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Isokawa, Masako and Bradley E. Alger. Ryanodine receptor regulates endogenous cannabinoid mobilization in the hippocampus. *J Neurophysiol* 95: 3001–3011, 2006. First published February 8, 2006; doi:10.1152/jn.00975.2005. Endogenous cannabinoids (eCBs) are produced and mobilized in a cytosolic calcium ($[Ca^{2+}]_i$)–dependent manner, and they regulate excitatory and inhibitory neurotransmitter release by acting as retrograde messengers. An indirect but real-time bioassay for this process on GABAergic transmission is DSI (depolarization-induced suppression of inhibition). The magnitude of DSI correlates linearly with depolarization-induced increase of $[Ca^{2+}]_i$ that is thought to be initiated by Ca^{2+} influx through voltage-gated Ca^{2+} channels. However, the identity of Ca^{2+} sources involved in eCB mobilization in DSI remains undetermined. Here we show that, in CA1 pyramidal cells, DSI-inducing depolarizing voltage steps caused Ca^{2+}-induced Ca^{2+} release (CICR) by activating the ryanodine receptor (RyR) Ca^{2+}-release channel. CICR was reduced, and the remaining increase in $[Ca^{2+}]_i$ was less effective in generating DSI, when the RyR antagonists, ryanodine or ruthenium red, were applied intracellularly, or the Ca^{2+} stores were depleted by the Ca^{2+}-ATPase inhibitors, cyclopiazonic acid or thapsigargin. The CICR-dependent effects were most prominent in cultured or immature acute slices, but were also detectable in slices from adult tissue. Thus we suggest that voltage-gated Ca^{2+} entry raises local $[Ca^{2+}]_i$ sufficiently to activate nearby RyRs and that the resulting CICR plays a critical role in initiating eCB mobilization. RyR may be a key molecule for the depolarization-induced production of eCBs that inhibit GABA release in the hippocampus.

INTRODUCTION

Brain cannabinoid receptors (CB1Rs) modulate synaptic transmission by altering the probability of neurotransmitter release (e.g., Hajos et al. 2000; Hoffman and Lupica 2000; Katona et al. 1999; Sullivan 1999; Takahashi and Linden 2000; see Howlett et al. 2004 for review). Natural ligands for CB1R are endogenous cannabinoids (eCBs). They are synthesized from the Ca^{2+}-dependent cleavage of phospholipid precursors in neurons as a result of depolarization- or neurotransmitter-induced increase of cytosolic Ca^{2+} or the activation of G-protein–coupled receptors (Di Marzo et al. 1994; Giuffrida et al. 1999; Jung et al. 2005; Piomelli 2003; Stella et al. 1997). Depolarization-induced suppression of inhibition (DSI; Llano et al. 1991; Pitler and Alger 1992) is an activity-dependent eCB response that modulates the release of γ-aminobutyric acid (GABA) from interneuron terminals that express CB1R (Diana et al. 2002; Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Trettel and Levine 2003; Wilson and Nicoll 2001; reviews by Alger 2002; Freund et al. 2003). Although the physiological significance of DSI has been questioned (Hampson et al. 2003), recent work stresses the importance of specific patterns of action potentials in regulating eCB release (Zhuang et al. 2005), suggesting that DSI may have a physiological role under the right circumstances. eCBs facilitate the induction of long-term potentiation (LTP) (Carlson et al. 2002) and metabotropic glutamate receptor (mGluR)–induced short-term plasticity (Rouach and Nicoll 2003). They also induce glutamatergic (Gerdeman et al. 2002; Robbe et al. 2002), GABAergic (Chevaleyre and Castillo 2003), and spike-timing–dependent (Sjostrom et al. 2003) long-term depression (LTD). The numerous roles of eCBs on synaptic signaling make understanding their synthesis and release a matter of great importance. Electrophysiological approaches have provided considerable insight into eCB mechanisms and yet, because eCB synthesis and release are not directly measured in physiological experiments, we use the more general term "mobilization" to refer to the underlying but indirectly detected synthesis and release of eCBs.

DSI is initiated by activation of voltage-gated calcium channels (VGCCs) (Lenz and Alger 1999; Llano et al. 1991; Pitler and Alger 1992). Although previous work suggested that Ca^{2+} influx through N-type Ca^{2+} channels was primarily responsible for DSI (Lenz et al. 1998), this could not be unambiguously demonstrated (Hoffman and Lupica 2000; Wilson et al. 2001; Yamasaki et al. 2006). Photolysis-induced release of caged Ca^{2+} within the CA1 pyramidal cell also mobilizes eCBs, without activation of VGCCs (Wang and Zucker 2001; Wilson and Nicoll 2001), and DSI is sensitive to the chelation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) (Lenz and Alger 1999; Pitler and Alger 1992). These observations suggest the possible existence of another source of Ca^{2+}, for example, a Ca^{2+} release from internal stores, that might be able to initiate hippocampal DSI. However, this possibility has not been thoroughly investigated. The objective of the present work is to test the hypothesis that intracellular Ca^{2+} channels that are activated by the increase in $[Ca^{2+}]_i$ are involved in this phenomenon.

The two major cytoplasmic Ca^{2+}-sequestering organelles are mitochondria and endoplasmic reticulum (ER). Release of Ca^{2+} from ER is thought to be mediated by two classes of Ca^{2+}-permeant channels: the ryanodine receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP_3R). In the present study, we focus on the RyR-mediated Ca^{2+} release from ER in the CA1 pyramidal cells because *1*) the hippocampus expresses high concentrations of all RyR isoforms (Sharp et al. 1993); *2*) the $[Ca^{2+}]_i$ that generates half-maximal DSI is 3.5–4 μM in

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CA1 pyramidal neurons (Wang and Zucker 2001), which far exceeds the optimal activation of the IP_3R and may even inhibit some IP_3R isoforms from binding to their ligands (Worley et al. 1987); *3*) the activation of the mGluRs and muscarinic acetylcholine receptors (mAChRs), both of which can stimulate Ca^{2+} release from IP_3-sensitive stores and enhance DSI without detectable elevation of $[Ca^{2+}]_i$ (Hashimotodani 2005; Kim et al. 2002; Maejima et al. 2001, 2005); and, finally, *4*) RyRs are involved in eCB-mediated responses in ventral striatum (Robbe et al. 2002), prefrontal cortex (Melis et al. 2004), dentate gyrus (Isokawa and Alger 2005), and cultured autaptic hippocampal neurons (Straiker and MacKie 2005).

