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Cannabinoid-Mediated Short-Term Plasticity in Hippocampus

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Abstract Endocannabinoids modulate both excitatory and inhibitory neurotransmission in hippocampus via activation of pre-synaptic cannabinoid receptors. Here, we present a model for cannabinoid mediated short-term depression of excitation (DSE) based on our recently developed model for the equivalent phenomenon of suppressing inhibition (DSI). Furthermore, we derive a simplified formulation of the calcium-mediated endocannabinoid synthesis that underlies short-term modulation of neurotransmission in hippocampus. The simplified model describes cannabinoid-mediated short-term modulation of both hippocampal inhibition and excitation and is ideally suited for large network studies. Moreover, the implementation of the simplified DSI/DSE model provides predictions on how both phenomena are modulated by the magnitude of the pre-synaptic cell's activity. In addition we demonstrate the role of DSE in shaping the post-synaptic cell's firing behaviour qualitatively and quantitatively in dependence on eCB availability and the pre-synaptic cell's activity. Finally, we explore under which conditions the combination of DSI and DSE can temporarily shift the fine balance between excitation and inhibition. This highlights a mechanism by which eCBs might act in a neuro-protective manner during high neural activity.

Keywords Calcium · DSI · DSE · Endocannabinoids · Hippocampus · Short-term Plasticity

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1 Introduction

The endocannabinoid (eCB) signalling system underlies a number of short- and long-term forms of synaptic plasticity at both excitatory and inhibitory synapses in various brain areas including the hippocampus (Kano et al, 2009). eCBs are retrograde messengers which are synthesised and released post-synaptically and bind to pre-synaptic cannabinoid (CB) receptors. CB_1 is the most abundant CB receptor subtype in the brain. The activation of pre-synaptic CB_1 receptors on interneurons and pyramidal cells transiently reduces the release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the excitatory neurotransmitter glutamate (GLU), respectively. In hippocampus, eCBs are mobilised by excitatory cells in response to neuronal activity via three main pathways: a Ca^{2+} dependent signalling cascade following strong Ca^{2+} influx through plasma membrane Ca^{2+} channels, a Ca^{2+} independent pathway resulting from strong activation of metabotropic G-protein coupled receptors, and a combination of both pathways when they are activated simultaneously (Kano et al, 2009). It has been recently reported that hippocampal fast-spiking interneurons can also mobilise eCBs required for long-term plasticity, albeit with a higher induction threshold (Peterfi et al, 2012). A form of CB-mediated short-term depression that is Ca^{2+} dependent is known as depolarisation-induced suppression of inhibition (DSI) or excitation (DSE) (Wilson and Nicoll, 2001; Ohno-Shosaku et al, 2001, 2002b). Hippocampal DSI and DSE share common biochemical signalling pathways. The CB_1 receptor is responsible for both DSI/DSE which can be induced by application of CB_1 receptor agonists and are blocked by CB_1 receptor antagonists (Ohno-Shosaku et al, 2002b; Wilson and Nicoll, 2001). Also, both DSI and DSE are

mediated by 2-arachidonoylglycerol (2-AG), one of the main eCBs in hippocampus (Gao et al, 2010; Tanimura et al, 2010). While DSI and DSE have been shown to exhibit qualitatively similar behaviour, their quantitative characteristics differ. DSE is less prominent than DSI and has a higher induction threshold in hippocampal cultures and slices, supposedly due to the lower sensitivity of CB_1 receptors expressed on excitatory rather than on inhibitory synaptic terminals (Ohno-Shosaku et al, 2002b). It should also be noted that the expression of CB_1 receptors on excitatory terminals is lower but more homogeneous than in inhibitory terminals (only expressed at cholecystokinin-positive perisomatic basket cells (Ohno-Shosaku et al, 2002b)).

We recently developed a biophysically realistic mathematical model for the eCB signalling pathway that underlies DSI at hippocampal synapses. The model describes major signalling molecules and cascades and successfully captures many of the experimentally observed key characteristics (Zachariou et al, 2013). Here, we go beyond this work by developing a corresponding model for CB-mediated short-term depression of excitation in hippocampus that accounts for the reduced magnitude and higher induction threshold of DSE versus DSI. We employ the same core model as for the CB_1 receptor on inhibitory terminals but modify key parameters based on fitting experimental data in order to describe the dynamics of the CB_1 receptor on excitatory terminals. Finally, we reduce the Ca^{2+}-mediated eCB synthesis subsystem in order to obtain a minimal model which is ideally suited for network studies. We then implement the DSI/DSE reduced model in order to obtain new insights on how the firing behaviour of the post-synaptic cell can be modulated by CBs when varying the pre-synaptic cell's firing rate and the sensitivity of the receptor to this activity. We also obtain new predictions on how DSI/DSE interact in a scenario of balanced excitation and inhibition input and how the same stimulus can result in a temporally ordered inducement of first DSI and then DSE.

1.1 Biophysics of Cannabinoid Signalling

The eCB signalling system that underlies both DSI and DSE is illustrated in Fig. 1. Post-synaptically the cytoplasmic Ca^{2+} concentration increases mainly due to depolarisation-induced Ca^{2+} entry through L-type voltage-gated Ca^{2+} channels (VGCCs). A leak current from the endoplasmic reticulum (ER) provides an additional yet minor Ca^{2+} influx to the cytoplasm. Ca^{2+} exits the cytoplasm through sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps and plasma membrane Ca^{2+} ATPase (PMCA) pumps. Activity-dependent intracellular Ca^{2+} rises in the post-synaptic (excitatory) cell upregulate Diacylglycerol (DAG) through an as-yet-unknown pathway. 2-AG is synthesised in the post-synaptic cell when DAG is metabolised by Diacylglycerol Lipase α (DGLα) and it partially undergoes oxidation by COX-2. 2-AG is then released by a putative eCB membrane transporter and activates pre-synaptic CB_1 receptors (found on inhibitory and excitatory synaptic terminals), thus causing the splitting of G protein heterotrimers into G_α and $G_{\beta\gamma}$ subunits. The $G_{\beta\gamma}$ subunit directly inhibits the pre-synaptic N-type VGCCs by setting them into a reluctant state, resulting in the inhibition of neurotransmitter (GABA and glutamate) release. 2-AG is then sequestered into the presynaptic cell by an as-yet-unknown eCB transporter, where most of it is degraded by MGL.

1.2 Modelling Setup

The main components of the model are (1) a pre-synaptic inhibitory cell with CB_1 receptor dynamics modulating inhibitory synaptic transmission, (2) a pre-synaptic excitatory cell with CB_1 receptor dynamics modulating excitatory synaptic transmission, and (3) a post-synaptic excitatory cell with Ca^{2+} mediated eCB synthesis dynamics, as illustrated in Fig. 1. The complete model description can be found in the Methods section.

