while the remainder of the cells had a tonic SS output (Fig. 7(B)). It should be noted that the recordings were performed extracellularly, which precludes the ability to determine whether the pausing output was the result of transitions to a hyperpolarized potential or patterns of afferent inhibitory inputs. However, these results suggest that tonic firing and toggling behavior are not mutually exclusive output patterns.
Fig. 7. Single unit recordings of Purkinje cell activity from awake cats reveal two distinct patterns of firing. (A), An example recording from a pausing Purkinje cell is characterized by long pauses in SS output (breaks in green lines above extracellular recording) and rapid changes in output that are sometimes coincident with a complex spike (red lines above extracellular recording). Voltage traces below the extracellular recordings show CS (red) and SS (green) waveforms. Raster plots (bottom) show single unit activity for an extended time period with pauses indicated (red lines). (B), Representative recordings from a second population of Purkinje cells that continuously fire, displaying no pauses or state transitions.

Source: Modified from Yartsev et al. (2009).

Fig. 8. \( I_h \) modifies the range over which bistability is evoked in Purkinje cells. (A), Representative spike output of a rat Purkinje cell in vitro in response to 2 nA current steps (15 ms) of positive and then negative polarity spaced 500 ms apart reveals toggling between an up- and down-state of firing. Applying 20 \( \mu \)M ZD-7288 to block \( I_h \) does not prevent toggling. (B), Bifurcation diagrams as a function of driving current for a 5-equation model under 3 different HCN conductance densities (gHCN): 0.03 (Control), 0, or 0.3 mS/cm\(^2\). Stable and unstable fixed points are denoted by a gray and black line, respectively. Lower limit of the spike (limit cycle) amplitude is denoted by the thick black line. Note that the magnitude of the bistable range (indicated by dashed lines) is dependent on gHCN. (C), Plot of the bistable range of the model (dashed lines) in (B) plotted as a function of gHCN.

Source: Modified from Fernandez et al. (2007).
Several in vivo studies have also shown that state transitions are associated with CF input (Kitamura & Hauser, 2011; Loewenstein et al., 2005; Yartsev et al., 2009) (Figs. 1(A), 7). Significant correlations were found between CF input and both up- and down-state transitions. While spontaneous CF inputs were examined, state transitions were also associated with sensory-evoked CF input using air puff stimulation of the vibrissa of anesthetized animals (Kitamura & Hauser, 2011; Loewenstein et al., 2005). Activity resembling state transitions has been recorded in awake, decerebrate ferrets during eye-blink conditioning (Jirenhed, Bengtsson, & Hesslow, 2007). However, similar transitions were not observed during optokinetic response or visuovestibular training in awake animals (Schonewille et al., 2006).

Therefore, while anesthesia has been shown to alter Purkinje cell firing in vivo and even increase the number of pauses in firing and apparent toggling following CF input (Bengtsson & Jorneell, 2007; Schonewille et al., 2006), the number of reports of state transitions in vivo continue to grow. However, it is not always clear if extended pauses in firing reflect bistable membrane properties of Purkinje cells or the loss of CF input. There is evidence that bistable behavior can be observed in vivo, although definitive evidence for toggling through bistable membrane properties in awake animals has not yet been reported. Recent studies in awake animals suggest that toggling will be confirmed in vivo, but may vary from cell-to-cell and potentially across different regions of cerebellum.

