Behavioral/Systems/Cognitive

Electrical Coupling among Irregular-Spiking GABAergic Interneurons Expressing Cannabinoid Receptors

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Anatomical studies have shown that the G-protein-coupled cannabinoid receptor-1 (CB1) is selectively expressed in a subset of GABAergic interneurons. It has been proposed that these cells regulate rhythmic activity and play a key role mediating the cognitive actions of marijuana and endogenous cannabinoids. However, the physiology, anatomy, and synaptic connectivity of neocortical CB1-expressing interneurons remain poorly studied. We identified a population of CB1-expressing interneurons in layer II/III in mouse neocortical slices. These cells were multipolar or bitufted, had a widely extending axon, and exhibited a characteristic pattern of irregular spiking (IS) in response to current injection. CB1-expressing-IS (CB1-IS) cells were inhibitory, establishing GABA_A receptor-mediated synapses onto pyramidal cells and other CB1-IS cells. Recently, electrical coupling among other classes of cortical interneurons has been shown to contribute to the generation of rhythmic synchronous activity in the neocortex. We therefore tested whether CB1-IS interneurons are interconnected via electrical synapses using paired recordings. We found that 90% (19 of 21 pairs) of simultaneously recorded pairs of CB1-IS cells were electrically coupled. The average coupling coefficient was ~6%. Signaling through electrical synapses promoted coordinated firing among CB1-IS cells. Together, our results identify a population of electrically coupled CB1-IS GABAergic interneurons in the neocortex that share a unique morphology and a characteristic pattern of irregular spiking in response to current injection. The synaptic interactions of these cells may play an important role mediating the cognitive actions of cannabinoids and regulating coherent neocortical activity.

Key words: cannabinoids; CB1; electrical synapses; irregular-spiking; interneuron; GABAergic

Introduction

CB1 is a G-protein-coupled cannabinoid receptor widely expressed throughout the brain (Matsuda et al., 1990). Cells expressing CB1 receptors are involved in a variety of actions, including regulating rhythmic activity (Hájos et al., 2000), mediating the depolarization-induced suppression of inhibition (DSI) in the hippocampus (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001), cerebellum (Kreitzer and Regehr, 2001), and neocortex (Trettel and Levine, 2003; Trettel et al., 2004), and participating in different forms of long synaptic plasticity (Gerdeeman et al., 2002; Chevaleyre and Castillo, 2003). In the cortical mantle, CB1 receptors are abundantly expressed, showing a particularly dense distribution in superficial layer II/III (Marsicano and Lutz, 1999; Egertova and Elphick, 2000). Although cortical CB1-expressing interneurons are thought to mediate many of the cognitive actions of marijuana and could play a key role in cortical function, very little is yet known about their basic physiology and synaptic connectivity.

Immunohistochemical studies have reported that, in the hippocampus and neocortex, CB1 receptors are present on axon terminals of specific GABAergic cells (Katona et al., 1999; Marsicano and Lutz, 1999; Tsou et al., 1999; Egertova and Elphick, 2000; Bodor et al., 2003). Specifically, CB1 expression has been shown to be mainly restricted to large cells containing the neuropeptide cholecystokinin (CCK) (Katona et al., 1999; Marsicano and Lutz, 1999; Tsou et al., 1999; Bodor et al., 2003). Other major classes of neocortical GABAergic interneurons, including those expressing parvalbumin, somatostatin, or VIP, lack CB1 immunoreactivity (Katona et al., 1999, 2000; Tsou et al., 1999; Bodor et al., 2003). To date, targeting CB1-expressing cells for systematic study has been difficult, and their physiology, morphology, and synaptic properties remain unknown.

