Research

Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala

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Previously, we found that in the lateral amygdala (LA) of the mouse, WIN55,212-2 decreases both glutamatergic and GABAergic synaptic transmission via activation of the cannabinoid receptor type 1 (CB1), yet produces an overall reduction of neuronal excitability. This suggests that the effects on excitatory transmission override those on inhibitory transmission. Here we show that CB1 activation by WIN55,212-2 and Δ^9 -THC inhibits long-term depression (LTD) of basal synaptic transmission in the LA, induced by low-frequency stimulation (LFS; 900 pulses/1 Hz). The CB1 agonist WIN55,212-2 blocked LTD via $G_{i/o}$ proteins, activation of inwardly rectifying K^+ channels (K_{ir} s), inhibition of the adenylate cyclase-protein kinase A (PKA) pathway, and PKA-dependent inhibition of voltage-gated N-type Ca^{2+} channels (N-type VGCCs). Interestingly, WIN55,212-2 effects on LTD were abolished in CB1 knock-out mice (CB1-KO), and in conditional mutants lacking CB1 expression only in GABAergic interneurons, but were still present in mutants lacking CB1 in principal forebrain neurons. LTD induction per se was unaffected by the CB1 antagonist SRI41716A and was normally expressed in CB1-KO as well as in both conditional CB1 mutants. Our data demonstrate that activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the LA. These findings suggest that CB1 expressed on either glutamatergic or GABAergic neurons play a differential role in the control of synaptic transmission and plasticity.

The amygdala integrates inputs from various brain regions such as the hippocampus, the prefrontal cortex, and the thalamus, providing a common output and playing an important role in fear memory, triggered reminders of past aversive experiences, and also pain processing (Le Doux 2000; Davis and Whalen 2001; Neugebauer et al. 2004). We previously showed that endocannabinoids released in the amygdala are important for the extinction of aversive memory (Marsicano et al. 2002). Most behavioral effects of exogenously applied cannabinoids (Manning et al. 2001; Pertwee 2001) are mediated by the $G_{i/o}$ protein-coupled cannabinoid type 1 (CB1) receptor (Zimmer et al. 1999), which is highly expressed in many brain regions, including the amygdala (Herkenham 1995). CB1 receptors have been identified mainly on GABAergic interneurons (Marsicano and Lutz 1999; Katona et al. 2001), but recently also glutamatergic neurons in the hippocampus were shown to contain functional CB1 receptors (Doménici et al. 2006; Kawamura et al. 2006; Monory et al. 2006; Takahashi and Castillo 2006). In the amygdala, electrophysiological findings also speak in favor of both glutamatergic and GABAergic localization of CB1 receptors. In the lateral amygdala (LA), the agonist WIN55,212-2 reduced isolated glutamatergic and GABAergic currents (Azad et al. 2003) via activation of CB1 receptors. Although it may be assumed that these effects may compensate each other, the application of WIN55,212-2 induced an overall decrease of basal synaptic transmission. This indicates that in the LA, cannabinoid-induced modulation of neuronal

activity is mainly determined by CB1 receptors located on excitatory pyramidal neurons.

Earlier studies showed that CB1 receptor activation also modulates synaptic plasticity, since cannabinoids influence long-term potentiation and long-term depression (LTD) of synaptic transmission in different brain regions (Misner and Sullivan 1999; Auclair et al. 2000). However, little is known about the mechanisms and the neuronal sites where these CB1-mediated effects are exerted. Low frequency stimulation (LFS) of afferents with 900 pulses at 1 Hz induces LTD of basal synaptic transmission, which involves the simultaneous activation of both excitatory and inhibitory inputs (Rammes et al. 2001; DeBock et al. 2003). The present study details the role and site of CB1 receptors in this form of synaptic plasticity.

Results

LTD of field potentials and excitatory postsynaptic currents in the LA is inhibited by exogenous application of a CB1 agonist in a fully CB1-dependent manner

LFS with 900 pulses at 1 Hz induced a robust LTD of both extracellularly recorded field potentials (FPs) and intracellularly recorded excitatory postsynaptic currents (EPSCs) in the LA. Responses were reduced to $70 \pm 5\%$ (FP; $n = 7$, $P < 0.05$; Fig. 1A) and $75 \pm 7\%$ (EPSCs; $n = 10$, $P < 0.05$; Fig. 1B) of control, respectively. Since the FP had short and constant latencies of ~ 3 msec and was reliably followed by a frequency stimulation up to 50 Hz, the evoked potentials appeared to reflect the activity of a mono-

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Figure 1. Cannabinoids block the induction of LTD of synaptic transmission in the LA. (A) Low frequency stimulation (LFS; 900 pulses/1 Hz) of afferents induces a LTD of extracellularly recorded excitatory postsynaptic field potentials (FPs) in the LA ($n = 7$; $P < 0.05$; filled circles). The CB1 receptor agonist WIN55,212-2 blocks the induction of LTD at the concentration of 2.5 μM (WIN 2.5 μM : $n = 7$; $P > 0.05$; open circles), while the lower concentration of 1.0 μM has no effect on LTD induction ($n = 6$; $P < 0.05$; open squares). (B) The same stimulation paradigm depresses intracellularly recorded EPSCs in the LA ($n = 10$; $P < 0.05$; filled triangles), without changing the paired-pulse ratio (interstimulus interval [ISI] 50 msec; $n = 10$; $P > 0.05$; filled squares). LTD of EPSCs is also blocked by WIN55,212-2 (2.5 μM ; $n = 7$; $P > 0.05$; open triangles). Representative traces are shown. All data are normalized to the respective control values (last 10 min before LTD induction). Asterisks mark the stimulation artifacts.

synaptic connection. LFS did not change paired-pulse ratio (PPR) of EPSCs (control: 1.13 ± 0.08 ; LFS: 1.17 ± 0.07 ; $n = 10$; $P > 0.05$; Fig. 1B), which is an index for an alteration of presynaptic transmitter release (Manabe et al. 1993).

Application of the CB1 receptor agonist WIN55,212-2 reduced the FP amplitudes at two different concentrations (1 μM : $66 \pm 4\%$; 2.5 μM : $60 \pm 8\%$) and also decreased the EPSCs (2.5 μM : $69 \pm 10\%$). As soon as the responses were stable for at least 20 min after WIN55,212-2 application, LFS was applied. WIN55,212-2 completely blocked the induction of LTD of FPs and EPSCs at the higher concentration (FPs: control + 2.5 μM WIN55,212-2: 100%; LFS: $103 \pm 5\%$; $n = 7$; $P > 0.05$; Fig. 1A; EPSCs: control + WIN55,212-2: 100%; LFS: $99 \pm 4\%$; $n = 7$; $P > 0.05$; Fig. 1B), while the low concentration had no effect on LTD induction (FPs: control + 1.0 μM WIN55,212-2: 100%; LFS: $72 \pm 3\%$; $n = 6$; $P < 0.05$; Fig. 1A); Reducing only afferent stimulation to attenuate synaptic responses had no effect on LTD induction per se (data not shown). In order to rule out whether WIN55,212-2 blocked or just occluded LTD, LFS was applied in the presence of riluzole (10 μM), a substance that exerts similar effects on excitatory and inhibitory synapses as the CB1 agonist:

It is known to inhibit the release of glutamate and, at concentrations $>3 \mu\text{M}$, also of GABA (Westphalen and Hemmings 2003). After application of this substance, which per se decreased synaptic transmission to $72 \pm 4\%$ and isolated GABA_A receptor-mediated IPSCs to 50% ($n = 5$), LFS still induced LTD (control + riluzole: 100%; LFS: $62 \pm 7\%$; $n = 4$; $P < 0.05$; data not shown). These experiments demonstrate the specificity of the WIN55,212-2-mediated effect on LTD.