5. Possible explanations for discrepancies in the literature

There are several possible explanations for the different observations regarding bistability in vivo. It is generally assumed that the properties of Purkinje cells are uniform across the cerebellum and thus equally likely to exhibit the capacity for bistable behavior. However, this is belied by the wide array of activities with which the cerebellum is involved. Rather, it is possible that Purkinje cells in different areas of the cerebellum will exhibit different ion channel complements. Indeed, differences in ligand-gated receptor activation of potassium currents and intrinsic firing patterns in Purkinje cells have been observed across different cerebellar lobules in the in vitro slice preparation (Ishii, Nakajo, Yanagawa, & Kubo, 2010; Kim et al., 2012). Heterogeneity in the size of CF inputs could also account for discrepancies in the ability to elicit toggling in vivo. Of direct importance here are findings that the amplitude of CF input has a nonmonotonic effect on the probability of generating state transitions (Fernandez et al., 2007; McKay & Turner, 2005). As mentioned, current injections simulating a CF input less than 0.25 nA or greater than 5 nA were unable to generate state transitions with high probability in vitro, whereas current amplitudes between 0.5 and 2.0 nA had a high probability of causing state transitions (Fernandez et al., 2007). These results indicate that there is a critical amplitude of CF input required to generate state transitions. Interestingly, in vivo calcium imaging shows that the dendritic calcium influx induced by CF input exhibits considerable variability, and that the probability of observing state transitions is dependent on the amplitude of calcium transients during the CF input (Kitamura & Hauser, 2011). The variability in the size of the CF-evoked calcium transient is controlled by the local inhibitory network (Kitamura & Hauser, 2011), suggesting that background network activity will regulate the occurrence of toggling behavior by its effect on the CF-induced postsynaptic current.

Purkinje cell activity will also be subject to neuromodulatory influence. \( I_H \) has a clear role in regulating the bistable regime in Purkinje cells (Fernandez et al., 2007) (Fig. 8) as well as responses to inhibitory inputs (Fig. 1(D)) and can be modulated by various agents, including serotonin (Fernandez et al., 2007; Oldfield et al., 2010; Williams et al., 2002). Therefore, modulation of \( I_H \) (or other contributing channels) could contribute to heterogeneity of Purkinje cell output patterns. In fact, electrical stimulation of the Raphe complex induces long pauses in Purkinje cell output (Strahle, Strahle, & Barnes, 1979; Weiss & Pellet, 1982), consistent with the ability for serotonin to increase toggling behavior and affect locomotor activity (Mendlin, Martin, Rueter, & Jacobs, 1996).

Dynamic clamp studies in layer III pyramidal cells of the medial entorhinal cortex show that the bistable region of a cell is reduced by an increase in leak conductance (Fernandez & White, 2009), a prediction that also applies to Purkinje cells (Fernandez et al., 2007) (Fig. 10(A)). Therefore, variability in the amount of network synaptic activity could affect the background conductance level and probability of observing bistable behavior. Indeed, different cerebellar regions appear to be correlated with different output patterns in vivo. Studies that reported bistable activity and toggling in Purkinje cells were carried out in the vermal regions (e.g. lobule VI (Yartsev et al., 2009) and cerebellar hemispheres, mainly Crus II (Kitamura & Hauser, 2011; Loewenstein et al., 2005; Yartsev et al., 2009)). However, when recordings were made from the occulo- or vestibulo-cerebellar regions, particularly the flocculus, bistable or pausing output was not observed (Schonewille et al., 2006). These differences could reflect region-specific differences in signal processing requirements. For example, whisker or perfusor stimulation results in short, high frequency bursts of mossy fibers in Crus I and Ila (Chadderton, Margrie, & Hauser, 2004; Rancz et al., 2007), while vestibular manipulation results in continuous, frequency modulated patterns of mossy fiber input to granule cells in the flocculus (Arenz, Bracey, & Margrie, 2009; Arenz, Silver, Schafer, & Margrie, 2008). Toggling of Purkinje cell firing may then be an inherent feature of processing signals in lobules that receive high frequency bursts in mossy fiber input. On the other hand, granule cells in the vestibulocerebellum show high frequency bursting behavior rather than frequency-modulated output, suggesting a non-linear transformation of mossy fiber