Recent studies have demonstrated that several classes of GABAergic interneurons in the cerebral cortex are interconnected via electrical synapses (Galarreta and Hestrin, 1999; Gibson et al., 1999). Cumulative data indicate that gap junctions are established specifically among interneurons belonging to the same type and may define functional classes of GABAergic interneurons (for review, see Galarreta and Hestrin, 2001a; Long and Connors, 2004). Moreover, electrical coupling promotes the coordinated firing of connected cells, contributing to the generation of rhythmic synchronous activity. Neurons expressing CB1...
Pyramidal cells and other CB1-IS cells. In addition, CB1-expressing irregular-spiking (CB1-IS) cells were inhibitory morphology and irregular pattern of firing after current injection. CB1-expressing interneurons in layer II/III with a characteristic irregular spiking. A population of layer II/III GABAergic interneurons is characterized by an irregular pattern of spiking. A, A multipolar EGFP-GAD65 fluorescing cell in a 17-d-old mouse. B, Same field visualized under infrared differential interference contrast (IR-DIC) video microscopy. The arrow points to the cell illustrated in A. C, Three examples of the firing pattern of the same neuron in response to a pulse of depolarizing current injection (200 pA, 600 msec). Note the characteristic irregular spiking. D, Firing pattern in response to a larger current injection (400 pA, 600 msec). E, F, Portions of the bottom traces in C and D (indicated by the boxes) are shown at an expanded scale to illustrate the oscillations of the membrane potential.

Receptors have been proposed to regulate rhythmic oscillatory activity (Hájos et al., 2000; Wilson and Nicoll, 2002), but whether these cells are interconnected via electrical synapses, which may coordinate their firing, remains to be determined.

We have addressed these issues using paired recordings in acute slices of mouse neocortex. We have identified a population of CB1-expressing interneurons in layer II/III with a characteristic morphology and irregular pattern of firing after current injection. CB1-expressing irregular-spiking (CB1-IS) cells were inhibitory and established GABA_A receptor-mediated synapses onto pyramidal cells and other CB1-IS cells. In addition, CB1-IS cells were electrically coupled, and these electrical synapses helped synchronize their firing activity.

Materials and Methods

Slice preparation and cell identification. We used a mouse strain expressing an enhanced green fluorescent protein (EGFP) under the control of the promoter for glutamic acid decarboxylase 65 (GAD65) (Erdélyi et al., 2002; Brager et al., 2003; López-Bendito et al., 2004). Juvenile mice of both sexes (14–20 d old) were anesthetized by an intraperitoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg) and were decapitated. Parasagittal cortical slices (300 μm thick, 30° angle) were obtained in an ice-cold extracellular solution. After dissection, the slices were incubated at 32–34°C for 30 min and then at room temperature (20–22°C) until transferred to a submersion-type recording chamber. The extracellular solution bathing the slices during the dissection, incubation, and recordings contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 MgSO_4, 2 CaCl_2, 26 NaHCO_3, 20 glucose, 4 lactic acid, 2 pyruvic acid, and 0.4 ascorbic acid, pH 7.4 (315 mOsm) and was continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2. Kynurenic acid (1 mM) was added during the dissection and incubation period.

Fluorescent neurons in the somatosensory cortex were visualized using an upright microscope (Axioskop; Zeiss, Thornwood, NY) illuminated with a xenon lamp (150 W; Opti Quip, Highland Mills, NY) equipped with a 40× water immersion lens and EGFP filters (XF100; Omega Optical, Brattleboro, VT). Once a fluorescent neuron was selected, it was visualized using infrared differential interference contrast video microscopy and recorded using conventional patch-clamp techniques (Stuart et al., 1993). EGFP-positive cells were classified as CB1-IS cells depending on their pattern of spiking in response to current injection. Fast-spiking (FS) cells were EGFP negative and were identified by their typical discharges of high-frequency nonaccommodating spikes in response to near-threshold current injection (McCormick et al., 1985).

Paired recording and data analysis. Simultaneous somatic whole-cell recordings were made with patch electrodes (3–4 MΩ) filled with a solution containing the following (in mM): 130 K-methylsulfate, 6.3 KCl, 10 HEPES, 4 MgATP, 20 phosphocreatine(Na), 0.3 NaGTP, 0.2 EGTA and 0.3% biocytin or 106 K-methylsulfate, 40 KCl, 10 HEPES, 4 MgATP, 20 phosphocreatine(Na), 0.3 NaGTP, 0.2 EGTA, and 0.3% biocytin, pH 7.3 (295 mOsm). Recordings were performed at 31–32°C. The error attributable to the liquid junction potential (~11 mV) was not corrected. We did not compensate for the series resistance that ranged between 10 and 25 MΩ. Both cells were recorded under current-clamp mode using two Axopatch 200B amplifiers (Axon Instruments, Union City, CA). The voltage and current output were filtered at 5 kHz and digitized at 16 bit.
resolution (ITC-18; InstruTech, Port Washington, NY), with a sampling frequency of 10 kHz.

