A biochemical mechanism for time-encoding memory formation within individual synapses of Purkinje cells

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Abstract

Within the classical eye-blink conditioning, Purkinje cells within the cerebellum are known to suppress their tonic firing rates for a well defined time period in response to the conditioned stimulus after training. The temporal profile of the drop in tonic firing rate, i.e., the onset and the duration, depend upon the time interval between the onsets of the training conditional and unconditional stimulus. Direct stimulation of parallel fibers and climbing fiber by electrodes was found to be sufficient to reproduce the same characteristic drop in the firing rate of the Purkinje cell. In addition, the specific metabotropic glutamate-based receptor type 7 ($mGluR_7$), which resides on the Purkinje cell synapses, was found responsible for the initiation of the response, suggesting an intrinsic mechanism within the Purkinje cell for the temporal learning. In an attempt to look for a mechanism for time-encoding memory formation within individual Purkinje cells, we propose a biochemical mechanism based on recent experimental findings. The proposed model tries to answer key aspects of the "Coding problem" of Neuroscience by focussing on the Purkinje cell's ability to encode time intervals through training. According to the proposed mechanism, the time memory is encoded within the dynamics of a set of proteins — $mGluR_7$, G-protein, G-protein coupled Inward Rectifier Potassium ion channel, Protein Kinase A and Protein Phosphatase 1 — which self-organize themselves into a protein complex. The intrinsic dynamics of these protein complexes can differ and thus can encode different times. Based on their amount and their collective dynamics within individual synapses, the Purkinje cell is able to suppress its own tonic firing rate for a specific time interval. Specifically, the time memory is encoded within the rate constants of the biochemical reactions and altering these rates constants means storing different time memory. The proposed mechanism is verified by a simplified mathematical model and corresponding dynamical simulations of the involved biomolecules, yielding testable experimental predictions.

Author summary

Hebbian plasticity is a widely accepted form of learning that can encode memories in our brain. Spike-timing dependent plasticity resulting in Long-term Potentiation or Depression of synapses has become a general consensus as a primary mechanism behind

the formation of a substrate for memory formation within a neuronal population. However, recent experiments of conditional eyeblink response in Purkinje cells have challenged this point of view by showing that these mechanisms alone cannot account for temporal memory formation in the Purkinje cell. To explain the underlying mechanism behind this novel synaptic plasticity, we introduce a biochemical mechanism based on protein interactions occurring within a single synapse. These protein interactions and the associated rate constants are sufficient to encode time delays by auto-induced inhibition on a single excitatory synapse, suggesting that synapses are capable of storing more information than previously thought.

Introduction

How do we store memories in our brain? How do we retrieve and edit them when required? Recent experimental findings have shed some light onto these fundamental questions. Experiments have shown that memories are held within specific neuronal populations [1–3]. Such populations, referred as *memory engram cells* [4, 5] store memory either by forming or eliminating synapses [6, 7] or by altering synaptic strengths between neurons [8, 9] within the population. These forms of learning and memory encoding fall under the widely accepted Hebbian learning paradigm [10]. However, the individual contribution of each synapse to the engrams, and how changes in synaptic strength affects memories, remain poorly understood. The problem of information encoding was raised by C.R. Gallistel [11] and termed as the "Coding Question", one of the fundamental open questions in Neuroscience today. Recent experiments on Purkinje cells, one of the major neuronal populations in the Cerebellum and essential for motor coordination, have shed some light on the Coding Problem. Those experimental results have illustrated that the memory of time interval duration can be encoded within individual Purkinje synapses, and does not require a whole neuronal population [12, 13]. In addition, the stored time memory can be accessed and changed anytime. This result has also challenged the prevailing doctrine of Hebbian learning by showing that traditional changes of synaptic strength alone cannot explain the Purkinje cell response after learning [14].

Purkinje cells can learn to encode a specific time memory through Classical or Pavlovian conditioning. This kind of Associative learning can occur when a biologically potent stimulus, such as food, is paired with a neutral stimulus, such as a bell, that precedes it. Depending upon the response the potent stimulus elicits, e.g., saliva flow, and the exact protocol followed, Classical Conditioning can be categorized into various kinds. One of them being classical motor conditioning, such as the eye blink conditioning, where a neutral conditional stimulus (CS) in the form of a light or a sound can trigger an eye blink response before the onset of an unconditional stimulus (US) that elicits a blink reflex response [15, 16]. In other words, the CS triggers a response which predicts the time of arrival of the US. The conditioned response appears after successive training sessions, where a CS is followed by an US after a fixed time interval "T", called the interstimulus time interval (ISI) [17]. At the cellular level, the eye blink response is causally related to a suppression of the tonic firing of individual Purkinje cells, which regulate the activity of ocular muscles [17, 18]. Because of such causal connection, the suppression of the firing rate of the Purkinje cell is termed as the conditional response of the Purkinje cell. Previous mechanistic explanations considered Long-term Depression (LTD) of selective parallel fibers-Purkinje cell (pf-PC) synapses as the main mechanism behind the conditional response in the Purkinje cell [19]. The Marr-Albus model suggests that the time memory of the response is encoded within the network dynamics of Granule cell neurons and inhibitory interneurons, found within the molecular layer of the Cerebellum between Mossy fibers and Purkinje cells. However,

recent experiments on ferrets were able to pinpoint the source of the conditional response by showing that the direct stimulation of parallel fibers and climbing fibers using electrodes was sufficient for Purkinje cells to learn the specific time interval duration [12]. These experiments also showed that a glutamate-based metabotropic receptor type 7 (mGluR7) which resides on Purkinje cells synapses, initiates the conditional response [13] by opening G-protein coupled Inward Rectifier Potassium (GIRK) ion channels [20]. As Purkinje cells suppress their tonic firing for a fixed duration, GIRK ion channels open, implying that there exists a specific biochemical mechanism within the Purkinje cell that can encode and store temporal information.

Unlike other memory formation mechanisms requiring neuronal assemblies, temporal signatures can be encoded within a single Purkinje cell, but the specific mechanism remains poorly understood. Here, we propose a biochemical description, based on past experimental findings, that is able to explain temporal memory formation, consolidation and access.

Biochemical description

Below we discuss the conditional response of the Purkinje cell and the associated biochemistry in detail in three subsections: during training, after training and Retraining.

During training

Activation of conditional response was found to be initiated by activation of $mGluR_7$ receptors [13]. Purkinje cells express $mGluR_7$ receptors on their entire cell body and dendritic branches [21], yet no conditional response was observed before training [12]. The fact that in the presence of CS, Purkinje cells show similar behavior before training and after training in the presence of $mGluR_7$ antagonist such as 6-(4-Methoxyphenyl)-5-methyl- 3-(4-pyridinyl)-isoxazolo[4,5-c]pyridin-4(5H)-one hydrochloride (MMPIP) or LY341495 implies that the learning of conditional response is associated with the expression of $mGluR_7$ receptors on the synapse. In other words, during training $mGluR_7$ receptors are being transported from perisynaptic zone to postsynaptic zone of the synapse via some biochemical mechanism which activates during training. Once placed on the synapse, $mGluR_7$ receptors in presence of glutamate from parallel fibers activate $G_{i/o}$ type G-proteins whose $G_{\beta\gamma}$ subunits activate GIRK ion channels [20, 22]. However, there can be two other possibilities which can prevent conditional response before training- 1) during training, instead of $mGluR_7$ receptor, GIRK ion channels are being transported to the synapse or 2) during training, the expression of $G_{i/o}$ type G-protein might increase on the synapse. However, we can rule out these alternatives. Immunohistochemistry analysis found the presence of GIRK subtypes GIRK2/3 ion channels on PC synapses which are innervated by parallel fibers and these results did not involve any kind of conditional training in prior [23]. The other possibility of changing G-protein expression before and after training would affect both conditional response profile as well as various other physiological properties of the Purkinje cell as different types of G-protein play essential roles in various signal transductions and physiological properties of the cell [24]. Since no change in the tonic firing rate has been observed before and after conditional training [12], we believe that other physiological properties of the cell may remain unaltered as well after conditional training. Thus, translocation of $mGluR_7$ receptors to the synapse is most likely the result of the training and we assume that the amount of other proteins- GIRK ion channels and G-protein are constant for all different conditional training.

To train a Purkinje cell for a specific duration "T" requires two stimuli separated by "T", also called Interstimulus Interval (ISI). The first stimulus must come from the parallel fibers and the second stimulus from the climbing fiber [17]. Generally, translocation of mGluR type receptors to and from the synapse happens by Clathrin-mediated Endocytosis (CME) [25]. Also, G-protein coupled receptor kinases (GRKs) and in some cases, protein kinases such as Protein kinase C (PKC) can phosphorylate receptors and initiate their endocytosis and translocation via CME [25]. Therefore, we propose that the PKC initiates trafficking of $mGluR_7$ receptor via CME. This is supported by the fact that the presence of two stimuli, one from the parallel fiber and the other one from the climbing fiber, also make PKC activation most favorable [31]. In particular, the presence of either one of the two stimuli is not enough for the Purkinje cell to learn conditional response [17]. While dendritic spines of Purkinje cell which parallel fibers are innervating express $mGluR_1$ receptors [26] and those receptors can potentially activate PKC [27], evidently PKC activation is not the downstream effect of $mGluR_1$ receptor activation [28, 29]. Similarly, climbing fiber stimulus alone cannot activate PKC. While Long-Term Depression (LTD) of parallel fiber-Purkinje cell synapses occurs via PKC activation [30], it only happens when both parallel fibers and climbing fiber are active [31]. Hence, PKC can become active during training and help in translocation of $mGluR_7$ receptors to the synapse. However, PKC alone is not responsible for the translocation of $mGluR_7$ receptors as Purkinje cells cannot be trained for ISI durations shorter than 100ms [32] but PKC can become active even when both CS and US occur at the same time. Currently, we cannot make any suggestion for proteins, which might be involved in addition to PKC during conditional learning.

To ensure storage of time memory of interest, such translocation process must stop after some time. This can happen by inhibiting PKC activation which can be achieved by either removal of $mGluR_1$ receptors from the synapse or by preventing rise in intracellular Ca^{+2} ion concentration within the synapse. The second option is most suitable one because (i) translocated $mGluR_7$ receptors at the synapse can open GIRK ion channels, which will drop the membrane potential and thus inhibit opening of Voltage-gated Ca^{+2} ion channels. Activation of GIRK ion channels causing a drop in tonic firing rate during training has been observed in the experiment [33]. (ii) If $mGluR_1$ receptors were to be removed from the synapse, then the retraining process with different ISI would not happen as there would be none or very few $mGluR_1$ receptors left on the synapse to produce Diacylglycerol (DAG), a necessary membrane-bound biomolecule for PKC activation [27]. As Purkinje cells can be retrained [34], the amount of $mGluR_1$ receptors cannot change on the synapse. Therefore, we conclude that as training progresses the intracellular Ca^{+2} ion concentration decreases to a level that is no longer sufficient to activate PKC, which prevents further translocation of $mGluR_7$ receptors to the synapse and a steady state is reached. When this steady state has been reached, then we can say that the Purkinje cell has learned the conditional response of duration "T" as shown in fig.(1). This mechanism of translocation also suggests that the training period will be longer for long duration conditional response as observed in the experiments [12]. As the net amount of the receptor translocated during training depends upon the net duration of training, long duration training means more transportation of receptors to the synapse, which produces a long duration of the conditional response. We will explain how a higher amount of receptors can produce a long duration conditional response below.