We now report that *1*) DSI is associated with an increase in $[Ca^{2+}]_i$ that comprises ryanodine-sensitive and -insensitive components, *2*) the magnitudes of DSI and the ryanodine-sensitive $[Ca^{2+}]_i$ signals are linearly correlated, *3*) the ryanodine-insensitive $[Ca^{2+}]_i$ signal was much less able to generate DSI, and *4*) ER Ca^{2+}-pump inhibitors block the caffeine-induced $[Ca^{2+}]_i$ increase and attenuate DSI. These experiments were mainly done in hippocampal slice cultures because they are thin and flat and thus optimal for optical imaging techniques. We have observed similar effects in acute hippocampal slices from immature and adult rats, although they are less marked in adult rats. Based on these findings, we suggest that voltage-gated Ca^{2+} entry raises local $[Ca^{2+}]_i$ sufficiently to activate nearby RyRs, and that the resulting CICR plays a critical role in initiating eCB mobilization, particularly in younger animals.

METHODS

Hippocampal slice culture

Six-day-old Sprague–Dawley pups were anesthetized by halothane and decapitated. All procedures involving animals were approved by the University of Maryland Institutional Animal Care and Use Committee. Brains were removed and both hippocampi were dissected free. Slices were cut perpendicular to the septotemporal axis, 400 μm thick, and placed in 12-mm Millicell inserts (Coster) according to the method of Stoppini et al. (1991). The slices were bathed with medium that consisted of: 50% Eagle's basal medium, 20% Hank's balanced salt solution, 20% horse serum, and 100 U/ml penicillin–streptomycin (all from GIBCO BRL), and incubated in 5% CO_2 in air at 35°C. For experiments, slices were transferred to a recording chamber and perfused with artificial cerebrospinal fluid (ACSF) consisting of (in mM): 124 NaCl, 3 KCl, 1.24 Na_2HPO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose (all from Sigma), bubbled with 95% O_2-5% CO_2. Recording was done at room temperature.

Acute slice preparations from adult and immature rat hippocampus

Sprague–Dawley pups (7–21 days) and adult rats (4–6 wk) were deeply anesthetized with halothane and decapitated. Brains were quickly removed and immersed in ice-cold ACSF (composition above). The hemispheres were separated, trimmed at the septal level, and mounted on the Vibrotome stage (Technical Products International) with the rostral end down. Slices were cut 300 μm thick and incubated in the oxygenated ACSF at 35°C for ≥1 h before experiments were conducted.

Whole cell recording and stimulation

CA1 pyramidal cells were visually identified and voltage clamped at −70 mV in the whole cell configuration (Axopatch 200A, Axon Instruments). Patch pipettes were filled with (in mM): 100 Cs methanesulfonate, 50 CsCl, 10 Hepes, 4 MgATP, 0.1 EGTA, and 5 QX-314 (all from Sigma), and had resistances of 3–5 MΩ in the bath. A field-stimulating electrode (concentric stainless steel, 100 μm in diameter) was placed on the *s. radiatum* or *s. oriens* to evoke inhibitory postsynaptic currents (IPSCs) (S8800, Grass). Extracellular ACSF contained 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide (NBQX) and 100 μM DL-aminophosphonovaleric acid (DL-APV) (Tocris Cookson) to block ionotropic glutamatergic excitatory postsynaptic currents (EPSCs), allowing extracellular stimulation to evoke monosynaptic IPSCs (eIPSCs). Caffeine (20 mM, Sigma, osmolarity adjusted to equal that of the external ACSF) was pressure applied locally to the pyramidal cell soma through a glass pipette (2 μm in diameter, 10–15 psi for 1.5 s) (Picospritzer, General Valve). Cyclopiazonic acid (CPA, 30 μM at a final concentration of 0.026% dimethylsulfoxide [DMSO] from 100 mM DMSO stock), thapsigargin (TG, 2 μM in ACSF), and ryanodine (10–40 μM at a final concentration of 0.01–0.02% DMSO from 100 mM DMSO stock) were bath applied. In some experiments, ryanodine (100 μM) and ruthenium red (20–40 μM) were included in the recording pipette.

Assessment of DSI

eIPSCs were elicited every 3 s. DSI was induced by applying depolarizing voltage steps through the recording pipette from −70 to 0 mV for variable durations (0.1 to 3 s, as indicated). A voltage step was given every 3–5 min. The magnitude of DSI (% DSI) was determined as follows

$$\%\ \mathrm{DSI} = \frac{(\text{mean amplitude of 5 eIPSCs before the voltage step}) - (\text{mean amplitude of 3 eIPSCs after the voltage step})}{\text{mean amplitude of 5 eIPSCs before DSI step}} \times 100$$

Calcium imaging

As noted in the text, different recording situations called for the use of different Ca^{2+} indicators. The Ca^{2+} indicator dyes, fura-2 (100 μM, K_d: 140 nM), fluo-3 (100 μM, K_d: 325 nM), or fura-FF (250–500 μM, K_d: 5.5 μM), all in cell-impermeant form (Molecular Probes), were dissolved in the intrapipette solution. A mercury arc lamp (100 W) and a filter wheel (Lambda 10–2, Sutter Instruments) were used to excite the indicators. Fluorescence signals were collected with a cooled CCD camera (Pixel-37, Photometrics) at a rate of 5–10 frames/s. Ten frames were acquired before a given depolarizing voltage step, and 150–250 frames were acquired after the step. Changes in the fluorescence intensity were calculated as $\Delta F/F$ or the ratio of responses produced by light flashes having wavelengths of 380 and 360 nm (the 380/360 ratio) within regions of interest for volume-averaged signals (Metafluor, Universal Imaging). Background subtraction was done by collecting the fluorescence in a region away from the neuron studied.

Ratiometric measurements were calibrated according to Grynkiewicz et al. (1985) to estimate $[Ca^{2+}]_i$ using a K_d of 131 nM for fura-2 and 5.5 μM for fura-FF. We measured R_{min} and R_{max} in situ. For the calibrations, a CA1 pyramidal cell was whole cell patch clamped with a pipette that contained 2 mM BAPTA and fura-2 or fura-FF in the recording pipette solution. Five pairs of 380/360 measurements were taken 5 and 10 min after the break-in. Extracellular ACSF was then switched to a nominally Ca^{2+}-free ACSF. The replacement of the extracellular ACSF was checked by the disappearance of eIPSCs (evoked every 3 s). When eIPSCs became undetectable, which occurred in 15–20 min, an additional five pairs of 380/360 measurements were taken. These last pairs were averaged and used to calculate R_{min}. Subsequently, 20 mM CaCl plus 100 μM ionomycin were pressure ejected for 120 s from a micropipette (2 μm diam), placed close to the soma. Measurements of fluorescence at 380 and

360 nm were made continuously during the entire ejection period. The 380/360 ratio decreased to 10–16% of the original values within 30 s and remained at that value until the end of the ejection. R_{max} was calculated from the lowest ratio value observed. The ratio recovered to 42–50% of the original within 25–30 s after the ejection ended, showing that the decrease was attributable to the rapid perfusion of cell interior with a high $[Ca^{2+}]_i$, rather than loss of the recording. We calculated β with 360 nm, which was 1.002 with fura-2 and 1.081 with fura-FF in slice culture, and 0.8992 with fura-FF in acutely prepared adult slices. In situ calibration was done in three cells with fura-2 and in six cells with fura-FF in a given type of slice preparation.