IPSPs were recorded in the presence of the AMPA and kainate receptor antagonist DNQX (10 μM, Sigma, St. Louis, MO). DNQX, bicuculline methiodide (Sigma), WIN55,212-2 (Tocris Cookson, Ellisville, MO), and AM-251 (Tocris Cookson) were applied in the bath. WIN55,212-2 and AM-251 were dissolved in DMSO (10 mM stock solution).

The input resistance and membrane time constant of CB1-IS cells were calculated by injecting long pulses of depolarizing current (50 pA, 300 msec). Spike amplitudes and the afterhyperpolarization potentials (AHPs) were measured relative to the spike threshold. The coupling coefficient was calculated as the ratio between the change of membrane voltage produced in the noninjected cell and that in the injected cell. Assuming a simple model of two isopotential neurons connected by a single electrical junction, the gap junction conductance (Gc) was calculated according to the equation Gc = 1/[R∞(CC) − Rmem], where R∞ is the input resistance of the postsynaptic neuron, and CC is the step coupling coefficient.

Data are given as mean ± SEM. Differences were considered statistically significant (Student’s t test) if p < 0.05.

Histology, immunohistochemistry, and morphology. To study the morphology of the recorded neurons, biocytin (0.3%) was included in the pipette solution during the physiological experiments. The slices containing biocytin-filled cells were fixed with 4% paraformaldehyde in 0.01 M phosphate buffer and 0.2% picric acid overnight at 4°C. Standard avidin–biotin–horseradish peroxidase complex (ABC; Vector Laboratories, Burlingame, CA) and the 3,3′-diaminobenzidine (DAB) reaction procedure were used. The slices were mounted in M01 media from Biomeda (Foster City, CA). Reconstructions of the neurons were done with Neurolucida (MicroBrightField, Williston, VT) using a 100× oil-immersion objective.

To detect the presence of CB1 receptors and parvalbumin in biocytin-filled electrophysiologically characterized neurons, the slices were fixed in 4% paraformaldehyde in 0.01 M phosphate buffer and 0.2% picric acid for 2 hr at 4°C. After washing the slices with Tris-buffered saline (TBS), tissue sections were incubated overnight at 4°C with a rabbit anti-CB1 antibody (1:2000; Ken Mackie, Department of Anesthesiology, University of Washington, Seattle, WA). Next, the slices were washed again in TBS and incubated for 3 hr in the secondary antibody goat anti-rabbit IgG Alexa Fluor 555 (1:500; catalog #A21428; Molecular Probes, Eugene, OR). After rinsing the tissue in TBS, the slices were incubated in streptavidin–Alexa Fluor 350 (1:300; catalog #S-11249; Molecular Probes) for 45 min to reveal the biocytin. The slices were rinsed again in TBS and mounted in M01. Those slices tested for parvalbumin were rinsed in TBS, incubated in a blocking solution containing 1% Triton X-100, 2% goat serum, and 2% bovine serum albumin for 4 hr, and incubated overnight at 4°C with a rabbit anti-parvalbumin antibody (1:3000; catalog #PV28; Swant, Bellinzona, Switzerland). Next, the slices were rinsed again in TBS and incubated for 3 hr in the secondary antibody goat anti-rabbit IgG Alexa Fluor 555 (1:500; catalog #A21428; Molecular Probes), rinsed again, and mounted in M01 media.