2 Results

2.1 CB_1 Receptor on Pre-synaptic Excitatory Cell

Experiments in cultured hippocampal neurons suggest that excitatory synapses are less sensitive than the inhibitory ones to both administered exogenous CB agonist (WIN55,212-2) and depolarisation-mobilised eCB (Ohno-Shosaku et al, 2002b) as shown with error bars in Fig. 2(a) and 2(b). In the case of WIN55,212-2 administration, EPSPs were 30-fold less sensitive than IPSPs, demonstrated by the WIN55,212-2 half-inhibition concentrations for the synaptic events (0.06 μM and 0.002 μM for the excitatory and inhibitory synapses respectively) (Ohno-Shosaku et al, 2002b). In order to account for the reduced sensitivity of the CB_1 receptor found on pre-synaptic excitatory terminals, we modified the half-inhibition concentration (IC_{50}^{WIN} from equation (28)) for inhibiting the pre-synaptic Ca^{2+} channels to match the experimental observation (see Table 1). We also modified the half-inhibition concentration for 2-AG (IC_{50}^{AG} from equation (27)) and the willing-to-reluctant transition rate constant κ_+^{A} (equation (25)) in order

to obtain the best fit for both experimental protocols (see Protocols I and II in subsection 4.9), as shown in Fig. 2(a)-2(b). The adapted model captures the reduced magnitude and higher induction required both in the case of depolarisation-induced eCB synthesis and the administration of exogenous CBs (Fig. 2(a)-2(b)). The time course of DSE is shown in Fig. 2(c) and exhibits a decay with a time constant of 10.2 sec which is similar but shorter than that for DSI (experiments 15.6 ± 1.5 sec, model 12.8 sec) as previously reported (Alger, 2002; Ohno-Shosaku et al, 2002b). Experimental CB concentration response curves for the exogenous CB agonist WIN55,212-2 and for the endogenous CB agonist 2-AG for DSI are illustrated by solid lines in Fig. 2(d). The equivalent CB concentration response curves for DSE are shown as dashed lines and were obtained from the DSE model presented here. As expected both the WIN and AG response curves are shifted to the right, consistent with experimental findings that DSE has a higher induction threshold than DSI.

2.2 Reduction of Calcium-Mediated eCB Synthesis in Post-synaptic Cell

2.2.1 Endocannabinoid Synthesis - Reduction

The eCB synthesis model can be reduced by eliminating the differential equations for DAG (D) and 2-AG (AG) (equations (30)-(31)). This can be achieved following a qualitative observation that the time scale of the dynamics of D and AG are much faster than that of the cytoplasmic Ca^{2+} concentration (c). This suggests that the variables D and AG rapidly approach their equilibrium value. Thus, they can be considered as instantaneous variables and be replaced by their respective steady-state values $AG(t) \to AG_\infty(c(t))$ and $D(t) \to D_\infty(c(t))$, where

$$D_\infty = \frac{v_c^{\mathrm{D}} c^2}{K_c^2 + c^2}, \quad v_c^{\mathrm{D}} = \frac{v_c}{k_d + k_{11} DL}, \tag{1}$$

$$AG_\infty = \frac{v_c^{\mathrm{AG}} c^2}{K_c^2 + c^2}, \quad v_c^{\mathrm{AG}} = \frac{k_{11} DL}{k_{12} COX} v_c^{\mathrm{D}}. \tag{2}$$

The simulation results for the eCB synthesis reduced model are the same quantitatively as for the original model, as shown in dashed lines in Fig. 2(a) - 2(c).

2.2.2 Intracellular Calcium - Reduction

The eCB synthesis model can be reduced even further by simplifying the intracellular Ca^{2+} dynamics. Initially we removed the minor constant Ca^{2+} influx from outside the cell J_{IN} resulting in a simplified equation for c,

$$\frac{\mathrm{d}c}{\mathrm{d}t} = J_{\mathrm{LEAK}} - J_{\mathrm{SERCA}} + J_{\mathrm{CHAN}} - J_{\mathrm{PMCA}}, \tag{3}$$

The reduced model in terms of eCB synthesis and the removal of J_{IN} is an excellent qualitative and in most cases quantitative fit for the original model in the depolarisation protocol (results not shown). In terms of the time course, the reduced model is similar to the original model (DSE decay time constant 9.6 sec - original model 10.2 sec, DSI decay time constant 11.1 sec - original model 12.8 sec).

The intracellular Ca^{2+} model can be simplified even further by removing the dynamics of the ER, which is an often used approach in modelling neuronal Ca^{2+} (De Schutter and Smolen, 1998). This approximation renders equation (34) obsolete, and equation (3) for the intracellular Ca^{2+} concentration becomes

$$\frac{\mathrm{d}c}{\mathrm{d}t} = J_{\mathrm{CHAN}} - J_{\mathrm{PMCA}}. \tag{4}$$

In order to reproduce the expected peak cytoplasmic Ca^{2+} concentration rise during the depolarisation protocol, we modified the parameters for the PMCA pump (see Table 1). Results for the overall reduced model, i.e. for both eCB synthesis and intracellular Ca^{2+}, are shown in Fig. 3 (dashed lines). The reduced model provides a better fit of the magnitude of DSI/DSE during the depolarisation protocol than the original model (calculated with euclidean distance), as seen in Fig. 3(a). However, there are subtle differences in terms of the cytoplasmic Ca^{2+} and eCB transient (Fig. 3(c)-3(d)) as the decay of the reduced model is relatively faster than in the original model. Although this results in a faster return to baseline of the IPSPs/EPSPs amplitude (8.3 sec for DSI and 8.4 sec for DSE), as shown in Fig. 3(b), the correct trend is captured. It should be noted that a wide range of decay time constants for DSI has been reported with mean values of 10-22 sec for experiments at $20 - 34$ °C (Lenz and Alger, 1999; Morishita and Alger, 1999; Ohno-Shosaku et al, 2001; Wang and Zucker, 2001; Glickfeld and Scanziani, 2006).

2.3 DSE Depends on Pre-Synaptic Activity

Our simulations with DSI in a previous study (Zachariou et al, 2013) have revealed that not only CB availability but also the firing rate of the pre-synaptic inhibitory cell modulates the magnitude of DSI, as also suggested experimentally (Földy et al, 2006). In essence, the magnitude of DSI increases due to CB availability (which results in depression of synaptic events) and decreases

due to the pre-synaptic cell's firing rate (which results in facilitation of the synaptic events). Here, we investigate the potential equivalent modulation of DSE by the pre-synaptic firing of the excitatory cell. Given the similarity between DSI and DSE, we tested if DSE exhibits a similar response to frequency modulation by pre-synaptic firing as DSI.

To test this prediction we run simulations (see Protocol III) varying the sensitivity of the CB_1 receptor to the pre-synaptic cells' firing rate (κ_-^A, κ_-) and the pre-synaptic cell frequency while the agonist WIN55,212 (5 μM) is applied for 1 min. Our results show that, as in the case of DSI (Fig. 4(a)), DSE is also modulated by the presynaptic firing rate and the sensitivity of the CB_1 receptor to this activity (Fig. 4(b)). A first observation is that for higher values of κ_-^A, κ_- there is a smaller range of pre-synaptic cell's frequencies that DSI/DSE can be induced. For example for $\kappa_-^A, \kappa_- = 0.3$, for frequencies larger than 10 Hz DSI/DSE cannot be induced whereas for $\kappa_-^A, \kappa_- = 0.005$, DSI/DSE can be induced across the whole range of frequencies tested here (0.2-100 Hz). A second observation is that the effect of the pre-synaptic cell's firing frequency on DSE decays faster than the effect on DSI for all κ_-^A, κ_- values. This can be explained by the overall lower sensitivity of DSE on CB availability compared to that of DSI.