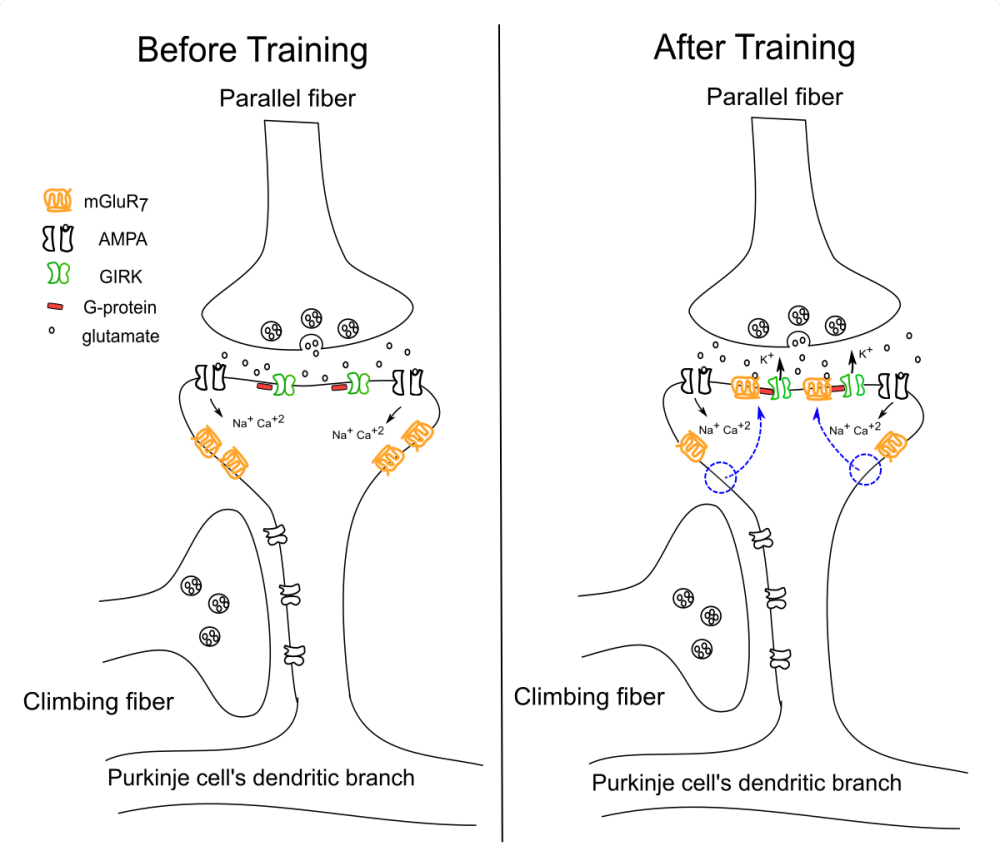

Fig 1. $mGluR_7$ receptor distribution before and after conditional training in the Purkinje cell. Before training, $mGluR_7$ receptors are localised at perisynaptic areas of the synapses. After training, as pointed out by the blue arrows, these receptors localised themselves at the postsynaptic areas of the synapse via CME.

After training

Conditional response of hundreds of milliseconds duration can be initiated by just 20 milliseconds duration of CS [12]. This means that just activation of $mGluR_7$ receptors by CS is enough to initiate the conditional response. In addition, the dynamics of the G-protein activation by $mGluR_7$ receptor and the binding of G-protein subunits to the GIRK ion channels are usually too slow to explain the fast dynamics of the conditional response observed in experiments [12]. Such fast activation of GIRK ion channels correlates with some past studies — it was proposed and later verified experimentally that the $mGluR_7$ receptor form a protein complex with the G-protein of $G_{i/o}$ type, which is in close association with the GIRK ion channel with the help of Regulator of G-protein signaling protein 8 (RGS8) [35, 36]. RGS8 protein is expressed in dendritic spines of the Purkinje cell [37] and has a special property to accelerate both activation and deactivation of G-protein causing fast opening and closing of GIRK ion channels [36]. It has two domains- RGS8's core domain is responsible for faster deactivation of the G-protein and an additional N-terminal domain helps in faster G-protein activation; possibly by enhancing the coupling between G-protein and the receptor [38]. Thus, fast dynamics of the conditional response is possible by forming a protein complex of $mGluR_7$ receptor, G-protein and GIRK ion channel facilitated by RGS8 protein.

There are two additional important properties of the conditional response which we

must consider- 1) repetitive CS causes loss of conditional response [12] and 2) it is independent of CS duration. These two properties are in fact related to each other. Loss of conditional response implies removal of $mGluR_7$ receptors from the synapse and independence of CS duration implies that some protein blocks receptor's active site to prevent reactivation of conditional response. Dephosphorylation of $mGluR_7$ receptors by Protein Phosphatase 1 (PP1) causing their rapid internalization [39] can explain both properties of the conditional response. Rapid internalization of any receptor is initiated by binding of Arrestin protein which prevents receptor to trigger any signaling further [27]. As CS activates conditional response implies that PP1 is inactive before conditional response. In addition, the conditional response is mediated via activation of $G_{i/o}$ type G-protein whose G_α subunit blocks the production of Cyclic adenosine monophosphate (cAMP) by Acetyl Cyclase (AC) and thus results in reduced Protein Kinase A (PKA) activity. Also, due to the tonic firing of the Purkinje cell, Calmodulin, which regulate intracellular Ca^{+2} ions concentration [40], can stimulate Acetyl cyclase (AC) [41] to produce cAMP molecules and increase PKA activity. It was also found that PKA can phosphorylate $mGluR_7$ receptors [42] as well as PP1 regulatory protein such as Dopamine- and cAMP-Regulated neuronal Phosphoprotein (DARPP-32) or Inhibitor-1 (I-1) [43] to prevent dephosphorylation of receptors. Thus, phosphorylation of receptors and inhibition of PP1 activity by PKA helps in the retention of the memory for a long time. As PKA is essential for the conditional response, this protein could bind to receptor via special PKA anchoring proteins called A-kinase anchoring proteins (AKAP). Similarly, PP1 can also bind close to the receptor via another scaffold protein such as Spinophilin [44] which is expressed in dendritic spines of neurons across various regions of the brain including Cerebellum [45]. Such a close association of various proteins together lead to the formation of a protein complex which is self-sufficient in its own regulation and dynamics.

In short, the whole conditional response can be described as follow- Before CS, the PP1 protein is inactive because of PKA activity. The release of glutamate during parallel fiber stimulation activates $mGluR_7$ receptors on the Purkinje cells synapse [step 1 of Fig. (2)], which in turn activate G-proteins bound to them [step 2 of Fig. (2)]. Each unit of G-protein splits into a G_α subunit and a $G_{\beta\gamma}$ subunit. One unit of G_α subunit binds to an AC enzyme to block the production of cAMP molecules to deactivate PKA via hydrolysis of cAMP molecules by Phosphodiesterase enzyme (PDE) [27] [step 3 of Fig. (2)], while the $G_{\beta\gamma}$ subunit binds to GIRK ion channel, which will become fully active upon binding of four $G_\beta\gamma$ subunits [22]. As PKA activity decreases, PP1 activity rises due to dephosphorylation of DARPP-32 or I-1 by Protein Phosphatases such as PP2A [43, 46] [step 4 of Fig. (2)] which causes dephosphorylation of $mGluR_7$ receptors [step 5 of Fig. (2)] and initiates their rapid internalization. However, rapid internalization of a receptor is a much slower process compared to the conditional response as it involves many protein interactions and hence the receptor does not remove from the synapse immediately after dephosphorylation. But after dephosphorylation, $mGluR_7$ receptor decouples quickly from the G-protein and its active site is blocked by Arrestin protein to prevent the receptor to activate G-protein further [47]. After receptor dephosphorylation, the active G-protein is deactivated by the RGS8 protein [step 6 of Fig. (2)]. As G-protein activity reduces, GIRK ion channels also close down. In the absence of active G-protein, PKA activity rises again [step 7 of Fig. (2)] due to the activity of AC enzyme in the presence of Calmodulin. Active PKA deactivates PP1 [step 8 of Fig. (2)] by phosphorylating DARPP-32 or I-1 and finally, PKA also phosphorylates $mGluR_7$ receptors [step 9 of Fig. (2)] to prevent their internalization and prepare the Purkinje cell for another conditional response. It is likely that reactivation of PKA takes some time, which might explain why CS cannot initiate another conditional response while CS is still on.

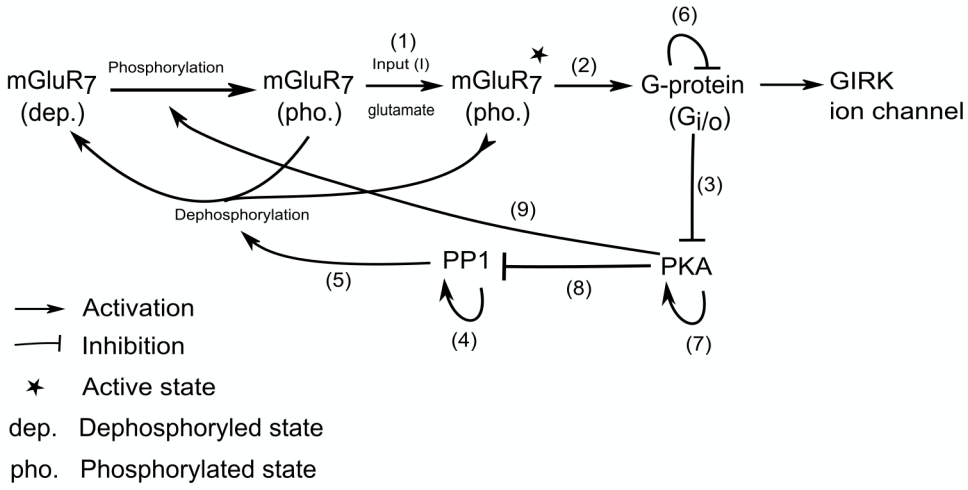

Fig 2. Interactions between different biochemicals involved in our proposed mechanism. The numbers on the top of the arrows highlight the order in which different reactions occur during the conditional response. Conditional response initiates with the release of glutamate from parallel fibers denoted by I as input in (1), which activates $mGluR_7$ receptors. In (2), active receptors activate G-proteins, which deactivate PKA through (3). As PKA activity reduces, PP1 activity rises through (4) causing dephosphorylation of the receptor (5). As receptor activity reduces, RGS8 reduces G-protein activity (6), which allows PKA activity to rise again (7). Active PKA will deactivate PP1 (8) and lastly phosphorylate dephosphorylated receptors to prevent their rapid internalization (9).

In fig.(2), the rate at which GIRK ion channels open or close depends upon the rate at which intermediate reactions occur. In other words, the time memory of the training is stored within the rate constants of reactions. In a complete cycle of GIRK ion channel activation and deactivation, altering only a few rate constants for both activation and deactivation process is sufficient to store different time memory of the conditional response. We will use this concept in our mathematical model to model different conditional responses as we will discuss in the next section.

Training with different ISI duration

Training with different ISI duration means storage of different time memory. As we have mentioned above that the time memory is encoded within the rate constants of the biochemical reactions which regulate gating dynamics of GIRK ion channels and altering these rates constants means storing different time memory. However, there are two additional questions we need to answer in order to get a complete understanding of time memory storage in biochemical reactions- 1) How do these biochemical reactions get tuned so finely to store specific time duration? 2) Among all possible rate constants of the proposed biochemical mechanism, which rate constants are most likely to get affected by choosing different ISI for the training.

