Endocannabinoids facilitate the induction of LTP in the hippocampus

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Exogenous cannabinoids disrupt behavioral learning and impede induction of long-term potentiation (LTP) in the hippocampus[1], yet endogenous cannabinoids (endocannabinoids)[2] transiently suppress inhibitory post-synaptic currents (IPSCs)[3,4] by activating cannabinoid CB1 receptors on GABAergic interneurons[5]. We found that release of endocannabinoids by a rat CA1 pyramidal cell during this depolarization-induced suppression of inhibition (DSI)[6,7] enabled a normally ineffective train of excitatory post-synaptic currents (EPSCs) to induce LTP in that cell, but not in neighboring cells. By showing that endocannabinoids facilitate LTP induction and help target LTP to single cells, these data shed new light on the physiological roles of endocannabinoids and may lead to a greater understanding of their effects on behavior and potential clinical use.

We simultaneously recorded whole-cell responses from pyramidal cells and field EPSPs (fEPSPs) from the CA1 region of the rat hippocampal slice (**Fig. 1**) and used stimulus trains that by themselves were incapable of inducing LTP in control solution (saline). Each 'weak' train (WT) consisted of 20 pulses at 50 Hz and at stimulus intensities roughly one-third of population spike threshold. Experiments (~10%) were discarded if the weak train induced LTP of the fEPSP. Single EPSCs and fEPSPs were evoked by the weak stimulus delivered at 0.1 or 0.05 Hz. One weak train, delivered 3 or 13 s after a DSI-inducing voltage step (1-s depolarization from the holding potential of –70 mV to 0 mV), was applied through a whole-cell pipette. This protocol (D + WT, **Fig. 1a**) produced LTP of the whole-cell EPSC, but not of the fEPSP, in eight of nine cases (**Fig. 1b** and **c**). Without a preceding depolarizing voltage step, the weak train did not induce LTP of either the EPSC or the fEPSP (**Fig. 1d** and **g**).

When the weak train was preceded by the voltage step, the envelope of the EPSCs during the train was larger than it was when no step was given; the increase of the second EPSC over the first was particularly apparent (**Fig. 1e**). We inferred that DSI-induced disinhibition[8] enabled the normally ineffective EPSCs evoked by the train to initiate NMDA (*N*-methyl-D-aspartate) receptor–dependent LTP[9] in the cell.