Overall, our results show how DSE is modulated by different sensitivities of the CB_1 receptor by the firing rate of the pre-synaptic excitatory cell. This prediction could be tested experimentally for glutamatergic synapses with a similar protocol as used in (Földy et al, 2006) for GABAergic synapses.

2.4 DSE Modulation of Firing Behaviour

Short-term plasticity mechanisms such as DSE modulate the firing rate and pattern of cells and can also act as a self-brake during excessive glutamatergic activity. DSE-like pre-synaptic inhibition of glutamatergic transmission can have neuro-protective effects as otherwise excessive activity can lead to high intracellular calcium increase and trigger excitotoxicity and neuronal death (Marsicano, 2003; Piomelli et al, 2000). Here, we further explore the role of DSE by considering a two-excitatory-cell protocol. By stimulating the pre-synaptic cell with varying frequencies, we explore under which conditions DSE occurs and affects the firing behaviour of the post-synaptic cell. We also vary the sensitivity of the CB_1 receptor to the pre-synaptic cell's firing rate by varying the maximal reluctant-to-willing transition rate κ_-^A. In Fig. 5(a)-(b) we observe that for high values of κ_-^A =0.3, regardless of the pre-synaptic rate (5 Hz for Panel (a) and 40 Hz for Panel (b)) DSE is not induced and the post-synaptic cell fires without interruptions, continuously responding to the input it receives from the pre-synaptic cell. Although high levels of eCB are produced neurotransmission is not affected due to the higher sensitivity of the CB_1 receptor to pre-synaptic firing, which keeps the variable w_A (the fraction of willing Ca^{2+} pre-synaptic channels) near its maximum value of 1. Note that $0 \leq w_A \leq 1$, where $w_A = 1$ corresponds to the maximal synaptic response and $w_A = 0$ indicates no synaptic transmission. When the same protocol is used (Protocol IV) but for a lower value of $\kappa_-^A = 0.005$, DSE is induced both for low (5 Hz in Fig. 5(c)) and high (40 Hz in Fig. 5(c)) pre-synaptic frequencies. In the case of 5 Hz (Fig. 5(c)) the synthesis of eCB results in the reduction of w_A below 0.77, which reflects an effective firing threshold. Hence, the post-synaptic cell ceases to fire temporarily and starts firing again once eCB has relaxed back to 0 and consequently w_A has recovered above the firing threshold of 0.77. DSE causes a temporal dampening of the activity, which results in a pattern of firing with intermittent pauses (while eCB recovers). For higher frequencies (40 Hz) as seen in Fig. 5(d) there is competition between pre-synaptic firing and the level of eCB, evident from the variable w_A which fluctuates close to the firing threshold of 0.77. The eCB does not recover to 0 and there are also spikes during the intermittent pauses, as opposed to Fig. 5(c). The above results are summarised in Fig. 5(e) where we plot the post- versus the pre- synaptic cell firing rate for different values of κ_-^A. We observe that the post-synaptic cell's firing rate is reduced for lower κ_-^A values. To illustrate the effect of DSE on the firing pattern, in Fig. 5(f) we plot the Skewness Coefficient of the post-synaptic cell (solid lines) and the pre-synaptic cell (dashed line) versus the stimulation frequency of the pre-synaptic cell. The skewness coefficient is a measure of the asymmetry of the interspike interval distribution (Shinomoto and Sakai, 1999) and is expected to be 2 for a Poisson process. We observe that for both the pre-synaptic cell firing pattern and for the post-synaptic cell with high κ_-^A values the skewness coefficient is approximately 2, suggesting that the pre-synaptic cell dictates the post-synaptic cell's firing and both have a Poisson-like firing pattern for all pre-synaptic frequencies. For lower values of κ_-^A the skewness coefficient is higher for a range of presynaptic frequencies. Once the pre-synaptic cell's firing frequency is increased sufficiently to take over the eCB effect on the overall amplitude of the synaptic events, the skewness coefficient decreases and eventually reaches the value 2. Depending on the value of κ_-^A

the cutoff frequency for which the skewness coefficient starts decreasing is different.

2.5 DSE and DSI interaction

Hippocampal DSI and DSE are two forms of plasticity which share common modes of induction and are mediated by the same type of eCB but are expressed with different magnitudes (Diana and Marty, 2004; Ohno-Shosaku et al, 2002b). Here, we explore how these closely related mechanisms interact in a simple cell network of three cells, one pre-synaptic excitatory cell, one presynaptic inhibitory cell and one post-synaptic excitatory cell. The post-synaptic cell receives a balanced input from the two pre-synaptic cells (which are both stimulated with a Poisson spike train at the same time and the synaptic conductances are set such that the inhibitory and excitatory inputs cancel out and the post-synaptic cell rarely fires). At first, at time 5 sec the post-synaptic cell is stimulated with a single 50 Hz pulse (1 sec duration). In Fig. 6(a) we observe that for a high value of $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.3$ neither DSI nor DSE are induced and the post-synaptic cell only fires during the 1 sec stimulation. However, for a low value of $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.005$ (Fig. 6(b)) DSI is induced first as indicated by the fast decay of w. This results in a dis-inhibition of the post-synaptic cell which starts to fire with the 1 sec stimulation and continuous to fire for about 10 sec (since it is now receiving only excitation from the presynaptic excitatory cell). This prolonged activity of the post-synaptic cell results in a further increase of eCB and eventually DSE is also induced, resulting in a pause of activity for the post-synaptic cell. Once the post-synaptic cell ceases to fire, the eCB level starts to relax back to zero, resulting in an increase to both w_{A} and w. However, w_{A} recovers first and the post-synaptic cell starts to fire again for a limited amount of time, resulting in intermittent firing, as seen in Fig. 5(c)-(d) where DSE was solely studied. Secondly, we test the same protocol when the stimulatory pulse duration is increased to 5 sec. We observe in Fig. 6(c) that for a high value of $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.3$, the results are similar to the case of 1 sec duration input. In contrast, for a lower value of $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.005$ (Fig. 6(d)), both DSI and DSE are induced after the 5 sec stimulation due to the prolonged activity and high resulting levels of eCB. Overall, these findings suggest that DSI and DSE can be induced either together or in isolation under different conditions and at different times and can result in a variety of firing behaviours in the post-synaptic cell. Large amounts of eCB can induce both DSI and DSE simultaneously whereas smaller amounts can induce DSI and DSE in a temporally organised manner (first DSI since it is more sensitive to eCB availability than DSE). This temporal order of induction is important as it highlights the role of DSE as a regulator of excessive activity as it can be induced after DSI and regulate any activity that has resulted from dis-inhibition of the post-synaptic cell due to DSI.