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Factors affecting Purkinje cell bistability.</th>
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<tr>
<td>Factor</td>
<td>Effect</td>
</tr>
<tr>
<td>Na(^+) current</td>
<td>Maintenance of the up-state; allows state transitions</td>
</tr>
<tr>
<td>HCN current</td>
<td>Reduces bistable range; increases probably of MLI invoked up-state transition; reduces DAP</td>
</tr>
<tr>
<td>Slow K(^+) current (K(_C) channels)</td>
<td>Regulate state transitions; adjust voltage level of firing and rest states; up-to-down state transitions</td>
</tr>
<tr>
<td>Ca(^{2+}) current</td>
<td>Activation of K(_C) channels; HVA Ca(^{2+}) channels not necessary for state transitions</td>
</tr>
<tr>
<td>DAP</td>
<td>Maintenance of the up-state</td>
</tr>
<tr>
<td>Fast K(^+) current</td>
<td>Increases DAP; necessary for maintenance of up-state</td>
</tr>
<tr>
<td>High membrane resistance</td>
<td>Increases DAP duration and amplitude; necessary for maintenance of up-state</td>
</tr>
<tr>
<td>Presence of dendritic compartment</td>
<td>Generation of DAP and stable up-state</td>
</tr>
</tbody>
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Fig. 9. Bistability in Purkinje cells relies on specific ionic factors. (A), Bistable behavior in Purkinje cell evoked in response to depolarizing and hyperpolarizing current steps is not prevented by a blocker of $I_H$ (ZD 7228 10 μM) or the HVA calcium channel blocker Cd$^{2+}$ (200 μM). Spike responses are greatly reduced in the presence of Cd$^{2+}$, presumably due to inactivation upon block of calcium-dependent potassium currents. (B), Sodium current contributes to the plateau depolarization underlying stable tonic firing during an up-state. Shown are spike plateau potentials evoked in a Purkinje cell model under different maximal sodium conductances. Under control conditions ($g_{Na_{max}} = 40$ mS/cm$^2$) a transient current step (1 μA/cm$^2$, 20 ms) is sufficient to take the system from rest to constant firing (plateau). Small reductions in $g_{Na_{max}}$ reduce the duration of the plateau potential to the point where the system can no longer shift permanently to the firing state. (C), Comparison of the time course of somatic membrane voltage (top trace) and slow potassium current (middle trace) in a Purkinje cell model in response to two CF-like stimuli (4.2 μA/cm$^2$, 15 ms) separated by 500 ms. Spike firing after the transition to a firing state ensures a greater background activation of potassium current when the second CF-like stimulus is applied (arrow). Lowest trace compares the rate of repolarization in a Purkinje cell recording after a current pulse (500 ms) depolarizes the cell to $-50$ mV (gray line) or $-55$ mV (black line) in the presence of 100 nM TTX. A spike independent and faster rate of repolarization is generated from the depolarized state (presumed slow potassium current).

Source: Modified from Fernandez et al. (2007) and Williams et al. (2002).

6. Bistability in circuit function

If bistability does occur in vivo, it remains to be seen what purpose this behavior would play in circuit function. Unlike many areas in the brain, the cerebellar cortex lacks recurrent excitatory networks, which can provide short-term memory capabilities. Single-cell multistability is a mechanism for short-term memory, as the state of the system can be used as a direct read-out of prior input which needs to be taken into consideration (Ruigrok, Hensbroek, & Simpson, 2011). Lastly, species-specific differences may contribute to the variability in Purkinje cell activity. Current studies have recorded from mice, rats, guinea pigs, and cats (Kitamura & Hausser, 2011; Loewenstein et al., 2005; Schonewille et al., 2006; Yartsev et al., 2009). While it is generally assumed that neuronal output is similar between species, differences in preparation should not be ignored.
Fig. 10. The incidence of establishing a saddle homoclinic bifurcation and bistability is modulated by several ionic factors. Shown are results from a 5-equation model in which different ionic factors are varied. (A) Bifurcation diagrams as a function of driving current under the 5 indicated conditions. Stable and unstable fixed points are denoted by a thin gray and thin black line, respectively. Lower limit of the spike (limit cycle) amplitude is denoted by the thick black line. To establish slow refractory dynamics, the time constant function for the somatic refractory variable was doubled. For high leak conductance, the base maximal leak was multiplied by 10. To establish a smaller window current for sodium conductance the steady-state inactivation parameter was shifted by $-6 \text{mV}$. Note that the bistable region is reduced when the dendritic compartment is removed and with slow refractory dynamics (area between dashed lines). The bistable region is eliminated in the presence of high leak conductance and reduced Na$^+$ window current. (B) Superimposed membrane voltage responses of the model under the different conditions indicated in (A) when driven with a brief current pulse (2 ms, 30 $\mu$A/cm$^2$). Note that the size of the DAP is reduced by all 4 experimental conditions.

