Endocannabinoids are emerging as potent modulators of neuronal activity throughout the brain, and activation of the type-1 cannabinoid receptor (CB1R) reduces sensory-evoked cortical responses in vivo, presumably by decreasing excitatory transmission. In the neocortex, CB1R is differentially expressed across neocortical laminae, with highest levels of expression in layers 2/3 and 5. Although we have shown that cannabinoid signaling in layer 2/3 of somatosensory cortex targets both γ-aminobutyric acid (GABA) and glutamate release, the predominant effect is a net increase in pyramidal neuron (PN) activity due to disinhibition. The role of endocannabinoid signaling in layer 5, the main output layer of the neocortex, remains unknown. We found that inducing activity in layer 5 PNs resulted in endocannabinoid-mediated depolarization-induced suppression of excitation (DSE), whereas the majority of inhibitory inputs were cannabinoid insensitive. Furthermore, in contrast to layer 2/3, the net effect of elevations in action potential firing of layer 5 PNs was an endocannabinoid-mediated decrease in PN spike probability. Interestingly, excitatory synaptic currents in layer 5 evoked by intralaminar stimulation were cannabinoid sensitive, whereas inputs evoked from layer 2/3 were insensitive, suggesting specificity of cannabinoid signaling across glutamatergic inputs. Thus, cannabinoids have differential effects on excitation and inhibition across cortical layers, and endocannabinoid signaling in layer 5 may serve to selectively decrease the efficacy of a subset of excitatory inputs.

Keywords: cannabinoid, DSE, DSI, neocortex, synapse

Introduction

The endocannabinoid system plays an important role in the modulation of synaptic transmission throughout the brain. In the neocortex, pyramidal neurons (PNs) synthesize and release the endogenous cannabinoid ligands anandamide and 2-arachidonylglycerol (Stella and Piomelli 2001). In addition, there are high levels of expression of the type-1 cannabinoid receptor (CB1R) (Matsuda and others 1993; Tsou and others 1998; Marsicano and Lutz 1999; Egertova and others 2003) and the endocannabinoid-metabolizing enzymes fatty acid amide hydrolase (Egertova and others 2003) and monoglycerol lipase (Gulyas and others 2004). Thus, it is not surprising that CB1R activation modulates cortical activity and may be involved in sensory processing. For example, administration of delta-9-tetrahydrocannabinol (Δ⁹-THC) decreases sensory-evoked cortical responses in anesthetized animals (Willison and others 1982; Pontzer and others 1986). Conversely, disruption of endocannabinoid signaling by the CB1R antagonist N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide enhances whisker-evoked hyperemic responses in somatosensory cortex (Patel and others 2002). Furthermore, Wallace and others (2003) demonstrated that Δ⁹-THC or the cannabinoid receptor agonist WIN55,212-2 completely eliminates recurrent epileptic activity in a rat pilocarpine model of epilepsy. Together, these results suggest that an endogenous endocannabinoid tone is present and that activation of CB1R in the cortex reduces excitatory synaptic transmission.

In vitro studies, however, suggest that endocannabinoid signaling in the cortex predominantly targets inhibitory synaptic transmission as CB1R staining appears to be primarily localized to GABAergic interneurons. In layer 2/3 of the neocortex, depolarization of principal neurons results in a retrograde endocannabinoid-mediated suppression of GABA release, termed depolarization-induced suppression of inhibition (DSI) (Fortin and others 2004; Trettel and others 2004; Bodor and others 2005), similar to earlier results in hippocampus and cerebellum (Kreitzer and Regehr 2001a; Maejima, Ohno-Shosaku, and Kano 2001; Wilson and others 2001). Although we previously showed that cannabinoids can also directly modulate excitatory transmission in layer 2/3, the net effect of CB1R activation by either endogenous or exogenous ligands is an increase in PN spike probability (Fortin and others 2004). This suggests that endocannabinoids, at least within layer 2/3, function to increase excitation within cortical networks by inducing disinhibition. Thus, the cellular basis for the net decrease in sensory responses produced by cannabinoid administration in vivo is unclear.