Application of the CB1 receptor antagonist SR141716A (2.5 μM) for at least 45 min neither changed synaptic transmission nor affected LTD induction (FP: control + SR141716A: 100%; LFS: $69 \pm 15\%$; $n = 5$; $P < 0.05$; Fig. 2A), suggesting that LTD is independent of endogenous synthesis and release of endocannabinoids. However, pretreatment of the slices with SR141716A

Figure 2. The effect of WIN55,212-2 on LTD is mediated by CB1 receptors. (A) Pretreatment of the slices with the CB1 receptor antagonist SR141716A (SR; 2.5 μM) prior to WIN55,212-2 application prevents the effect of WIN55,212-2 and restores LTD induction ($n = 6$; $P < 0.05$; open circles). Pretreatment of the slices with the antagonist SR141716A per se does not affect LTD induction ($n = 5$; $P < 0.05$; filled circles). (B) WIN55,212-2 (2.5 μM) does not inhibit LTD of EPSCs in mice lacking CB1 receptors (CB1-KO; $n = 5$; effect of LFS: $P < 0.05$; open circles) but in slices from their wild-type littermates ($n = 4$; effect of LFS: $P > 0.05$; filled circles). (C) In the presence of Δ^9 -THC (1 μM), LFS only induced weak LTD ($n = 6$; $P > 0.05$; open circles). In control slices, LFS induced LTD ($n = 6$; $P < 0.05$; filled circles). Representative traces are shown. All data are normalized to the respective control values (last 10 min before LTD induction). Asterisks mark the stimulation artifacts.

(2.5 μ M) 30 min prior to WIN55,212-2 application prevented the effect of WIN55,212-2 on synaptic transmission (FP: control + SR141716A: 100%; WIN55,212-2: $99 \pm 5\%$; $n = 6$; $P > 0.05$) and restored LTD induction (FP: control + SR141716A + WIN55,212-2: 100%; LFS: $75 \pm 7\%$; $n = 6$; $P < 0.05$; Fig. 2A).

Accordingly, LFS induced LTD in both CB1-KO mice and their wild-type littermates (data not shown). However, in the CB1-KO mice, WIN55,212-2 (2.5 μ M) neither decreased synaptic currents (EPSC: control: 100%; WIN55,212-2: $97 \pm 2\%$; $n = 5$; $P > 0.05$) nor blocked the induction of LTD (EPSC: control + WIN55,212-2: 100%; LFS: $79 \pm 9\%$; $n = 5$; $P < 0.05$; Fig. 2B), whereas the expected effects were observed in the wild-type mice (EPSC: control: 100%; WIN55,212-2: $65 \pm 8\%$; $n = 4$; $P < 0.05$; control + WIN55,212-2: 100%; LFS: $101 \pm 3\%$; $n = 4$; $P > 0.05$; Fig. 2B).

We also tested whether Δ^9 -tetrahydrocannabinol (Δ^9 -THC) affects LTD induction. The application of Δ^9 -THC (1 μ M) decreased FP amplitude to $74 \pm 13\%$ ($n = 6$) (data not shown). LTD expression was remarkably reduced in the presence of Δ^9 -THC and almost returned to baseline levels (control: 100%; LFS: $61 \pm 3\%$; $n = 6$; $P < 0.05$; control + Δ^9 -THC: 100%; LFS: $88 \pm 3\%$; $n = 6$; $P < 0.05$; LTD control vs. LTD Δ^9 -THC, $P < 0.01$; Fig. 2C).

Altogether, these results show that LTD is independent of CB1 activation by endogenously synthesized endocannabinoids, but it is blocked by exogenous CB1 agonists via activation of CB1.

The effect of WIN55,212-2 on LTD exclusively depends on CB1 expressed on GABAergic interneurons

In the amygdala, CB1 is expressed both on GABAergic and glutamatergic neurons (Marsicano and Lutz 1999). Therefore, we wondered which neuronal subpopulation is involved in these pharmacological effects of WIN55,212-2 on LTD. To clarify this question, we used recently generated conditional mutant mice lacking the expression of CB1 exclusively either in all principal forebrain neurons (CB1^{f/f};CaMKII α Cre) (Marsicano et al. 2003) or in GABAergic interneurons (CB1^{f/f};Dlx5/6Cre) (Monory et al. 2006).

In slices of CB1^{f/f};CaMKII α Cre mice and their respective littermates, the application of WIN55,212-2 inhibited LTD induction (CB1^{f/f} EPSC: control: 100%; LFS: $76 \pm 7\%$; $n = 6$; $P < 0.05$; WIN55,212-2: 100%; LFS: $104 \pm 8\%$; $n = 6$; $P > 0.05$; CB1^{f/f};CaMKII α Cre EPSC: control: 100%; LFS: $74 \pm 7\%$; $n = 6$; $P < 0.05$; WIN55,212-2: 100%; LFS: $97 \pm 4\%$; $n = 6$; $P > 0.05$; Fig. 3A). Interestingly, however, in slices of CB1^{f/f};Dlx5/6Cre mice, WIN55,212-2 was not able to block LTD anymore (CB1^{f/f} EPSC: control: 100%; LFS: $80 \pm 6\%$; $n = 6$; $P < 0.05$; WIN55,212-2: 100%; LFS: $98 \pm 3\%$; $n = 6$; $P > 0.05$; CB1^{f/f};Dlx5/6Cre EPSC: control: 100%; LFS: $79 \pm 6\%$; $n = 6$; $P < 0.05$; WIN55,212-2: 100%; LFS: $78 \pm 7\%$; $n = 6$; $P < 0.05$; Fig. 3B). These results strongly indicate that the cannabinoid agonist WIN55,212-2 blocks LTD via the selective activation of CB1 exclusively expressed on GABAergic interneurons.