Source: Modified from Fernandez et al. (2007). 

input (Durstewitz, Seamans, & Sejnowski, 2000). Thus, a bistable output cell could provide the basis for a memory bank. A similar theory is presented in a model of the olivo-cerebellar system as a temporal pattern generator (Jacobson et al., 2008). In this model, synaptic input configures populations of Purkinje cells into a set of up- and down-states, which then converge onto neurons in the deep cerebellar nuclei (DCN). Neurons in the inferior olive are selectively decoupled by GABAergic output from neurons of the DCN. These olivary neurons then provide excitatory output to excitatory neurons of the same nuclei that give input to the various cerebellar targets (Jacobson et al., 2008). This theory has yet to be tested empirically, but raises some interesting possibilities for bistability in the cerebellum.

Traditional models of the cerebellum have used the CF input as an “error signal” that acts to correct Purkinje cell output by causing PF long-term depression. However, this correction takes place over a long period of time, while the ability for CF input to toggle the Purkinje cell between states might allow for instantaneous correction of erroneous PC output (Loewenstein et al., 2005). Because bistability provides two distinct forms of output, rather than information carried through rate modulation, a Purkinje cell that is providing erroneous output can be quickly switched to the opposite state by a CF acting as the traditional “error signal”. Furthermore, the voltage dependence of calcium influx in Purkinje cell dendrites ensures the calcium signal is larger when the cell is in the upstate as opposed to the down-state (Kitamura & Hausser, 2011). Long-term depression of the PF synapse would be more likely to occur in the up-state as it is a calcium-dependent process. This would allow for firing state-dependent synaptic plasticity and binary control over learning via synaptic weight changes.

Despite the interesting possibilities bistability presents for circuit function, it is not without limitations. State transitions occur in a probabilistic fashion and are highly sensitive to noise (unpublished observations). Furthermore, the ability for virtually any synaptic input to generate state transitions (Fig. 1) suggests that state transitions would occur at a high rate and would be difficult to control for the purpose of network processing. The lack of state transitions observed during the optokinetic response or visuovestibular training in awake mice (Schonewille et al., 2006) also suggests that bistability may not play an important role in certain types of cerebellar output.

The firing dynamics of neurons are responsible for the different properties of neuronal spike output. While a neuron’s membrane properties allow for a wide array of output behaviors that can be observed in vitro through experimental manipulation, only a subset of these behaviors may be present in vivo. Nevertheless, those membrane properties are still present in vivo and influence all aspects of a neuron’s output. Therefore, even if Purkinje cells...
do not exhibit state transitions in vivo, the bistable nature of their firing dynamics may provide many of the properties observed in vivo.

7. Predictions for future studies

In this review, we have given an overview of the in vitro mechanisms underlying bistability as well as the differing opinions that exist regarding bistable behavior in vivo. Based on the experimental and computational results reviewed here, we propose several studies that would be useful in elucidating the role of Purkinje cell bistability.