RESULTS

Depolarization induced CICR in CA1 pyramidal cells

Caffeine can release Ca^{2+} from intracellular stores in CA1 pyramidal cells in acute hippocampal slices (Garaschuk et al. 1997). Whether these stores are functional in cells of cultured hippocampus and whether they are activated during depolarization that produces DSI have not been studied. In cultured hippocampal slices (Fig. 1*A*), we used a puffer pipette (2 μm in diameter) to apply brief pressure pulses of caffeine locally to voltage-clamped CA1 pyramidal cell somata. Unless otherwise noted the pressure pulses used to eject caffeine were always 1.5 s long and the holding potential for all experiments was −70 mV. Caffeine elicited an elevation of $[Ca^{2+}]_i$ (Fig. 1*B*), and subsequent bath application of ryanodine (20 μM for 25 min) reduced the amplitude of caffeine-induced Ca^{2+} signals. We tested a variety of Ca^{2+} indicators to be certain that the results were not dependent on the nature of the dye and observed qualitatively the same results with all of them (Ca green-1N, $n = 8$; Ca green-5N, $n = 8$; fluo-4FF, $n = 9$; and fura-2, $n = 59$). We did note that caffeine interferes optically with fura-FF (cf. Muschol et al. 1999) and thus used fura-2 in experiments with caffeine.

We next tested whether RyR-mediated Ca^{2+} release occurred during DSI-inducing depolarizing voltage steps lasting from 0.25 to 3 s to 0 mV. $[Ca^{2+}]_i$ signals peaked at the end of the depolarizations and were higher with longer step durations. Within the center of the cytosol, including the nucleus, the Ca^{2+} signal relaxed slowly and clearly outlasted the duration of the depolarization (Fig. 1*C*, *left two columns*), whereas the $[Ca^{2+}]_i$ increase near the edge of the cell (the "shell Ca^{2+}") relaxed promptly and was temporally more closely linked to the duration of depolarization per se (Fig. 1*C*, *right two columns*). The appearance of higher nuclear fluorescence could be partially attributable to a difference in the binding ratios of the Ca^{2+} indicator in the cytoplasm and the nucleoplasm (Perez-Terzic et al. 1997; Power and Sah 2002), and thus may not necessarily indicate a greater increase in the nucleus. Bath-applied ryanodine reduced the depolarization-induced Ca^{2+} signals in both the cell center and the shell. The presence of ryanodine-sensitive Ca^{2+} signals in the shell suggests that RyR-mediated Ca^{2+} release occurs near the plasma membrane.

Because ryanodine could not be washed out of the slices within the time course of our experiments, we performed several kinds of controls to ensure that the decline in peak Ca^{2+} signals seen with bath-applied ryanodine were mediated by RyR. In the absence of ryanodine, the peak Ca^{2+} signals from both the center and the shell remained essentially constant, with the average difference, at the end of 90 min, being a decrease of 4.03% from control values ($t = 1.03$, $P = 0.671$). As noted above, the ryanodine-sensitive Ca^{2+} signals were observed with several different Ca^{2+} indicators including fura-2 and fura-FF (see Figs. 2 and 5). In contrast to a previous report (Alonso et al. 2003), we did not see obvious antagonism of CICR by fura-2. We also monitored basal $[Ca^{2+}]_i$ levels with fura-2 and found that the average basal $[Ca^{2+}]_i$ was reduced from 64.5 ± 1.67 nM in control to 50.5 ± 4.27 nM in

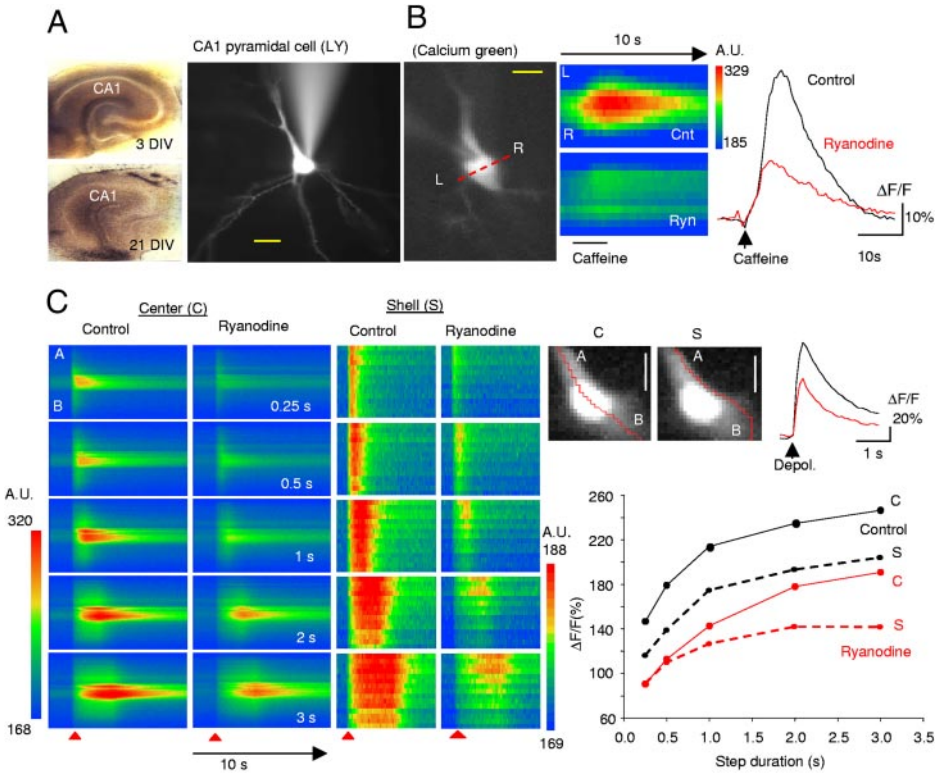

FIG. 1. Cytosolic Ca^{2+} ($[Ca^{2+}]_i$) signals elicited by local application of caffeine or to direct depolarization in CA1 pyramidal cells in hippocampal slice culture. *A*: examples of hippocampal slice cultures at 3 and 21 days in vitro (DIV) (typically cultures 9–24 DIV were used), and a CA1 pyramidal cell filled with Lucifer yellow (23 DIV, cal: 35 μm). *B*: caffeine-induced Ca^{2+} responses line scanned and averaged to show the relative increases in control and 20 μM ryanodine (calcium green-1N, cal: 20 μm). *C*: $[Ca^{2+}]_i$ signals evoked by direct depolarizations (lasting from 250 ms to 3 s, given at red triangles) of the cell were line scanned along the cell center (C) and the shell (S) in control and ryanodine (20 μM) (cal: 15 μm). Magnified scales in the 2 *right columns* reveal the ryanodine-sensitive component in the shell. Signals determined along the line were integrated and used as a region of interest (ROI) to calculate ΔF/F (graph).