3 Discussion

In this paper, we developed a model of DSE based on a recently published model of DSI (Zachariou et al, 2013) and experimental data (Ohno-Shosaku et al, 2002b). Our results show that by modifying the sensitivity of CB_1 receptors on excitatory synaptic terminals (compared to the inhibitory ones) the model can account for both the reduced magnitude and higher induction threshold of DSE versus DSI. The model successfully reproduces experimental observations of DSE both due to endocannabinoid-mobilising stimulation (depolarising voltage pulses) and due to CB agonist administration (WIN55,212-2) (Ohno-Shosaku et al, 2002b). The fact that both DSI and DSE can be described by the same equations but with different key parameters highlights an efficient design principle. Cells can use the same biochemical pathway, but have to fine-tune molecular properties to achieve complementary functions.

Furthermore, we reduced the post-synaptic part of the model for Ca^{2+} driven synthesis of eCB underlying the eCB-mediated short-term modulation of inhibition and excitation in hippocampus. Initially, we simplified the dynamics of DAG and 2-AG and obtained the same quantitative results as with the original model. In addition, we simplified the dynamics of the intracellular Ca^{2+} and removed the contribution of the ER. The overall reduced model successfully captures the main characteristics of DSI/DSE. In fact, in terms of the depolarisation evoked DSI/DSE, the reduced model is more precise than the original model. It also captures the time course of the depression of synaptic responses during DSI albeit with a faster decay time. We should note that by removing the ER, as is often done in single cell neuron models, the Ca^{2+} transient and, as a result, the eCB transient and the DSI/DSE time course decay faster. It has been suggested that although DSI/DSE are Ca^{2+} mediated, their duration is not rate-limited by the duration of the intracellular Ca^{2+} transient (Lenz and Alger, 1999; Wang and Zucker, 2001), but degradation of 2-AG by MGL and COX-2 has been shown to determine the DSI/DSE time course (Kim and Alger, 2004; Hashimotodani et al, 2007; Pan et al, 2011; Straiker et al, 2011). Since the ER does not contribute to intracellular Ca^{2+} during DSI/DSE (Kano

et al, 2009) and since the basic features of the short-lived DSI/DSE model are preserved without it, we removed the ER in order to obtain a model which is more appropriate for network studies due to its lower computational demand. The reduced eCB signalling modulation model of both excitation and inhibition in hippocampus opens the way for networks studies including both DSI and DSE in order to decipher the role of CBs in synaptic transmission in network emergent behaviour related to memory and learning (Kano et al, 2009). Nevertheless, the detailed model of DSI/DSE is more appropriate for expanding and investigating subcellular factors at the level of intracellular Ca^{2+} and eCB synthesis; e.g. the cooperative eCB synthesis, which depends on both depolarisation-evoked Ca^{2+} increase and the activation of metabotropic glutamate receptors (which can in turn engage the ER in eCB synthesis).

Following the development of the DSE model and the overall reduction of the complete DSI/DSE model we proceeded with implementing them and obtaining new predictions and insights in their physiological role in the brain. Firstly, our simulations suggest that both DSI and DSE are modulated by the pre-synaptic cell activity. Moreover, the particular sensitivity of the CB_1 receptor to the pre-synaptic activity can determine whether DSE will be induced and affects both qualitatively and quantitatively the behaviour of the post-synaptic cell. The CB retrograde signalling system which underlies plasticity phenomena such as DSI and DSE is also subject to adaptation and plasticity (for a review see (Iremonger et al, 2013)) induced by e.g. neuronal activity, neuromodulators, drugs, and stress. Hence a varying range of sensitivities of the CB_1 receptor to pre-synaptic firing could be a result of such meta-plasticity in the brain and natural heterogeneity in expression in a brain region.

Finally, our findings on the interaction of DSI and DSE in a three-cell protocol show that factors, such as the duration of an incoming stimulation and the sensitivity of the receptor to pre-synaptic firing, can shift the fine balance between excitation and inhibition. This has possible implications in pathological manifestations of inhibition and excitation imbalance such as epilepsy (Blair, 2006). Interestingly, CBs have been found to exert both convulsant and anticonvulsant effects (Monory and Lutz, 2008). A CB-mediated reduction in excitation during epileptiform activity would be expected to provide protection against excessive excitability whereas a reduction in inhibition would supposedly oppose this effect and result in pro-convulsant effect. Indeed, an up-regulation of CB_1 receptors on GABAergic terminals but not on glutamatergic terminals has been observed due to febrile seizures, suggesting a pathological enhancement of DSI (Chen et al, 2007). Also, it has been hypothesised that suppression of excitation is the mode of action of the anti-convulsant effects of CBs (Blair, 2006; Hajos and Freund, 2002). This hypothesis has been supported in a study by Monory et *al.* (Monory et al, 2006) where deletion of CB_1 receptors from glutamatergic terminals was shown to be pro-convulsant whereas the deletion of CB_1 receptors from GABAergic cells did not change seizure behaviour. Monory et al (2006) suggest that either DSI is not induced due to high pre-synaptic firing or because it is overwritten by DSE. Our simulations suggest that although DSI can increase excitability momentarily, DSE acts as a temporal brake to overall excitability since DSE is induced for amounts of eCBs that are equal or larger than those needed to induce DSI. Overall, our work provides new insights into the dynamic repertoire of eCB and shows how it might act in a neuro-protective manner during epileptogenic activity in hippocampus.

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4 Methods

4.1 Inhibitory Single Cell Model

For the inhibitory cell we use the single compartment model of Wang-Buzsáki (WB) (Wang and Buzsaki, 1996), as in (Zachariou et al, 2013). The membrane potential V_I is described by

$$C_\mathrm{m}\frac{\mathrm{d}V_\mathrm{I}}{\mathrm{d}t} = -I_\mathrm{ion,I}(V_\mathrm{I}) - I_\mathrm{syn,I}(V_\mathrm{I}) + I_\mathrm{ext,I}, \tag{5}$$

where C_m is the membrane capacitance, $I_\mathrm{syn,I}$ is the total synaptic input current, $I_\mathrm{ext,I}$ is the external current applied and $I_\mathrm{ion,I}(V_\mathrm{I})$ is the total ionic current given by the expression

$$I_\mathrm{ion,I}(V_\mathrm{I}) = I_\mathrm{L}(V_\mathrm{I}) + I_\mathrm{Na}(V_\mathrm{I}, h_\mathrm{I}) + I_\mathrm{K}(V_\mathrm{I}, n_\mathrm{I}), \tag{6}$$

where

$$I_\mathrm{L}(V_\mathrm{I}) = \overline{g}_\mathrm{L}(V_\mathrm{I} - V_\mathrm{L}), \tag{7}$$

$$I_\mathrm{K}(V_\mathrm{I}, n_\mathrm{I}) = \overline{g}_\mathrm{K} n_\mathrm{I}^4 (V_\mathrm{I} - V_\mathrm{K}), \tag{8}$$

$$I_\mathrm{Na}(V_\mathrm{I}, h_\mathrm{I}) = \overline{g}_\mathrm{Na} m_{\mathrm{I},\infty}^3(V_\mathrm{I}) h_\mathrm{I}(V_\mathrm{I} - V_\mathrm{Na}). \tag{9}$$

