

Bergmann glia modulate cerebellar Purkinje cell bistability via Ca^{2+} -dependent K^+ uptake

Fushun Wang¹, Qiwu Xu, Weishan Wang, Takahiro Takano, and Maiken Nedergaard¹

Division of Glial Disease and Therapeutics, Center for Translational Neuromedicine, University of Rochester, Rochester, NY 14642

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Recent studies have shown that cerebellar Bergmann glia display coordinated Ca^{2+} transients in live mice. However, the functional significance of Bergmann glial Ca^{2+} signaling remains poorly understood. Using transgenic mice that allow selective stimulation of glial cells, we report here that cytosolic Ca^{2+} regulates uptake of K^+ by Bergmann glia, thus providing a powerful mechanism for control of Purkinje cell-membrane potential. The decline in extracellular K^+ evoked by agonist-induced Ca^{2+} in Bergmann glia transiently increased spike activity of Purkinje cells in cerebellar slices as well as in live anesthetized mice. Thus, Bergmann glia play a previously unappreciated role in controlling the membrane potential and thereby the activity of adjacent Purkinje cells.

two-photon laser scanning microscopy | ion-sensitive microelectrode

Bergmann glial cells in cerebellum are electrically nonexcitable cells that in many ways serve the same functions as protoplasmic astrocytes in forebrain. Bergmann glia are chiefly responsible for glutamate uptake and extracellular K^+ homeostasis (1). Their highly negative resting membrane potential of -80 to -85 mV combined with a large number of inwardly rectifying K^+ channels helps maintain a tight control of extracellular K^+ concentration (2). Bergmann glial cells also display Ca^{2+} transients in response to glutamate or stimulation of climbing and parallel fibers in slices and to motor activity in vivo (3). However, although glial Ca^{2+} signaling has been shown to regulate synaptic transmission in several brain regions, it is presently not established whether Bergmann glia can modulate the activity of Purkinje cells.

Membrane potentials of almost all neurons in mammalian brain fluctuate between a hyperpolarized state (down state) and depolarized state (up state) during sleep/anesthesia and quiet wakefulness (4, 5). These fluctuations of the membrane potentials are synchronized between neighboring cells and detected as slow (~ 0.5 – 1 Hz), large-amplitude delta waves (4). Even though the existence of bistability of Purkinje cells in awake mice has been questioned, it is generally agreed that bistability is a common phenomenon in both anesthetized and sleeping animals (5). The bistability of the resting membrane potential in Purkinje cells was first discovered by Llinás and Sugimori in cerebellar slices (6, 7). The current model predicts that bistability of Purkinje cells, similar to thalamic neurons, relies on the balance between noninactivating inward currents (persistent Na^+ currents and/or T-type Ca^{2+} currents) (4, 8, 9) and an outward K^+ leak current (5–7, 10). However, Purkinje cell bistability is not simply an expression of intrinsic membrane properties because complex spikes generated by climbing fiber activation can toggle the membrane potential between up and down states in vivo (5). Recently, glutamate uncaging on distal dendrites of striate spiny neurons was shown to induce up states that lasted hundreds of milliseconds (11), and small hyperpolarization currents or interneuronal activity can toggle Purkinje cells between up and down states (12). Moreover, stimulating a single astrocyte can trigger up-state synchronizations of neighboring neurons in cortical slices, in a mechanism that depends on purinergic and glutamatergic signaling (13).

Our earlier studies have shown that astrocytes in hippocampus and cortex increase K^+ uptake in response to agonist-induced Ca^{2+} signaling (14). Because the extracellular concentration of K^+ is a critical determinant of Purkinje cell-membrane potential, we here asked whether Bergmann glia can modulate Purkinje cell

bistability. We found that selectively induced Ca^{2+} increases in Bergmann glia also evoked a transient reduction of extracellular K^+ in cerebellar slices and cerebellum in live adult mice. Moreover, Bergmann glial Ca^{2+} signaling consistently triggered a switch to up states of longer duration. All of the effects of Bergmann glial Ca^{2+} signaling on Purkinje cell bistability could be reproduced by simply reducing K^+ in the bath solution or depolarizing a single Bergmann glial cell. Thus, our observations indicate that Bergmann glia can regulate cerebellar network activity by a pathway involving Ca^{2+} -mediated K^+ uptake. Because Purkinje cells fire continuously in up state, Ca^{2+} signaling in Bergmann glia is associated with a sharp increase in output from the cerebellar cortex.