Results
Physiology of IS cells
To target CB1-expressing neurons in neocortical slices, we used a line of transgenic mice in which the EGFP-expressing cells include most of the CCK-positive neurons (López-Bendito et al., 2004). Because large, but not small, CCK-containing cells express CB1 receptors (Bodor et al., 2003), we selected large EGFP-positive cells in upper layer II/III for our recordings (Fig. 1A). Cells were visualized under infrared video microscopy (Fig. 1B) and recorded using conventional patch-clamp techniques. We selected relatively large neurons with multipolar or bifurcated appearance. Using this approach, we identified a population of neurons with a characteristic pattern of irregular spiking in response to depolarizing current injections that we termed IS cells. In response to near-threshold current injections, IS cells typically produced a small number of action potentials with variable interspike intervals (ISIs) (Figs. 1C, 2A). When the same current injection was repeated, the responses were highly variable from trial to trial (Fig. 1C). When larger current injections were used, the spiking was characterized by an initial burst of high-frequency action potentials, followed by a variable number of spikes at irregular interspike intervals (Fig. 1D). The average coefficient of variation of the ISI in traces containing 10 or more spikes was 0.58 ± 0.05 (n = 21 cells) (Fig. 2C). Other observations among

Figure 3. IS cells are immunoreactive to CB1 receptors. A, Characteristic irregular spiking of a layer II/III neuron. B, EGFP fluorescence of the cell whose response is shown in A (arrow). The arrowhead points to the axon of this neuron. The double arrowhead points to an unidentified IS cell. C, Photograph of the recorded cell filled with biocytin and revealed with streptavidin–Alexa Fluor 350, D, CB1 immunoreactivity of the cell shown in A–C. E, F, FS cells lack CB1 immunoreactivity. E, Pattern of firing of a representative FS cell filled with biocytin (G) during the recording (arrow). This cell was EGFP negative (F) and did not show immunoreactivity for CB1 receptors (H). The arrowheads in F and H point to a nonrecorded EGFP-positive cell immunoreactive for CB1 receptors.
Expression of CB₁ receptors in FS cells (Kawaguchi, 1993, 1995; Galarreta and Hestrin, 2002).

were wider and exhibited smaller, slower AHPs than those described in FS (Fig. 2). The membrane conductance of paired-pulse responses (coefficient of variation, 0.81). Bottom trace, Average of 50 responses. The paired-pulse ratio (IPSP2/IPSP1) was 0.94. The decay of the average IPSP was fitted with an exponential function with a time constant of 21 ms.

Next, we determined whether IS cells express CB₁ receptors. CB₁ receptors, and, hereinafter, we will refer to this neuronal population as CB₁-IS cells.

Morphology of CB₁-IS cells

To study the morphology of layer II/III CB₁-IS cells, we reconstructed electrophysiologically identified neurons filled with biocytin (n = 14 cells) (Figs. 4A, C, 5A). Typically the somatodendritic morphology of these cells was either multipolar (polygonal-shaped soma with four or more radial dendrites) or bitufted (ovoid-shaped soma with multiple dendrites extending from the upper and lower poles). They had relatively large cell bodies, with average somatic horizontal and vertical diameters of 12.6 ± 0.4 and 17.5 ± 0.7 μm, respectively (n = 30 cells). Their dendrites were smooth (aspy) and extended radially into layers I, II/III, IV, and V. The total average horizontal and vertical spreads of the dendrites were 360 ± 40 and 315 ± 35 μm, respectively (n = 7). In most cases, the axon originated from the lower region of the cell body or from a primary dendrite. Typically, the axon branched profusely in the vicinity of the soma within layer II/III and extended horizontally for several hundred micrometers. In contrast to the dendrites that often extended into layer I and approached the piamater, the axons of CB₁-IS cells branched densely in upper layer II but only rarely extended into layer I. In addition, some axonal collaterals descended vertically or obliquely spanning occasionally the entire thickness of the neocortex and reaching layer VI. The total average horizontal and vertical spreads of the axonal arbor were 1085 ± 170 and 920 ± 115 μm, respectively. We observed some putative axonal boutons surrounding the somata of other cells, suggesting that CB₁-IS cells include basket cells. CB₁-IS cells are therefore characterized by radial dendrites and a wide axonal arborization that expands horizontally over several hundred of micrometers and spans multiple layers from upper layer II to layer VI.