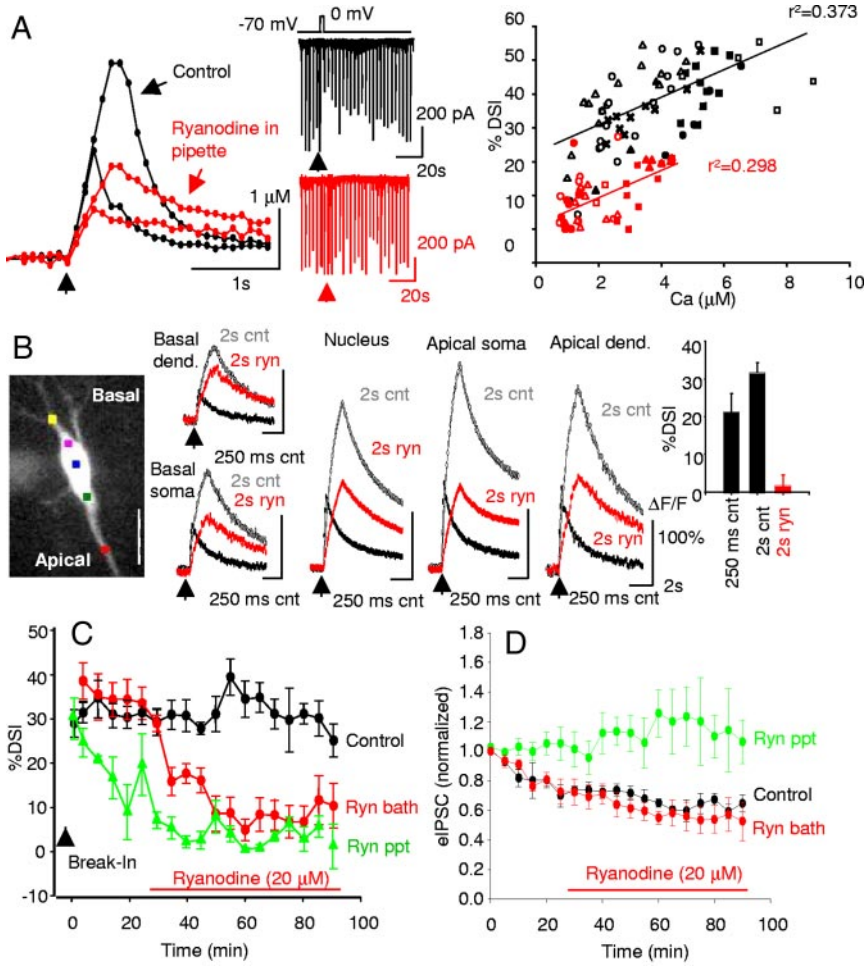

FIG. 2. Ryanodine depressed peak Ca^{2+} responses and altered the relationship between depolarization-induced suppression of inhibition (DSI) and $[Ca^{2+}]_i$. *A*: depolarization-induced increase in $[Ca^{2+}]_i$ (*left plot*, fura-FF) and DSI (*middle traces*) were measured in a control cell (black) and in a cell in which ryanodine had been intracellularly applied (red). Depolarizing voltage steps of 250 ms and 1 s were given to each cell at arrows. Ca^{2+} signals and DSI were recorded simultaneously. *Right graph*: group data for the $[Ca^{2+}]_i$–DSI relationship in control ($n = 7$, black) and ryanodine ($n = 6$, red). Each cell has a different symbol. *B*: cellular distribution of ryanodine-sensitive Ca^{2+} signals, measured with a high-affinity indicator, fluo-3, from 5 ROIs (each ROI indicated by a colored square on the image of the cell) before and after bath application of ryanodine (20 μM) (cal: 30 μm) in response to 250-ms and 2-s depolarizing steps. In all 5 ROIs, a 2-s voltage step in ryanodine caused a larger peak $[Ca^{2+}]_i$ than did a 250-ms step in control. However, the magnitude of DSI was greater with the 250-ms step in control than with the 2-s step in ryanodine (bar graph). *C*: time-dependent changes in the magnitude of DSI caused by intracellular or bath application of ryanodine. *D*: time-dependent changes in evoked inhibitory postsynaptic currents (eIPSCs) in control, with ryanodine in the pipette, and with ryanodine in the bath.

ryanodine ($n = 32$, $t = 10.88$, $P < 0.001$), which suggested that RyR-sensitive stores probably contributed to basal $[Ca^{2+}]_i$. This decrease in basal $[Ca^{2+}]_i$ by ryanodine also argues that the ryanodine-induced reduction in the peak Ca^{2+} signals observed with the nonratiometric dye, fluo-3, was not attributable to an increase of basal $[Ca^{2+}]_i$. Because ryanodine affected the Ca^{2+} signals from the cell center and shell in a similar way, in subsequent experiments, except as noted, we measured volume-averaged Ca^{2+} signals in the cell center.

Induction of DSI depends on the ryanodine-sensitive $[Ca^{2+}]_i$

Induction of half-maximal DSI reportedly requires a $[Ca^{2+}]_i$ increase of 3.5–15 μM in CA1 cells (Wang and Zucker 2001) and cerebellar Purkinje cells (Brenowitz and Regehr 2003). To avoid potential saturation of high-affinity indicators by these levels of $[Ca^{2+}]_i$, we used a low-affinity ratiometric dye, fura-FF (K_d: 5.5 μM; Molecular Probes) for the next experiments. The volume-averaged somal $[Ca^{2+}]_i$ increased by several micromolar in response to DSI-inducing voltage steps in cultured hippocampus (Fig. 2*A*). In control conditions, the $[Ca^{2+}]_i$–DSI relationship was linear over the range of the voltage steps used ($r^2 = 0.373$).

To restrict the site of action of ryanodine, we applied it intracellularly (100 μM in the pipette) and examined its effect on depolarization-induced $[Ca^{2+}]_i$ signals and DSI. Although ryanodine is membrane permeant, intrapipette application creates a localized high concentration of ryanodine in the recorded cell, while minimizing its effect on other cells. The effects of intracellularly applied ryanodine cannot be reversed and thus we tested for DSI within 5 min of the establishment of the whole cell configuration, selecting only cells that showed DSI for the experiments. Both the peak $[Ca^{2+}]_i$ signals and DSI decreased with time as ryanodine diffused from the pipette. With internal ryanodine, the identical durations (0.25–3 s) of depolarization increased $[Ca^{2+}]_i$ by 0.2 to 4.2 μM, i.e., only 3/8 of the $[Ca^{2+}]_i$ increase that was observed in control cells (graph in Fig. 2*A*). Moreover, a given $[Ca^{2+}]_i$ increase caused less DSI in ryanodine-treated cells than in control cells. Indeed, a 3-μM increase of $[Ca^{2+}]_i$ in the presence of ryanodine induced less DSI than a 1-μM increase of $[Ca^{2+}]_i$ in the absence of ryanodine. Again, ryanodine had the same effects on $[Ca^{2+}]_i$ and DSI regardless of the type of Ca^{2+} indicator, or even in the absence of any indicator. The data show that CICR is particularly effective in initiating DSI.