Results

Bergmann Glial Ca^{2+} Transients Changed Purkinje Cell Bistability. To address a possible role of Bergmann glia in regulation of Purkinje cell activity, we combined whole-cell recordings of Purkinje cells with imaging of intracellular Ca^{2+} signaling in Bergmann glia loaded with the Ca^{2+} indicator rhod2/am in cerebellar slices. Whole-cell recordings showed that the membrane potentials of almost all recorded Purkinje cells spontaneously fluctuated with a frequency of 0.5 ± 0.1 Hz between two stable states of 63.4 ± 2.16 mV (down state) and 47.2 ± 3.04 mV (up state), giving rise to a bimodal time distribution ($n = 83$; Fig. S1). Similar oscillations were recorded by extracellular electrodes, demonstrating that the oscillations are not a result of dialysis of intracellular fluid with the patch electrode (Fig. S1). To selectively stimulate Bergmann glial Ca^{2+} transients, cerebellar slices were prepared from mice selectively expressing the MrgA1 receptor under the human GFAP promoter (15). The MrgA1 receptor is a member of the G_q -coupled receptor family with expression restricted to a subset of nociceptive sensory neurons outside the CNS. Thus, the peptide ligand Phe-Met-Arg-Phe amide (FMRF) does not activate endogenous brain receptors (15). As previously reported, bath application of FMRF peptide induced robust Ca^{2+} increases in EGFP⁺ Bergmann glia (Fig. 1A) concomitant with a sharp increase in the duration of Purkinje cell up state (Fig. 1B and C). The FMRF-induced changes in Purkinje cell-membrane potential were transient, lasting 20–120 s. The regular pattern of bistability returned with a delay of ~ 10 – 60 s after normalization of Ca^{2+} in surrounding Bergmann glial cells. Of note, FMRF did not evoke Ca^{2+} increases in Bergmann glia and did not reduce extracellular K^+ in slices prepared from WT mice (Fig. S2). We next assessed the effect of ATP because it has previously been shown that ATP mediates Ca^{2+} increases in Bergmann glia after parallel fiber stimulation. Bath application of ATP also triggered elevations in Bergmann glial Ca^{2+} accompanied by a transient increase in Purkinje cell up state in both MrgA1⁺ mice and WT

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¹To whom correspondence may be addressed. E-mail: fushun_wang@urmc.rochester.edu or nedergaard@urmc.rochester.edu.

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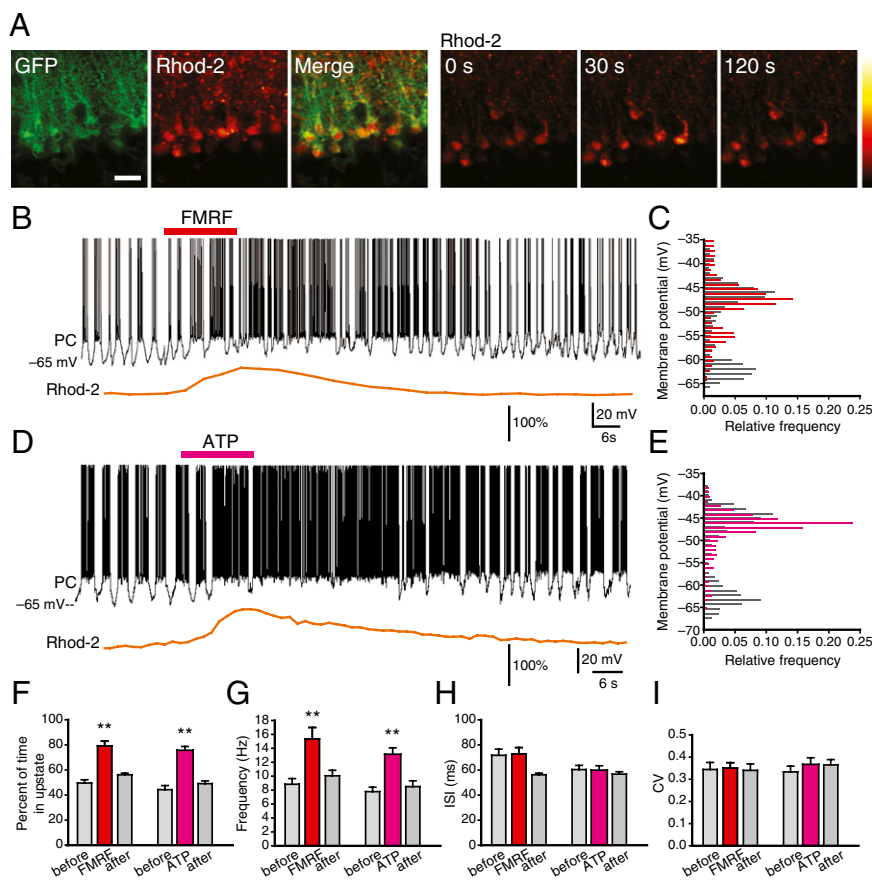