Processing of sensory inputs, however, depends on the integration of information across the layers within a cortical column, which is thought to represent a distinct functional element within the neocortex (Mountcastle 1997). In general, excitation, originating from the appropriate thalamic relay nuclei, projects to excitatory spiny neurons that reside in layer 4 (Sherman and Guillery 1996; Stratford and others 1996). These neurons then send excitatory projections to PNs residing in layer 2/3 (Feldmeyer and others 1999; Thomson and others 2002) that spread excitatory spiny neurons to PNs in layer 5, the main output layer of the neocortex (Dodt and others 2003). Interestingly, CB1R is also highly expressed in layer 5, where its expression is diffuse and localized to fibers (Egertova and others 2003), in contrast to the staining pattern observed in layer 2/3, where CB1R shows dense perisomatic staining.

Consistent with CB1R expression in layer 5, WIN55,212-2 has been shown to suppress evoked excitatory post synaptic responses recorded from layer 5 PNs in rat prefrontal cortex (Auclair and others 2000; but see Ferraro and others 2001). Presynaptic CB1Rs have also been implicated in the induction of spike timing-dependent plasticity between synaptically connected pairs of layer 5 PNs (Stjostrom and others 2003, 2004). In the present study we therefore explored the differential effects of endocannabinoids on inhibitory and excitatory transmission.

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in layer 5, as well as the net effect of endocannabinoid release on spike probability.

Materials and Methods

Slice Preparation
All experiments were performed on postnatal days 16–22 Swiss CD-1 mice (Charles River, Wilmington, MA) using protocols that were approved by the University of Connecticut Health Center Animal Care Committee. Briefly, animals were killed by rapid decapitation without anesthesia, and whole brains were removed and immersed in ice-cold "cutting and incubating solution" composed of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 4 MgCl2, 1 MgSO4, 4 lactic acid, 2 pyruvic acid, 20 glucose, 0.4 ascorbic acid, and 0.25 kynurenic acid equilibrated with 95% O2/5% CO2 (pH 7.3, 310 mOsm/kg). Transverse slices (300 μm) containing somatosensory cortex (Paxinos and Franklin 2001) were cut with a Dosaka EM (Kyoto, Japan) DTK-1000 vibratome and incubated at room temperature. After 30–40 min, slices were transferred to a heated recording chamber (32–33 °C) fixed to the stage of an Olympus BX51WI upright microscope equipped with a 40× vibratome and incubated at room temperature. After 30–40 min, slices were continuously perfused at 2 ml/min with oxygenated bath solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 2 MgCl2, 17.5 glucose, pH 7.3, equilibrated by continuous bubbling with 95% O2 and 5% CO2.

All drugs were delivered by bath perfusion unless specified otherwise. The GABAα antagonist GABAzine (10 μM; Tocris Cookson, Ellisville, MO) was used to pharmacologically isolate excitatory responses, and the ionotropic glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX) (10 μM) and 3-(2-carboxypropylin-4-ylpropyl)-1-phosphonic acid (CPP) (3 μM; Sigma-Aldrich, St Louis, MO) were used to isolate inhibitory activity. Stock solutions of the cannabinoid receptor agonist WIN55,212-2 (Sigma-Aldrich) and the CB1R antagonists SR141716A (RTI International, Research Triangle Park, NC) and 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-1-methyl-N1-piperidinyl-1H-pyrazole-3-carboxamide (AM251) (Tocris Cookson) were dissolved in 100% dimethyl sulfoxide (DMSO) at 103 times their final concentration and stored at −20 °C. Intracellular changes in postsynaptic [Ca2+] were expressed in terms of ΔF/F, where F is the average background-subtracted fluorescence intensity when the cell was at rest and ΔF is the change in fluorescence intensity immediately following the delivery of individual depolarizing stimulus pulses delivered through the whole-cell recording pipette. Fluorescence intensity was measured in an approximately 2 × 4-μm region within the soma. A similar size region of interest was used to detect changes in background fluorescence.

Data Analysis
The amplitudes of evoked responses were measured offline using Clampfit (v9.2, Axon Instruments, Union City, CA) by averaging a 5- to 10-ms period around the peak and subtracting the average baseline value during a 10-ms period taken just before stimulus onset. The magnitude of depolarization-induced suppression of excitation (DSE) or DSi of evoked currents was used as the measure of changes in amplitude following depolarization at compared with baseline. DSi of sIPSCs was calculated as the percent suppression in synaptic charge integrated in 3–5 s periods following depolarization compared with baseline. Drug-induced changes in the amplitude of evoked currents were quantified as the mean percent change in amplitudes of the last 12 consecutive responses acquired during each drug application relative to the drug-free baseline.