If the DSI Ca^{2+} sensors are closely associated with intracellular Ca^{2+} stores, then the $[Ca^{2+}]_i$ signals necessary for DSI could be highly spatially restricted. Although we observed ryanodine-sensitive $[Ca^{2+}]_i$ signals throughout the soma (Fig. 1), localized ryanodine-sensitive $[Ca^{2+}]_i$ signals might be induced by the DSI stimulation protocol. DSI predominantly affects GABAergic synapses that terminate on the cell somata and probably originate from basket cells (Martin et al. 2001; Wilson et al. 2001). Dendritic IPSPs are also weakly affected

by DSI (Morishita and Alger 2001) unless mGluRs are also activated (Chevaleyre and Castillo 2004). Accordingly, we sampled the depolarization-induced $[Ca^{2+}]_i$ signals from five regions of interest (ROIs) in different parts of the cell, i.e., the proximal basal dendrite, basal soma, the nucleus, apical soma, and proximal apical dendrite (Fig. 2*B*), before and during ryanodine application, to determine whether the ryanodine-sensitive signals covary with the strength of DSI in a given region. However, there were no marked differences across these cellular regions. In all ROIs, longer voltage steps produced larger Ca^{2+} signals. That is, the amplitude of Ca^{2+} signals elicited by 2-s voltage steps was larger than the amplitude of Ca^{2+} signals elicited by 0.25-s steps, and ryanodine reduced the amplitudes of both Ca^{2+} signals ($P < 0.001$, $n = 5$ for both groups). However, when Ca^{2+} signals were compared between control condition and during ryanodine application, in all ROIs, the Ca^{2+} signals elicited by 2-s steps in ryanodine were similar to, or greater than, the Ca^{2+} signals induced by the 0.25-s step in control ACSF. Nevertheless, 0.25-s steps in control ACSF elicited greater DSI than did 2-s steps in ryanodine (bar graph in Fig. 2*B*). We conclude that Ca^{2+} signals are not qualitatively different between the CA1 cell soma and dendrites, and that the apparent difference in DSI between soma and dendrites is probably attributable to the decrease in numbers of CB1R-expressing interneuron terminals in the dendritic regions of the hippocampus (Freund et al. 2003). Figure 2*C* summarizes time-dependent changes in DSI in control cells, in cells treated with bath-applied ryanodine, and in cells loaded intracellularly with ryanodine. The data suggest that the Ca^{2+} necessary for DSI was in large part derived from ryanodine-sensitive internal Ca^{2+} stores.

Conceivably, DSI would appear reduced if ryanodine decreased DSI-susceptible eIPSCs without actually affecting the DSI process. Indeed, a high concentration (100 μM) of ryanodine can decrease GABA release from interneuron terminals (Galante and Marty 2003). Therefore we examined time-dependent changes in eIPSCs in control and ryanodine-containing ACSF, and in control ACSF when ryanodine was present in the recording pipette (Fig. 2*D*). The eIPSCs were slightly greater with ryanodine in the pipette ($n = 5$) than in control ACSF ($n = 5$) ($F = 45.52$, $P < 0.001$); nevertheless DSI was significantly reduced by intracellular ryanodine ($t = 7.69$, $P < 0.001$, $n = 6$). When ryanodine was bath applied, the reduction in the mean eIPSC amplitude was slightly greater, 9.2% ($n = 4$) than that in control cells ($n = 5$) ($F = 99.01$, $P < 0.001$), although bath-applied ryanodine reduced DSI by 70.7% ($t = 4.03$, $P < 0.003$, $n = 4$, paired *t*-test). These results show that ryanodine's reduction of DSI cannot be explained by reduction in eIPSCs.

The preceding results suggested that the Ca^{2+} responsible for eliciting DSI comes predominantly from RyR-regulated CICR, although because this was a potentially controversial result, we performed two additional kinds of tests. To be certain that the use of the low-affinity Ca^{2+} indicator dye was not somehow biasing the data, we also tested the high-affinity ratiometric dye, fura-2 ($n = 6$). A 0.5-s depolarizing voltage step increased $[Ca^{2+}]_i$ to 246 ± 0.044 μM in control ACSF, and 28.0% DSI was induced. In the presence of ryanodine, $[Ca^{2+}]_i$ increased to 284 ± 0.042 μM in response to a 2-s depolarization, and yet only 19.0% DSI was induced, i.e., significantly less than in control ACSF ($t = 3.48$, $P < 0.02$). The lack of measurable difference between the increase in $[Ca^{2+}]_i$ produced by 0.5-s steps in control and 2-s steps in ryanodine was not a result of dye saturation because a 2-s depolarization in control ACSF increased $[Ca^{2+}]_i$ to 783 μM in the same cell. Thus these results also argue that global $[Ca^{2+}]_i$ is not the most important factor for DSI induction.

Finally, we asked whether ryanodine acted specifically on RyRs in affecting DSI. Ryanodine is an open-channel blocker (Simkus and Stricker 2002) and prevents Ca^{2+} release from only those RyR channels that have been activated. A prediction of the internal Ca^{2+} store model is that even in the presence of a low concentration of ryanodine it should be possible to elicit a Ca^{2+} signal and DSI by opening as-yet-unblocked release channels. We therefore examined the effect of prolonging the depolarization on DSI during ryanodine application. First, DSI was produced in control ACSF with depolarizing steps of 1, 2, or 3 s, and then 20 μM ryanodine was bath applied, whereas 1-s steps were repeated every 5 min for 20 min. At the end of 20 min, % DSI declined to 33.9 ± 15.2% of control ($n = 6$, $P < 0.001$). When DSI had been significantly reduced by ryanodine, the duration of the depolarizing step was increased to 3 s. The first 3-s voltage step produced detectable, although small, DSI (89.6 ± 22.5% of control, $P < 0.001$). However, DSI declined again when the 3-s step was repeatedly applied (to 18.8 ± 10.6% of control, $P < 0.001$). Similar results were obtained with intracellular ryanodine. These data indicate that the reduced rise in $[Ca^{2+}]_i$ and DSI reflect the specific blocking action of ryanodine on Ca^{2+} store release channels.

Ca^{2+}-ATPase inhibitors modulate DSI induction

If DSI depends on CICR, it should be affected by other manipulations that affect the intracellular Ca^{2+} stores. Cyclopiazonic acid (CPA) and thapsigargin (TG) inhibit Ca^{2+}-ATPase and deplete Ca^{2+} stores. CPA is a reversible, competitive antagonist, and TG is an irreversible, noncompetitive antagonist (Kostyuk and Verkhratsky 1994). We first tested the state of Ca^{2+} store filling in cultured hippocampal slices by applying caffeine puffs. As noted earlier, caffeine interfered with fura-FF, so we used fura-2 in these experiments.