The gating variables $h_{\rm I}$, $n_{\rm I}$ are given by first order differential equations of the form

$$\frac{{\rm d}\psi}{{\rm d}t} = \phi_{\rm I}\frac{(\psi_\infty(V)-\psi)}{\tau_\psi(V)}, \tag{10}$$

where $\psi = h_{\rm I}, n_{\rm I}$ and

$$\psi_\infty = \frac{\alpha_\psi}{\alpha_\psi+\beta_\psi}, \qquad \tau_\psi = \frac{1}{\alpha_\psi+\beta_\psi}. \tag{11}$$

The gating variable $m_{\rm I}$ is assumed to be activated instantaneously and is substituted by its steady-state function

$$m_{\rm I,\infty} = \frac{\alpha_{\rm m_I}}{\alpha_{\rm m_I}+\beta_{\rm m_I}}. \tag{12}$$

Further details about α_ψ and β_ψ ($\psi = h_{\rm I}, n_{\rm I}, m_{\rm I}$) and the parameters of the WB model can be found in (Wang and Buzsaki, 1996). The temperature adjustment variable $\phi_{\rm I}$ is given by

$$\phi_{\rm I} = 5^{(T_0-T_{\rm I})/10}, \tag{13}$$

where $T_{\rm I} = 27$ °C denotes the temperature of the original electrophysiological experiment for constructing the WB model and $T_0 = 25$ °C denotes the temperature of the model during simulations.

4.2 Excitatory Single Cell Model

For the excitatory cell we consider a single compartment model with Hodgkin and Huxley like dynamics (Huguenard and Mccormick, 1994; Traub and Miles, 1991) as in (Zachariou et al, 2013). The membrane potential V_E is described by

$$C_{\rm m}\frac{{\rm d}V_{\rm E}}{{\rm d}t} = -I_{\rm ion,E}(V_{\rm E}) - I_{\rm syn,E}(V_{\rm E}) + I_{\rm ext,E}, \tag{14}$$

where $C_{\rm m}$ is the membrane capacitance, $I_{\rm ext,E}$ is the external current applied, $I_{\rm syn,E}$ is the total synaptic input current and $I_{\rm ion,E}(V_{\rm E})$ is the total ionic current given by the expression

$$\begin{aligned} I_{\rm ion,E}(V_{\rm E}) =& I_{\rm L}(V_{\rm E}) + I_{\rm Na}(V_{\rm E},h_{\rm E},m_{\rm E}) + I_{\rm K}(V_{\rm E},n_{\rm E}) \\ &+ I_{\rm Ca,L}(V_{\rm E},c), \end{aligned} \tag{15}$$

where

$$I_{\rm L}(V_{\rm E}) = \overline{g}_{\rm L}(V_{\rm E}-V_{\rm L}), \tag{16}$$

$$I_{\rm K}(V_{\rm E},n_{\rm E}) = \overline{g}_{\rm K}n_{\rm E}^4(V_{\rm E}-V_{\rm K}), \tag{17}$$

$$I_{\rm Na}(V_{\rm E},h_{\rm E},m_{\rm E}) = \overline{g}_{\rm Na}m_{\rm E}^3(V_{\rm E})h_{\rm E}(V_{\rm E}-V_{\rm Na}). \tag{18}$$

The gating variables $h_{\rm E}, n_{\rm E}, m_{\rm E}$ are also given by first order differential equations of the form (10). Further details about α_ψ and β_ψ ($\psi = h_{\rm E}, n_{\rm E}, m_{\rm E}$) and the parameters of the model can be found in (Huguenard and Mccormick, 1994) and were obtained from ModelDB (accession number: 3808) (Hines et al, 2004). The Ca^{2+} current $I_{\rm Ca,L}$ through the L-type VGCC is described by a Goldman-Hodgkin-Katz (GHK) equation for Ca^{2+} (Hemond et al, 2008; Zachariou et al, 2013). The equations describing concentration of Ca^{2+} in the cytoplasm (c) can be found in subsection 4.5.1. This model is adjusted for temperature with the factor

$$\phi_{\rm E} = 3^{(T_0-T_{\rm E})/10}, \tag{19}$$

where $T_{\rm E} = 36$ °C denotes the temperature for the original electrophysiological experiment for constructing the model and $T_0 = 25$ °C denotes the temperature of the model during the simulations.

4.3 CB_1 Receptor on Excitatory Synaptic Terminal

The model developed for CB_1 receptors on inhibitory synaptic terminal (Zachariou et al, 2013) is adapted to describe the CB_1 receptor on excitatory terminal. The excitatory synaptic current for the post-synaptic cell is given by

$$I_{\rm AMPA} = \overline{g}_{\rm A}g_{\rm A}(V_{\rm E}^{\rm post}-V_{\rm A}), \tag{20}$$

where $V_{\rm E}^{\rm post}$ is the post-synaptic membrane potential, $\overline{g}_{\rm A}$ denotes the peak conductance, $g_{\rm A}$ presents the fraction of bound post-synaptic receptors and $V_{\rm A}$ corresponds to the reversal potential for AMPA receptors. The fraction of willing pre-synaptic Ca^{2+} channels ($w_{\rm A}$) is incorporated directly into the expression for the fraction of bound postsynaptic AMPA receptors

$$\frac{{\rm d}g_{\rm A}}{{\rm d}t} = \frac{g_{\rm A}^\infty(V_{\rm E}^{\rm pre},w_{\rm A})-g_{\rm A}}{\tau_{\rm A}}, \tag{21}$$

where

$$g_{\rm A}^\infty(V_{\rm E}^{\rm pre},w_{\rm A}) = \frac{1}{1+{\rm e}^{-(V_{\rm E}^{\rm pre}-kd_{\max}(1-w_{\rm A}))/5}}. \tag{22}$$

Here, $V_{\rm E}^{\rm pre}$ is the pre-synaptic excitatory cell membrane potential and $w_{\rm A}$ is described by

$$\frac{{\rm d}w_{\rm A}}{{\rm d}t} = k_-^{\rm A}(1-w_{\rm A}) - k_+^{\rm A}w_{\rm A}, \tag{23}$$

where $k_-^{\rm A}$ is the reluctant-to-willing transition rate (reflecting the voltage-dependent dissociation of the inhibiting $G_{\beta\gamma}$ subunit from the pre-synaptic Ca^{2+} channel) and is given by the expression

$$k_-^{\rm A} = \frac{\kappa_-^{\rm A}}{1+{\rm e}^{-V_{\rm pre}^{\rm E}/5}}, \tag{24}$$

where κ_-^{A} is the maximal reluctant-to-willing transition rate. The k_+^{A} in equation (23) is the willing-to-reluctant transition rate, reflecting the concentration of activated G proteins. Hence, k_+^{A} depends on the fraction of bound G proteins (denoted by q_{A}) and is given by the expression

$$k_+^{\mathrm{A}} = \kappa_+^{\mathrm{A}} q_{\mathrm{A}}, \tag{25}$$

where κ_+^{A} is the willing-to reluctant rate constant and q_{A} evolves according to

$$\frac{\mathrm{d}q_{\mathrm{A}}}{\mathrm{d}t} = \frac{q_{\mathrm{A}}^{\infty} - q_{\mathrm{A}}}{\tau_{q_{\mathrm{A}}}}. \tag{26}$$