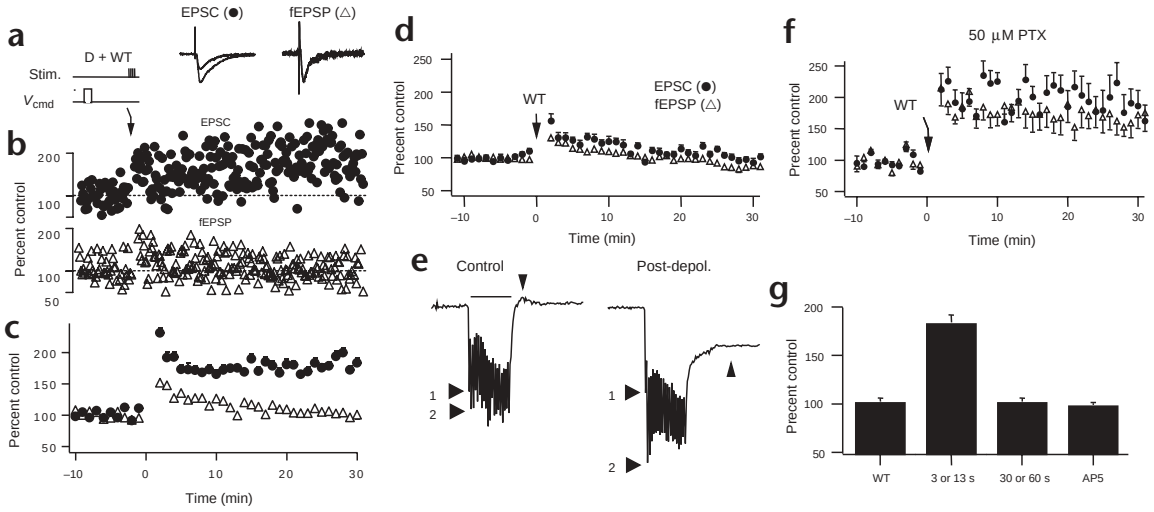

Fig. 1. Induction of LTP in single CA1 pyramidal cells by a weak train preceded by a DSI-inducing voltage step. (**a**) The D + WT protocol. A 1-s depolarizing voltage step to 0 mV (D) preceded a 20-pulse train (50 Hz) of stimuli (WT). V_{cmd}, voltage command. Sample traces of EPSCs and fEPSPs recorded when indicated in (**b**). (**b**) Top, EPSC amplitudes (●); bottom, the simultaneously recorded fEPSP (Δ), both evoked at 0.1 Hz. (**c**) Group data (n = 9). EPSCs were potentiated (to 183 ± 8.7% of control); fEPSPs were not potentiated (101 ± 6.2%). (**d**) Neither EPSCs nor fEPSPs were potentiated (94 ± 2.2% and 102 ± 4.4% of control, respectively, n = 6) by a WT if no depolarizing step was given to the cell. (**e**) Traces of EPSCs recorded during a 400-ms WT (at bar) in control or after a depolarizing voltage step. The integral of the waveform over 500 ms was larger (122 ± 15%, $P < 0.05$) after the step (n = 4) than before (n = 5); the ratio of the second EPSC to the first increased by 116 ± 25% ($P < 0.05$). (**f**) Both the EPSC and fEPSP were potentiated (to 175 ± 29.7% and 187 ± 24.6%, n = 5) by a WT in picrotoxin (PTX), even though no depolarizing step was given (n = 5). (**g**) Summary of control experiments showing that EPSC LTP was produced when intervals between the weak train and the depolarizing pulse were 3 or 13 s, but not 30 or 60 s (108 ± 5.7%, n = 6). In addition, 50 μM AP5 prevented EPSC LTP induction (98 ± 3.4%, n = 4). *, significant difference from controls (ANOVA, $P < 0.05$). Conventional hippocampal slices were used[10]; procedures approved by University of Maryland School of Medicine Institutional Animal Care and Use Committee. Extracellular solution contained 120 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose and 2.5 mM $CaCl_2$, and was bubbled (30°C) with 95% O_2/5% CO_2, pH 7.4. The whole-cell pipette contained 140 mM $CsCH_3SO_3$, 10 mM HEPES, 5 mM sodium phosphocreatine, 0.2 mM BAPTA, 0.3 mM Tris-GTP, 2 mM Mg-ATP, 3 mM KCl, 1 mM $MgCl_2$ and 5 mM QX-314; pH 7.25. Electrode resistance in the bath was 4–7 MΩ. Field potential electrodes were filled with bubbled extracellular solution.