The cause behind fine-tuning of conditional response is that there are several GIRK ion channels present at the synapse. Each GIRK ion channel requires four units of $G_{\beta\gamma}$ subunits to open completely [22]. This means that each GIRK ion channel forms a protein complex with four units of G-protein, receptor and RGS8 protein along with PKA and PP1 proteins with their anchoring proteins together result in the formation a protein complex. As each of these protein complexes has their own intrinsic dynamics which regulate how fast GIRK ion channel opens and closes upon stimulation, we can

call each of these protein complexes as "Time Encoding protein Complexes" (TEC). Within each TEC, the rate of G-protein activation by receptor and the rate of binding of G-protein subunits to the GIRK ion channel decide the overall rate of opening of GIRK ion channels i.e., the onset of conditional response while after onset of conditional response, rate of PKA deactivation, rate of PP1 activation, rate of dephosphorylation of the receptor and the deactivation of G-protein by RGS8 decide the overall duration of conditional response as after this duration GIRK ion channels begin to close. Thus, each TEC encodes complete time information of the conditional response in terms of rate constants between different biochemicals and stores this memory by forming a protein complex. Formation of a protein complex as TEC ensures strong consolidation of memory with least chances of error in the information storage. If the rates were to be changed so does the memory as well. The rates can be affected by translocation of extra $mGluR_7$ receptors to the synapse during conditional training. These extra $mGluR_7$ receptors can form clusters with receptors which are part of TEC with the help of scaffold protein- Protein Interacting with C Kinase - 1 (PICK1) [48]. Such cluster formation can affect TEC intrinsic dynamics properties by influencing protein interactions of $mGluR_7$ receptor with the G-protein facilitated by RGS8. As a result, RGS8's ability to accelerate the dynamics of the conditional response might be affected, which results in a delayed onset of the conditional response. Such clustering of receptors can also affect the dynamics of PKA proteins anchored close to the receptor via AKAP proteins, thus affecting the time duration of the conditional response. In overall, we proposed that the interaction of extra $mGluR_7$ receptors with TECs can affect the dynamics of TECs in discrete amount and collectively these TECs units help to produce the conditional response of specific duration in the Purkinje cell.

Retraining can happen via two ways, (i) erase the memory first and then store another memory by retraining with different ISI interval, or (ii) retrain the Purkinje cell with different ISI without erasing the initial memory. Experimentally, loss of memory is accompanied by repeating unpaired representation of CS and US several times. Our proposed mechanism can explain this phenomenon as well. Due to the action of PP1 on the receptor, every time a CS initiates the conditional response, receptors at the synapse might undergo into a phase from which phosphorylation of the receptor by PKA cannot bring it back from rapid internalization process and hence the receptor will be removed from the synapse. Because of rapid internalization, retraining with same or different ISI will be faster as many receptors are close to the synapse. This rapid relearning phenomenon is called "Saving" and takes only a few minutes to recall old memory [17]. If retraining with different ISI is performed without erasing old memory then the conditional response profile differ in terms of the onset of the conditional response while the duration remains intact when compared with the case where retraining does involve erasing of memory in prior retraining [17, 49]. This difference implies that there might be some membrane-bound proteins or scaffold protein such as PICK1 might be interacting with TECs. If the two ISIs duration differs significantly then surprisingly, Purkinje cell pauses twice in the presence of continuous CS of sufficient long duration [12]. Depending upon the time difference between two different ISIs, different conditional responses were observed implying that there might be additional interactions among TECs which give rise to a wide variety of conditional responses [50].

Mathematical model

To model the conditional response behavior in the Purkinje cell, we start with an established dynamical model of the Purkinje cell which incorporates many properties of the cell within a realistic biophysical framework [51], see Materials and Methods for details. Before training, GIRK ion channels cannot open because $mGluR_7$ receptors are

not present at the synapse. However, after training $mGluR_7$ receptors are present at the synapse to open GIRK ion channels. Therefore, adding an additional term for the gating of the GIRK ion channel in the Purkinje cell model will allow it to show conditional response. As GIRK ion channels reside at synapses, an additional term of GIRK ion channel gating must be added in the dendritic equation of the Purkinje cell model. Eq.(1) defines the gating of the GIRK ion channels, in which g_{GIRK} is the net conductance of GIRK ion channels per unit area, h_{GIRK} is the gating parameter and V_{GIRK} is the voltage dependence of the GIRK ion channel obtained from the I-V characterstics curve [52]

$$\begin{aligned} I_{GIRK} &= -g_{GIRK} h_{GIRK} V_{GIRK}(V_d) \\ V_{GIRK}(V_d) &= -0.02(1.3V_d + 50.0)/(1.0 + exp((V_d + 40)/10.0)) \end{aligned} \tag{1}$$

Gating of the GIRK ion channel depends upon the availability of the Phosphatidylinositol 4,5-bisphosphate (PIP_2) molecules [22]. This molecule has low affinity for the GIRK ion channel but binds efficiently after binding of $G_{\beta\gamma}$ subunits to the GIRK ion channel. The amount of PIP_2 on the synaptic membrane is low but its quantity is often replenished by various biochemical processes to maintain its concentration fairly constant upon consumption or degradation [53]. Therefore, the amount of active $G_{\beta\gamma}$ subunits can determine gating of GIRK ion channels. As G-protein is closely associated with the GIRK ion channel, we can assume fast binding of $G_{\beta\gamma}$ subunit to the GIRK ion channel. With these assumptions, we can equate normalized G-protein activity with the GIRK ion channel gating parameter h_{GIRK} as shown in eq.(6) below.

G-protein activity depends upon the activity of $mGluR_7$ along with other proteins as shown in fig.(2) which self-orgainze to form discrete units of TECs. As we don't know the amount of TEC and different time durations it can encode, we choose to model collective dynamics of TECs and different biochemical interactions within it. As a result, instead of using discrete variables for different biochemical's activity, we can use continuous variables as an average dynamics of different biochemicals by considering all TECs together. Even within each TEC, we don't know how strongly different biochemicals are interacting with each other. In addition, AKAP proteins which anchor PKA close to cAMP production machineries does accelerate its activation, but not fast enough as required for the conditional response [54, 55]. It is possible that other proteins such as Homer Proteins might be involved in the TEC, facilitating cross-talk between target proteins [56]. Thus, due to lack of knowledge of various protein interactions and their strengths within TEC, we only attempt to create a working model whose aim is to reproduce features of conditional response - 1) conditional response should be independent of CS duration and 2) changing the dynamics of PKA and G-protein should be sufficient to produce conditional response of different durations. Our working model consist of four main biochemicals - $mGluR_7$, G-protein, PKA and PP1. In order to simplify and minimise number of parameters to fit, we used nonlinear terms to model their overall behaviour as observed *in vivo*.

The nondimensional dynamical equations for the proposed biochemical mechanism within individual TECs are as follows:

$$\tau_1 \frac{du}{dt} = \frac{1}{\alpha + x} u(u_0 - u) - \beta u, \tag{2}$$

$$\tau_2 \frac{dv}{dt} = (v - v_2)(v_1 - v)(v - v_0) - \gamma w v + I, \tag{3}$$

$$\tau_3 \frac{dw}{dt} = -\delta u w + \eta(w_0 - w) + w(w_0 - w), \tag{4}$$

$$\tau_4 \frac{dx}{dt} = (v - x) \tag{5}$$

$$h_{GIRK} = v/v_0 \tag{6}$$

where u, v, w and x are the activities of PKA, mGluR_7 receptor, PP1 and G-protein respectively. In the above model, all the parameters and variables are positive and dimensionless quantities except for τ_i for $i = 1, 2, 3, 4$, which have dimension of time.

Eqs.$(2 - 5)$ match the pictorial diagram shown in Fig. 3 which depicts various variables and their dependencies. In order to understand various terms within each equation, let us focus on each equation individually. Before CS, when G-protein is still inactive, i.e., $x \sim 0$, AC enzyme produces cAMP molecules facilitated by the Calmodulin protein. As the activity of AC enzyme increases, cAMP production also increases, which increases PKA activity. This behaviour of PKA activity is modeled in eq.(2) by the first term $u(u_0 - u)/\alpha$ for $x = 0$, where u_0 is the maximum PKA activity possible. Active PKA phosphorylates phosphodiesterase enzyme (PDE) which hydrolyses cAMP molecules to Adenosine monophosphate (AMP) [57]. Activity of PDE depends upon the activity of PKA as it can be dephosphorylated by Protein Phosphatases such as PP2A. Thus, PDE activity depends upon PKA activity to reduce net PKA activity. This behaviour of PDE is modeled by the second term $-\beta u$ in eq.(2), where β parameter signifies the strength of the PDE action on PKA activation. Upon parallel fiber stimulation, glutamate activates mGluR_7 receptor which activates G-protein to produce G_α subunit to blocks cAMP molecule production. This behaviour is modeled by the prefactor $1/(\alpha + x)$ of $u(u_0 - u)$ in eq.(2), where x denotes G-protein activity. The prefactor corresponds to blocking of AC enzyme which is obtained from the Hill's equation with Hill's coefficient equal to 1 as only one unit of G_α protein binds to AC. For more details on Hill's equation, see Materials and Methods. The constant α denotes the disassociation constant K_d of AC and G_α subunit and has a small value due to their strong bonding. τ_1 signify overall time scale of the PKA dynamics. As, according to proposed mechanism, different conditional response is the result of change in the dynamics of PKA activation and deactivation, the value of τ_1 will increase or decrease for conditional response of longer or shorter durations.

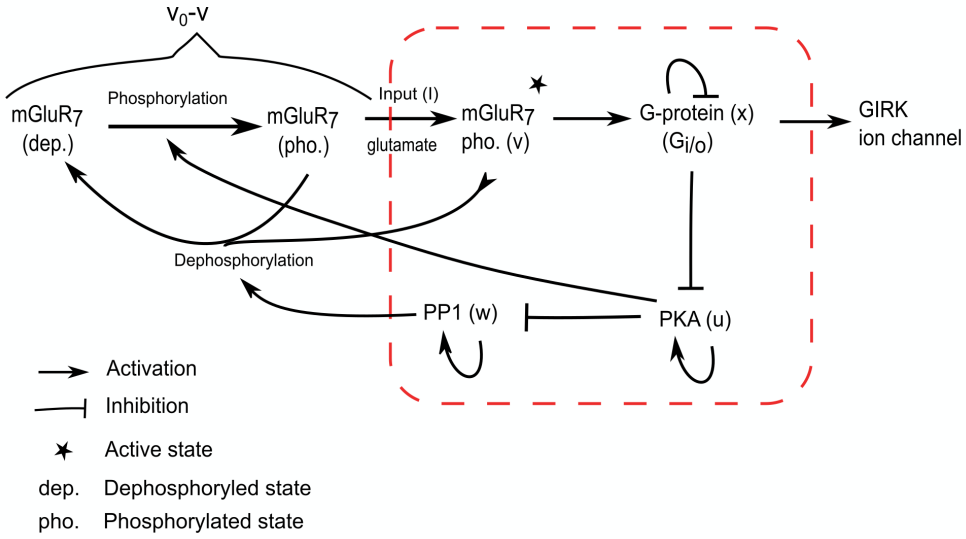

Fig 3. A conceptual minimal model of conditional response in the Purkinje cell.

Eq.(3) models the activity of the $mGluR_7$ receptor. The first term is a cubic polynomial, which captures the switching property of $mGluR_7$ receptors corresponding to the unaltered conditional response with changing the stimulus durations. In the cubic polynomial $v_0 \gg v_1 \gtrsim v_2$, where v_0 signifies the amount of receptors which are associated with the G-protein, v_1 is the threshold activity that needs to be crossed to initiate the conditional response and v_2 is the net finite intrinsic activity of receptors [58]. As each $mGluR_7$ receptor can form a complex with only unit of G-protein, value of v_0 remains constant as we assumed that the amount of G-protein is constant for all different conditional response trainings. While values of v_1 and v_2 depend upon intrinsic properties of the receptor itself which we also assumed to be constant. After deactivation of PKA by G-protein activity, activity of PP1 rises, which dephosphorylate the receptors resulting in the blockade of receptor's active site to activate G-proteins further. This is modeled as $-\gamma wv$ in eq.(3), which denotes the lowering of net receptor activity due to dephosphorylation by PP1. It is a product because PP1 interacts with the $mGluR_7$ receptor during dephosphorylation. The factor γ denotes the strength of the influence of PP1 on $mGluR_7$ receptors. As Spinophilin binds PP1 close to the $mGluR_7$ receptor by binding to RGS8 and not the receptor, the strength of influence of PP1 on $mGluR_7$, i.e., the value of γ, can be assumed constant for different conditional responses. Finally, I denotes the strength of the signal in the form of glutamate release from parallel fibers to activate $mGluR_7$ receptors and τ_2 signifies the overall time scale of receptors activation and deactivation. The value of τ_2 is assumed to be smaller than fastest onset of conditional response observed in the experiment [12]. Eq.(4) models the activity of the PP1 protein. Its activity is regulated by PKA as a suppressor by phosphorylating DARPP-32 or I-1 protein which is modeled as $-\delta uw$. It is a product because PKA interacts directly with the PP1 regulatory protein, which binds to PP1 to block its activity. The factor δ signifies strength of PKA influence on PP1 activity. As the activity of PKA decreases, PP1 activity rises by the action of other Phosphatase proteins such as PP2A/B [43] and by itself [59]. The rise due to other Phosphatase proteins is given by $\eta(w_0 - w)$, while the rise of PP1 by itself is given by $w(w_0 - w)$, where w_0 is the maximum activity of PP1 and the factor η controls the strength of the influence of other Phosphatase proteins on the rise of PP1

activity. τ_3 signifies the overall time scale of PP1 activation and deactivation. For simplicity, we assume all the variables in this equation to be constant.