Results

Endocannabinoid-Mediated DSE in Neocortical Layer 5 PNs
Layer 5 PNs in acute hemislices of mouse somatosensory cortex were recorded using whole-cell voltage clamp techniques. EPSCs were evoked by intralaminar stimulation at a frequency of 0.2 Hz in the presence of GABA (10 μM), a GABAa receptor antagonist. After a stable baseline of short latency (1.5–2.5 ms) EPSCs was obtained, PNs were depolarized via the patch electrode using 10-ms pulses from −70 mV to 0 mV. In the example shown in Figure 1A, a train of 60 pulses delivered at a frequency...
of 20 Hz induced a marked decrease in EPSC amplitude that was reversible and repeatable. In 9 cells, this depolarization protocol resulted in a significant reduction in the amplitude of the first 2 EPSCs following the train (65.3 ± 7.4% of baseline, Fig. 1B, P < 0.05). Here and in subsequent figures, the number of cells tested is indicated in parentheses.

DSE Is Associated with Decreased Glutamate Release
The reduction in EPSC amplitude following postsynaptic depolarization could be due to changes in presynaptic glutamate release, changes in postsynaptic glutamate receptors, or both. To address
this issue we used paired-pulse stimulation to assess changes in the probability of neurotransmitter release. During DSE, the mean PPR increased from 1.29 ± 0.14 to 1.67 ± 0.22 (Fig. 2F; n = 6, P < 0.05). In these same cells, depolarization in the presence of SR141716A failed to alter the PPR (pretrain = 1.29 ± 0.13, posttrain = 1.28 ± 0.21). These results indicate that endocannabinoid-mediated DSE in layer 5 is most likely due to a CB1R-mediated reduction in release probability of glutamatergic afferents.

**DSE Can Be Induced with a Single High-Frequency Burst of Postsynaptic APs**

We next performed current clamp recordings to determine whether the DSE observed under voltage clamp conditions could also be induced by physiologically relevant AP firing patterns. It is well established that layer 5 PNs, in addition to firing single APs, can fire brief high-frequency AP bursts (Larkum and others 1999), and these bursts may play an important role in encoding sensory information (Lisman 1997). APs were generated by somatic current injection of a train of 1-nA current pulses (pulse duration = 2–3 ms). An example time course of DSE induced by a brief high-frequency burst is shown in Figure 3A. On average, a 100-Hz burst of 5 APs reduced evoked EPSPs amplitudes to 65.3 ± 3.1% of baseline with significant suppression lasting 2 s (Fig. 3B; n = 6, P < 0.05). In 5 out of 5 of these cells, DSE was prevented by subsequent bath application of SR141716A (Fig. 3B). We tested the effects of a single burst...
of APs at different frequencies and only found significant DSE at 80 or 100 Hz. A train of three 100-Hz AP bursts did not produce more DSE than a single burst (Fig. 3C, n = 6). In order to verify the necessity of APs, we included QX-314 (5 μM) in the whole-cell recording pipette to block sodium channel activation. Under these conditions, no DSE was observed in response to five 3-ms/1-nA current pulses delivered at 100 Hz (94.5 ± 2.1% of baseline, n = 9, P > 0.6). In order to determine if the magnitude of DSE was correlated with the elevation in postsynaptic [Ca2+]i, we included Oregon Green 488 BAPTA-1 in the recording pipette. Bursts of 5 APs at either 80 or 100 Hz, which were sufficient to induce DSE, elicited greater peak elevations in postsynaptic [Ca2+]i, compared with 5 AP bursts at 20 or 40 Hz (Fig. 3D, n = 4, P < 0.05), which failed to induce DSE. Thus, with the same number of pulses, changes in the frequency of stimulation result in differences in the magnitude of the calcium increase that were correlated with the magnitude of DSE.