Pharmacological CB1 receptor-mediated effects on LTD depend on G_{i/o}-protein activation

CB1 receptors are coupled to G_{i/o} proteins (Pertwee 2001). However, they may also couple to G_s proteins under certain conditions (Glass and Felder 1997; Maneuf and Brothie 1997). To investigate whether the effect of CB1 receptor activation on LTD induction involves pertussis toxin-sensitive G_{i/o} proteins, slices were incubated with pertussis toxin (5 μ g/mL) for 5–7 h at 37°C. Pertussis toxin per se did not inhibit LTD induction (control + pertussis toxin: 100%; LFS: $80 \pm 5\%$ of control; $n = 3$;

Figure 3. Activation of CB1 receptors located on GABAergic interneurons are necessary for the WIN55,212-2-induced inhibition of LTD. (A) In slices of wild-type (CB1^{f/f}) and CB1^{f/f};CaMKII α Cre mice, low frequency stimulation (LFS; 900 pulses/1 Hz) induces a LTD of EPSCs in the LA ($n = 6$; $P < 0.05$; filled circles). The CB1 receptor agonist WIN55,212-2 (WIN; 2.5 μ M) blocks the induction of LTD ($n = 6$; $P > 0.05$; open circles) in both genotypes. (B) In slices of CB1^{f/f} and CB1^{f/f};Dlx5/6Cre mice, LFS (900 pulses/1 Hz) of afferents induces a LTD of EPSCs in the LA ($n = 6$; $P < 0.05$; filled squares). WIN55,212-2 (WIN; 2.5 μ M) blocks the induction of LTD ($n = 6$; $P > 0.05$; open squares) only in slices of CB1^{f/f} mice but not in slices of CB1^{f/f};Dlx5/6Cre mice. All data are normalized to the respective control values (last 10 min before LTD induction).

$P < 0.05$) but prevented the effect of WIN55,212-2 (2.5 μ M) on synaptic transmission (control + pertussis toxin: 100%; WIN55,212-2: $97 \pm 3\%$; $n = 7$; $P > 0.05$) and LTD. In the presence of WIN55,212-2, LFS depressed FP amplitude to $83 \pm 10\%$ of control ($n = 7$; $P < 0.05$) in slices preincubated with pertussis toxin (Fig. 4A), indicating that CB1 receptor-mediated inhibition of LTD in the LA involves the activation of G_{i/o} proteins.

Pharmacological CB1 receptor activation blocks LTD via inhibition of the adenylate cyclase-protein kinase A pathway

CB1 receptor activation is known to inhibit the adenylate cyclase (AC)-protein kinase A (PKA) pathway (Childers and Deadwyler 1996; Pertwee 2001). In order to reveal whether this mechanism is also involved in cannabinoid-induced inhibition of LTD, we investigated the effect of WIN55,212-2 on LTD in the presence of either the AC activator forskolin or the PKA activator Sp-cAMPS.

Figure 4. CB1 receptor-mediated inhibition of LTD in the LA involves activation of $G_{i/o}$ proteins and inhibition of the adenylyl cyclase-protein kinase A pathway. (A) Preincubation of the slices with pertussis toxin (PTX; 5 μ M) for 5–7 h at 37°C prevents the inhibiting effect of WIN55,212-2 (WIN; 2.5 μ M) on LTD induction ($n = 7$; $P < 0.05$). (B) The selective activation of the adenylyl cyclase with 10 μ M forskolin (FSK) abolishes the effect of WIN55,212-2 (WIN; 2.5 μ M), and restores LTD ($n = 5$; $P < 0.05$). (C) In accordance with this result, WIN55,212-2 (WIN; 2.5 μ M) also fails to block LTD when the protein kinase A is activated by 25 μ M Sp-cAMPs ($n = 5$; $P < 0.05$). Representative traces are shown. All data are normalized to the respective control values (last 10 min before LTD induction). Asterisks mark the stimulation artifacts.

As control experiments, we also tested the effect of both substances on the induction of LTD per se.

Forskolin enhanced the FP amplitudes to $128 \pm 9\%$ ($n = 5$) but did not affect LTD induction (control + forskolin: 100%; LFS: $73 \pm 7\%$; $n = 5$; $P < 0.05$). However, the selective activation of the AC abolished the effect of WIN55,212-2 on LTD: In the presence of forskolin (10 μ M) and WIN55,212-2 (2.5 μ M), LFS again significantly reduced FP amplitude to $79 \pm 10\%$ ($n = 5$; $P < 0.05$; Fig. 4B). We previously found that activation of the PKA is essential for the induction of LTD in the basolateral amygdala (BLA) of FVB/N mice (DeBock et al. 2003). We, therefore, assumed that cannabinoid-induced inhibition of this enzyme might play an important role in the effect of WIN55,212-2 on LTD. In fact, we found that Sp-cAMPs (25 μ M) per se affected neither basal synaptic transmission (data not shown) nor LTD induction in the LA (control + Sp-cAMPs: 100%; LFS: $88 \pm 5\%$; $n = 5$; $P < 0.05$), but inhibited the effect of WIN55,212-2 on LTD

induction (control + Sp-cAMPs + WIN55,212-2: 100%; LFS: $89 \pm 5\%$; $n = 5$; $P < 0.05$; Fig. 4C).

Pharmacological CB1 receptor activation inhibits LTD through activation of K_{ir} channels

Cannabinoids modulate voltage-dependent and voltage-independent K^+ channels (Deadwyler et al. 1995; Mackie et al. 1995; Schweitzer 2000). We recently found that, in the LA, voltage-dependent K^+ channels and K_{ir} s play a major role in WIN55,212-2-induced effects on synaptic transmission (Azad et al. 2003). Therefore, we studied the effect of WIN55,212-2 (2.5 μ M) in the presence of 4-aminopyridine (4-AP; 100–300 μ M), a blocker of voltage-dependent K^+ channels (Mathie et al. 1998; Bordey and Sontheimer 1999), and $BaCl_2$ (300 μ M), which blocks K_{ir} channels (Coetzee et al. 1999; Takigawa and Alzheimer 1999). In the presence of 4-AP (100–300 μ M), WIN55,212-2 (2.5 μ M) no longer decreased FP amplitudes (control + 4-AP: 100%; WIN55,212-2: $99 \pm 4\%$; $n = 7$; $P > 0.05$) but still inhibited LTD (control: 4-AP + WIN55,212-2: 100%; LFS: $106 \pm 5\%$; $n = 7$; $P > 0.05$; Fig. 5A). In contrast, blockade of K_{ir} with $BaCl_2$ (300 μ M) impeded the effect of WIN55,212-2 (2.5 μ M) and restored LTD induction (control: $BaCl_2$ + WIN55,212-2: 100%; LFS: $86 \pm 3\%$; $n = 5$; $P < 0.05$) (Fig. 5B). K^+ channel blockers may prolong the presynaptic action potential by diminishing repolarization of the presynaptic terminal (Hoffman and Lupica 2000) and thus enhance presynaptic Ca^{2+} influx. As the latter has been shown to be necessary for induction of LTD in the BLA (Rammes et al. 2001; DeBock et al. 2003), this effect could counteract the effect of WIN55,212-2 on LTD. We, therefore, investigated the effects of 4-AP (4-AP) and $BaCl_2$ on WIN55,212-2-induced modulation of LTD after lowering the extracellular Ca^{2+} concentration from 2 mM to 1 mM (Mg^{2+} concentration was increased from 1 mM to 2 mM to obtain equal osmolarity). Under these conditions, blockade of voltage-gated K^+ channels had no effect, whereas blockade of K_{ir} channels still abolished the actions of WIN55,212-2 (2.5 μ M) on LTD (control: 4-AP + WIN55,212-2: 100%; LFS: $105 \pm 2\%$; $n = 7$; $P > 0.05$; Fig. 5A; control: $BaCl_2$ + WIN55,212-2: 100%; LFS: $79 \pm 10\%$; $n = 4$; $P < 0.05$; Fig. 5B). Lowering extracellular Ca^{2+} concentration to 1 mM influenced LTD induction (control: 100%; LFS: $103 \pm 7\%$; $n = 5$; $P > 0.05$; Fig. 5C). However, in the presence of both 4-AP (100–300 μ M) and $BaCl_2$ (300 μ M), LFS again induced a robust LTD (control + 4-AP: 100%; LFS: $86 \pm 4\%$; $n = 9$; $P < 0.05$; control + $BaCl_2$: 100%; LFS: $82 \pm 6\%$; $n = 4$; $P < 0.05$; Fig. 5C).