Eq.(5) models G-protein activity. As we assumed the amount of G-protein on the synapse is constant, net G-protein activity will be same for all different conditional training. As a result, G-protein activity will be limited to 'v_0' which is the total amount of G-protein present at the synapse and modeled as $(v - x)$. τ_4 signifies the time scale of activation and deactivation of the G-protein. As different training involves different amount of $mGluR_7$ receptors, net dynamics of G-protein activation and deactivation by RGS8 is affected by extra $mGluR_7$ receptors interactions with TECs. Therefore, depending upon training, the value of τ_4 can be small or big will result in short or long delayed onset of the conditional response respectively. When v reduces due to PP1 activity, $(v - x) < 0$ which signifies the deactivation of G-protein due to the action of RGS8 protein.

In eqs. (3) and (4), the terms $-\gamma wv$ and $-\delta uw$ signify the interaction of PP1 with $mGluR_7$ and PKA with PP1, respectively. Yet, there are no corresponding terms in eq.(2) of PKA and eq.(4) of PP1 because those interactions are enzymetic in nature and have very short time scales compared to the response, which we are trying to model. Hence, the activity of PKA and PP1 does not change when they interact with other proteins. Note that the τ_i factors on the LHS of each equation make them nondimensional.

Results

Properties of the model

Since experimental results have shown that the conditional response is independent of stimulus durations, the activation of the G-protein must also satisfy this property as it regulates GIRK ion channels. This behavior is indeed captured by our mathematical model. It also successfully captured the dynamics of other biochemicals - PKA, $mGluR_7$, PP1 and G-protein as proposed in the mechanism which is shown in Fig. (4). As per our proposed mechanism, before CS, PKA activity is high while activity of $mGluR_7$ receptor, G-protein and PP1 is low. Upon CS, $mGluR_7$ receptors become active, which in turn activate G-protein. Due to activation of G-protein, PKA activity drops down, which causes a rise in the PP1 activity. When PP1 activity is high enough, it causes deactivation of $mGluR_7$ receptors which then causes deactivation of the G-protein by RGS8. When the stimulus is turned off, activities of various proteins return back to their original states as shown in Fig. (4) top panel. However, if the stimulus remains on for a long time, then even very weak G-protein activity can prevent rise of PKA activity high enough to block PP1. As a result, PP1 activity will be significant to cause dephosphorylation of $mGluR_7$ receptors and blocks their active sites to prevent initiation of another conditional response as shown in Fig. (4) middle panel. Very faint G-protein activity in case of long duration stimulus can be observed in Fig. (4) bottom panel, is enough to prevent reactivation of conditional response in the presence of stimulus.

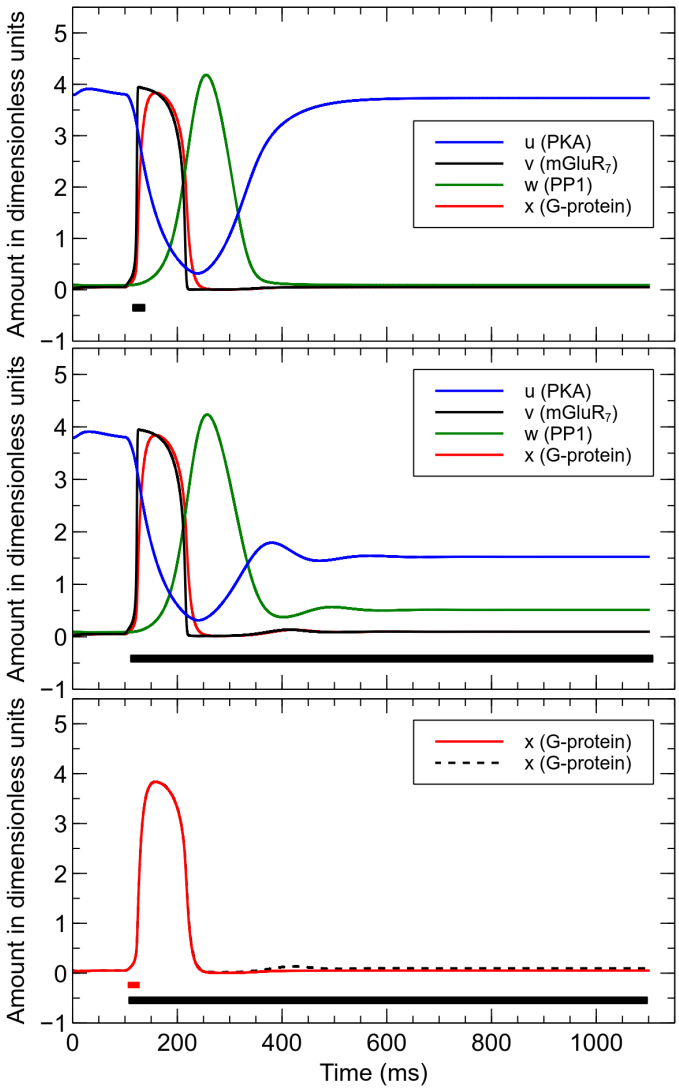

Fig 4. Temporal behaviour of PKA, mGluR$_7$, PP1 and G-protein. Time varying dimensionless quantities of PKA, mGluR$_7$, PP1 and G-protein upon short (top panel) and long (middle panel) stimulus durations represented by black horizontal bar at the bottom of each figure. At the bottom panel, activity of G-protein is shown as above two panels but with two different stimulus durations together, indicated by the red and black bars at the bottom. Both responses are almost identical implying that the G-protein activity is indeed independent of stimulus duration. Parameters for our dynamical model of PKA, mGluR$_7$, G-protein and PP1 are: $\alpha = 0.1, u_0 = 11.0$, $\beta = 48, v_0 = 4.0, v_1 = 1.01, v_2 = 1.0, \gamma = 2.0, \delta = 5.0, \eta = 0.2, w_0 = 6.0, I = 0.1, \tau_1 = 2100ms, \tau_2 = 6ms, \tau_3 = 60ms, \tau_4 = 7.9ms$.

Specifically, the chosen parameter values will determine the specific extreme values of the various variables and their temporal profiles. While these values vary with the chosen parameters, the overall properties of model will not be affected as long as two features are preserved: i) The G-protein activation remains largely independent of the stimulus duration, and ii) no oscillatory response emerges. These two features are essential in order to reproduce experimental results. We have verified that these features are preserved over a large range of parameter values for our model. Specifically,

changes in size of up to 100% of the values we use in the different figures will render above mentioned two features unchanged. Thus, our conceptual model can robustly capture the main experimental results.

By combining the dynamical equations of $mGluR_7$, G-protein, PKA and PP1 with the Purkinje cell model, we can generate the conditional response dynamics of the Purkinje cells as shown in Figs. (5,6). In Fig.(5) we have shown that the suppression of firing rates during conditional response of ISI = 150ms is independent of stimulus durations as observed in the experiments [12]. Result shown in Fig.(5) can be considered an average response of the firing rate during the conditional response given the deterministic nature of our model.

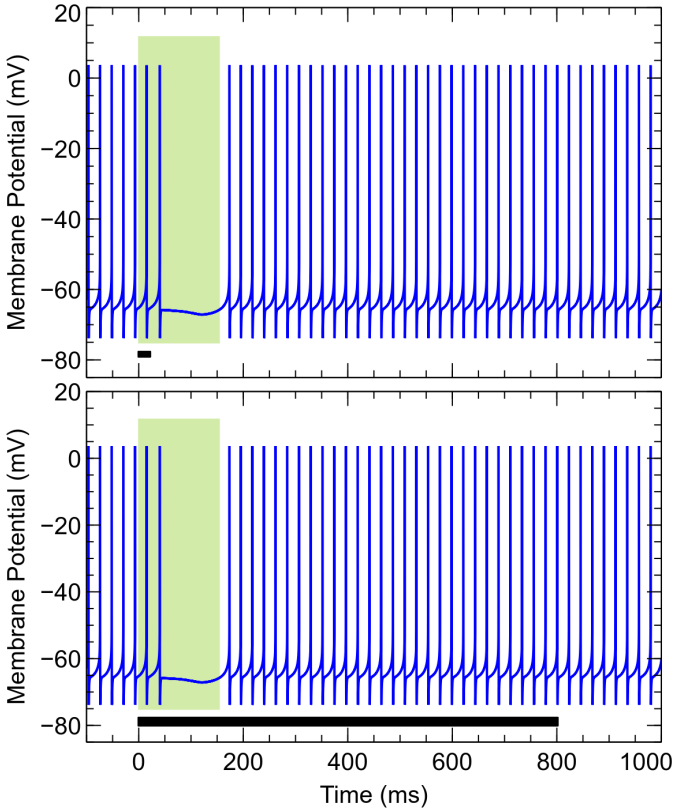

Fig 5. Conditional response from the model is independent of stimulus duration. The width of the light green vertical bar corresponds to the duration of the ISI = 150 msecs and the black bar at the bottom signifies the conditional stimulus duration. Parameters for the dynamical model of PKA, $mGluR_7$, G-protein and PP1 are the same as in Fig. 4. For all other parameters of the Purkinje cell model, see Materials and Methods.

In order to obtain a longer duration conditional response, more $mGluR_7$ receptors need to be inserted into the synapse. These extra receptors cause a rise in the value of τ_1 and τ_4 as discussed earlier. Different τ_1 and τ_4 values, which we have used for reproducing different conditional response, are summarized in Table 1. Fig.(6) shows different long duration conditional responses, which matche with the experimental results [12]. Fig.(6) shows the drop in firing rate for ISI = 200ms, while Fig.(6) shows the drop in firing rate for ISI = 400ms as an additional case. Indeed, the three conditional response firing patterns obtained from our model shown in the left panel of Fig. (7) match with the experimental results [13]. In addition, our proposed mechanism

also explains why the time-memory remains unaffected in the presence of $mGluR_7$ antagonist 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)isoxazolo[4,5-c]pyridin-4(5H)-one hydrochloride (MMPIP) as observed in experiments [13]. Specifically, because of the presence of MMPIP, fewer $mGluR_7$ receptors are left to activate GIRK ion channels, which leads to a smaller drop in firing rate. However, reducing the net amount of active $mGluR_7$ does not inhibit internal interactions between receptor and other proteins involved in our proposed mechanism. Hence, the time-memory, which is encoded within rate constants of biochemical reactions, is unaffected by MMPIP as shown in Fig. (7).