Activation of CB_1 receptors by the exogenous CB agonist WIN55,212-2 (WIN) and the endogenously produced CB agonist 2-AG (AG) have different concentration response curves for inhibition of the pre-synaptic Ca^{2+} channels (Guo and Ikeda, 2004). This differential effect is incorporated in $q_{\mathrm{A}}^{\infty} = h_{\mathrm{AG}}(\overline{AG}) + h_{WIN}(WIN)$ where

$$h_{\mathrm{AG}}(\overline{AG}) = \frac{B_{\max}^{\mathrm{AG}}}{1 + (IC_{50}^{\mathrm{AG}}/\overline{AG})^{n_h}}, \tag{27}$$

$$h_{\mathrm{WIN}}(WIN) = \frac{B_{\max}^{\mathrm{WIN}}}{1 + (IC_{50}^{\mathrm{WIN}}/WIN)^{n_h}}. \tag{28}$$

Here, $B_{\max}^{\mathrm{AG}}/B_{\max}^{\mathrm{WIN}}$ and $IC_{50}^{\mathrm{AG}}/IC_{50}^{\mathrm{WIN}}$ denote the maximum inhibition and half-inhibition concentration for WIN55,212-2 and 2-AG, respectively. n_h presents the Hill coefficient and $\overline{AG} = (1 - MGL) \times AG$ corresponds to the fraction of AG (mobilised by the post-synaptic cell - see equation (31)) which is available post-degradation by MGL.

4.4 CB_1 Receptor on Inhibitory Synaptic Terminal

The inhibitory synaptic current for the post-synaptic cell is expressed by

$$I_{\mathrm{GABA_A}} = \overline{g}_{\mathrm{GA}} g_{\mathrm{GA}} (V_{\mathrm{E}}^{\mathrm{post}} - V_{\mathrm{GA}}), \tag{29}$$

where $\overline{g}_{\mathrm{GA}}$ denotes the peak conductance, g_{GA} presents the fraction of bound post-synaptic receptors and V_{GA} corresponds to the reversal potential for $GABA_A$ receptors. The dynamics of g_{GA} is governed by equations that have the same form as equations (21)–(28), but with different parameter values as listed in Table 1.We denote variables for the inhibitory synapse by the same name as for the excitatory synapse, but without the subscript or superscript A. For notational convenience, we suppressed a subscript A in equations (27) and (28), but note that each constant in these equations has a different value for inhibitory and excitatory synapses.

4.5 Endocannabinoid Synthesis

In the original model (Zachariou et al, 2013) synthesis of 2-AG through the Ca^{2+} mediated DAG (D) - DGLα (DL) pathway and its oxidation by COX-2 (COX) in the post-synaptic cell are described by the equations

$$\frac{\mathrm{d}D}{\mathrm{d}t} = J_{\mathrm{CA}}(c) - J_{\mathrm{DEG}}(D) - J_{\mathrm{AG}}(D), \tag{30}$$

$$\frac{\mathrm{d}AG}{\mathrm{d}t} = J_{\mathrm{AG}}(D) - J_{\mathrm{COX}}(AG). \tag{31}$$

Here, $J_{\mathrm{CA}}(c)$ denotes Ca^{2+} dependent DAG production and is modelled by a Hill function with a Hill coefficient of 2,

$$J_{\mathrm{CA}}(c) = \frac{v_c c^2}{K_c^2 + c^2}\,. \tag{32}$$

$J_{\mathrm{DEG}}(D) = k_d D$ presents the degradation of DAG, $J_{\mathrm{AG}}(D) = k_{11} D \cdot DL$ models metabolising of DAG by DGLα to 2-AG and $J_{\mathrm{COX}}(AG) = k_{12} AG \cdot COX$ presents the loss of 2-AG due to oxidation by COX-2.

4.5.1 Intracellular Calcium

The concentration of Ca^{2+} in the cytoplasm (c) and in the ER (s) is described by the following differential equations (Zachariou et al, 2013)

$$\frac{\mathrm{d}c}{\mathrm{d}t} = J_{\mathrm{LEAK}} - J_{\mathrm{SERCA}} + J_{\mathrm{IN}} + J_{\mathrm{CHAN}} - J_{\mathrm{PMCA}}, \tag{33}$$

$$\frac{\mathrm{d}s}{\mathrm{d}t} = \frac{1}{\beta}(J_{\mathrm{SERCA}} - J_{\mathrm{LEAK}}), \tag{34}$$

where β denotes the ratio of the effective volumes of the ER to the cytoplasm. J_{IN} is a constant influx of Ca^{2+} from the cell exterior and $J_{\mathrm{LEAK}} = k_2(s - c)$ presents a leak Ca^{2+} flux from the ER to the cytoplasm. J_{SERCA} and J_{PMCA} present the fluxes through SERCA and PMCA pumps, respectively, and are described by Hill functions with a Hill coefficient of 2,

$$J_{\mathrm{Y}} = \frac{v_{\mathrm{Y}} c^2}{K_{\mathrm{Y}}^2 + c^2}, Y \in \{\mathrm{SERCA}, \mathrm{PMCA}\}. \tag{35}$$

J_{CHAN} corresponds to the L-type Ca^{2+} channel current $I_{\mathrm{Ca,L}}$ and is given by $J_{\mathrm{CHAN}} = -f_{\mathrm{Ca,L}} I_{\mathrm{Ca,L}}$ where $f_{\mathrm{Ca,L}}$ is the current-to-flux conversion factor (Zachariou et al, 2013).

4.6 External Input

The synaptic current for the pre-synaptic cell, which is an AMPA synapse for both the excitatory (E) and the inhibitory (I) cells, is given by

$$I_{\mathrm{syn,X}} = \overline{g}_{\mathrm{X}} n_{\mathrm{X}} g_{\mathrm{X}} (V_{\mathrm{X}}^{\mathrm{pre}} - V_{\mathrm{A}}), \quad X \in \{\mathrm{E}, \mathrm{I}\}, \tag{36}$$

where g_{X} is the synaptic conductance and is given by an alpha function, $\overline{g}_{\mathrm{X}}$ denotes the peak conductance, $n_{\mathrm{X}} = \mathrm{e}^1 \tau_{\mathrm{X}}$ is a normalization factor, $V_{\mathrm{X}}^{\mathrm{pre}}$ is the pre-synaptic membrane potential and V_{A} corresponds to the reversal potential for AMPA receptors. The synaptic current for the post-synaptic excitatory cell is given by

$$I_{\mathrm{syn,E}} = I_{\mathrm{AMPA}} + I_{\mathrm{GABA_A}} \tag{37}$$

where $I_{\mathrm{AMPA}}, I_{\mathrm{GABA_A}}$ are described by equations (20) and (29).