These results together with the finding that WIN55,212-2 blocks LTD only in $CB1^{f/f;Dlx5/6Cre}$ mice (Fig. 3) suggest that the cannabinoid agonist blocks LTD via activation of K_{ir} channels expressed on interneurons. However, a differential sensitivity of glutamatergic and GABAergic synapses to $BaCl_2$ might also explain the effects observed. To investigate this possibility, we performed additional experiments in slices of $CB1^{f/f;CaMKII\alpha Cre}$ mice, where CB1 is expressed exclusively on interneurons but not on glutamatergic principal neurons. We found that under the conditions of both 1 and 2 mM Ca^{2+} concentrations, $BaCl_2$ inhibited the effect of WIN55,212-2 on LTD (1 mM: control + $BaCl_2$ + WIN55,212-2: 100%; LFS: $83 \pm 4\%$; $n = 6$; $P < 0.05$; Fig. 5B; 2 mM: control: $BaCl_2$ + WIN55,212-2: 100%; LFS: $71 \pm 4\%$; $n = 6$; $P < 0.05$; Fig. 5B).

Since these results point to a major role of K_{ir} channels in CB1-mediated inhibition of LTD, we tested the effect of WIN55,212-2 (2.5 μ M) on isolated K_{ir} channel-mediated currents in interneurons of the LA. CB1 receptor activation increased the membrane current evoked by a depolarization ramp in the LA in a voltage-dependent manner (Fig. 6A). At a membrane potential of -100 mV, WIN55,212-2 (2.5 μ M) increased the membrane cur-

Figure 5. CB1 receptor-mediated inhibition of LTD in the LA involves the activation of K_{ir} s. (A) When voltage-dependent K^{+} -channels are blocked with 100–300 μM 4-AP, WIN55,212-2 (2.5 μM) still inhibits LTD induction under the condition of standard extracellular Ca^{2+} concentration of 2 mM ($n = 7$; $P > 0.05$; open circles). Similar results are obtained when the extracellular Ca^{2+} concentration is decreased to 1 mM ($n = 7$; $P > 0.05$; filled circles) to eliminate the problem of a possible interaction of K^{+} -channel blockade with presynaptic Ca^{2+} influx. (B) However, blockade of K_{ir} s with BaCl_2 (300 μM) prevents the effects of WIN55,212-2 (WIN; 2.5 μM) and restores LTD induction. This is the case under the conditions of both standard (2 mM; $n = 5$; $P < 0.05$; open circles) and decreased (1 mM; $n = 4$; $P < 0.05$; filled circles) extracellular Ca^{2+} concentration. In slices of CB1^{f/f};CaMKIIaCre mice, where CB1 is expressed exclusively on interneurons, but not on glutamatergic principal neurons, BaCl_2 inhibits the effect of WIN55,212-2 on LTD under the conditions of both 1 and 2 mM Ca^{2+} concentration (1 mM; $n = 6$; $P < 0.05$; open squares; 2 mM; $n = 6$; $P < 0.05$; filled squares). (C) Control experiments show that an extracellular Ca^{2+} concentration of 1 mM is not sufficient to induce LTD ($n = 5$; $P > 0.05$; filled circles). However, in the presence of both 100–300 μM 4-AP ($n = 9$; $P < 0.05$; open circles) and 300 μM BaCl_2 ($n = 4$; $P < 0.05$; filled triangles), LFS again induces LTD similar to control values. Representative traces are shown. All data are normalized to the respective control values (last 10 min before LTD induction). Asterisks mark the stimulation artifacts.

rent (control; trace a) to $148 \pm 15\%$ ($n = 4$; trace b). The resting K_{ir} current was subtracted from that additionally seen in the presence of WIN55,212-2 (Fig. 5B). When using slices of CB1-KO mice, WIN55,212-2 did not potentiate K_{ir} -mediated currents (Fig. 6A; trace e). In the presence of the K_{ir} channel blocker Ba^{2+} (300 μM ; Fig. 6A; trace c), WIN55,212-2 did not affect the membrane

current anymore ($n = 4$; Fig. 6A; trace d). Due to the elevated extracellular K^{+} concentration, the current activated had a reversal potential of $-33 \text{ mV} \pm 2 \text{ mV}$ (calculated by the Nernst equation = 35.6 mV).

Pharmacological CB1 receptor activation reduces currents through N-type voltage-gated calcium channels in the LA via inhibition of the PKA

We previously found that the induction of LTD in the BLA depends on the activation of N-type but not on the activation of P/Q-type, L-type, or T/R-type Ca^{2+} channels (Rammes et al. 2001; DeBock et al. 2003). Since cannabinoid actions are known to involve voltage-gated calcium channels (VGCCs) (Twitchell et al. 1997; Hoffman and Lupica 2000), it seems likely that WIN55,212-2-mediated inhibition of LTD induction in the LA also involves the inhibition of N-type Ca^{2+} channels.