Excitatory Inputs Show Differential Sensitivity to Cannabinoids

In the neocortex, it is not known if the expression of CB1Rs is confined to a subset of glutamatergic synapses in the neocortex. To address this issue, we recorded from layer 5 PNs and compared the cannabinoid sensitivity of EPSCs evoked from stimulation within layer 5 (see Fig. 4A) to stimulation in layer 2/3 (see Fig. 4D). We found that the mean amplitude of EPSCs evoked by layer 5 stimulation was significantly reduced by the cannabinoid agonist WIN55,212-2 (5 μM) (Fig. 4B, baseline = 178.1 ± 48.3 pA vs. WIN55,212-2 = 90.3 ± 22.3 pA, n = 5, P < 0.05). In all cells tested, subsequent application of SR141716A reversed the WIN55,212-2-induced decrease in EPSC amplitudes, confirming that the effect was most likely mediated by CB1Rs (Fig. 4B,C). Layer 5 PNs responded to layer 2/3 stimulation with EPSCs with slightly longer latencies (range: 2.6–4.5 ms) and slower rise times than for EPSCs within layer 5 (P < 0.05). In contrast to the suppression of EPSCs evoked by layer 5 stimulation, WIN55,212-2 had no effect on EPSCs evoked from layer 2/3 (baseline = 115.3 ± 35.8 pA, WIN55,212-2 = 105.6 ± 23.6 pA, Fig. 4E,F), indicating that only a subset of glutamatergic inputs received by layer 5 PNs is sensitive to cannabinoid modulation.

Although CB1Rs appear to mediate neocortical DSE, it is not clear whether they are expressed pre- or postsynaptically. The marked increase in PPR during bouts of DSE suggests that CB1Rs...
are presynaptically expressed (see Fig. 2F). To confirm this result, we also examined the effects of WIN55,212-2 on PPR and on the frequency of miniature postsynaptic currents. We found that the WIN55,212-2-induced decrease in evoked EPSCs was paralleled by an increase in PPR (baseline = 1.22 ± 0.24, WIN55,212-2 = 1.84 ± 0.28, P < 0.05) that returned to baseline levels following the application of SR141716A (Fig. 5A, n = 4). WIN55,212-2 also reduced mEPSC frequency (see example in Fig. 5B) from 3.5 ± 0.9 to 2.5 ± 0.7 Hz (Fig. 5C, n = 6, P < 0.05), an effect that was reversed by SR141716A (Fig. 5C). WIN55,212-2, however, had no effect on the mean amplitude of mEPSCs (Fig. 5D) or the distribution of mEPSCs. Taken together, these results suggest that CB1Rs are most likely expressed presynaptically on a subset of glutamatergic terminals.

**Inhibitory Inputs onto Layer 5 PNs Are Predominantly DSI and Cannabinoid Insensitive**

In many brain regions where DSE occurs (e.g., hippocampus, cerebellum, and ventral tegmental area), depolarization of principal cells also results in the suppression of inhibitory synaptic currents (DSI, reviewed in Alger 2002). Therefore, we wanted to determine whether DSE and DSI are coexpressed within layer 5. To isolate IPSCs, the ionotropic glutamate receptor antagonists DNQX (10 μM) and CPP (3 μM) were added to the bath perfusate. We first examined the effect of depolarization on spontaneous inhibitory activity (sIPSCs). As shown in the example in Figure 6A and summary data in Figure 6B, depolarization failed to alter the frequency or amplitude of sIPSCs. Because it has been shown that the activity of DSI-sensitive inputs can be increased by muscarinic receptor activation (Kim and others 2002; Fortin and others 2004) we also performed experiments in the presence of the muscarinic receptor agonist carbachol (CCh). Surprisingly, in the majority of cells tested (9 out of 17), 5 μM CCh caused a marked reduction in the frequency of sIPSCs (baseline = 3.5 ± 1.0 Hz, CCh = 0.89 ± 0.5 Hz), and we were thus unable to examine DSI in these cells. In 5 cells where CCh did not alter the frequency of sIPSCs, no DSI was observed (Fig. 6C,D). However, in 3 of the 17 cells tested, CCh caused a marked increase in sIPSC activity (baseline = 1.9 ± 0.1 Hz, CCh = 6.3 ± 1.6 Hz), and a subsequent train of depolarizing pulses suppressed sIPSC synaptic charge to 24.6 ± 5.3% of baseline, an effect that was blocked by SR141716A (data not shown).