Fig. 1. Agonist-induced Ca^{2+} signaling in Bergmann glia modifies Purkinje cell bistability and increases their spiking activity. (A) Two-photon imaging of freshly prepared vibratome sections from a MrgA1^+ mouse expressing EGFP under the GFAP promoter (green; *Left*) loaded with the Ca^{2+} indicator rhod2/am (red). EGFP and rhod2 signal overlap, indicating that rhod2 primarily is taken up by Bergmann glia. *Right* three images display rhod2 emission changes in response to exposure to the MrgA1 receptor agonist FMRF (15 μM). (B) Whole-cell recording of a Purkinje cell (PC) combined with imaging rhod2 in Bergmann glia in a slice from a MrgA1^+ mouse. The durations of up states increase concurrently with a Ca^{2+} increase in Bergmann glia evoked by FMRF. (C) Histogram depicting the bimodal distribution of Purkinje cell-membrane potential before (gray) and after (red) FMRF exposure (10 s were analyzed, bin = 1 mV). (D) ATP (100 μM) increased Purkinje cell spiking and rhod2 signal in neighboring Bergmann glial cells. (E) Histogram depicting the bimodal distribution of Purkinje cell-membrane potential before (gray) and after (pink) ATP exposure (10 s were analyzed, bin = 1 mV). (F) Percentage of time Purkinje cells spent in up state before, during, and after exposure to FMRF or ATP (** $P < 0.01$, $n = 6-7$). (G) Action potential frequency before, during, and after exposure to FMRF and ATP (** $P < 0.01$, $n = 6-7$). (H) Comparison of ISIs of action potentials before, during, and after exposure to FMRF or ATP. ISI was analyzed during up state only ($P = 0.13$ for FMRF, $P = 0.9$ for ATP, $n = 6-7$). (I) CVs for action potentials before, during, and after application of FMRF and ATP ($P = 0.6$ for FMRF, $P = 0.3$ for ATP, $n = 6-7$).

mice (Fig. 1 D and E and Fig. S2). Of note, Purkinje cells only fired action potentials during up state. As a consequence, Ca^{2+} signaling in Bergmann glia led to a significant increase in Purkinje cell output detected as an increased frequency of spiking (Fig. 1 F and G). Interspike interval (ISI) and coefficient of variance (CV) of action potential firing during up state did not change significantly after application of either FMRF or ATP (Fig. 1 H and I and Fig. S2), suggesting that neither FMRF nor ATP directly modified the properties of the voltage-gated channels generating Purkinje cells action potentials.

Because it cannot be excluded that ATP activates Purkinje cell P2Y_1 receptors, the P2Y_2 and P2Y_4 receptor-specific agonist UTP was also evaluated. UTP-induced Ca^{2+} increases in Bergmann glia were directly comparable to those of ATP and FMRF and were also associated with a transient increase in the duration of Purkinje cell up states (Fig. 2 A and B). To address whether agonist-induced increases in cytosolic Ca^{2+} of Bergmann glia was required for receptor-mediated changes in Purkinje cell output, UTP was next applied to slices prepared from inositol 1,4,5-trisphosphate (IP_3) receptor subtype 2 knockout mice ($\text{IP}_3\text{R2}^{-/-}$). In contrast, Bergmann glia did not exhibit Ca^{2+} transients in response to UTP in slices prepared from $\text{IP}_3\text{R2}^{-/-}$ mice (Fig. 2C), consistent with the report that Bergmann glia only expresses $\text{IP}_3\text{R2}$ and not subtypes 1 or 3 (16). UTP also failed to trigger significant changes in Purkinje cell bistability in slices prepared from $\text{IP}_3\text{R2}^{-/-}$ mice ($P = 0.8$, $n = 6$; Fig. 2 C–E). Similarly, whereas the spiking frequency increased in $\text{IP}_3\text{R2}^{+/+}$ mice ($P < 0.01$), no significant change was observed in $\text{IP}_3\text{R2}^{-/-}$ mice ($P = 0.437$; Fig. 2F). Purkinje cell up state ISI and CV were unaffected by the UTP exposure in both genotypes, suggesting that UTP did not alter the membrane properties of Purkinje cells (Fig. 2 G and H). Similarly, ATP increased bistability in Purkinje cells from WT mice but failed to induced changes in $\text{IP}_3\text{R2}^{-/-}$ mice too (Fig. S2).

Overall, the analysis showed that agonist-induced Ca^{2+} increases in Bergmann glia were linked to a sharp increase in the duration of Purkinje cell up state and thereby in spiking activity. The increase in up state was transient because normalization of Ca^{2+} in Bergmann glia after a delay of 10–60 s was followed by a return to normal periodic oscillations of the membrane potential in Purkinje cells.

Ca^{2+} Signaling in Bergmann Glia Reduced Extracellular K^+ Concentration.

Because buffering extracellular K^+ is a well-known function of astrocytes, and Ca^{2+} signaling in hippocampal astrocytes has been shown to increase their uptake of K^+ (14, 17, 18), we next tested whether agonist-induced Ca^{2+} signaling in Bergmann glia triggered a transient decrease in extracellular K^+ in cerebellar slices. We combined two-photon imaging of Ca^{2+} and whole-cell recording of Bergmann glia with measurements of extracellular K^+ by using ion-sensitive microelectrodes.