We, therefore, tested whether WIN55,212-2 has any effect on VGCCs, particularly on N-type VGCCs in interneurons of the LA. In these experiments, Ba^{2+} was used as an additional charge carrier. Whole-cell VGCC currents were evoked by step depolarization from a holding potential of -70 mV to 0 mV for 300 msec. Cadmium (Cd^{2+} ; 10 μM) abolished these currents, proving that they were Ca^{2+} channel-mediated ($n = 4$; Fig. 7A,Aa). WIN55,212-2 (2.5 μM) reduced the VGCC-mediated currents to $63 \pm 7\%$ of control ($n = 4$; Fig. 7A, Aa). The voltage dependence of currents for control and in the presence of WIN55,212-2 was very similar, as seen in a plot of normalized peak currents (Fig. 7Ab). In both conditions, current activation was first detected with a test depolarization beyond -50 mV , and the current

Figure 6. CB1 receptor activation increases K_{ir} currents in interneurons of the LA. (A) Current-voltage (I - V) curves of membrane currents elicited by depolarization ramps at time points of control (a), WIN55,212-2 (WIN; 2.5 μM) (b), BaCl_2 (300 μM) (c), BaCl_2 plus WIN55,212-2 (WIN; 2.5 μM) (d), and using slices of CB1-KO mice (WIN; 2.5 μM) (e) are shown. At a membrane potential of -100 mV , WIN55,212-2 (WIN; 2.5 μM) increases the membrane current (a) to $148 \pm 15\%$ (b; $n = 4$; $P < 0.05$). In the presence of the K_{ir} -channel blocker BaCl_2 (300 μM ; c) and in CB1-KO (e), WIN55,212-2 does not affect the membrane current anymore ($n = 4$; $P > 0.05$). (B) The I - V curve of the WIN55,212-2-induced current is obtained by subtracting the current in the control from that during exposure to the CB1 receptor agonist WIN55,212-2 (b - a).

Figure 7. CB1 receptor activation decreases Ca²⁺ influx through presynaptic N-type VGCCs of interneurons via inhibition of the PKA. (A) Using Ba²⁺ as the charge carrier, voltage-gated calcium channel (VGCC) currents are evoked by step depolarization from a holding potential of -70 mV to 0 mV for 300 msec. WIN55,212-2 (WIN; 2.5 μ M) reduces the VGCC-mediated currents significantly ($n = 4$; $P < 0.05$; left). In the presence of cadmium (Cd²⁺; 10 μ M), step depolarization from -70 mV to 0 mV does not evoke any currents anymore, proving that the measured currents are mediated by VGCCs ($n = 3$; $P > 0.05$; right and Aa). The WIN55,212-2-mediated effect on VGCC was voltage-independent ($n = 5$; Ab). (B) N-type VGCCs are isolated by application of the T/R-type VGCC blocker nickel (Ni²⁺; 50 μ M), the L-type VGCC blocker nifedipine (NIF; 20 μ M), and the P/Q-type VGCC blocker ω -agatoxin IVA (AgTx; 200 nM). WIN55,212-2 (WIN; 2.5 μ M) clearly reduces the remaining current ($n = 5$; $P < 0.05$). This effect of WIN55,212-2 is abolished in the presence of the PKA blocker Rp-cAMPs (25 μ M; $n = 3$; $P > 0.05$), which itself already reduces N-type VGCC currents ($n = 3$; $P < 0.05$). (C,D) WIN55,212-2 (2.5 μ M) does not reduce N-type VGCC currents in slices pretreated with the CB1 antagonist SR141716A (2.5 μ M; $n = 5$; $P > 0.05$) or in slices of CB1-KO mice ($n = 4$; $P > 0.05$).

reached maximum amplitude near 10 mV. After blockade of P/Q-type, L-type, and T/R-type Ca²⁺ channels with ω -agatoxin IVA (200 nM), nifedipine (20 μ M), and nickel (Ni²⁺; 50 μ M), respec-

tively, the remaining N-type Ca²⁺ channel-mediated current was set to 100%. WIN55,212-2 (2.5 μ M) reduced this current to $70 \pm 8\%$ of control ($n = 5$; $P < 0.05$; Fig. 7B). Since WIN55,212-2-induced inhibition of LTD involves the PKA (Fig. 3C), we tested whether CB1 receptor activation reduces N-type Ca²⁺ channel-mediated currents directly or via inhibition of the AC-PKA pathway. The effect of WIN55,212-2 (2.5 μ M) on N-type Ca²⁺ channels was, therefore, also tested after blockade of the PKA with Rp-cAMPs (25 μ M). As shown in Figure 7B, application of the PKA blocker already reduced the isolated currents to $79 \pm 4\%$ of control ($n = 4$). As soon as the effect of Rp-cAMPs was stable for at least 10 min, WIN55,212-2 (2.5 μ M) was applied. When the PKA activity was blocked, WIN55,212-2 (2.5 μ M) did not reduce the remaining current anymore (control + Rp-cAMPs: 100%; WIN55,212-2: $101 \pm 6\%$; $n = 4$; $P > 0.05$; Fig. 7B). In order to rule out whether WIN55,212-2 acts directly on the N-type Ca²⁺ channels or via activation of CB1, we investigated the effect of the agonist on these channels both in the presence of the CB1-antagonist SR141716A (2.5 μ M) as well as in slices of CB1-KO mice. We found that under both conditions, WIN55,212-2 (2.5 μ M) did not reduce the Ca²⁺ currents anymore (control + SR141716A: 100%; WIN55,212-2: $107 \pm 7\%$; $n = 5$; $P > 0.05$; CB1-KO control: 100%; WIN55,212-2: $99 \pm 9\%$; $n = 4$; $P > 0.05$; Fig. 7C,D), indicating that the actions of the agonist on the N-type Ca²⁺ currents were CB1 receptor-mediated.

Discussion

In the present study, we showed that the induction of LTD in the LA was completely inhibited by the CB1 receptor agonist WIN55,212-2. This effect could be blocked by the CB1 antagonist SR141716A, which did not influence the induction of LTD per se. In mice lacking CB1 receptors, LTD was still inducible but was not blocked by WIN55,212-2, showing that the action of the agonist depends on CB1 receptors. Furthermore, WIN55,212-2 completely blocked LTD in mice lacking CB1 receptors in principal glutamatergic neurons but, interestingly, had no effect on LTD in the LA of conditional CB1 mutant mice lacking CB1 receptors in GABAergic interneurons. Our experiments therefore strongly indicate that the effect of WIN55,212-2 exclusively depends on the presence of CB1 receptors located on GABAergic interneurons.

Many investigations have focused on the effects of cannabinoids on basal synaptic transmission recorded by FPs or EPSCs (Misner and Sullivan 1999; Auclair et al. 2000; Katona et al. 2001; Robbe et al. 2002; Freund et al. 2003), which reflect combined excitatory and inhibitory synaptic responses. Previously, we found that in the LA and BLA, WIN55,212-2 reduces both isolated glutamatergic and GABAergic transmission through CB1 activation (Azad et al. 2003; Domenici et al. 2006). However, these functionally opposite effects do not seem to compensate each other, as revealed by the agonist-induced decrease of EPSCs and FPs. This suggests that in the LA, the inhibitory effects of cannabinoids on excitatory glutamatergic transmission override those on inhibitory GABAergic transmission. In contrast, WIN55,212-2-induced inhibition of LTD is probably the sole consequence of a CB1-mediated decrease of the activity of GABAergic interneurons and is not mediated via an effect on glutamatergic transmission. These findings support the results of a previous investigation of our group showing that LTD in the LA-BLA involves the activation of interneurons and depends on an increase of GABAergic transmission, rather than a presynaptic inhibition of glutamate release (Rammes et al. 2000, 2001). Together with these previous findings, our present data indicate that LFS with 900 pulses at 1 Hz increases GABAergic transmission, which is not controlled by the activation of the endocan-

nabinoid system. However, WIN55,212-2 is able to directly stimulate CB1 receptors on GABAergic interneurons, thereby reducing the release of GABA and blocking the induction of LTD.