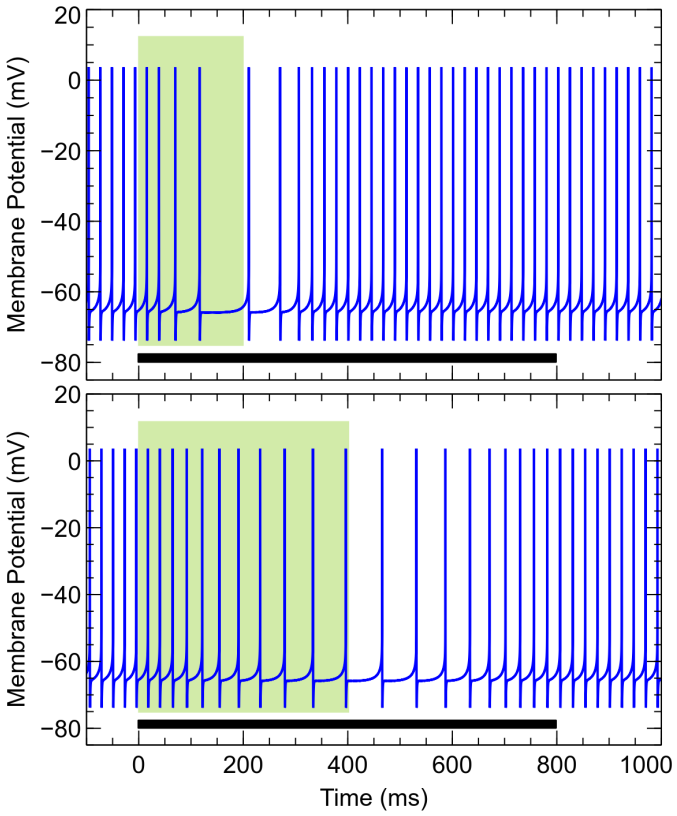

Fig 6. Different conditional responses of the Purkinje cell obtained from the mathematical model. For ISI = 200ms (top panel), the firing rate drops and then rises slowly, which is consistent with the experimental results. The values of τ_1 and τ_4 are 7.2s and 33.3ms, respectively. For higher ISI = 400ms (bottom panel), the drop and rise of the firing rate is observed to be even slower compared to ISI = 200ms at the top panel. The values of τ_1 and τ_4 for ISI = 400ms are 18.0s and 120.0ms, respectively. The width of the light green vertical bar corresponds to the duration of the ISI interval. The black horizontal bar at the bottom represents the conditional stimulus duration.

Table 1. Model parameters for different conditional responses of the Purkinje cell.

ISI (ms)	τ_1 (s)	τ_4 (ms)
150.0	2.1	7.9
200.0	7.2	33.3
300.0	15.0	79.0
400.0	18.0	120.0

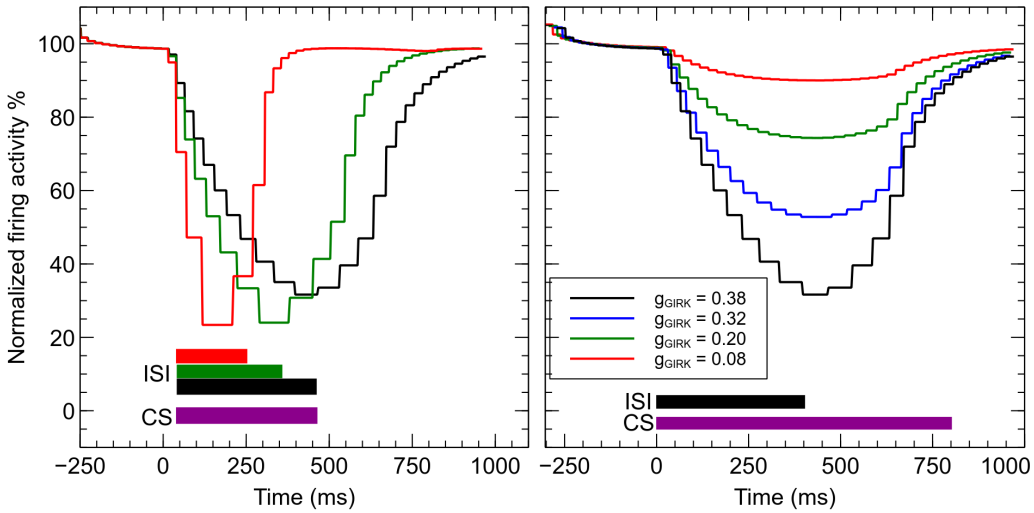

Fig 7. Conditional response profiles for different ISIs and different amounts of MMPIP. Conditional response profiles obtained from the model for different values of τ_1 and τ_4 (see Table 1, all other parameters as in Fig. 5) (left panel), and in the presence of $mGluR_7$ receptor's antagonist MMPIP (right panel). The latter leads to a decrease in the net amount of active $mGluR_7$ and, hence, the amount of active GIRK ion channels, which corresponds to smaller values of g_{GIRK} (see Eq. 1). Here, $\tau_1 = 18.0$s, $\tau_4 = 120.0$ms and all other parameters as in Fig. 5. Note that the normalized firing activity is calculated here by taking the inverse of the time interval between two successive spikes and dividing it by the firing frequency before the onset of the conditional response.

Changing values of both τ_1 and τ_4 simultaneously is one possibility to model different conditional responses within the framework of our model. We would like to point out that changing either one of the two alone does not reproduce the experimental behavior. Based on existing studies, we have no clear evidence for changes in the value of any other model parameter given our proposed mechanism. Therefore as a first approximation, we have assumed them to not change at all.

Model predictions

Based on our proposed model, we can make two predictions that can be tested easily in experiments. 1) If PP1 is knocked out then active $mGluR_7$ receptors will never deactivate after they become active from the conditional stimulus and hence G-protein will remain active. This implies that the Purkinje cell will not fire again after receiving the conditional stimulus as shown in Fig. (8). 2) On the other hand, as PKA regulates PP1 activity, knocking out PKA activation will activate PP1, which will dephosphorylate $mGluR_7$ receptors and hence G-protein cannot be activated. This

implies that the Purkinje cell will not exhibit a conditional response as shown in Fig. (8).

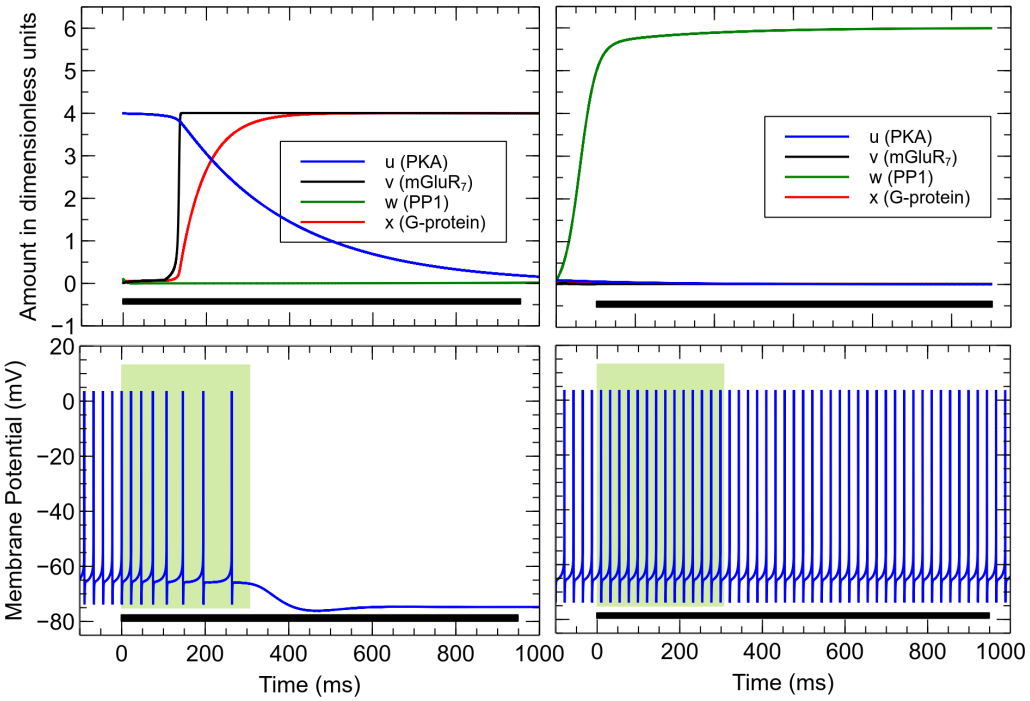

Fig 8. Model predictions for knockout experiments. In our mathematical model, PP1 can be knocked out by setting $w_0 = 0.1$ (top left panel), which prevents the Purkinje cell to fire again after the initiation of the conditional response (bottom left panel). PKA can be knocked out by setting $u_0 = 0.1$ in our model (top right panel), which prevents the Purkinje cell to initiate a conditional response (bottom right panel).

However, in reality biological cells are very robust and have redundancy mechanisms to overcome such behaviours. As a result, there might be still a weak conditional response observed after knocking out PKA or a slow deactivation of G-protein after knocking out PP1, but in both cases significant effects on the conditional response should be observable.

Specific experimental options to test proposed model

There are various experimental options to check whether our proposed mechanism for the conditional response is valid or not.

1. As PKA is an essential biochemical for the resensitization of the receptor and maintaining low PP1 activity, reducing PKA activity in the cell will prevent the Purkinje cell from suppressing its firing rate as PP1 will desensitize the receptor and therefore GIRK ion channels will not be activated. This can be verified by using cAMPS-Rp or triethylammonium salt which will block the cAMP production and hence PKA.

2. As PP1 desensitizes the receptor during conditional response, blocking of PP1, using Okadaic acid, must affect the deactivation rate of GIRK ion channels during the conditional response.

3. If $mGluR_1$ receptors are activating PKC then blocking of $mGluR_1$ receptors using Cyclothiazide during training will not initiate trafficking of $mGluR_7$ receptors and thus no conditional response should be observed even after extensive training.

4. Use of RGS8 knockout specimen should allow only long duration conditional response: Without RGS8 protein, the activation and deactivation of G-protein will be much slower and will produce only long conditional response durations. In addition, only sufficient long CS will be able to initiate the conditional response as $mGluR_7$ receptors will take longer time to activate G-protein in the absence of RGS8 protein which help in the formation of protein complex.

Discussion

In this article, we introduced a potential biochemical mechanism to explain time-encoding memory formation within a single synapse of a Purkinje cell. This time-encoding memory is stored in an excitatory synapse, but it is associated with an inhibitory response, i.e., the suppression of the Purkinje cell's tonic firing rate in the presence of an excitatory stimulus, namely a glutamate discharge from the parallel fiber. During conditional training, Purkinje cells imprint the time information by expressing an appropriate amount of $mGluR_7$ receptors on the synapse, while encoding time information in the form of rate constants. The memory is stored by forming a protein complex, i.e., a Time-Encoding protein Complex (TEC). Alterations of rate constants within TECs will change its temporal signature, while the removal of receptors from the synapse will cause memory loss. However, during retraining, the previous memory can quickly be reacquired and become accessible again. Our idea of TEC is similar to the "Timer Proteins" previously proposed by Ref. [12], but in contrast, it does not require an active selection of feedforward protein activations to produce a specific conditional response. Recently, a different biochemical mechanism was proposed for time memory learning, which uses Ca^{+2} ion dynamics for storing different time information [60]. That model does not incorporate the documented role of GIRK ion channels and it also predicts faster learning for long duration conditional responses, which is not compatible with previous experimental findings [12].

As previously mentioned, in our model the time information of the conditional response is stored in the TECs found on individual synapses, implying that the substrate or the Engram of a time memory can reside at individual synapses, not in a cell or a cell assembly. This result is in line with the synaptogenic point of view of memory substrates [10], where single synapses play a large role in memory formation. In contrast, another point of view puts more emphasis on the intrinsic plasticity of a whole neuronal cell compared to the synaptic plasticity of individual synapses [61]. Intrinsic plasticity considers changes in the electrophysiological properties of the cell by changing the expression of Voltage-dependent Ca/K ion channels and many other kinds of ion channels, which are expressed by neurons and which decide neural firing rate as well as the sensitivity of the cell upon stimulation. However, neither points of view can fully account for the development of the conditional response in the Purkinje cell, since it neither involves the formation or elimination of pf-PC synapses [12, 13], nor LTD of pf-PC synapses [14] nor any change in the electrophysiological properties of the cell [12]. Thus, Purkinje cells show a novel form of synaptic plasticity and provide an example of monosynaptic memory encoding. In addition, considering this fact and that each Purkinje cell makes many different synapses with different parallel fiber bundles, the storage capacity of a Purkinjce cell might be much higher than previously thought and the Purkinje cell might be considered as a multi-information storage device. Specifically, one might be able to encode a specific time interval by stimulating only a subset of

parallel fibers and encode another time interval by stimulating a separate subset of fibers. In this case, specific time information out of the whole set can be selectively retrieved when specific parallel fiber bundles become active upon stimulation, producing the conditional response for the previously encoded time interval. While this might be a very difficult task using electrodes, it is conceivable that other protocols for conditional training [33] are more promising. This remains an exciting challenge for the future.