Bath application of FMRF in MrgA1^+ mouse cerebellar slices triggered a robust increase in Bergmann glial Ca^{2+} accompanied by a transient reduction of extracellular K^+ and a minor hyperpolarization of Bergmann glial membrane potential (Fig. 3A). Similarly, application of ATP consistently triggered Ca^{2+} increases and hyperpolarization of Bergmann glia concomitant with a significant drop in extracellular K^+ (Fig. 3B), providing strong support for a role of Bergmann glia in active K^+ uptake. To address whether Bergmann glial Ca^{2+} was required for receptor-mediated K^+ uptake, UTP was applied to slices prepared from $\text{IP}_3\text{R2}^{-/-}$ mice and WT littermates ($\text{IP}_3\text{R2}^{+/+}$). UTP-induced Ca^{2+} increases in Bergmann glia were also linked to a transient decrease in extracellular K^+ and hyperpolarization of Bergmann glia in $\text{IP}_3\text{R2}^{+/+}$ mice (Fig. 3C). Consistent with the notion that agonist-induced K^+ uptake in Bergmann glia is Ca^{2+} -dependent, slices prepared from $\text{IP}_3\text{R2}^{-/-}$ mice failed to exhibit UTP-mediated increases in Ca^{2+} and reduction in extracellular K^+ concentration