In a previous investigation, we found that LFS of afferents in the LA with 100 pulses at 1 Hz activates the endogenous cannabinoid system and, thereby, induces a LTD of inhibitory GABAergic synaptic transmission (LTDi) in the BLA (Marsicano et al. 2002; Azad et al. 2004). The results of the present study showed that in the same pathway, LTD of basal synaptic transmission most conventionally induced by prolonged LFS with 900 pulses at 1 Hz did not require the activation of the endocannabinoid system. The fact that LTD is blocked by an exogenous synthetic agonist but is unaffected by a CB1 antagonist is a very interesting result. This observation adds to the growing evidence that the pharmacological effects of CB1 agonists are sometimes different from the endogenous physiological functions of the endocannabinoid system. This is likely due to the lack of temporal and spatial specificity of agonist treatments compared with the “on demand” activation of the endogenous system (Marsicano and Lutz, 2006).

The obvious differential role of CB1 in LTD and LTDi is surprising. There might be several possible explanations for this observation: First, these data suggest that endocannabinoids are only transiently released during the early phase of LFS. It might be possible that the blocking effect of endocannabinoids on GABAergic transmission during these first 100 pulses is counteracted with ongoing stimulation, which activates PKA-regulated N-type VGCCs in principal neurons projecting onto interneurons and thus indirectly enhances presynaptic GABA release. The resulting LTD evoked by this prolonged LFS can be blocked by CB1 receptor agonists via a $G_{i/o}$ protein-mediated activation of K_{ir} channels and an inhibition of PKA-regulated N-type VGCCs. Second, there is the possibility of a differential role of Ca^{2+} in the two phases of stimulation. In our previous investigation, we showed that LTDi induced by 100 pulses at 1 Hz is independent of intracellular Ca^{2+} . In contrast, in the present study, we found that lowering extracellular Ca^{2+} concentration from 2 to 1 mM inhibits the induction of LTD by 900 pulses, indicating a differential role of Ca^{2+} in LTDi and LTD. Based on the latter result, it seems likely that the ongoing long-term stimulation with 900 pulses during LTD leads to the release of Ca^{2+} , which again triggers processes (i.e., activation of the AC-PKA cascade) underlying the expression of LTD. Third, our results might also be explained by a different sensitivity of GABAergic and glutamatergic synapses to endocannabinoids as observed in the hippocampus (Ohno-Shosaku et al. 2002). In this structure, a group of interneurons highly sensitive to endocannabinoids could be distinguished from principal neurons, which were shown to be less sensitive to endogenously released cannabinoids. Fourth, it might also be possible that a low level of CB1 occupancy during 1-Hz stimulation is enough to induce LTDi but not to block LTD. In order to test this hypothesis, we also used a lower concentration of WIN55,212-2 (1 μ M), which decreased synaptic transmission but did not block LTD. Together with our previous finding that 100 pulses of 1 Hz do not affect FP amplitude and do not induce LTD, our data suggest that a low level of CB1 occupancy during 1-Hz stimulation for 100 sec is enough to induce LTDi, while blockade of LTD requires higher concentrations. The final results support the hypothesis of a differential, activity-dependent role of CB1 in LTDi and LTD and indicate that other mechanisms such as the activation of GABA receptors are involved in LTD (see also Rammes et al. 2001).

These results raise the following question: Which exact intracellular mechanisms control the expression of LTD and the inhibition of this form of synaptic plasticity by CB1 activation. The present study suggests that WIN55,212-2 blocks LTD via ac-

tivation of $G_{i/o}$ protein-gated K_{ir} channels, which inhibits LFS-induced presynaptic GABA release. WIN55,212-2 also markedly reduced presynaptic N-type VGCC-mediated currents in the LA via inhibition of the PKA. Interestingly, it has been found that Ca^{2+} influx through N-type Ca^{2+} channels is a prerequisite for the induction of LTD in the BLA (DeBock et al. 2003), suggesting that these channels might also be involved in the effects of cannabinoids on this form of synaptic plasticity.

Activation of $G_{i/o}$ proteins (Mackie and Hille 1992; Misner and Sullivan 1999) and the subsequent inhibition of the AC-PKA cascade (Childers and Deadwyler 1996) are established mechanisms underlying cannabinoid-induced effects. However, cannabinoid receptors can also couple to G_s proteins (Glass and Felder 1997) to stimulate the AC-PKA pathway under certain conditions (Maneuf and Brothie 1997). In the present study, WIN55,212-2-induced inhibition of LTD in the LA required the activation of $G_{i/o}$ proteins and an inhibition of the AC-PKA pathway. These data strongly suggest that inhibition of LTD in the amygdala is a specific action of $G_{i/o}$ -linked receptors, including CB1. Interestingly, WIN55,212-2-mediated modulation of synaptic transmission in the LA was previously found to involve activation of $G_{i/o}$ proteins but to be independent of the AC-PKA signaling cascade (Azad et al. 2003). These data suggest that cannabinoid actions on synaptic transmission and synaptic plasticity in the amygdala involve different cellular mechanisms. PKA activation may lead to a modulation of both Ca^{2+} channels and voltage-dependent K^+ channels (Fraser and Scott 1999; Brosenitsch and Katz 2001). In the present study, CB1 receptor activation reduced N-type VGCC-mediated currents in the LA in a PKA-dependent manner. Although CB1 receptors can inhibit Ca^{2+} channels directly via activation of $G_{i/o}$ proteins (Pertwee 2001), this does not seem to be the case in the LA, as WIN55,212-2 did not reduce Ca^{2+} currents anymore when PKA signaling was blocked with Rp-cAMPS. Since we previously found that LTD in the BLA requires the activation of metabotropic glutamate receptors (mGluRs) (Rammes et al. 2001), it might be assumed that the effect of WIN55,212-2 on LTD also involves this group of receptors. However, this is unlikely, because of our finding that the effect of the CB1 agonist is specifically mediated by CB1.