IS cells were a reduction in the amplitude of the later spikes in the response (Fig. 1D) and small oscillations of the membrane potential between the spikes (Fig. 1E,F). IS cells had an average input resistance of 127.7 ± 7.2 MΩ and a time constant of 12.2 ± 1.0 msec (n = 21 cells). Their spikes had an average amplitude of 67.4 ± 1.9 mV, a half-width of 0.80 ± 0.05 msec, and were followed by an AHP with an amplitude of 13.5 ± 0.5 mV and a decay of 55.8 ± 5.2 msec (Fig. 2A,E). The minimum value of the AHP occurred on average 7.1 ± 0.8 msec after the spike peak (Fig. 2E). Figure 2 compares the firing pattern and the properties of individual spikes in IS cells (Fig. 2A,C,E) and in FS (Fig. 2B,D,F) cells. Typically, action potentials in IS cells were wider and exhibited smaller, slower AHPs than those described in FS cells (Kawaguchi, 1993, 1995; Galarreta and Hestrin, 2002).

Expression of CB₁ receptors

Next, we determined whether IS cells express CB₁ receptors. CB₁ immunostaining revealed a dense network of immunopositive fibers in layer II/III, with some scattered immunoreactive somata. We found that 78% (7 of 9) of physiologically characterized IS cells reacted to the CB₁ antibody (Fig. 3A–D). Immunoreactivity was located in the cell body, with a perinuclear distribution, and also in the axonal process. Some putative boutons of the CB₁-positive axonal processes surrounded the cell bodies of CB₁-negative cells. Other types of GABAergic interneurons, including the parvalbumin-positive cells, have been shown to be CB₁ immunonegative (Katona et al., 1999; Tsou et al., 1999). Consistent with these results, CB₁-IS cells were immunonegative for parvalbumin (data not shown). Furthermore, we recorded from layer II/III FS cells (n = 5), known to contain parvalbumin, and found that none of the tested cells were immunopositive for CB₁ receptors (Fig. 3E–H). These results suggest that IS cells express CB₁ receptors, and, hereinafter, we will refer to this neuronal population as CB₁-IS cells.

CB₁-IS cells are GABAergic

To confirm that CB₁-IS cells establish GABAergic synapses, we performed simultaneous whole-cell recordings among CB₁-IS cells and between CB₁-IS cells and layer II/III pyramidal neurons (n = 65 pairs). We found nine pairs in which a presynaptic CB₁-IS cell was connected via chemical synapses to a pyramidal cell (n = 5 of 44 pairs) (Fig. 4A,B) or to another CB₁-IS cell (n = 4 of 21 pairs) (Fig. 4C,D). Anatomical reconstructions of two of these pairs are shown in Figure 4, A and C. Note that the axons of the CB₁-IS cells branched profusely in layer II/III, as well as projected descending branches into layer V and VI. We generated presynaptic action potentials with brief (2–3 msec) suprathreshold current injections in the CB₁-IS cells and recorded the
CB₁ receptor activation blocks CB₁-IS IPSPs

Activation of CB₁ receptors reduces GABA release in GABAergic axonal terminals of the hippocampus (Katona et al., 1999; Hajas et al., 2000; Hoffman and Lupica, 2000) and the neocortex (Trettel and Levine, 2002). GABAergic interneurons are heterogeneous, and only those expressing CB₁ receptors are selectively targeted by cannabinoid agonists. Thus, paired recordings have shown that, whereas some unitary IPSCs are dramatically depressed by cannabinoid agonists, others remain unaffected (Ohno-Shosaku et al., 2001; Wilson et al., 2001). To examine whether CB₁-IS cells express functional CB₁ receptors in their axonal terminals, we tested the effect of the cannabionid receptor agonist WIN55,212-2 (1 µM) on CB₁-IS-mediated IPSPs. In all tested pairs (n = 4), WIN55,212-2 completely abolished (to 1.6 ± 2.2% of control) the IPSP generated by a CB₁-IS cell onto a pyramidal neuron (Fig. 5). The inhibitory transmission was recovered after the addition of the CB₁ selective antagonist AM-251 (10 µM; to 91.6 ± 25.2% of control). These results indicate, therefore, that CB₁-IS cells express functional CB₁ receptors whose activation can completely block CB₁-IS mediated IPSPs.