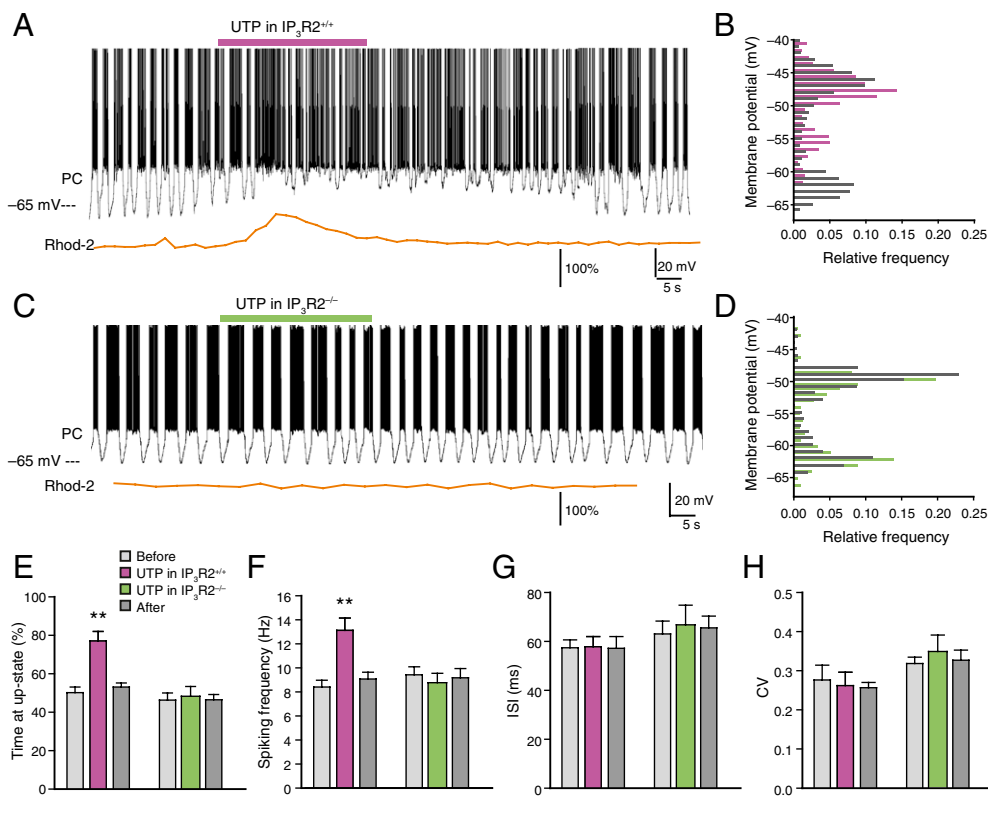


Fig. 2. Ca^{2+} dependency of Bergmann glial modulation of Purkinje cell bistability. (A) The P2Y_2 and P2Y_4 receptor agonist UTP ($100 \mu\text{M}$) induced increases in cytosolic Ca^{2+} in Bergmann glia and an increase in up state of Purkinje cells (PC) in a $\text{IP}_3\text{R2}^{+/+}$ (WT) mouse. (B) Histogram plots the membrane potential distributions before (gray) and after (pink) UTP exposure (10 s , bin = 1 mV). (C) UTP failed to induce increases in Ca^{2+} in an $\text{IP}_3\text{R2}^{-/-}$ mouse and failed to change the regular pattern of bistability in Purkinje cells. (D) Histogram shows the membrane potential distributions before (gray) and after (green) UTP exposure in the $\text{IP}_3\text{R2}^{-/-}$ recording in C (10 s , bin = 1 mV). (E) Time spent in up state before, during, and after exposure to UTP in $\text{IP}_3\text{R2}^{+/+}$ and $\text{IP}_3\text{R2}^{-/-}$ littermates (** $P < 0.01$, $n = 6-7$). (F) The spiking frequency before, during, and after exposure to UTP in $\text{IP}_3\text{R2}^{+/+}$ and $\text{IP}_3\text{R2}^{-/-}$ littermates (** $P < 0.01$, $n = 6-7$). (G) Comparison of ISIs of action potentials during up states before, during, and after exposure to UTP in $\text{IP}_3\text{R2}^{+/+}$ and $\text{IP}_3\text{R2}^{-/-}$ littermates ($P = 0.14$ for $\text{IP}_3\text{R2}^{+/+}$, $P = 0.29$ for $\text{IP}_3\text{R2}^{-/-}$, $n = 6-7$). (H) CVs before, during, and after exposure to UTP in $\text{IP}_3\text{R2}^{+/+}$ and $\text{IP}_3\text{R2}^{-/-}$ littermates ($P = 0.65$ for $\text{IP}_3\text{R2}^{+/+}$, $P = 0.81$ for $\text{IP}_3\text{R2}^{-/-}$, $n = 6-7$).

(Fig. 3D). A comparison of FMRF, ATP, and UTP showed comparable increases in Ca^{2+} , extracellular K^+ , and hyperpolarization of Bergmann glia (Fig. 3E–G). In addition, UTP had no significant effect on extracellular K^+ or Bergmann glial membrane potential in slices prepared from $\text{IP}_3\text{R2}^{-/-}$ mice (Fig. 3F and G). UTP also failed to alter bistability and thereby the output of Purkinje cells in $\text{IP}_3\text{R2}^{-/-}$ mice (Fig. 2). Importantly, the Ca^{2+} -mediated change in extracellular K^+ was positively linked to hyperpolarization of Bergmann glia ($R^2 = 0.486$, $n = 24$; Fig. 3H). Thus, these experiments showed that agonist-induced Ca^{2+} signaling in Bergmann glia is linked to a transient reduction of extracellular K^+ . Of note, the relative large tip of the K^+ -selective microelectrode ($\sim 3 \mu\text{m}$) creates an artificially large extracellular space, and the actual K^+ decreases might be of a larger amplitude than those recorded, as suggested in refs. 19 and 20.

Hyper- and Depolarization of Bergmann Glia Modulate Purkinje Neuron Bistability. The passive membrane permeability of Bergmann glial cells is predominantly attributable to K^+ . Hyper- and depolarization currents are almost exclusively carried by K^+ influx or efflux and are accompanied by changes of extracellular K^+ in a symmetrical manner. Previous analysis shows that clamping the resting membrane potential of Bergmann glial cells offers an alternative approach to assessing the role of glial K^+ buffering on Purkinje cell bistability (2). We repeated the original observations by Hounsgaard and Nicholson (2) and confirmed that injecting a hyperpolarizing current (-100 pA) in Bergmann glial cells caused a drop in the resting membrane potential, averaging $-7.04 \pm 0.69 \text{ mV}$ ($n = 6$), which was paralleled by a decrease in extracellular K^+ of $0.288 \pm 0.017 \text{ mM}$ ($n = 6$; Fig. S3A). Conversely, injecting a depolarizing current ($+100 \text{ pA}$) triggered a symmetric increase in membrane potential, $-7.00 \pm 0.68 \text{ mV}$ ($n = 6$), and an increase in extracellular K^+ , averaging $0.290 \pm 0.021 \text{ mM}$ ($n = 6$; Fig. S3A).

To assess the effect of Bergmann glial cell-membrane potential on Purkinje cell up and down states, we next used dual whole-cell recordings of neighboring Purkinje cells and Bergmann glial

cells. As shown in Fig. S3B and C, the relative time Purkinje cells spent in up state increased from $39.1\% \pm 8.5\%$ to $68.2\% \pm 11.4\%$ ($n = 6$) when an adjacent Bergmann glial cell was hyperpolarized by injecting a negative current (-100 pA). Depolarization of Bergmann glial cells ($+100 \text{ pA}$ current) had the opposite effect (Fig. S3D–F). Concomitantly, the gap in membrane potential between the two states narrowed when Bergmann glial cells were hyperpolarized and widened when Bergmann glial cells were depolarized (Fig. S3G). The ISI and CV of action potentials did not change significantly in response to either hyperpolarization or depolarization of Bergmann glial cells (Fig. S3H).