Cannabinoids also modulate voltage-dependent K^+ channels in hippocampal neurons via a cAMP-dependent process (Deadwyler et al. 1995). These channels are important for the effects of cannabinoids on synaptic transmission in the LA (Azad et al. 2003). However, they obviously do not play a major role in cannabinoid-induced inhibition of LTD, since their modulation by 4-AP did not affect WIN55,212-2 actions in the present investigation. Cannabinoids can also activate K_{ir} conductance (Mackie et al. 1995). In the present study, the effect of WIN55,212-2 on LTD was clearly inhibited when K_{ir} channels were blocked with Ba^{2+} . This was the case under the conditions of standard (2 mM), as well as decreased (1 mM) extracellular Ca^{2+} concentrations. The latter experiments were performed in order to detect a possible increase of presynaptic Ca^{2+} influx due to K^+ channel blockade, which might counteract the effect of WIN55,212-2 on LTD. In fact, we found that decreasing extracellular Ca^{2+} concentration per se inhibited the induction of LTD, which, however, was restored when K^+ channels were blocked by 4-AP or Ba^{2+} . These experiments support the idea that K^+ channel blockade enhances presynaptic Ca^{2+} influx, and suggest that this effect can be counteracted by lowering extracellular Ca^{2+} concentration. Under the conditions of both normal and decreased Ca^{2+} concentrations, WIN55,212-2 inhibited LTD induction only in the presence of 4-AP but not of Ba^{2+} , demonstrating that G protein-gated K_{ir} s, but not voltage-gated K^+ channels, are important for cannabinoid-induced inhibition of LTD in the LA. In accordance with this finding, WIN55,212-2 significantly enhanced isolated Ba^{2+} -

sensitive K_{ir} channel-mediated currents in interneurons of the LA.

However, there is also one obvious caveat in the interpretation of the present data. For the investigation of WIN55,212-2-mediated effects on K_{ir} s and VGCCs, presumably somatic currents from identified interneurons were recorded, although it might not be excluded that channels, which are located on axon terminals, are just as relevant.

There is evidence that the mechanisms underlying CB1 receptor-mediated modulation of synaptic transmission (Misner and Sullivan 1999; Hoffman and Lupica 2000; Azad et al. 2003) and synaptic plasticity (Misner and Sullivan 1999; Gerdeman et al. 2002; Robbe et al. 2002) vary in different brain regions. In the hippocampus, CB1 receptor activation was shown to inhibit the induction of LTD through presynaptic mechanisms with blockade of NMDA receptors being of major importance (Misner and Sullivan 1999). In the BLA of the mouse, LTD was reduced when presynaptic Ca^{2+} influx was blocked, whereas LTD was independent of a rise in postsynaptic Ca^{2+} . In addition, in the BLA, LTD was only partly reduced after blockade of NMDA receptors (Rammes et al. 2001). These results clearly suggest a presynaptic site of LTD induction. Since blockade of GABA_A receptors inhibits LTD induction in the BLA (Rammes et al. 2000, 2001) and PPR (an index for an alteration of presynaptic transmitter release; see Manabe et al. 1993) of glutamatergic synaptic transmission did not change after LFS in the present study, it seems likely that LTD per se is not mediated by a presynaptic inhibition of glutamate release, but by a presynaptic activation of GABAergic inputs.

Our findings are also interesting in view of the clinical application of cannabinoids and the use or abuse of marijuana. Cannabinoids are known to have various wanted and unwanted effects, including analgesia and impairment of learning and memory. Both the development of chronic pain as well as learning and memory are based on processes of synaptic plasticity. The most important clinically applied cannabinoid is Δ^9 -THC. Among others, this substance is known to have analgesic, sedative, and mood improving properties, which are useful in cancer patients suffering from pain and fear (Azad and Rammes 2005). In the present study we found that Δ^9 -THC significantly reduces LTD induction. Our results might, therefore, also partly reflect the mechanisms underlying the effect of cannabinoids on pain and cognition.

In conclusion, we found that, in the LA of the mouse, cannabinoids inhibit LTD via CB1 receptors specifically located on GABAergic interneurons, whereas they modulate synaptic transmission in the same structure by CB1 expressed on principal neurons. Our previous and present data therefore indicate a distinct role of CB1 expressed on either excitatory or inhibitory neurons in the control of synaptic transmission and plasticity.

Materials and Methods

Slice preparation

For all experiments, male 42- to 63-d-old mice were used. The number of experiments (*n*) reflects the number of different animals used. C57BL6/JOlaHsd mice were purchased from Harlan Winkelmann (Borchen, Germany). CB1-KO mice (lacking CB1 expression in all cells of the body) were generated as described by Marsicano et al. (2002). Conditional mutant CB1^{f/f};CamKII α -Cre mice lacking CB1 expression in all forebrain principal neurons and, consequently expressing CB1 in the forebrain, only in cortical GABAergic interneurons, were generated as described (Marsicano et al. 2003). The generation of conditional mutant CB1^{f/f};Dlx5/6-Cre mice lacking CB1 expression in all GABAergic neurons is described by Monory et al. (2006). All mutant mice used were littermates and were genotyped before and after the experiment by polymerase chain reaction (PCR). All electrophysiological

experiments were performed in accordance with the guidelines of the Ethical Committee on the Use and Care of Animals (Government of Bavaria, Germany). For slice preparation, the animals were anaesthetized with isoflurane and decapitated. Slices were prepared from animals during the light-phase. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 2 mM CaCl₂, 1 mM MgCl₂, 25 mM D-glucose, 1.25 mM NaH₂PO₄ (pH 7.4) and bubbled with a 95% O₂/5% CO₂ mixture. Coronal slices of the amygdala (400 μ m thick) were prepared using a vibroslicer (FTB). After incubation in a holding chamber with ACSF (22°C–25°C) for at least 60 min, the slices were placed in the recording chamber and superfused with ACSF at a flow rate of 1.5 mL/min.

Recording of FPs and EPSCs

In the LA, excitatory FPs and postsynaptic currents (EPSCs) were evoked by square pulse stimuli (0.066 Hz, 5–12 mA, 200 μ sec) delivered via bipolar tungsten electrodes insulated to the tip (5- μ m tip diameter) and positioned in the LA. All experiments were carried out at room temperature (22°C–25°C). FPs were recorded using glass microelectrodes (1–2 M Ω) filled with ACSF. The stimulus intensities were adjusted to produce a FP of ~50% of the maximum amplitude. LFS (900 pulses/1 Hz) was applied to induce LTD at the same stimulus intensity. Before LFS was applied, the responses to single stimuli had to remain stable for at least 20 min. The voltage differences between onset and peak (a), and between this negative peak and the succeeding positive peak (b), were measured, and the amplitudes of the FPs were calculated as (a + b)/2. The FPs were averaged before analysis. We stimulated every 15 sec and averaged four consecutive FPs into one response. Therefore, each dot in the graph represents the normalized average of four responses and covers a recording time of 1 min.