4.7 DSI/DSE Measure

The magnitude of depolarisation-induced suppression of inhibitory/excitatory events was measured as the percentage of the minimum amplitude of IPSPs/EPSPs acquired after the end of the depolarisation ($\mathrm{Amp_{post}}$) relative to the average amplitude of IPSPs/EPSPs acquired before the depolarisation ($\mathrm{Amp_{pre}}$). The depression due to agonist administration was estimated similarly as the percentage of the minimum IPSP/EPSP amplitude during drug application relative to the average IPSP/EPSP before drug application. This is expressed as a percentage by

$$\mathrm{Amp} = 100 \frac{\mathrm{Amp_{post}}}{\mathrm{Amp_{pre}}}. \tag{38}$$

The DSI/DSE measure is given by

$$\mathrm{DSI/DSE} = 100 - \mathrm{Amp}. \tag{39}$$

Note that the terms DSI/DSE are used in principle only for the case of depolarisation induced suppression whereas a more appropriate term in the case of drug administration is eCB-iSTD/eCB-eSTD (eCB-mediated short-term plasticity of inhibition/excitation). However, for notational convenience we here use the terms DSI/DSE for both cases.

4.8 Spiketrain Statistics

4.8.1 Skewness coefficient

The skewness coefficient (SC) is a measure of the asymmetry of the interspike interval (ISI) distribution (Shinomoto and Sakai, 1999). Let $\mu = \langle ISI \rangle$ denote the mean of the ISI distribution, then

$$SC = \left\langle (ISI - \mu)^3 \right\rangle \left\langle (ISI - \mu)^2 \right\rangle^{-3/2}. \tag{40}$$

Note that $SC = 2$ for a Poisson process.

4.9 Simulation Protocols

Protocol I. This experimental protocol is adapted from (Ohno-Shosaku et al, 2002a). Experiments were performed in hippocampal cultures at room temperature. Both the pre- and post-synaptic cells are clamped at -80 mV. The pre-synaptic cell is then depolarised to 80 mV for 2 msec with 0.2 Hz to evoke inhibitory neurotransmitter release. The agonist WIN55,212 of $\mathrm{CB_1}$ receptors is applied for 1 min (until the steady state of DSI is reached).
Protocol II. This experimental protocol is adapted from (Ohno-Shosaku et al, 2002b, 2001). Experiments were performed in hippocampal cultures at room temperature. Both the pre- and post-synaptic cells are clamped at -80 mV. The pre-synaptic cell is then depolarised to 80 mV for 2 msec with 0.2 Hz to evoke inhibitory neurotransmitter release. The post-synaptic cell is depolarised to 0 mV for varying length of time (0.5-5 sec).
Protocol III. Simulations are performed at room temperature. Both the pre- and post-synaptic cells are clamped at -80 mV. The pre-synaptic cell is then depolarised to 80 mV for 2 msec with a certain frequency in the range of [0.2-100] Hz to evoke inhibitory (for DSI) or excitatory (for DSE) neurotransmitter release while the agonist 5 μM of WIN55,212 of $\mathrm{CB_1}$ receptor is applied for 1 min. The simulation is repeated for different values of $\kappa_-^{\mathrm{A}}, \kappa_-$ [0.005,0.01,0.05,0.3].
Protocol IV. A pre-synaptic excitatory cell contacts a post-synaptic excitatory cell. The pre-synaptic cell is stimulated with a Poisson spike train of various frequencies (5-80 Hz) for 1 min. The maximum synaptic conductances are $\overline{g}_{\mathrm{E}} = \overline{g}_{\mathrm{A}} = 1\mathrm{mS/cm^2}$. The simulation is repeated for different values of $\kappa_-^{\mathrm{A}} = [0.005, 0.01, 0.05, 0.3]$.
Protocol V. One excitatory and one inhibitory pre-synaptic cell are connected to a post-synaptic excitatory cell. Both pre-synaptic cells are stimulated with a Poisson spike train of 5 Hz frequency. The maximum synaptic conductances are $\overline{g}_{\mathrm{E}} = \overline{g}_{\mathrm{A}} = 1\mathrm{mS/cm^2}$, $\overline{g}_{\mathrm{I}} = \overline{g}_{\mathrm{GA}} = 0.5\mathrm{mS/cm^2}$. The simulation is repeated for different values of $\kappa_-^{\mathrm{A}}, \kappa_-$ [0.005,0.3].

4.10 Simulations

For Figs. 2-4 the system of ordinary differential equations was solved numerically using a fourth-order Runge-

Kutta algorithm (time step = 0.01 msec) and was implemented in XPPAUT [100] and the Python interface for XPP (XXPy) (Nowacki, 2011). The system was initialised with the same set of values (reflecting the steady state of the system). For Figs. 5-6 the system was simulated with the BRIAN simulator (Goodman, 2009) with a time step = 0.03 msec and random initial conditions. Simulations' results were plotted and analysed in MATLAB (MATLAB, 2010). Upon publication, the model will be made available for public download from the ModelDB model repository of the SenseLab database (senselab.med.yale.edu/SenseLab/ModelDB).

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Fig. 1 Schematic representation of the DSI/DSE model. Key factors underlying the synthesis and release of the eCB 2-AG at post-synaptic sites are illustrated. Also pre-synaptic signalling by CB_1 receptors at hippocampus excitatory and inhibitory synaptic terminals in included. For more information see text - section 1.1.

Fig. 2 (a)-(c) Experimental results (Ohno-Shosaku et al, 2001, 2002b,a) with error bars and simulation results from the detailed DSI/DSE model (solid lines) and the eCB synthesis reduced model (dashed lines). **(a)** The relative EPSP/IPSP amplitude reduction in response to administration of CB agonist (WIN55,212-2). Plotted with a logarithmic scale for the x axis (see Protocol I). **(b)** The relative EPSP/IPSP amplitude reduction in response to voltage clamp depolarisation of the post-synaptic cell (see Protocol II). **(c)** The time course of DSI/DSE due to a 5 sec depolarisation of the post-synaptic cell at time 0 sec. The amplitude (normalised to the value before depolarisation) of the IPSPs/EPSPs is plotted just before, during and after the depolarising pulse (see Protocol II). **(d)** Experimentally obtained concentration response curves (solid lines) from (Guo and Ikeda, 2004) for the effect of exogenous CB agonist, WIN55,212-2 (WIN) and of endogenous CB agonist, 2-AG (AG), on the pre-synaptic N-type VGCCs on inhibitory CB sensitive terminals and response curves obtained through fitting (dashed lines) for excitatory CB sensitive terminals. Plotted with a logarithmic scale for the x axis.

Fig. 3 (a)-(d) Experimental results (Ohno-Shosaku et al, 2001, 2002b) with error bars and simulation results from the detailed DSI/DSE model (solid lines) and the overall reduced model in terms of both eCB synthesis and intracellular Ca^{2+} (dashed lines) (see Protocol II). **(a)** The relative EPSP/IPSP amplitude reduction in response to voltage clamp depolarisation of the post-synaptic cell. **(b)** The time course of DSI/DSE due to a 5 sec depolarisation of the post-synaptic cell at time 0 sec. The amplitude (normalised to the value before depolarisation) of the IPSPs/EPSPs is plotted just before, during and after the depolarising pulse. **(c)** The evoked cytoplasmic Ca^{2+} transient (c) due to a 5 sec depolarisation of the post-synaptic cell at time 0 sec. **(d)** The evoked eCB transient (AG) due to a 5 sec depolarisation of the post-synaptic cell at time 0 sec.