Extracellular K^+ Directly Modulated Purkinje Cell Bistability. The analysis above indicates that Ca^{2+} signaling in Bergmann glia induced both a decrease in extracellular K^+ and an increase in the duration of Purkinje cell up states. To critically evaluate whether a reduction in extracellular K^+ , in the absence of Bergmann glial Ca^{2+} signaling, is sufficient to trigger an increase in the duration of up states, we next systematically changed extracellular K^+ while continuously recording from Purkinje cells. Whole-cell recording from Purkinje cells showed that, by decreasing bath K^+ from 2.5 mM to 1 mM , the duration of up state increased ($P < 0.01$, $n = 6$; Fig. 4A and B). The increase in duration of up state was linked to a twofold increase in the frequency of simple spikes (Fig. 4C and D) without affecting ISI or CV of action potentials during up state (Fig. 4E and F). The normal pattern of bistability returned with a delay of $10-30 \text{ s}$ after bath K^+ was returned to 2.5 mM (Fig. 4A). Testing a range of physiological K^+ concentrations ($1-5 \text{ mM}$) revealed that up state was prevalent when K^+ concentration was reduced in the bath solution (Fig. 4G). The prevalence of up state with lower K^+ was associated with a narrowed gap between up and down states of Purkinje cell-membrane potentials (Fig. 4H). Of note, K^+ -sensitive microelectrodes inserted $\sim 80-100 \mu\text{m}$ below the surface of the slice showed that changes in extracellular K^+ within cerebellar slices were of much smaller amplitude than the

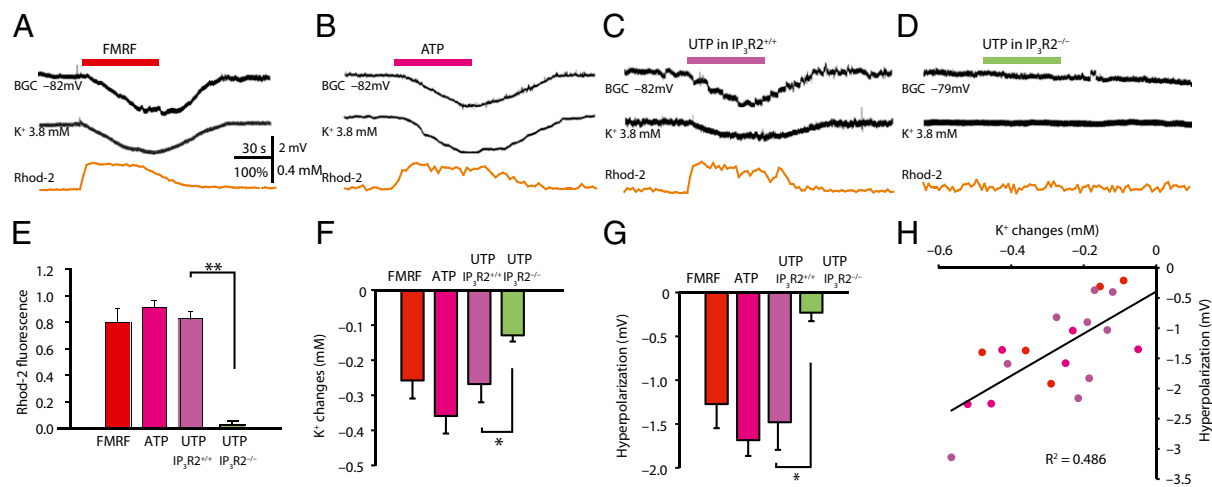


Fig. 3. Ca^{2+} signaling in Bergmann glia is linked to membrane hyperpolarization and a reduction of extracellular K^+ . (A) Combined whole-cell recordings of Bergmann glia with measurement of extracellular K^+ and cytosolic Ca^{2+} in slices from a MrgA1^+ mouse. The MrgA1 agonist FMRF (15 μM) triggered hyperpolarization of the Bergmann glia concomitantly with a decrease in extracellular K^+ and an increase in rhod2 signal. (B) Exposure to ATP (100 μM) also triggered Ca^{2+} signaling and hyperpolarization of Bergmann glia concomitant with a decrease in extracellular K^+ . (C) UTP evoked increases in cytosolic Ca^{2+} and a hyperpolarization of Bergmann glia as well as a decrease in extracellular K^+ in slices prepared from $\text{IP}_3\text{R}2^{+/+}$ mice. (D) UTP had little effect in slices prepared from $\text{IP}_3\text{R}2^{-/-}$ littermates. (E) Histogram comparing increases in rhod2 signal in slices from MrgA1^+ mice exposed to FMRF (15 μM , $n = 19$), slices from WT mice exposed to ATP (100 μM , $n = 16$), and slices from either $\text{IP}_3\text{R}2^{+/+}$ ($n = 13$) or $\text{IP}_3\text{R}2^{-/-}$ ($n = 13$) mice exposed to UTP (100 μM) (** $P < 0.01$, $n = 12$). (F) Changes in extracellular K^+ in slices from MrgA1^+ mice exposed to FMRF ($n = 9$), slices from WT mice exposed to ATP ($n = 9$), and slices from either $\text{IP}_3\text{R}2^{+/+}$ ($n = 9$) or $\text{IP}_3\text{R}2^{-/-}$ ($n = 6$) mice exposed to UTP (* $P < 0.05$, $n = 6-9$). (G) Changes in Bergmann glia membrane potential in slices from MrgA1^+ mice exposed to FMRF, slices from WT mice exposed to ATP, and slices from either $\text{IP}_3\text{R}2^{+/+}$ or $\text{IP}_3\text{R}2^{-/-}$ mice exposed to UTP (* $P < 0.05$, $n = 6-9$). (H) Scatter histogram displaying Bergmann glia membrane potential as a function of changes in extracellular K^+ . Data are combined from the agonists mentioned before: ATP, FMRF, and UTP ($R^2 = 0.486$, $n = 24$).