Electrical coupling among CB₁-IS cells

Recent studies have shown that interneurons belonging to the same type are electrically coupled via gap junctions (for review, see Galarreta and Hestrin, 2001a; Long and Connors, 2004). To study whether CB₁-IS cells are interconnected via electrical synapses, we recorded from pairs of CB₁-IS cells in layer II/III (Fig. 6A, B) and injected pulses of subthreshold depolarizing or hyperpolarizing current into one of the cells. The distance between the somata of the two cells ranged from 37 to 218 µm, with an average value of 106 ± 16 µm. When the cells were electrically coupled, we observed a change in the membrane potential of the noninjected cell (Fig. 6C). We found that 90% of the pairs examined (19 of 21 pairs) were electrically coupled. The mean coupling coefficient was 5.6 ± 0.8% (range, 0.6–10.9%; n = 19 pairs) (Fig. 6D). Electrical coupling was always bidirectional. Furthermore, we observed that, although the strength of the electrical coupling varied significantly among different pairs of CB₁-IS cells, it was similar in both directions (Fig. 6D). Assuming a model of isopotential cells, the estimated coupling conductance between pairs of CB₁-IS cells was 427 ± 67 pS (range, 44–941 pS; n = 19 pairs). Electrical coupling was not found between CB₁-IS cells and pyramidal neurons (20 pairs tested).

Next, we studied how action potentials in CB₁-IS cells are transmitted through the electrical synapses. We examined this issue in trials in which the presynaptic cell fired spontaneously, after being depolarized to near threshold with a prolonged current injection (Fig. 7A). Individual spikes produced a biphasic signal in the postsynaptic cell. Typically, the hyperpolarizing component reflecting the spike AHP was larger (0.8 ± 0.15 mV; n = 7 pairs) and slower than the brief depolarizing component (0.48 ± 0.11 mV; n = 7 pairs) reflecting the spike itself. The average latency between the peak of the presynaptic spike and that of the postsynaptic spikelet was 0.88 ± 0.20 msec (range, 0.51–2.03 msec; n = 7 pairs) (Fig. 7C). The hyperpolarizing component reached its minimum 20.90 ± 1.97 msec after the peak of the presynaptic spike and decayed to baseline over tens of milliseconds (half-width, 49.1 ± 7.1 msec) (Fig. 7B). These slow kinetics reflect the relatively slow AHP of CB₁-IS cells (Fig. 2).

Electrical synapses promote coordinated firing of CB₁-IS cells

Electrical coupling has been shown to promote coordinated firing of different classes of GABAergic neocortical interneurons
We found that a high percentage of spikes occurred within a window of 5 msec from a spike in the second cell, relative to the total number of spikes. We defined firing of both cells was correlated by detecting spikes that occurred pairs). Under these conditions, we examined whether the firing of an electrically coupled CB1-IS cell. We recorded current in cell 1 simultaneously affected the membrane voltage of the noninjected cell 2. The injection of current in cell 2 similarly affected the membrane potential of cell 1. The coupling coefficient was 10.2%. Traces are the average of 80 – 100 responses. D, Bi-directionality of electrical coupling. Plot showing the coupling coefficient when current is transmitted from cell 1 to cell 2 and from cell 2 to cell 1. Data from the same pair are connected by a line.

Next, we examined in further detail the temporal relationship between coordinated spikes. We observed that typically a spike in one cell produced a spikelet in the second cell that peaked between 0.5 and 1.5 msec. If the membrane voltage of the second cell reached the spike threshold during the rise or peak of the spikelet, a spike was then generated in that cell (Fig. 9B). Thus, these data suggest that the electrically transmitted spikelet, together with the rise time of the postsynaptic spike, accounted for the brief delay measured between the peaks of correlated spikes in electrically coupled CB1-IS cells. Altogether, these results suggest that, under these conditions, transmission of spikelets underlies the coordinated firing activity of CB1-IS cells.