A local puff of caffeine to a CA1 pyramidal cell soma transiently increased $[Ca^{2+}]_i$, and five sequential puffs given at 0.1 Hz caused a gradual reduction in the peak $[Ca^{2+}]_i$ responses (*black traces* in Fig. 3*A*). Evidently the Ca^{2+} stores are at least partially filled at rest and are depleted by repeated caffeine application. Ten seconds after the last caffeine puff, two 1-s depolarizing voltage pulses to 0 mV, given at 1 Hz, were applied to reload the stores. These pulses generated a large double-peaked $[Ca^{2+}]_i$ signal. Twenty seconds after the twin pulses, a single caffeine puff generated a detectable $[Ca^{2+}]_i$ increase, showing that VGCC-dependent Ca^{2+} influx partly refilled the stores. Five minutes later, a 1-s depolarizing voltage step elicited DSI (Fig. 3*A*, control). DSI was tested at 5-min intervals to avoid direct effects of caffeine on GABAergic transmission (Taketo et al. 2004).

Having confirmed that CICR in hippocampal CA1 cells in slice culture has similar features to those of CICR in other cells, we then asked whether store depletion could affect DSI by applying CPA (30 μM) for 20 min. In CPA, repeated caffeine application produced only a small $[Ca^{2+}]_i$ signal, suggesting that the stores had been nearly emptied (*red traces*

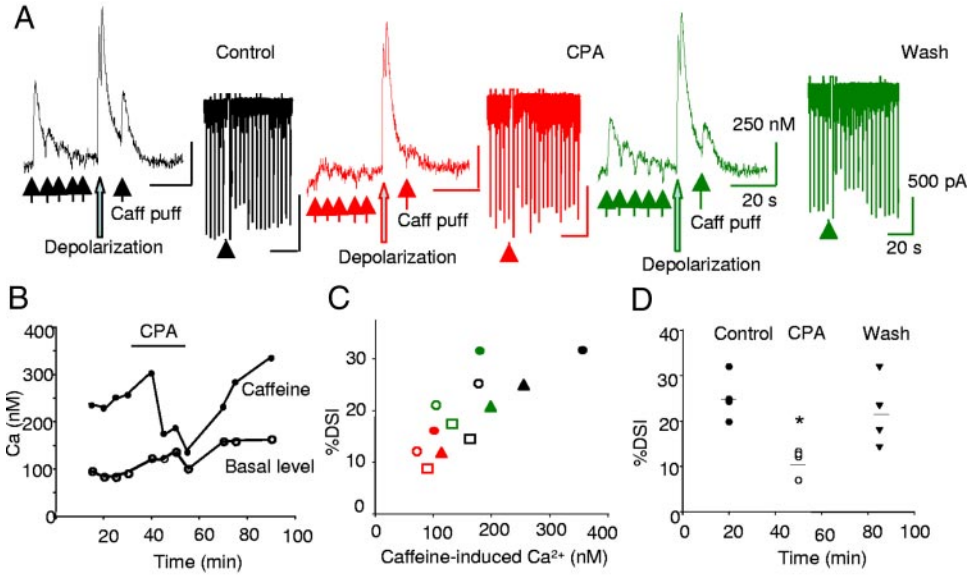

FIG. 3. Effects of Ca^{2+}-store depletion on DSI. *A*: caffeine-induced $[Ca^{2+}]_i$ increase and DSI, recorded simultaneously in control conditions (black), during application of the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid (CPA, red), and after washing out CPA (green). Small arrows indicate 1.5-s, local pressure applications (puffs) of 20 mM caffeine given at 0.1 Hz. Large arrows indicate a pair of voltage steps from −70 to 0 mV for 1 s at 1 Hz. A 3rd voltage step of 1 s was given 5 min after this series of caffeine applications to test the magnitude of DSI (see text for details). *B*: example of the time course of reversible reduction in caffeine-elicited $[Ca^{2+}]_i$ increases by CPA. *C*: magnitude of DSI varied with the magnitude of the caffeine-induced Ca^{2+} response ($n = 4$, each cell is indicated by a different symbol). *D*: reversible reduction of DSI in CPA ($n = 7$, 4 of which are the same as in *C*).

in Fig. 3*A*). The loading pulses generated a double-peaked rise in $[Ca^{2+}]_i$, and a single caffeine puff 20 s later generated a minimal increase in $[Ca^{2+}]_i$, suggesting that 30 μM CPA effectively prevented store refilling. DSI was also reduced in CPA. When CPA was washed from the bath, the $[Ca^{2+}]_i$ signals and DSI recovered (*green traces* in Fig. 3*A*).

To determine whether the filling state of the stores normally remains stable, we monitored the $[Ca^{2+}]_i$ signals produced by caffeine puffs given every 5 min for 90 min (Fig. 3*B*). Although the caffeine-induced $[Ca^{2+}]_i$ signals did not decline in the absence of CPA, they did once CPA was applied ($t = 6.17$, $P < 0.001$). Basal $[Ca^{2+}]_i$ levels increased gradually during the CPA experiments ($t = 17.93$, $P < 0.001$, paired t-test); nevertheless, the shift in the basal $[Ca^{2+}]_i$ was small and did not affect the changes caused by CPA on the caffeine-induced Ca^{2+} signals. Removal of CPA restored the caffeine-induced $[Ca^{2+}]_i$ signals spontaneously (between CPA and wash, $t = 3.09$, $P < 0.03$, Fig. 3*B*). These observations suggested that the stores in CA1 pyramidal neurons are stable under control conditions and become depleted when Ca^{2+}-ATPase is blocked. Most important, the magnitude of DSI correlates well with the store filling state, as assayed by the caffeine-induced increase in $[Ca^{2+}]_i$ (Fig. 3*C*). TG, the other Ca^{2+}-ATPase inhibitor, also ($n = 6$) decreased the depolarization-initiated Ca^{2+} signals (Fig. 4*A*) and DSI (Fig. 4*B*), without affecting eIPSCs (Fig. 4*C*).

Finally we tested ruthenium red (RR), a cell-impermeant RyR blocker on the depolarization-induced Ca^{2+} signals and DSI by including it in the recording pipette at 20–40 μM. Only cells that showed DSI within 5 min of whole cell break-in were analyzed. Intracellular RR reduced DSI ($n = 6$, $t = 14.18$, $P < 0.001$, Fig. 4*D*) but not eIPSCs (Fig. 4, *E* and *F* = 0.07, $P = 0.8$, one-way ANOVA with repeated measures).