changes in bath K^+ (Fig. 4H, compare bottom axes) and the depth of the recordings affected how much changes in bath K^+ changed extracellular K^+ (Fig. S4). The most significant effect on Purkinje cell bistability occurred at 3 mM extracellular K^+ , which is within the physiological concentration range (2).

To address the alternative hypothesis that Bergmann glia modulate Purkinje cell bistability via Ca^{2+} -mediated release of gliotransmitters, we analyzed the effect of glutamatergic, GABAergic, and purinergic receptor antagonists on Purkinje cell bistability. Mixtures of glutamate, GABA, and purinergic receptors had no detectable effects on Purkinje cell bistability in rat cerebellar slices ($P > 0.5$; Fig. S5). Moreover, the same combination of receptor antagonists did not significantly reduce FMRF-induced changes in Purkinje cell bistability in slices prepared from MrgA1^+ mice ($P > 0.5$; Fig. S5), suggesting that FMRF-induced Ca^{2+} signaling is not associated with gliotransmitter release. This finding is consistent with a study that found that FMRF-induced Ca^{2+} signaling in hippocampal astrocytes did not result in gliotransmitter release (15).

Agonist-Induced Bergmann Glial Ca^{2+} Signaling in Vivo Triggered a Reduction in Extracellular K^+ and Increased Neural Activity. Bergmann glia exhibit spontaneous Ca^{2+} events in vivo that are expressed as highly localized Ca^{2+} increases within individual processes or radially expanding waves encompassing up to ~ 40 cells (3). The functional role of Ca^{2+} signaling in Bergmann glia has not yet been established, although locomotion is linked to an increased frequency of Ca^{2+} events (3). We here investigated the physiological significance of agonist-induced Ca^{2+} increases in Bergmann glia in vivo, as far as Purkinje cell bistability/slow oscillations are concerned. Acute cranial windows were prepared over the vermis, and the cerebellar cortex bolus was loaded with the Ca^{2+} indicator rhod2/am. Ca^{2+} imaging was combined with recordings of cerebellar electrocortical activity (ECoG). To identify processes of Bergmann glia, MrgA1 receptor mice expressing EGFP under the human GFAP promoter (15) or reporter mice expressing EGFP under the glutamate transporter 1 (GLT1) promoter (GLT1-EGFP) (21) were used. The EGFP⁺

palisading Bergmann glial processes labeled more intensively with rhod2 than the surrounding neuropil (Fig. 5A). Time-lapse imaging documented that FMRF (15 μM) in MrgA1^+ mice or ATP (100 μM) in GLT1-EGFP mice triggered robust increases in Ca^{2+} . The Ca^{2+} increases engaged all EGFP⁺ glial processes (Fig. 5A–C). Concurrent with the increase in Ca^{2+} , extracellular K^+ decreased from 3.98 ± 0.05 mM to 3.56 ± 0.07 mM ($n = 5$) in response to FMRF and from 3.91 ± 0.04 mM to 3.45 ± 0.05 mM for ATP ($n = 7$; Fig. 5B and D). Thus, agonist-induced K^+ in the live intact cerebellum was directly comparable to observations in cerebellar slices (Fig. 3). Recording of cerebellar ECoG revealed that agonist exposure triggered a transient decrease in the power of low-frequency activity (≤ 4 -Hz delta waves), whereas higher frequencies ($> 4-32$ Hz) increased (Fig. 5E, Right) (22). Agonist-induced changes in ECoG were transients as the relative power of slow and high-frequency activity before and after the Ca^{2+} increases did not differ (Fig. 5B and C; $P > 0.01$, paired t test, $n = 7$ for ATP or FMRF). Thus, in vivo observations in adult mice (Fig. 5) replicated all of the major findings in cerebellar slices (Figs. 1–4).