Discussion

In this paper, we identified a population of CB1-expressing GABAergic neurons in the neocortex. These cells are characterized by an irregular pattern of firing after current injection and a widely spread axonal arborization. Furthermore, CB1-IS cells are electrically coupled, forming a network in which electrical synapses promote their coordinated spiking.

The endocannabinoids are lipid-soluble messengers that interact with cell surface receptors that are also activated by Δ9-tetrahydrocannabinol, the principle active component in marijuana (Piomelli, 2003). Endocannabinoids are thought to function as retrograde messengers released by neurons to suppress the strength of their synaptic inputs by activating presynaptic CB1 receptors. In the hippocampus (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001), the cerebellum (Kreitzer and Regehr, 2001), and the neocortex (Trettel and Levine, 2003), endocannabinoid activation of CB1 receptors has been shown to mediate DSI, a transient depression of inhibition after the depolarization of the postsynaptic cell (Llano et al., 1991; Pitler and Alger, 1992). Endocannabinoids have also been proposed to regulate rhythmic activity (Hájos et al., 2000) and be involved in some forms of long-term depression (Gerdeman et al., 2002; Chevaleyre and Castillo, 2003). CB1 modulation of synaptic efficacy may have significant functional consequences in the cerebral cortex, and it is therefore important to identify and study the cells containing CB1 receptors. Here we selected and recorded for the first time from a population of interneurons expressing CB1 receptors in the neocortex. The expression of CB1 receptors was detected immunohistochemically and confirmed pharmacologically. GABA<sub>A</sub>-mediated synaptic responses generated by CB1-IS cells onto pyramidal neurons were blocked by a cannabinoid agonist, and this effect was reversed by a selective CB1 receptor antagonist. CB1-IS cells were characterized by an irregular pattern of spiking in response to depolarizing current injection and a unique morphology.

CB1-IS cells exhibited a characteristically irregular pattern of firing different from those previously identified as fast spiking, late spiking, and low-threshold spiking (LTS)/burst-spiking non-pyramidal cells (Kawaguchi and Kubota, 1997). Irregular firing similar to the one we describe here has been observed in a popu-
lation of VIP-containing bipolar interneurons of the rat somatosensory cortex (Cauli et al., 1997; Porter et al., 1998; Cauli et al., 2000). In contrast with CB1-IS cells, which are multipolar or bitufted and have a wide horizontal axonal arborization (Figs. 4, 5), VIP-containing IS cells were characterized by a bipolar cell body and very narrow vertically oriented dendritic and axonal trees. This suggests that IS cells are heterogeneous, and CB1-IS are different from VIP bipolar interneurons. Furthermore, some CCK-containing interneurons in the hippocampus exhibited a firing pattern that may resemble the one described here for CB1-IS cells [Pawelzik et al. (2002), their Figs. 7, 8E, 9]. Additional studies are necessary to establish whether neocortical CB1-IS cells correspond to the presumed CB1-containing neurons described as regular spiking in the hippocampus (Wilson and Nicoll, 2001, 2002) or whether they represent two different populations of CB1-containing cells. In any case, we believe that the term “regular spiking,” also used to describe noncategorized interneurons (Kawaguchi, 1995).

Immunohistochemical studies indicate that the expression of CB1 receptors in the cerebral cortex is present in a population of CCK-containing interneurons (Katona et al., 1999, 2000; Tsou et al., 1999; Bodor et al., 2003). CCK-expressing cells have been shown to be heterogeneous, including subpopulations with different morphology, electrophysiology, and expression of neuropeptides and calcium-binding proteins (Kubota and Kawaguchi, 1997; Cope et al., 2002; Kawaguchi and Kondo, 2002; Pawelzik et al., 2002; Losonczy et al., 2004). Thus, small CCK-containing neocortical cells are usually positive for VIP and calretinin (Kawaguchi and Kondo, 2002) but lack CB1 expression (Bodor et al., 2003). In contrast, large CCK-containing basket neurons are generally negative for VIP and calretinin (Kawaguchi and Kondo, 2002) but express CB1 receptors (Bodor et al., 2003). We suggest that CB1-IS cells may include large CCK-positive cells (Kubota and Kawaguchi, 1997). Interestingly, CB1 expression has also been reported in a population of calbindin-expressing neurons (Marsicano and Lutz, 1999; Tsou et al., 1999; Bodor et al., 2003). Additional experiments are necessary to determine whether other interneuron populations, in addition to CB1-IS cells, express functional CB1 receptors in the cerebral cortex.