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Fig. 4 DSI **(a)** and DSE **(b)** as modulated for different firing frequencies of the pre-synaptic cell (0.2-100 Hz) during CB agonist WIN212,55 (5 μM) administration (see Protocol III). Results are plotted for different values of the parameters $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = [0.005, 0.01, 0.05, 0.3]$ msec^{-1}.

Fig. 5 Panels **(a)-(d)**: Simulations of two excitatory cells (PRE, POST) connected with a CB sensitive synapse (see Protocol IV). In each panel, the top graph shows the spiketrains of the two cells and the fraction of willing Ca^{2+} channels w_{A}. The bottom graph shows the eCB transient (AG) of the post-synaptic cell. **(a)** The pre-synaptic cell is stimulated with 5 Hz Poisson input and $\kappa_{-}^{\mathrm{A}} = 0.3$. **(b)** The pre-synaptic cell is stimulated with 40 Hz Poisson input and $\kappa_{-}^{\mathrm{A}} = 0.3$. **(c)** The pre-synaptic cell is stimulated with 5 Hz Poisson input and $\kappa_{-}^{\mathrm{A}} = 0.005$. **(d)** The pre-synaptic cell is stimulated with 40 Hz Poisson input and $\kappa_{-}^{\mathrm{A}} = 0.005$. **(e)** The post- versus the pre- synaptic cell firing rate for different values of κ_{-}^{A}. **(f)** The Skewness Coefficient (SC) for the spiketrain of the post-synaptic cell (in solid lines) is plotted versus the stimulation frequency of the pre-synaptic cell for different values of κ_{-}^{A}. Note that the results for κ_{-}^{A}=0.05, 0.3 lie on top of each other. For reference we also plot the SC for the pre-synaptic cell (dashed line).

Fig. 6 Simulations of an excitatory cell (PRE-E) and an inhibitory cell (PRE-I) connected with CB sensitive synapses to an excitatory cell (POST). The pre-synaptic cells are both stimulated with a 5 Hz Poisson input (see Protocol V). In each panel, the top graph shows the spiketrains of the three cells. The middle graph shows the eCB transient (AG) of the post-synaptic cell, and the bottom graph shows the fraction of willing Ca^{2+} channels for the pre-synaptic inhibitory (w) and excitatory terminals (w_{A}). **(a)** The post-synaptic cell is stimulated briefly with 50 Hz for 1 sec and $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.3$. **(b)** The post-synaptic cell is stimulated briefly with 50 Hz for 1 sec and $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.005$. **(c)** The post-synaptic cell is stimulated briefly with 50 Hz for 5 sec and $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.3$. **(d)** The post-synaptic cell is stimulated briefly with 50 Hz for 5 sec and $\kappa_{-}^{\mathrm{A}}, \kappa_{-} = 0.005$.

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Table 1 Parameters for the CB_1 receptor, plastic excitatory and inhibitory synapses, eCB synthesis and intracellular Ca^{2+} dynamics. The parameters of the CB_1 receptor that shift the model from DSI to DSE are indicated in the DSE column. Parameters with no entry in the DSE column have the same value as in the DSI column. In the REDUCTION column the modified parameter for the PMCA pump for the reduced model (final reduction incorporating both eCB synthesis and calcium dynamics for both the DSI and DSE cases) is shown. Variables that are set to 0 are denoted by a star ($\star$). No entry in this column indicates the same value as in the DSI column.

PARAMETER	DESCRIPTION	DSI	DSE	REDUCTION
CB_1 Receptor				
κ_+, κ_+^{A} (msec^{-1})	Maximal willing-to-reluctant transition rate	0.0006	0.0004	
κ_-, κ_-^{A} (msec^{-1})	Reluctant-to-willing transition rate constant	0.3		
$B_{\max}^{\mathrm{AG}}$	Maximum inhibition (due to AG)	0.5		
$B_{\max}^{\mathrm{WIN}}$	Maximum inhibition (due to WIN)	0.48		
IC_{50}^{WIN} (μM)	Half-inhibition concentration (WIN)	0.002	0.06	
IC_{50}^{AG} (μM)	Half-inhibition concentration (AG)	0.48	16	
n_h	Hill coefficient	1.2		
$\tau_{q_{\mathrm{A}}}$ (msec)	CB_1 receptor unbinding time constant	1000		
Synapses				
$kd_{\max}$ (mV)	Scaling factor	100		
$\overline{g}_{\mathrm{GA}}$ ($\mathrm{mS/cm}^2$)	GABA peak conductance	0.3		
V_{GA} (mV)	GABA reversal potential	-80		
$\overline{g}_{\mathrm{A}}$ ($\mathrm{mS/cm}^2$)	AMPA peak conductance	0.3		
V_{A} (mV)	AMPA reversal potential	0		
τ_{A} (msec)	AMPA synaptic time constant	1		
τ_{GA} (msec)	GABA synaptic time constant	1		
$\overline{g}_{\mathrm{E}}$ ($\mathrm{mS/cm}^2$)	AMPA peak conductance (input on pre-synaptic excitatory cell)	1		
$\overline{g}_{\mathrm{I}}$ ($\mathrm{mS/cm}^2$)	AMPA peak conductance (input on pre-synaptic inhibitory cell)	0.5		
τ_{E} (msec)	AMPA synaptic time constant (input on pre-synaptic excitatory cell)	1		
τ_{I} (msec)	AMPA synaptic time constant (input on pre-synaptic inhibitory cell)	1		
2-AG Synthesis				
v_{c} ($\mu\mathrm{Mmsec}^{-1}$)	Maximal Ca^{2+} evoked DAG synthesis rate	0.7		
K_{c} (μM)	Half activation Ca^{2+} evoked DAG synthesis	10		
k_{d} ($\mu\mathrm{Mmsec}^{-1}$)	DAG degradation rate	0.66×10^{-3}		
k_{11} ($\mu\mathrm{Mmsec}^{-1}$)	2-AG synthesis rate	0.5		
k_{12} ($\mu\mathrm{Mmsec}^{-1}$)	2-AG degradation rate	0.01		
DL (μM)	DGLα availability	50		
COX (μM)	COX-2 availability	1		
Calcium Dynamics				
v_{PMCA} ($\mu\mathrm{Mmsec}^{-1}$)	Maximal PMCA pump rate	0.01×10^{-3}		0.55×10^{-3}
K_{PMCA} (μM)	PMCA pump half-activation	0.12		
v_{SERCA} ($\mu\mathrm{Mmsec}^{-1}$)	Maximal SERCA pump rate	0.9×10^{-3}		$\star$
K_{SERCA} (μM)	SERCA pump half-activation	0.1		$\star$
J_{IN} ($\mu\mathrm{Mmsec}^{-1}$)	Constant influx	0.0004×10^{-3}		$\star$
k_2 ($msec^{-1}$)	Ca^{2+} leak rate	0.0203×10^{-3}		$\star$
ϵ	Strength of plasma membrane fluxes	1		$\star$
β	Ratio of effective volume ER/cytocol	0.185		$\star$