Caffeine stimulates Ca^{2+} release from ryanodine-sensitive intracellular stores, and these stores were involved in DSI; thus it seemed possible that caffeine would suppress eISPCs in an eCB-dependent manner. We found that a puff application of caffeine onto the soma of DSI-inducing CA1 pyramidal cells indeed reversibly reduced the eIPSCs by 73.2 ± 6.98%, with a time course of IPSC recovery similar to that of DSI. However,

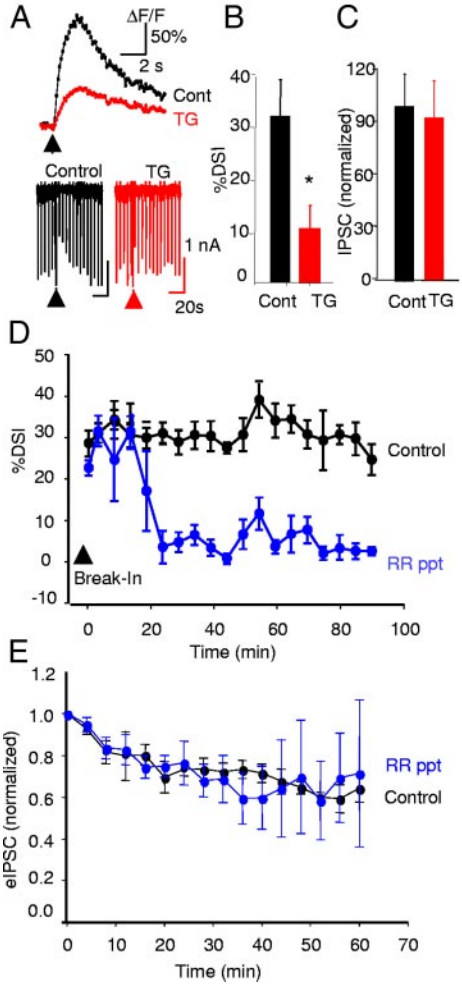

FIG. 4. Thapsigargin (TG) and ruthenium red (RR) decrease DSI. *A*: Ca^{2+}-ATPase inhibitor, TG, reduced depolarization-induced $[Ca^{2+}]_i$ increases and DSI (fluo-3). *B* and *C*: group data ($n = 6$) showing TG caused a significant reduction in the magnitude of DSI ($P < 0.01$), but not in eIPSC amplitudes. *D*: intracellular application of RR, a RyR channel blocker, depressed DSI. Time-dependent changes in DSI in control and RR ($n = 6$); break-in to whole cell recording mode was at $t = 0$. *E*: mean eIPSC amplitude was unaffected by intracellular RR ($n = 6$).

neither of the CB1 antagonists, AM 251 (4 μM) nor SR141716A (1 μM), bath applied for 20 min, altered the caffeine response. The eIPSCs were reduced by 69.88 ± 11.62% in the antagonists, i.e., not significantly different from the reduction observed in control ACSF ($n = 4$, $t = 0.50$, $P = 0.65$, paired t-test). Although this result shows that eCB is not the major mediator of caffeine-induced eIPSC suppression, it does not preclude the possibility of an eCB-dependent component. Caffeine's well-known and robust Ca^{2+}-independent inhibitory actions on $GABA_A$ IPSCs (Fiumelli et al. 2005; Kano et al. 1995; Taketo et al. 2004) could have masked eCB-mediated effects. Nevertheless this is clearly a topic for future investigation.

In summary, the data obtained with ryanodine, CPA, TG, and RR strongly support the conclusion that internal stores are the predominant Ca^{2+} source for DSI initiation in cultured slices. We next investigated the role of internal stores in DSI initiation in acute slices.

RyR regulates $[Ca^{2+}]_i$ and DSI in acutely prepared hippocampal slices

The hippocampus expresses high concentrations of RyRs during development as well as in the adult. We first tested hippocampal slices prepared acutely from pups age-matched to our cultured slices. Bath-applied ryanodine (20–40 μM) reduced depolarization-induced $[Ca^{2+}]_i$ signals and DSI ($t = 8.61$, $P < 0.001$, $n = 4$), without altering eIPSCs (354.8 ± 64.0 pA in control and 273.7 ± 19.6 pA in ryanodine, $t = 3.12$, $P < 0.053$) in the immature slices.

We next investigated CA1 pyramidal cells in young adult (4–6 wk) hippocampal slices (e.g., Fig. 5*A*). Local caffeine puffs generated transient increases in $[Ca^{2+}]_i$ in CA1 cells (e.g., Fig. 5*B*), indicating that the stores in these cells are not empty at rest. Moreover, depolarizing voltage steps to 0 mV, 1 or 3 s in duration, induced duration-dependent increases in peak $[Ca^{2+}]_i$ in adult neurons. These Ca^{2+} signals remained constant in response to a given duration of depolarization in control ACSF (Fig. 5*C*, black lines), but declined when 40 μM ryanodine was applied (Fig. 5*C*, red lines) ($F = 61.35$, $P < 0.001$, one-way ANOVA with repeated measures, $n = 5$).

To test for a role for CICR in DSI in the adult cells, we applied ryanodine (100 μM) intracellularly, accepting only those cells that showed DSI within 5 min of break-in for the analysis. Both DSI and the eIPSCs declined after break-in, but after 35 min DSI was reduced to a significantly greater extent than were the eIPSCs (Fig. 5*D*; $F = 1.95$, $P < 0.05$: two-way ANOVA with repeated measures). Figure 5*E* shows representative examples of the depolarization-induced Ca^{2+} signals and associated DSI at three different time points shown by the arrows in *D*. Ryanodine reduced the apparent magnitude of DSI by half, i.e., from 40% at the beginning of the ryanodine perfusion (black) to 20% at 35–50 min of ryanodine perfusion