To explore the possibility that DSI-sensitive inputs are present in layer 5 but are not spontaneously active or CCh sensitive, IPSCs were evoked using the same intralaminar stimulation paradigm that was used to evoke EPSCs. Under these conditions, a 60-pulse/20-Hz train that reliably produced DSE had no effect on IPSCs (Fig. 7A, n = 6). Consistent with the lack of DSI, no change in mean PPR values was observed (pre- and posttrain values were 1.33 ± 0.19 and 1.24 ± 0.06, respectively). We then repositioned the stimulating electrode from layer 5 to layer 2/3. In 10 out of 10 layer 5 PNs, inhibitory responses evoked from layer 2/3 stimulation also failed to demonstrate DSI following a brief train of depolarizing pulses (Fig. 7B).

The general lack of DSI of evoked IPSC and sIPSC could be due to a lack of CB1R expression at inhibitory synapses or insufficient endocannabinoid release. We therefore examined the effects of WIN55,212-2 on evoked IPSC and sIPSCs. Application

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**Figure 4.** WIN55,212-2 (WIN), a cannabinoid receptor agonist, suppresses EPSCs evoked from layer 5 but not from layer 2/3. (A) Diagram indicating the location of the stimulating electrode relative to the patched neuron for experiments shown in (B) and (C). (B) Representative time course illustrating the effect of WIN (5 μM) on EPSCs evoked from layer 5 and subsequent reversal following the addition of SR141716A (SR). (C) Group data demonstrating the effect of WIN on normalized EPSC amplitudes and after subsequent addition of SR. (D) Diagram illustrating the location of the stimulating electrode for experiments shown in (E) and (F). (E) Representative time course showing the lack of effect of WIN and SR on EPSCs evoked from layer 2/3. (F) Summary data for WIN and SR on the amplitude of EPSCs evoked from layer 2/3. *P < 0.05.
Synaptic depolarization would reduce AP probability in served DSE, but not DSI, in layer 5, we predicted that post-
(Fortin and others 2004). Given that we predominately ob-
synaptic depolarization is a transient increase in PN excitability
inhibitory and excitatory inputs, and the net effect of post-
In layer 2/3 of the neocortex, WIN55,212-2 suppresses both
DSE in Layer 5
Functional Significance of Endocannabinoid-Mediated
endogenous and exogenous cannabinoid modulation.

**Discussion**

The present results demonstrate that glutamatergic and GA-
BAAergic inputs to layer 5 PNs of mouse somatosensory cortex
are differentially regulated following depolarization-induced
endocannabinoid release, with DSE much more prominent
than DSI. The observed DSE was dependent on AP firing,
required elevations in postsynaptic intracellular Ca²⁺,
and was mediated by endocannabinoids as it was blocked by the CB1R
agonists Sr141716A and AM251. Similarly, we found that the
cannabinoid receptor agonist WIN55,212-2 depressed EPSCs
but not IPSCs recorded from layer 5 PNs. The selective
reduction in EPSC amplitudes resulting from depolarization or
WIN55,212-2 was expressed presynaptically because it was
accompanied by a decrease in the PPR of evoked EPSCs and
a reduction in the frequency, but not amplitude, of mEPSCs.
Thus, CB1R activation most likely triggers a decrease in the
probability of presynaptic glutamate release. Interestingly, we
found that stimulation within layer 5, but not layer 2/3, evoked
cannabinoid-sensitive synaptic currents, indicating that only
a subset of glutamatergic inputs express cannabinoid receptors.
Taken together, these results suggest that endocannabinoids
may provide a mechanism by which individual layer 5 PNs can
reduce their sensitivity to specific excitatory synaptic inputs,
thereby influencing synaptic integration and neuronal output.