Discussion

It is generally acknowledged that Ca^{2+} signaling in many brain regions can modulate synaptic activity. We here describe that in cerebellum, Ca^{2+} signaling in Bergmann glia transiently increased their K^+ uptake, reduced the extracellular concentration of K^+ , and prolonged the duration of Purkinje cell up states that resulted in an increase in spike activity. By combining measurements of extracellular potassium with two-photon imaging in genetically altered mice, we show that selectively stimulating Ca^{2+} transients in Bergmann glia expressing MrgA1 receptors lowers extracellular K^+ with concomitant increases in Purkinje cell up state (Fig. 1). The process is Ca^{2+} -dependent as the agonist-induced increase in Purkinje cell up state (Fig. 2) or reduction in extracellular K^+ (Fig. 3) was minimal or absent when Bergmann glia cells were unable to mobilize intracellular Ca^{2+} stores ($\text{IP}_3\text{R}2$ deletion). Simply lowering extracellular K^+ (Fig. 4) or depolarizing a single Bergmann glial cell (Fig. S3) replicated all of

tent currents (6, 7), neocortex is extensively interconnected by recurrent excitatory collaterals, and bistability operates through a balance between excitation and inhibition (8). Moreover, cerebellar Purkinje cells are inhibitory neurons that do not express adenosine A1 receptors (1, 23). It is, however, of interest that both studies concluded that glia Ca^{2+} signaling triggers an increase in up states and may reflect that glia use different strategies to modulate neural circuits depending on their location in CNS. Of note, our study excludes that gliotransmitter played a role in the increase in up states during Ca^{2+} signaling in Bergmann glia cells, because a broad range of receptor antagonists applied either individually or as a mixture failed to affect Bergmann glia modulation of bistability (Fig. S5).

Extracellular K^+ Modulates Purkinje Cell Bistability. During sleep and anesthesia, the membrane potentials of Purkinje cells oscillate between up and down states (5), whereas Purkinje cells in awake animals operate primarily in the up state (24). The bistability persists without synaptic input and is also present in cerebellar slices, suggesting that it is driven by intrinsic membrane properties (6, 7). Similar to other neurons, it is thought that Purkinje cell bistability is generated by the balance between inward currents, consisting of T-type Ca^{2+} currents and/or persistent Na^+ currents in combination with K^+ leak currents, and hyperpolarization-activated cation channels (6, 7, 12).

The Nernst equation predicts that reducing extracellular K^+ will result in membrane hyperpolarization by increasing the driving force for K^+ leaking out of the cell. Why then, does a reduction in extracellular K^+ lead to an increase in up states in Purkinje cells in both cerebellar slices and in vivo preparations? A recent study showed that hyperpolarization of Purkinje cells paradoxically increased the duration of up states by activating I_h channels (12). However, it is unlikely that this mechanism is responsible for the observations reported here because Ca^{2+} signaling in Bergmann glia was linked to hyperpolarization of up states and depolarization in down states (Figs. 1 and 2). A more likely explanation is that the hyperpolarization of up states (Fig. 4H) decreased the activity of the Ca^{2+} -dependent K^+ channels, which are necessary for driving the membrane potential back to the down state. Conversely, the depolarization of down states increased T-type Ca^{2+} and Na^+ channel activation and thereby destabilized, and shortened, the duration of down state (10). In all, although a reduction in extracellular K^+ results in membrane hyperpolarization at up states and depolarization at down states, the net effect is a stabilization and prolongation of up state combined with depolarization and destabilization of down state.

Implication of Ca^{2+} -Dependent Glial K^+ Buffering. Our results show that Bergmann glia cells have the ability to transiently boost their K^+ uptake capacity via receptor-mediated increases in cytosolic Ca^{2+} . We have shown in a previous study that the Na^+ , K^+ -ATPase activity is increased during Ca^{2+} signaling in hippocampal glia cells, resulting in a reduction in extracellular K^+ (14). We speculate that a similar mechanism exists in cerebellum and that the transient reduction of extracellular K^+ hyperpolarized Purkinje cells, which paradoxically prolonged up states. An in vivo analysis in anesthetized mice confirmed that agonist-induced Ca^{2+} signaling in Bergmann glia was linked to a transient reduction in extracellular K^+ and an increased duration of time spent in up state (Fig. 5). We speculate that the increased spontaneous Ca^{2+} signaling in nonanesthetized mice may cause a persistent reduction in extracellular K^+ that contributes to the lack of bistability in awake animals (24). If these observations can be generalized to other brain regions, our report provides a framework for dissecting the role of Ca^{2+} -dependent K^+ uptake on synaptic transmission and neural network activity. Neurotransmitters are released diffusely across most of the forebrain during arousal, and neurotransmitters directly induce Ca^{2+} signaling in cortical astrocytes (25). Ca^{2+} -dependent K^+ uptake in glia may therefore support the tonic up state characteristic of the awake state and affect functional networks on a global scale.

Materials and Methods

Full details of all methods are available in *SI Materials and Methods*.

Animals. All slice experiments used cerebellum from 14- to 23-d-old Sprague-Dawley rat pups or C57BL/6 mouse pups (Charles River).

Two-Photon Laser Scanning Microscopy. A custom-built microscope attached to a Tsunami/Millennium laser (10 W; Spectra-Physics) and scan box (FV300; Olympus) using FluoView software was used for two-photon laser scanning microscopy.

K^+ -Sensitive Microelectrodes. Ion-sensitive microelectrodes were fabricated from double-barreled pipette glass (PB150F-6; WPI).

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