For whole-cell patch-clamp recordings of EPSCs, principal neurons and interneurons of the LA were visualized using infrared video-microscopy and the gradient contrast system (Zeiss). Interneurons were distinguished by their morphological properties and by their spiking frequencies when applying depolarizing current pulses in bridge mode (Rammes et al. 2001). For recording of EPSCs, glass electrodes (4–5 M Ω) contained the following: 130 mM K-D-glucuronate, 5 mM KCl, 2 mM Mg-ATP, 10 mM D-glucose, 0.5 mM EGTA, 10 mM HEPES (pH 7.4).

Currents were recorded using a switched voltage-clamp amplifier (SEC-10L; npi Electronics) with switching frequencies of 75–80 kHz (25% duty cycle). Series resistance was monitored continuously and compensated in bridge mode. Neuronal input resistance was monitored by injecting hyperpolarizing current pulses (300 msec, –10 mV, 0.066 Hz) through the patch electrode (no current was injected during LTD induction). Neurons were clamped at a holding potential of –70 mV. When recording EPSCs, LFS (900 pulses/1 Hz) was applied in bridge mode. Before LFS, the amplitudes to single stimuli had to remain stable for at least 15 min. The recordings were filtered (3 kHz), and the digitized (9 kHz) data were stored to a Power Macintosh G3 computer by a data acquisition and evaluation program (Pulse v. 8.5; Heka electronic GmbH). Paired-pulse stimulation was performed by delivering the same stimulus at 50-msec interpulse intervals. PPR was determined by dividing the second amplitude by the first one (EPSC2/EPSC1).

The neuronal distribution in the amygdala is, unlike in the hippocampus, not laminar. Therefore, the recorded FP is most presumably a mixture of population spikes and EPSPs. However, the amplitudes of FPs and EPSCs depend on AMPA, NMDA, and GABA receptors (Rammes et al. 2000, 2001) and is, therefore, well suitable for investigating possible long-term changes of synaptic activity in a population of neurons.

Recording of Ca^{2+} currents

The solution for preparation of slices contained 130 mM NaCl, 3 mM KCl, 26 mM NaHCO₃, 1 mM CaCl₂, 5 mM MgCl₂, 1.25 mM NaH₂PO₄, 10 mM D-glucose, bubbled with a 95% O₂/5% CO₂ mixture to obtain a final pH of 7.4. Slices were maintained in a

similar solution, but with 2 mM CaCl_2 and 2 mM MgCl_2 . For voltage-clamp recordings of Ba^{2+} currents through VGCCs, we used an external HEPES-based Ringer solution bubbled with a 95% O_2 /5% CO_2 mixture that contained 145 mM NaCl, 3.5 mM KCl, 10 mM HEPES, 2 mM BaCl_2 , 2 mM MgCl_2 , 25 mM D-glucose, and 0.001 TTX (tetrodotoxin) (pH 7.3 using NaOH); the internal solution contained (in mM) the following: 30 mM TEACl, 100 mM CsCH_3SO_3 , 4 mM NaCl, 1 mM MgCl_2 , 0.5 mM CaCl_2 , 10 mM HEPES, 10 mM EGTA, 5 mM MgATP, 0.3 mM GTP-Tris, 10 mM Creatine- PO_4 -tris salt (pH 7.3 using TEAOH). Transmembrane currents were evoked by stepping the holding potential from -80 mV to $+40$ mV for 300 msec in 10-mV steps.

Prior to the analysis of Ca^{2+} currents, a standard P/4 protocol was used for the subtraction of leakage currents. This protocol provides the option to generate leak pulses while averaging to eliminate slow capacitive currents arising from the jump from holding to leak holding.

Recording of K^+ currents

After whole-cell access was established, K_i currents were investigated in standard ACSF bubbled with a 95% O_2 /5% CO_2 mixture. Patch electrodes (3.5–4.5 M Ω) were filled with the following: 120 mM KCH_3SO_3 , 0.01 mM CdCl_2 , 4 mM NaCl, 0.5 mM MgCl_2 , 0.5 mM CaCl_2 , 10 mM HEPES, 10 mM EGTA, 0.3 mM GTP-Tris, 20 mM Na_2 -creatinephosphate (pH 7.3 using NaOH). TTX (1 μM) was added to block Na^+ channels. Cells were held at -70 mV, and K_i currents were elicited by ramp voltage commands from -130 to 0 mV. To enhance K_i currents, controls and drug exposure were carried out in solutions with elevated K^+ (30 mM) by substitution for Na^+ .

Statistical analysis

The paired Student's *t*-test was used to compare control (10-min stable response) and the values after LFS. The latter values (10-min stable response) were chosen 30 min after termination of LFS at the earliest. Data are presented as mean $\pm$ SEM.

Chemicals

Drugs were applied via the superfusion system. The following pharmacological compounds were used: WIN55,212-2, pertussis toxin, forskolin (7 β -acetoxy-6 β -hydroxy-8,13-epoxy-labd-14-en-11-one), Sp-cAMPs (sp-adenosine 3',5'-cyclic monophosphothionate), Rp-cAMPs (8-bromoadenosine-3',5'-cyclic monophosphothioate), BaCl_2 , 4-AP, TEA (tetraethylammonium), Na_2 -creatinephosphate, riluzole, and Nifedipine from RBI/Sigma (RBI), tetrodotoxin, ω -agatoxin IVA (Tocris, Biotrend), and SR141716A (NIMH Chemical Synthesis and Drug Supply Program). Stock solutions of WIN55,212-2 (10 mM), SR141716A (10 mM), and Nifedipine (50 mM) were prepared in DMSO and stored at -20°C . Final DMSO concentrations were $\leq 0.05\%$.

Δ^9 -THC was stored as a stock solution in ethanol (10 mg/mL) at -20°C in the dark. The ethanol/ Δ^9 -THC solution was diluted in ACSF to give the required end concentration of 1 μM Δ^9 -THC. The same amount of ethanol ($\sim 0.03\%$) was applied to induce control LTD. Before the experiments, the system was rinsed with fatty acid free bovine serum albumin (1 mg/mL) to avoid binding of WIN55,212-2, Δ^9 -THC, and SR141716A to the walls of the tubing.

As the WIN55,212-2 concentrations applied in experiments using slice preparations vary between 1 μM and 10 μM throughout the literature, we performed a dose-finding study (1 μM ; 2.5 μM ; 5 μM ; 10 μM WIN55,212-2) in the LA of C57BL6/J OlaHsd mice (data not shown), which revealed a submaximal concentration of 2.5 μM in inhibiting LTD induction. We, therefore, chose this concentration for the present study.

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Effects of study task on the neural correlates of source encoding

Heekyeong Park, Melina R. Uncapher, and Michael D. Rugg

For the reference Rugg et al. 2008, the book title was incorrectly cited. The correct book title is *Progress in brain research*. The corrected reference should read:

Rugg, M.D., Johnson, J.D., Park, H., and Uncapher, M.R. 2008. Encoding-retrieval overlap in human episodic memory: A functional neuroimaging perspective. In *Progress in brain research*, 169 (eds. W. Sossin et al.), Section IV. Elsevier, Amsterdam.

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