Materials and Methods

Purkinje cell model

To model the conditional response behavior of the Purkinje cell, we start with an established dynamical model of the Purkinje cell [51] as summarized by eqs.(7) to (11). Specifically, it aims to model the dynamics of the Purkinje cell by incorporating many properties of the Purkinje cell within a realistic biophysical framework. In contrast to the original formulation [51], eqs.(7) to (11) already incorporate the features specific to our situation: In eq.(7), the input current term I_i, which originally signified an external electrical stimulus, now signifies the intrinsic current causing the tonic firing of the Purkinje cell [62, 63]. Moreover, we added the influence of the GIRK ion channel in eq.(8), which only becomes relevant after training — see also eq.(1). Here, g_{GIRK} is the net conductance of GIRK ion channels per unit area, h_{GIRK} is the gating parameter and V_{GIRK} is the voltage dependence of the GIRK ion channel obtained from the I-V characterstics curve of the ion channel [52].

Somatic voltage equation:

$$\begin{aligned} C_s\frac{dV_s}{dt} = \frac{(V_d - V_s)}{R} - g_{Na}m_\infty h(V_s - E_{Na}) - g_K(1-h)(V_s - E_K) \\ - g_{leak}(V_s - E_{leak}) - g_{I_H}ih(V_s - E_{ih}) + I_i \end{aligned} \tag{7}$$

Dendritic voltage equation:

$$\begin{aligned} C_d\frac{dV_d}{dt} = \frac{(V_s - V_d)}{R} - g_{leak}(V_d - E_{leak}) - g_{Kd(slow)}n_d(V_d - E_K) \\ - g_{GIRK}h_{GIRK}V_{GIRK}(V_d) \\ V_{GIRK}(V_d) = -0.02(1.3V_d + 50.0)/(1.0 + exp((V_d + 40)/10.0)) \end{aligned} \tag{8}$$

Na^+ activation equation:

$$\begin{aligned} m_\infty &= \frac{1}{1 + exp[-(V - V_{1/2})/k]}, \quad V_{1/2} = -40.0mV, \quad k = 3.0mV \\ \frac{dh}{dt} &= \frac{h_\infty - h}{\tau_h} = \frac{1}{1 + exp[-(V - V_{1/2})/k]}, \quad V_{1/2} = -40.0mV, \quad k = -3.0mV \\ \tau_h(V) &= \frac{295.4}{4(V + 50)^2 + 400} + 0.012 \end{aligned} \tag{9}$$

Hyperpolarizing activated cation current (I_h):

$$\begin{aligned} \frac{dI_h}{dt} = \frac{I_{h_\infty} - I_h}{\tau_{I_h}} = \frac{1}{1 + exp[-(V - V_{1/2})/k]}, \quad V_{1/2} = -80.0mV, \quad k = -3.0mV, \\ \tau_{I_h} = 100ms \end{aligned} \tag{10}$$

Slow K^+ activation equation:

$$\begin{aligned} \frac{dn_d}{dt} = \frac{n_{d\infty} - n_d}{\tau_{nd}} = \frac{1}{1 + exp[-(V - V_{1/2})/k]} \quad V_{1/2} = -35.0mV, \quad k = 3.0mV \\ \tau_{ih} = 15ms \end{aligned} \tag{11}$$

Parameters value of the Purkinje cell model

$$C_s = 1.5\mu F/cm^2, C_d = 1.5\mu F/cm^2, R = 0.75, g_{Na} = 40mS/cm^2,$$
$$g_{Ks} = 8.75mS/cm^2, g_{I_H} = 0.03mS/cm^2, g_{Kd(slow)} = 12mS/cm^2$$
$$g_{leak} = 0.032mS/cm^2, E_{Na^+} = 45mV, E_{K^+} = -95mV, E_{I_H} = -20.0,$$
$$E_{leak} = -77mV, g_{GIRK} = 0.38mS/cm^2, I_i = 0.198\mu A$$

Hill's equation

Hill's equation gives the fraction of protein saturated by a ligand at a given concentration of ligand in a solution [64]. Since, in the case of Acetyl cyclase (AC), only one unit of G_α subunit binds to it, Hill's coefficient will be 1. The fraction of AC bound by a G_α subunit to the total available amount of AC at a concentration $[G_\alpha]$ is given by

$$\frac{[Blocked\ AC]}{[AC]} = \frac{[G_\alpha]}{K_d + [G_\alpha]}, \tag{12}$$

where, K_d is the disassociation constant of AC and G_α subunit. However, we are interested in free AC. So, the fraction of free AC will be given by

$$\frac{[Active\ AC]}{[AC]} = 1 - \frac{[Blocked\ AC]}{[AC]} = \frac{K_d}{K_d + [G_\alpha]}. \tag{13}$$

Since the concentration of $[G\alpha]$ is proportional to its activity, eq.(13) can be written as

$$\frac{[Active\ AC]}{[AC]} = \frac{K_d}{K_d + x}, \tag{14}$$

where x denotes the G-protein activity.

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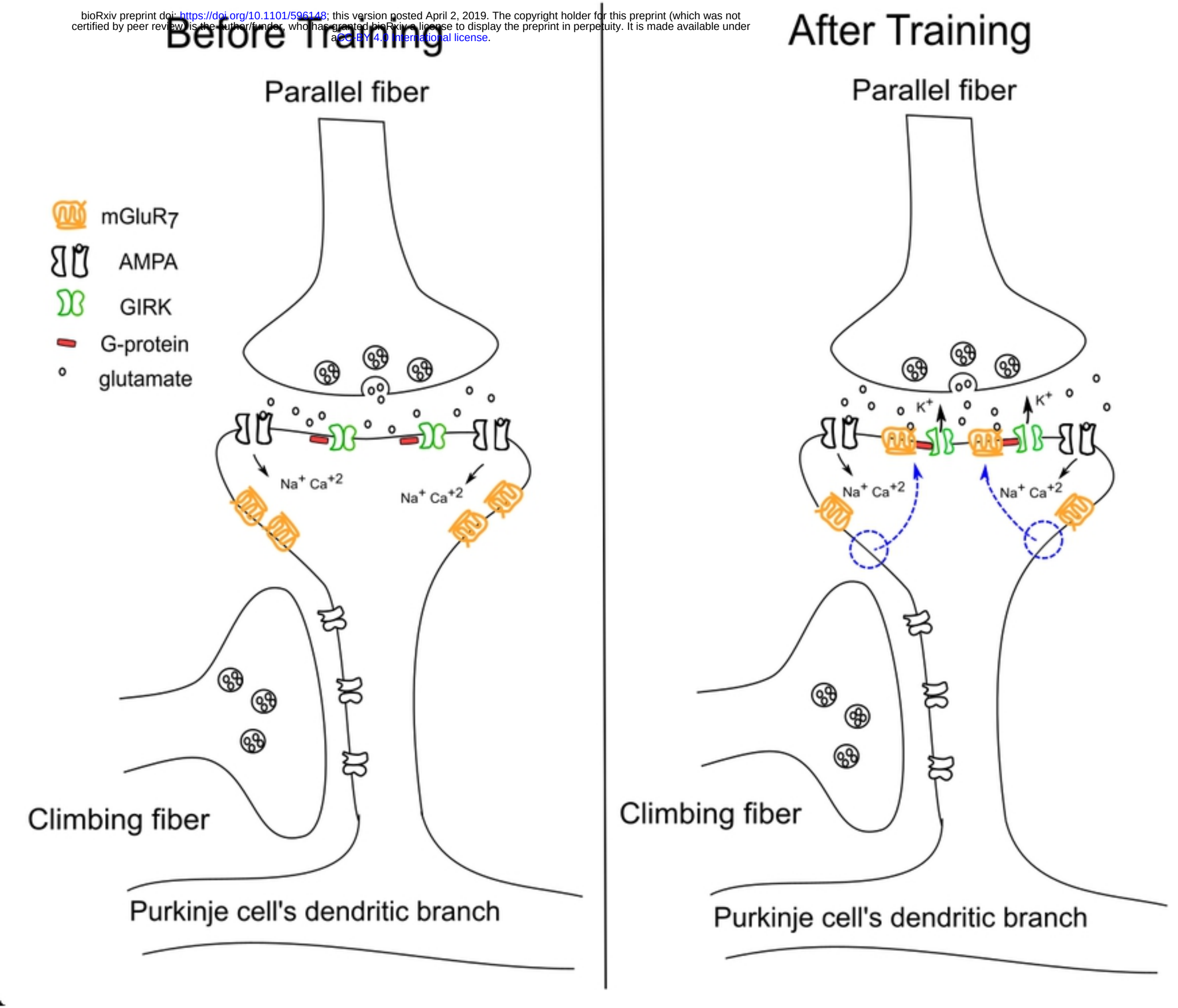