These findings also suggest that 2 different modes of activity
can mobilize endocannabinoids and induce DSE. Several sec-
onds of sustained AP firing at 20 Hz are required to elevate
postsynaptic [Ca²⁺]i to the levels that are sufficient to produce
DSE. Maintaining the increase in calcium by prolonging activity
extends the duration of DSE, presumably by producing sus-
tained endocannabinoid mobilization. DSE can also be induced
by a brief high-frequency (>80 Hz) burst of as few as 5 APs,
which produces a transient but greater elevation in postsynap-
tic [Ca²⁺]i compared with the same number of APs delivered
at a lower frequency. Thus, DSE in layer 5 PNs appears to
be dependent on the magnitude as well as the duration of
postsynaptic [Ca²⁺]i. Alternatively, in the absence of postsynap-
tic AP firing, a sufficient calcium elevation to induce DSE may
also be produced by temporal summation of multiple inputs, as
was recently demonstrated in the hippocampus (Zhuang and
others 2005).

The effects of both endocannabinoids as well as the exoge-
nous cannabinoid agonist appeared to be mediated by CB1R

**Functional Significance of Endocannabinoid-Mediated
DSE in Layer 5**

In layer 2/3 of the neocortex, WIN55,212-2 suppresses both
inhibitory and excitatory inputs, and the net effect of post-
synaptic depolarization is a transient increase in PN excitability
(Fortin and others 2004). Given that we predominately ob-
served DSE, but not DSI, in layer 5, we predicted that post-
synaptic depolarization would reduce AP probability in
response to mixed synaptic potentials evoked by layer 5
intralaminar stimulation (in the absence of neurotransmitter
receptor antagonists). The baseline stimulus intensity was
adjusted to produce a high probability (>70%) of evoking an
AP. Under these conditions, depolarization of layer 5 PNs with
a single 20-Hz train of 50 APs resulted in a transient decrease in
spike probability (baseline = 0.84 ± 0.09, posttrain = 0.07 ± 0.07,
Fig. 8A-C, P < 0.05) that endured for 10 s before returning to
baseline values. In these same cells, subsequent application of
Sr141716A prevented the depolarization-induced decrease in
spike probability (Fig. 8C). No change in Vm was observed
following the AP train (baseline = -63.5 ± 2.5 mV, 5 s posttrain
= -64.0 ± 2.6 mV), indicating that the transient decrease in spike
probability was not simply due to membrane hyperpolarization.
Therefore, postsynaptic depolarization and subsequent endo-
cannabinoid mobilization reduces spike probability as a result of
inhibiting presynaptic glutamate release.
activation as these effects were blocked by CB1R antagonists. There is, however, evidence suggesting that some cannabinoid effects on glutamate release may be mediated by a novel, unidentified cannabinoid receptor. In both the neocortex and hippocampus, for example, CB1R staining appears to be almost exclusively localized to GABAergic neurons. It has also been shown in the hippocampus that the effect of WIN55,212-2 on EPSCs persists in CB1R–/– mice (Hajos and others 2001; but see Ohno-Shosaku, Tsubokawa, and others 2002), whereas the effects on GABA release are clearly absent in the knockout. Interestingly, the same group showed that the effect of WIN55212-2 on EPSCs was blocked by SR141716A, but not by AM251, suggesting that AM251 may be selective for CB1R versus the novel receptor.

In the present study, however, we found that the WIN55,212-2-induced suppression of EPSCs was antagonized by either SR141716A or AM251, indicating that CB1Rs are most likely responsible for the modulation of glutamatergic transmission in the neocortex. AM251 also blocks the endocannabinoid-mediated long-term depression among layer 5 PNs (Sjostrom and others 2003, 2004). In support of a role for CB1R in neocortex, PNs have been shown to express CB1R mRNA (Matsuda and others 1993; Tsou and others 1998; Marsicano and Lutz 1999), and CB1R protein is widely expressed throughout the neocortex including layer 5 (Egertova and others 2003). In fact, the staining pattern in layer 5 is very different from that seen in layer 2/3—in layer 2/3, CB1R staining shows clear perisomatic labeling consistent with expression in GABAergic basket cell terminals (Katona and others 1999) and cannabinoids potently suppress GABA release in layer 2/3 (Trettel and Levine 2002). The staining in layer 5 lacks clear perisomatic labeling, consistent with the lack of effect of cannabinoids on GABA release, and therefore is suggestive of CB1R expression in glutamatergic terminals.