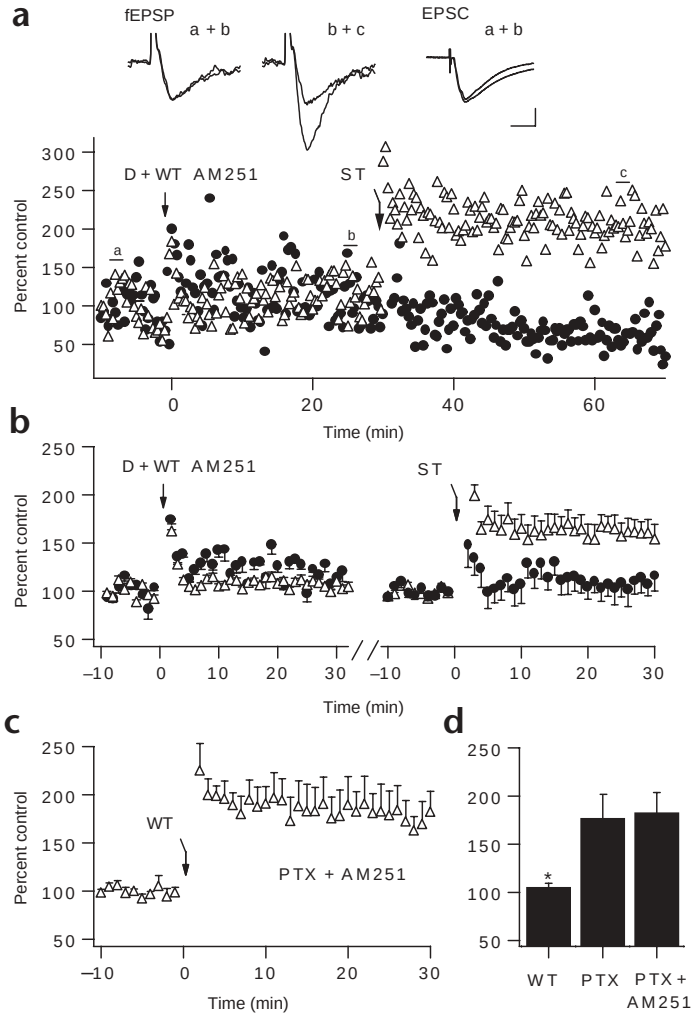

Fig. 2. The CB1R antagonist, AM-251 (2 μM), prevents LTP induced by the D + WT protocol, without affecting LTP induction. (**a**) AM-251 solution perfused for at least 20 min before delivery of D + WT (**Fig. 1a**) at time 0. Traces taken when indicated. Thirty min after D + WT, still in AM-251, a strong train (ST, 100 Hz for 1 s, repeated three times) produced LTP in the fEPSP. EPSC LTP was prevented by washout. (**b**) Group data from experiments as in (**a**). Neither EPSC LTP (98.2 ± 18.6%) nor fEPSP LTP (107 ± 3.3%) were produced (n = 6) in AM-251. (**c**) Group data (n = 5) in which the WT alone produced LTP (182 ± 21.4%; **Fig. 1f**) in 100 μM picrotoxin (PTX) and AM-251. (**d**) Summary of LTP induction caused by a WT in either control, PTX, or PTX and AM-251.

We tested whether a weak train delivered in the presence of picrotoxin, an antagonist of the γ-aminobutyric acid (GABA) type A receptor, could induce LTP of both EPSC and fEPSP, even with no depolarizing voltage step. This was confirmed (**Fig. 1f**), showing that IPSPs normally maintained the weak train below threshold for LTP induction. As DSI decays in 45–60 s (ref. 10), the ability of D + WT to facilitate LTP induction should depend on the interval between the step and the weak train. Indeed, LTP was produced when the interval was 3 or 13 s, but not when it was 30 or 60 s (**Fig. 1g**). Finally, we confirmed that NMDA receptor antagonism (by AP5) prevented the induction of EPSC LTP (**Fig. 1g**).

The critical question was whether CB1R antagonists could block LTP induced by the D + WT protocol, because DSI-induced disinhibition is mediated by activation of CB1Rs on interneuron terminals. To test this, we delivered D + WT to cells in slices pretreated for at least 20 min with AM-251 (2 μM), which blocks CB1Rs and DSI[3,4], and found neither EPSC LTP nor fEPSP LTP (**Fig. 2a** and **b**). AM-251 did not block LTP induction *per se*, because fEPSP LTP was subsequently induced by a strong stimulus protocol (strong train, ST, **Fig. 2b**) delivered to the same slices in AM-251, or by a weak train alone given in the presence of picrotoxin (**Fig. 2c**). 'Washout' of the LTP process caused by prolonged whole-cell recordings[11] explains the lack of EPSC LTP in **Fig. 2a** and **b**. Therefore, in D + WT, AM-251 prevented EPSC LTP induction by blocking DSI, not by affecting the LTP process directly.

We conclude that by mediating DSI, endocannabinoids facilitate LTP induction in single neurons activated within an unpotentiated population of neighboring neurons. A burst of action potentials in a single cell can induce DSI[6,12], and individual hippocampal cells *in vivo* often fire bursts independently[13]. Because released endocannabinoids travel ≤20 μm^3, DSI-mediated LTP facilitation must be restricted in space. For example, if incoming excitatory inputs affect a neuronal population, LTP induction should be enabled only in those neurons undergoing DSI. Such targeted LTP could underlie behavioral learning associated with LTP induction in limited subsets of cells, such as the establishment of 'place fields' during maze learning in rats[14]. Our results do not contradict reports that exogenous cannabinoids antagonize LTP induction[1] because exogenous cannabinoids applied globally affect all cells and types of cannabinoid receptors, including those on excitatory synapses[15]. We propose that global activation of cannabinoid receptors, by disrupting the exquisite temporal and spatial selectivity of coding and recall mediated by endocannabinoids, contributes to the learning and memory deficiencies associated with cannabinoid drug abuse.

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Competing interests statement

The authors declare that they have no competing financial interests.

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