Our model of Purkinje cell firing dynamics (Fernandez et al., 2007) provides several key predictions regarding regulation of bistability. First of all, as discussed, any conditions that affect the DAP will reduce bistability (Fig. 10). One primary determinant of DAP amplitude is background conductance, which could be regulated by the level of synaptic input. Indeed, differences in the amount of background synaptic input could explain why bistability is sometimes observed only in the anesthetized state when synaptic activity is low (Loewenstein et al., 2005; Schonewille et al., 2006). Similarly, slowing of refractory dynamics will affect the DAP. Decreases in potassium conductances or cell-specific differences in the expression of potassium currents would affect the repolarization of action potentials, reducing the DAP and the ability to maintain a stable depolarized state. Studies to examine lobule-specific differences in the DAP, both in vitro and in vivo, could determine whether bistability is regulated either by the network level of synaptic input or by the expression of ion channels. In particular, dynamic clamp provides a straightforward method to test the effects of background synaptic input on bistability, as has been done in other systems (Fernandez & White, 2009). It is interesting to note that previous dynamic clamp studies in Purkinje cells make no mention of bistable behavior when synaptic inputs were added to the cell soma (Jaeger & Bower, 1999).

In a similar manner, \( I_H \) exerts a strong control over the bistable region. The dynamics of \( I_H \) are dependent on the subunit composition of the channel and are subject to modulation by extrinsic and intrinsic sources (Biel, Wahl-Schott, Michalakis, & Zong, 2009). A systematic examination of the expression level and subunit distribution of \( I_H \) in the cerebellum may show that the amount of \( I_H \) is negatively correlated with bistable activity in vivo. Furthermore, modeling studies examining the effects of different HCN channel activation kinetics and voltage dependencies would be important in elucidating the regulation of bistable behavior.

We previously predicted that down-state transitions are dependent on increased activation of potassium current during the complex spike, possibly due to increased calcium influx and activation of a \( K_Ca \) channel. However, the identity of this \( K_Ca \) channel has not been determined. While we have shown that blocking SK or BK channels prevents CF regulation of SS output, the resultant bursting prevents any determination of the role of these channels in bistability (McKay et al., 2007). We recently demonstrated the expression of a novel Cav3-KCa3.1 ion channel complex in Purkinje cells which is activated by synaptic inputs (Engbers et al., 2012). We predict that this complex will play an important role in regulating bistability and the ability for CF inputs to control the expression of trimodal activity.

Lastly, bistable patterns have been implicated as the method by which the cerebellum controls DCN output (Jacobson et al., 2008). This differs strongly from other theories which have prevailed in the field, including simple spike pauses and temporal patterns in Purkinje cell simple spike output (Shin et al., 2007; Steuber et al., 2007). The potential role of bistability in the cerebellar network is difficult to determine experimentally in vitro, as it involves many different cell types, with many disconnected during in vitro slice preparation. However, computational models exist for many of the cells in the cerebellum, including granule cells (D’Angelo et al., 2001; Diwakar, Magistretti, Goldfarb, Naldi, & D’Angelo, 2009; Solinas, Nieuw, & D’Angelo, 2010), Golgi cells (Solinas et al., 2007a, 2007b), Purkinje cells (De Schutter & Bower, 1994a, 1994b; Fernandez et al., 2007), molecular layer interneurons (Anderson et al., 2010), DCN neurons (Engbers et al., 2011; Steuber, Schultheiss, Silver, De Schutter, & Jaeger, 2011) and cells of the inferior olive (Davies, Neil, & Whitaker, 1995). Many of these models are reduced models with few equations, making network simulations a real possibility. A computational study examining the effect of bistable Purkinje cell output on a simple cerebellar network would be instrumental in determining the role of spike patterns in the cerebellar network.

While the bistable properties of Purkinje cells have been demonstrated extensively in vitro, the presentation of toggling behavior in vivo is still an open question. The factors governing the bistable dynamics of Purkinje cells may be modulated by various extrinsic factors, such as network activity and anesthetics, and intrinsic factors, such as ion channel expression. Therefore, bistability may vary between cells, cerebellar regions, and experimental preparations. While the role of bistability in network function is unclear, the potential for bistable Purkinje cell output to play an important physiological role still exists.

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