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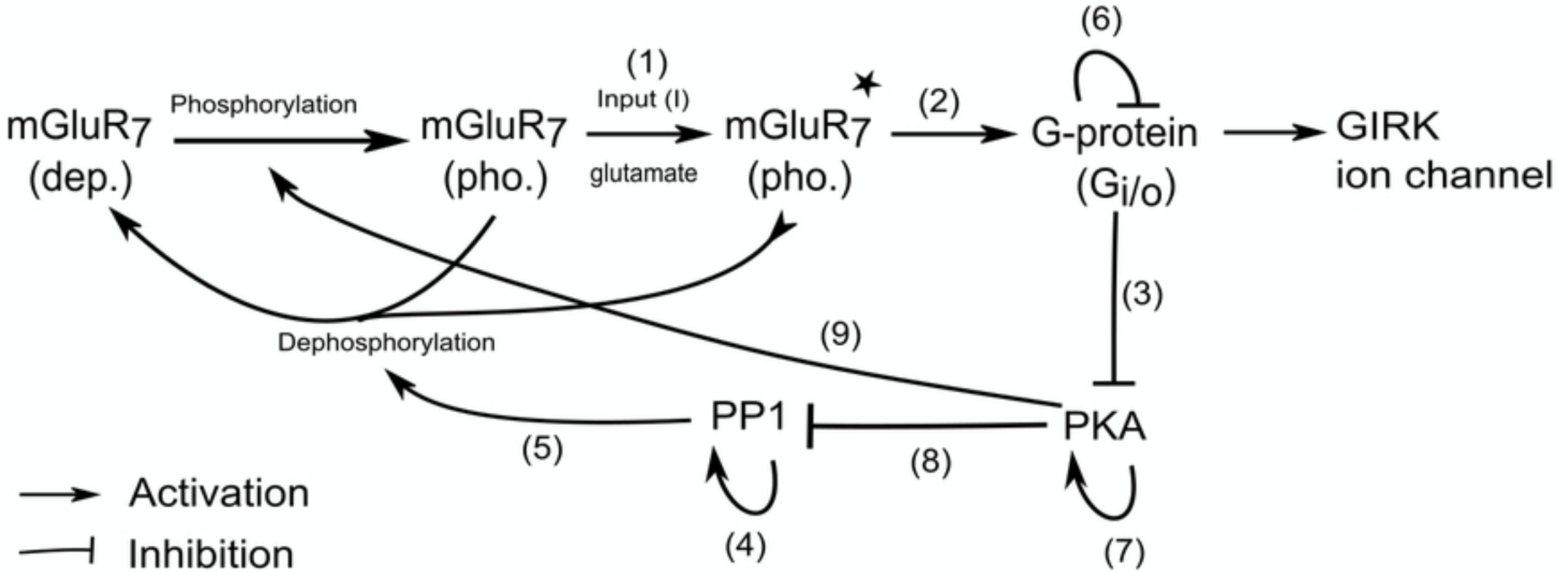
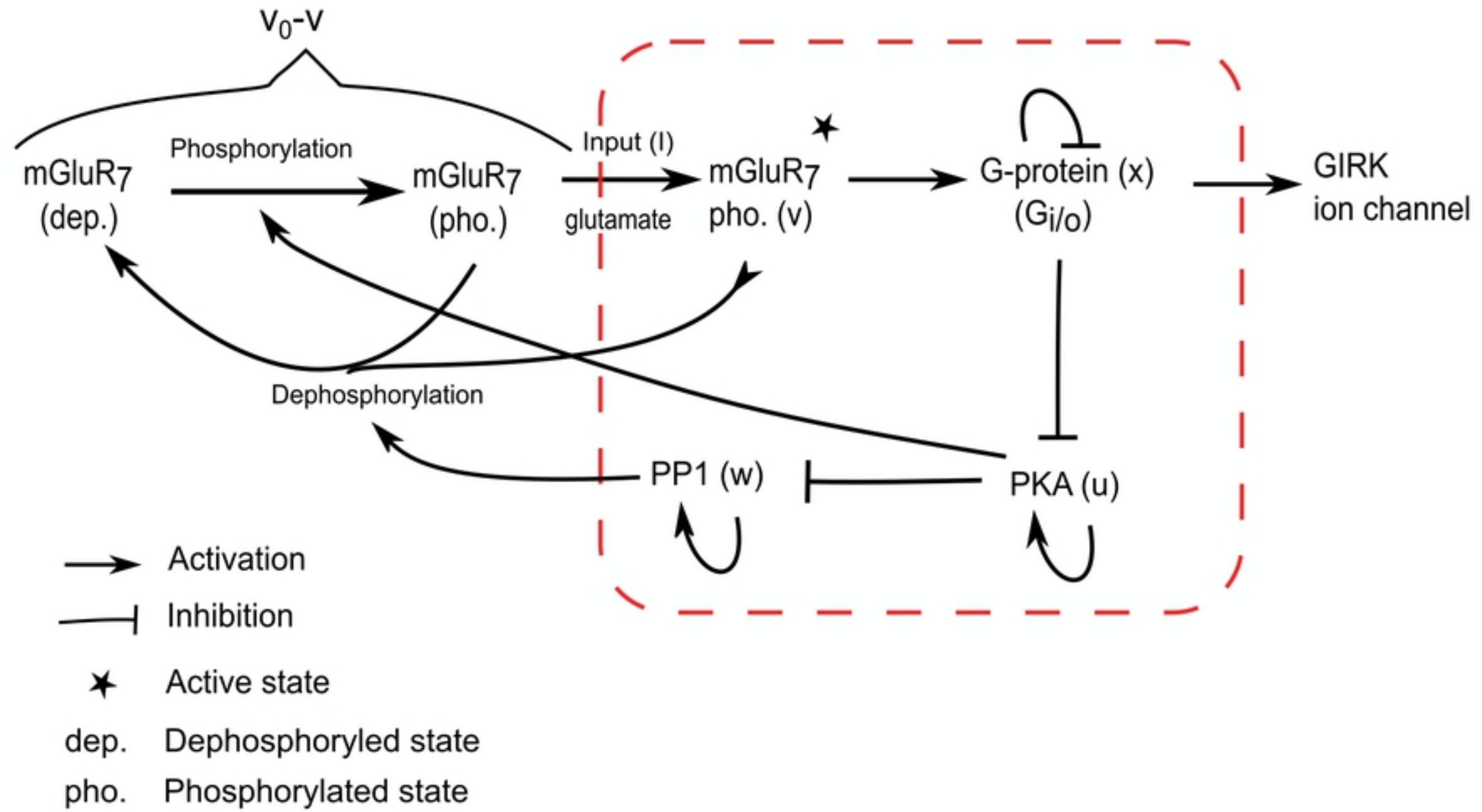

mGluR7
(dep.)
Phosphorylation
mGluR7
(pho.)
(1)
Input (I)
glutamate
mGluR7
(pho.)
(2)
G-protein
(Gi/o)
(6)
GIRK
ion channel
(3)
(9)
Dephosphorylation
PP1
PKA
(5)
(8)
(4)
(7)
Activation
Inhibition
Active state
dep. Dephosphoryled state
pho. Phosphorylated state

Fig2


v_0-v
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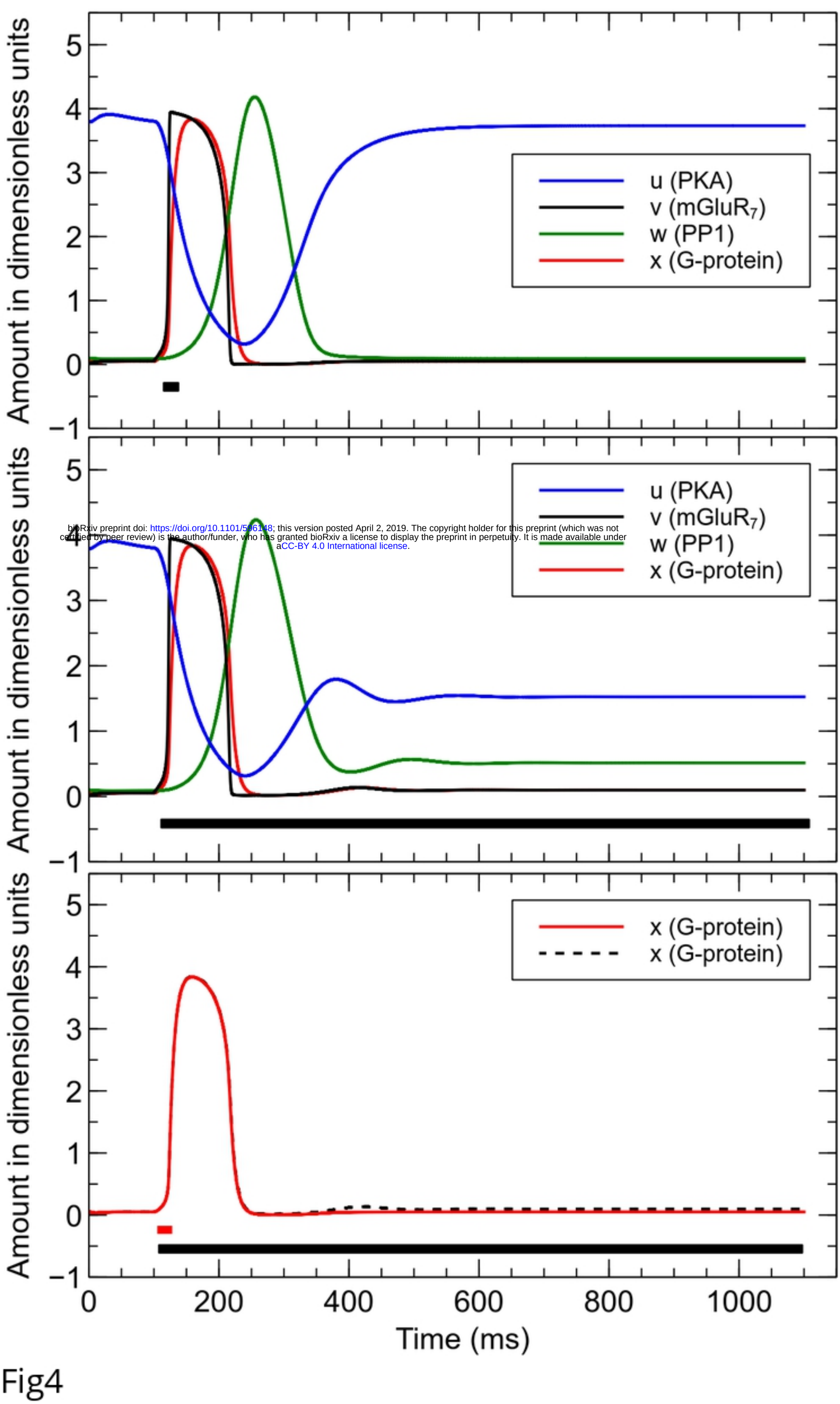
(dep.)
mGluR7
(pho.)
Input (I)
glutamate
mGluR7
pho. (v)
G-protein (x)
($G_{i/o}$)
GIRK
ion channel
Dephosphorylation
PP1 (w)
PKA (u)
Activation
Inhibition
Active state
dep. Dephosphoryled state
pho. Phosphorylated state

Fig3



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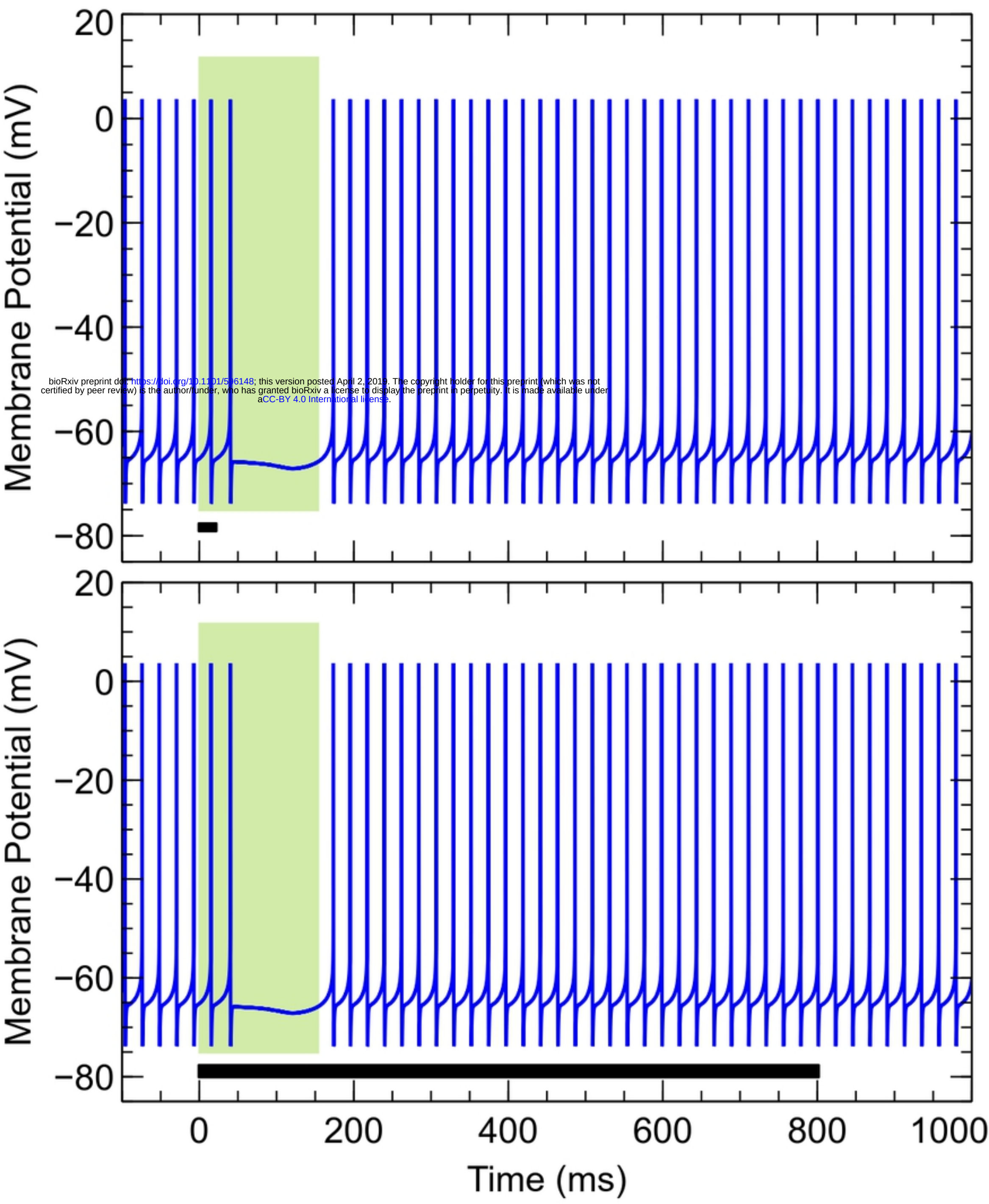