We found that DSE induced by brief high-frequency AP bursts was associated with a reduction in spike probability. Previous studies have shown that neocortical PNs can fire high-frequency AP bursts in vivo (Connors and others 1982; Llinas 1988; Larkum and others 1999) and that layer 5 PNs in somatosensory cortex fire such bursts following whisker stimulation (Helmchen and others 1999). This bursting activity has been implicated in enhancing the reliability of synaptic transmission (Lisman 1997; Swadlow and Gusev 2001), inducing burst-firing-associated long-term depression (Birtoli and Ulrich 2004), and in mediating cortical synchronization and oscillations (Silva and others 1991). Burst generation in vivo is thought to depend on the coincident activation of proximal and distal synaptic inputs (Larkum and others 1999). Therefore, in addition to associating inputs arriving from different cortical layers, bursts may also provide a regulatory signal (by way of endocannabinoid release) that allows layer 5 PNs to transiently decrease the synaptic strengths of a particular set of glutamatergic inputs. This also raises the possibility that cannabinoid-insensitive inputs may be influenced by other retrograde signals. For example, PNs in layer 2/3 appear to utilize different types of retrograde messengers (namely, endocannabinoids, and/or glutamate) to regulate the release of GABA from different subpopulations of inhibitory interneurons (reviewed in Zilberter and others 2005).

Previous in vivo studies have suggested that CB1R activation decreases cortical sensory responses by inhibiting excitatory transmission (Wilkison and others 1982; Pontzer and others 1986; Patel and others 2002). It is clear, however, that cannabinoids exert multiple effects within the cortex and demonstrate a great degree of laminar specificity. In layer 2/3, both GABAergic and glutamatergic inputs to PNs are cannabinoid sensitive, and the net result of depolarization-induced endocannabinoid release is an enhancement of PN activity (Fortin and others 2004). By contrast, in layer 5, GABAergic...
inputs onto layer 5 PNs are largely cannabinoid insensitive, whereas glutamate inputs are sensitive, and depolarization-induced endocannabinoid release decreased PN spike probability. Endocannabinoids have also been shown to induce a slow self-inhibition of low-threshold spiking interneurons in layer 5 (Bacci and others 2004). The differential effects of endocannabinoids within and across the different neocortical lamina underscore the potential complexity of cannabinoid effects on sensory responses.

The endocannabinoid system has also recently been shown to play a neuroprotective role in models of excitotoxicity (Marsicano and others 2003). Specifically, mice selectively lacking CB1Rs in glutamatergic neurons are more prone to kainate-induced neurotoxicity in vivo than their wild-type littermates (Marsicano and others 2003). Moreover, exogenous cannabinoids have been shown to exert neuroprotective effects in a variety of in vitro and in vivo models of neuronal injury (reviewed in van der Stelt and others 2002). Thus, under
pathological conditions, such as prolonged periods of depolarization resulting from excessive glutamate receptor activation, endocannabinoids could reduce the level of glutamate release at CB1R-expressing terminals, thereby protecting against excitotoxicity. In support of the neuroprotective effects of endocannabinoids, Wallace and others (2003) recently found that levels of the endocannabinoid 2-arachidonylglycerol are significantly increased during seizure activity. Moreover, they reported that a single injection of SR141716A in epileptic animals markedly increases seizure frequency, suggesting that under certain pathological conditions the activity-dependent release of endocannabinoids may play an anticonvulsant role in the central nervous system.

In summary, the results of the present study demonstrate that layer 5 PNs, which provide the major output from the cortex, are capable of modulating glutamatergic inputs via presynaptic CB1Rs. The finding that only a subset of glutamatergic inputs is modulated via CB1Rs is novel and may provide a mechanism by which individual layer 5 PNs can adjust the synaptic weights of specific glutamatergic inputs. A small population of CCh-sensitive GABAergic inputs can also be modulated by endocannabinoids, an effect that may be significant during periods of increased cholinergic drive. Taken together with our previous results, these data suggest that endocannabinoid mobilization produces a net increase in the activity of intracortically projecting layer 2/3 PNs, while dampening activity of the primary cortical output neurons in layer 5. Thus, endocannabinoid-mediated plasticity is a complex modulator of synaptic communication and could have important consequences with respect to the flow of sensory information within cortical networks.

Notes
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