Recent reports have proposed that electrical synapses connect GABAergic interneurons belonging to the same class, establishing functional networks embedded within the neocortex. Thus, parvalbumin-expressing FS cells, which account for ~50% of the total number of GABAergic cells in the neocortex, are highly coupled to other parvalbumin-FS cells but only very rarely coupled to other types of GABAergic interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999). Similarly, somatostatin-
expressing LTS cells are highly coupled among themselves but only exceptionally to parvalbumin-FS cells (Gibson et al., 1999). More recently, another network of multipolar-bursting cells expressing calbindin and parvalbumin has been described (Blatow et al., 2003). Multipolar bursting cells are strongly coupled to other multipolar bursting cells but not to parvalbumin-FS cells. Likewise, in layer 1, neurogliaform-LS cells were highly coupled via electrical synapses among themselves but not to other interneurons exhibiting different patterns of firing (Chu et al., 2003). In addition, other studies have reported electrical coupling between pairs of similar GABAergic interneurons, including, bipolar, fusiform, and regular-spiking nonpyramidal cells (Tamás et al., 2000; Venance et al., 2000; Szabadics et al., 2001). The high rate of electrical coupling among CB₁-IS cells reported here (90%) suggests that these cells establish a distinct network of GABAergic interneurons. Altogether, cumulative evidence including our results indicates that electrical synapses are formed between functionally similar GABAergic interneurons.

**Functional implications**

Electrical coupling among GABAergic interneurons has been shown to promote the coordinated firing of connected cells, contributing to the generation of rhythmic synchronous activity. A wide range of frequencies of oscillatory rhythmic activity has been observed in the neocortex, and it has been proposed that specific GABAergic cells underlie the different types of synchronous oscillations (Buhl et al., 1998; Galarreta and Hestrin, 1999; Beierlein et al., 2000; Szabadics et al., 2001; Blatow et al., 2003).

GABAergic interneurons exhibit very heterogeneous physiological properties, and both their intrinsic membrane properties as well as the specific characteristics of their synaptic connections (chemical and electrical) could affect the frequency of the synchronous oscillatory activity they promote (Traub et al., 1996; Wang and Buzsáki, 1996; Beierlein et al., 2000; Tamás et al., 2000; Szabadics et al., 2001; Bartos et al., 2002; Blatow et al., 2003). FS cells are characterized by low input resistance, brief spikes, fast AHPs, and relatively fast IPSCs. The peak of their spikelets typically follows the peak of the presynaptic spike by ~0.3 msec, and the minimum value of the hyperpolarizing component of the spikelet reflecting the AHP occurs 4–10 msec after the presynaptic spike (Galarreta and Hestrin, 1999, 2001b, 2002). Relative to FS cells, CB₁-IS cells showed higher input resistance, wider action potentials, and slower AHPs. In addition, the peak of the presynaptic spikelet followed the postsynaptic spike peak by ~1 msec, and the hyperpolarizing component of the spikelet reached its minimum ~20 msec after the presynaptic spike. These differences suggest that CB₁-IS cells may synchronize their activity at lower frequencies than FS cells. However, more experiments are necessary to examine the interaction among CB₁-IS cells and how this network orchestrate the activity of other neocortical populations.

By acting presynaptically on GABAergic interneurons expressing CB₁ receptors, it has been proposed that endocannabinoids may block GABAergic transmission at specific inputs and could disrupt synchronized oscillations in certain frequency ranges (Katona et al., 2000). Based on our results, it seems reasonable to speculate that the activity-dependent release of endocannabinoids could selectively diminish the action of a network of electrically coupled CB₁-IS cells. The possible functional consequences of this dynamic change in cortical circuitry remain to be explored.

**References**