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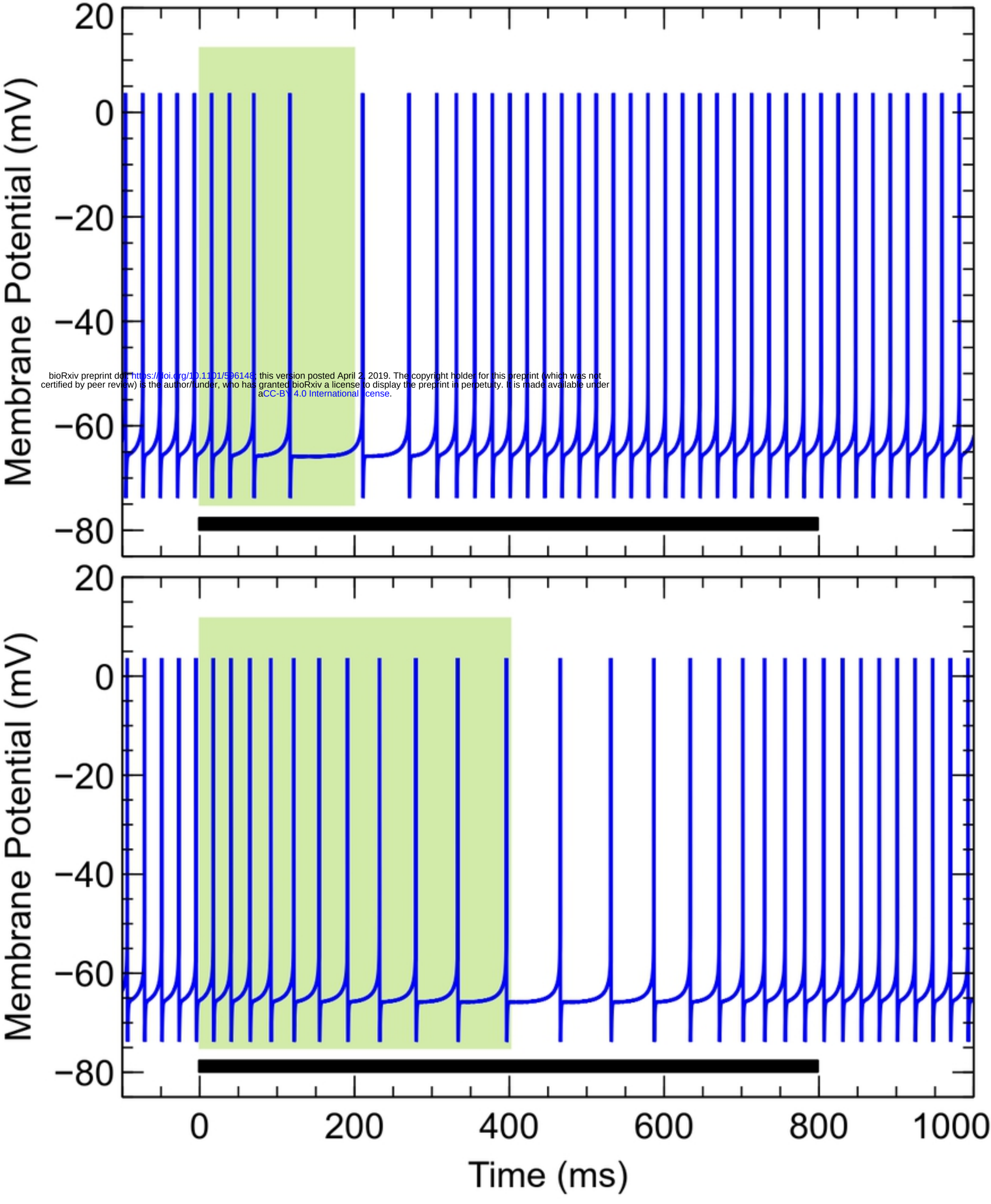

Fig6

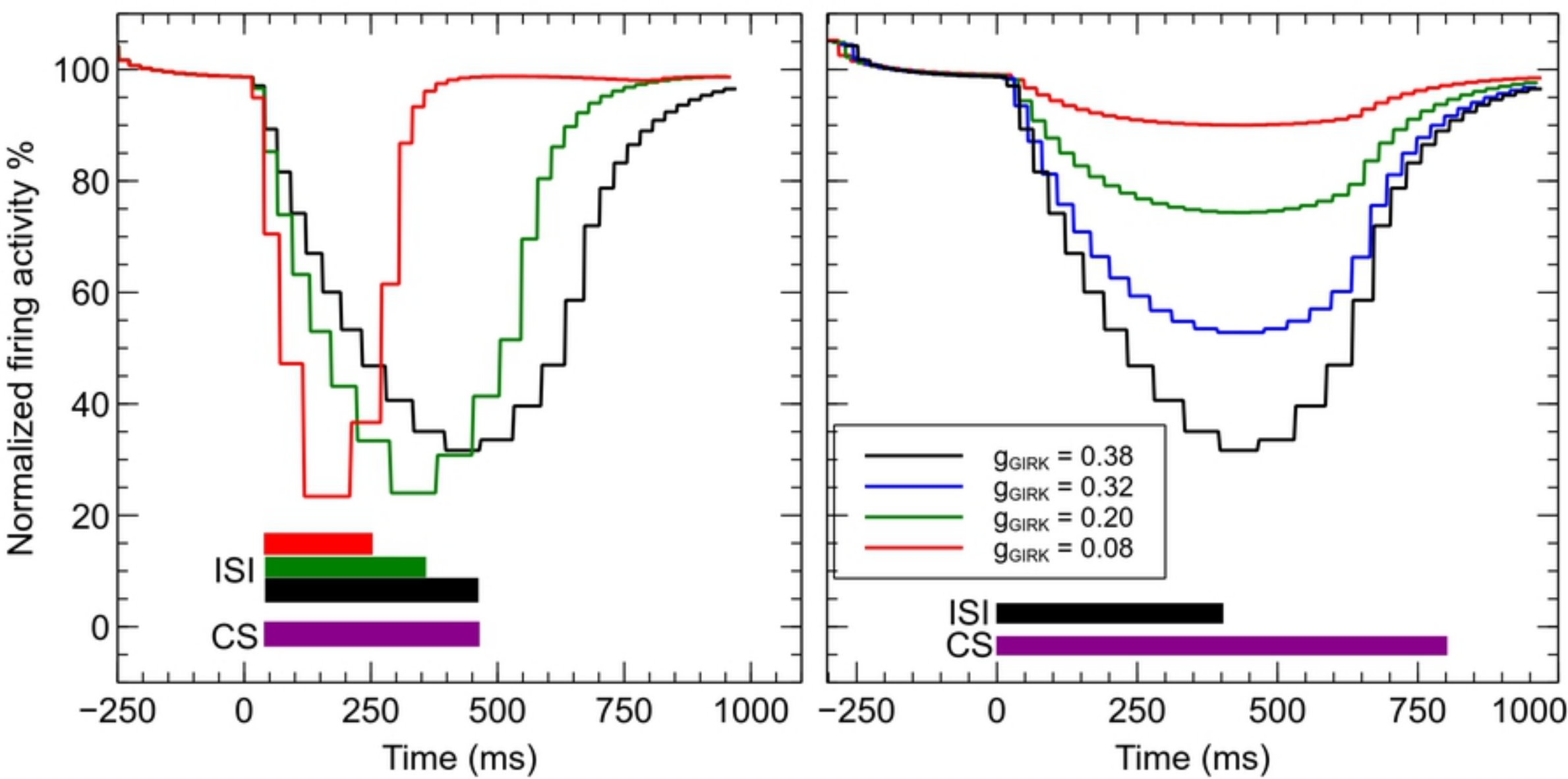
Normalized firing activity %
100
80
60
40
20
0
ISI
CS
−250
0
250
500
750
1000
Time (ms)
g_{GIRK} = 0.38
g_{GIRK} = 0.32
g_{GIRK} = 0.20
g_{GIRK} = 0.08
ISI
CS
−250
0
250
500
750
1000
Time (ms)

Fig7

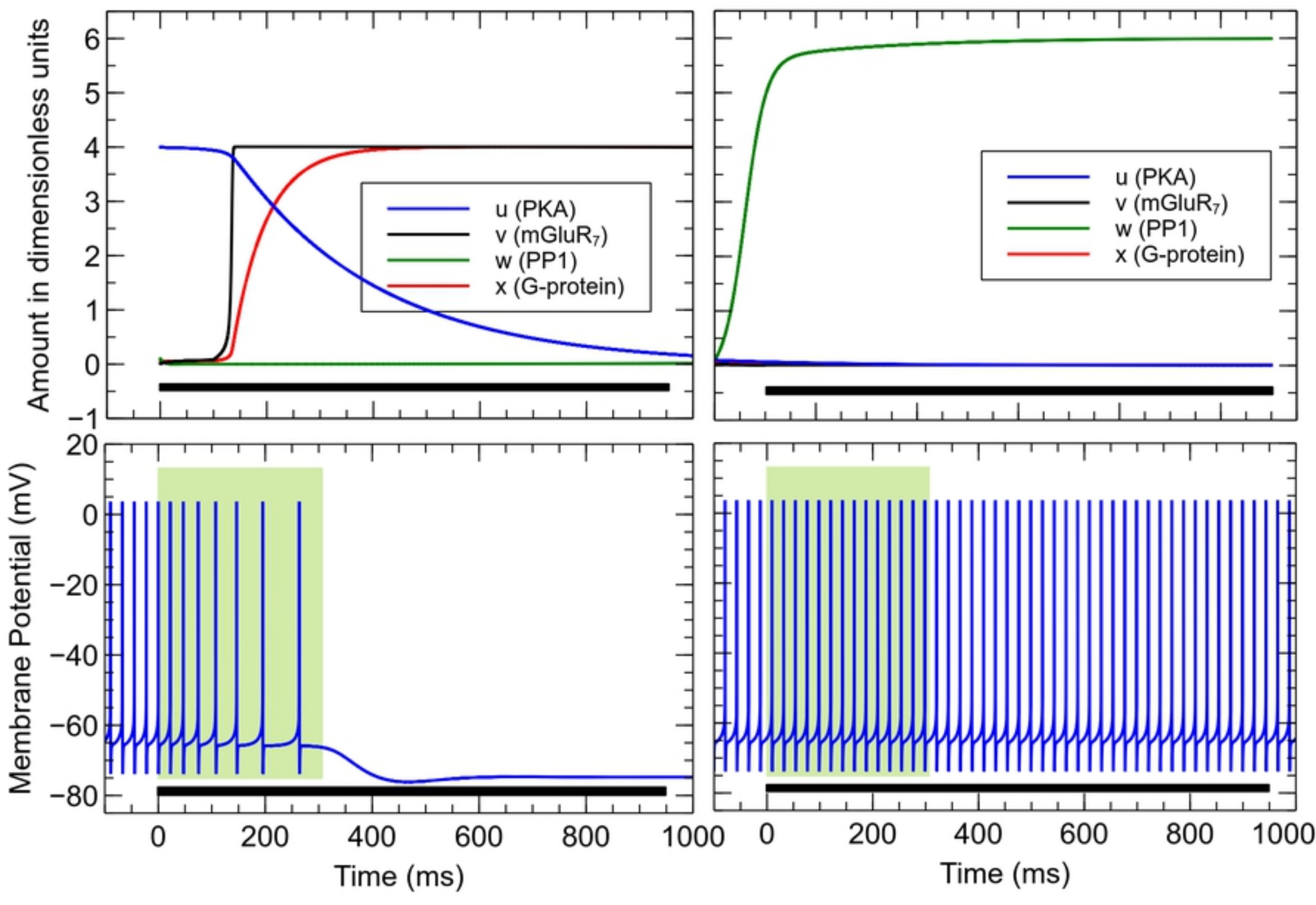
Amount in dimensionless units
u (PKA)
v (mGluR7)
w (PP1)
x (G-protein)
Membrane Potential (mV)
Time (ms)
Time (ms)

Fig8