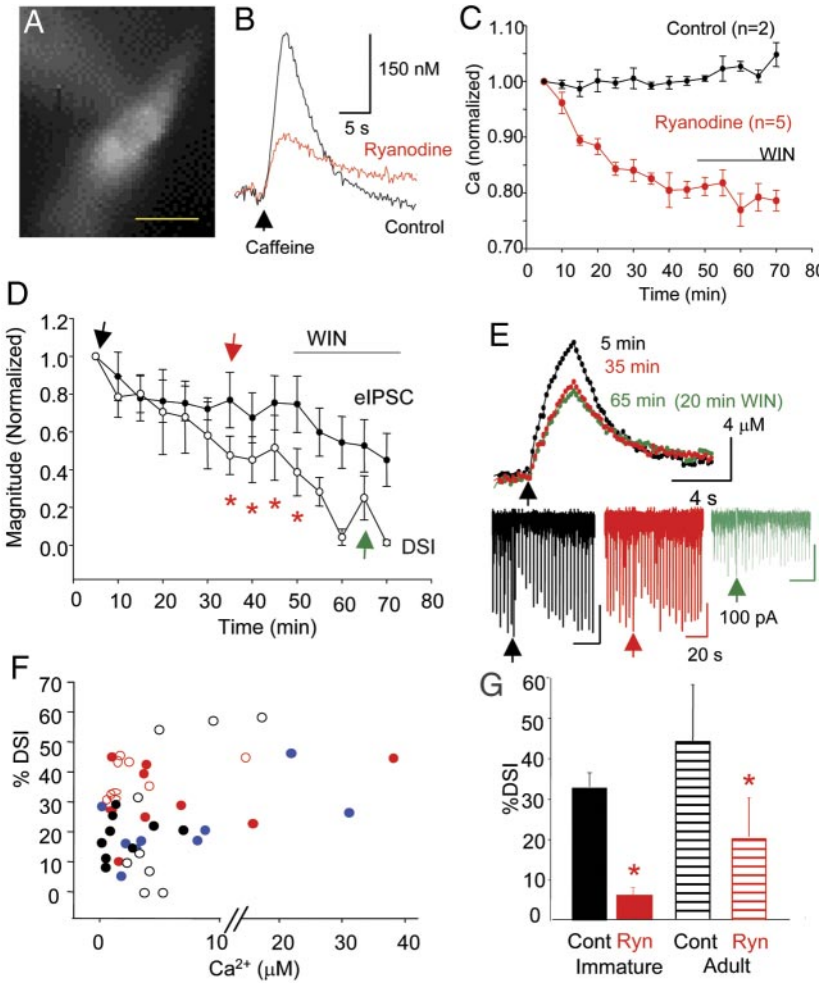

FIG. 5. Effects of store-released Ca^{2+} on the generation of DSI in acutely prepared adult rat hippocampal slices. *A*: adult rat CA1 pyramidal cell visualized with fura-FF. *B*: a caffeine-elicited $[Ca^{2+}]_i$ increase measured with fura-2 in a CA1 cell in an adult acute slice. Ryanodine reduced the peak amplitude of the Ca^{2+} signals. *C*: time-dependent changes of $[Ca^{2+}]_i$ signals in response to DSI-inducing depolarizations in control and cells treated with bath-applied 40 μM ryanodine ($n = 5$). CB1R agonist, WIN 55212–2 (4 μM), did not affect the $[Ca^{2+}]_i$ signals. *D*: time-dependent changes in DSI and eIPSCs during intracellular ryanodine application through the recording pipette (depression of DSI became significantly greater than the eIPSC depression at the red asterisks, $P < 0.05$, $n = 5$). *E*: samples of depolarization-induced $[Ca^{2+}]_i$ increases and DSI from the graph in *D* at specified time points (black, red, and green arrows). *F*: $[Ca^{2+}]_i$–DSI relationship, measured by fura-FF in 5 cells (each cell has a different symbol). Patch pipette also contained ryanodine, which reduced the peak $[Ca^{2+}]_i$ signal and the magnitude of DSI. *G*: summary data showing that ryanodine reduces DSI in acute slices from immature (2–3 wk old, $n = 4$) and adult rats (4–6 wk old, $n = 5$). In adult rats, % DSI in control was calculated at 5 min (1st), and % DSI in ryanodine was calculated as the mean DSI from the 7th to the 10th min.

(red). However, ryanodine also reduced the eIPSC, and loss of eCB-sensitive eIPSCs could produce a measured reduction in DSI on its own. To determine whether ryanodine diminished DSI independently of an effect on eCB-sensitive IPSCs, we bath applied the CB1 agonist, WIN 55212–2, 4 μM, in the presence of ryanodine and found that indeed WIN 55212–2 continued to reduce the eIPSCs by 30% ($P < 0.05$, Fig. 5, *D* and *E*), having no significant effect on the depolarization-induced increase in $[Ca^{2+}]_i$ (e.g., Fig. 5*C*). WIN 55212–2 can reduce hippocampal eIPSCs by somewhat more than 30% normally (Hoffman and Lupica 2000; Varma et al. 2001; Wilson and Nicoll 2001), and therefore our data argue that a decrease in eCB-sensitive eIPSCs could have played a partial role in the reduction of DSI by ryanodine.

In summary, our results show that DSI in acutely prepared slices is regulated by RyR-mediated release of Ca^{2+} from intracellular stores in both immature and adult hippocampus, although the contribution of CICR to DSI appears to be greater in immature than in adult cells ($t = 8.4$, $P < 0.01$, Fig. 5*G*), suggesting the possibility of developmental regulation in this process.

DISCUSSION

RyR-mediated Ca^{2+} release from stores often generates CICR and contributes to many neuronal functions, such as the spike afterhyperpolarization (Akita and Kuba 2000), synaptic plasticity (Lu and Hawkins 2002; Svoboda and Mainen 1999), and gene expression (Bito et al. 1996). Presynaptic RyRs and CICR participate in neurotransmitter release (see Bouchard et al. 2003 for review), including spontaneous (Bardo et al. 2002) and evoked (Galante and Marty 2003) GABA release, and also the release of glutamate (Emptage et al. 2001), ACh (Gonzalez Burgos et al. 1995), and the neuropeptides LHRH (Peng 1996), vasopressin (von Spreckelsen et al. 1990), and oxytocin (Wang et al. 1999).

RyR-mediated Ca^{2+} release is becoming increasingly recognized as a crucial element in certain eCB-mediated responses. For example, the induction of LTD in the striatal neurons is mediated by eCBs (Gerdeman et al. 2002; Robbe et al. 2002). This LTD requires the activation of mGluRs and the subsequent mobilization of Ca^{2+} from ryanodine-sensitive stores (Robbe et al. 2002). Depolarization-induced suppression of excitation (DSE) in the ventral tegmental dopamine neurons (Melis et al. 2004) and cultured autapic hippocampal neurons (Straiker and MacKie 2005) is also induced by eCBs as a result of Ca^{2+} release from ryanodine-sensitive intracellular stores, and we have provided evidence that RyR-mediated CICR is involved in the induction of DSI in the dentate gyrus granule cells (Isokawa and Alger 2005). In the present study, we investigated RyR-mediated CICR in hippocampal CA1 pyramidal cells in detail, and now show that it significantly increases the pool of somatodendritic Ca^{2+} after neuronal depolarization and plays a major role in eCB mobilization.

CICR occurs during the DSI-inducing depolarization

Previous work suggested that somatic $[Ca^{2+}]_i$ correlated linearly with the magnitude of DSI (Lenz and Alger 1999; Wang and Zucker 2001). Effective induction of DSI requires a train of action potentials or strong depolarization (Llano et al. 1991; Pitler and Alger 1992) and appears to have a threshold level of $[Ca^{2+}]_i$ for initiation (Lenz and Alger 1999). CICR is proportional to the intensity of depolarization and has an apparent threshold for initiation (Kostyuk and Verkharatsky 1994). Thus the functional properties of the RyR and CICR can explain some properties of DSI.

CICR occurs in the somata and dendrites of hippocampal and cortical neurons during action-potential generation (Jacobs and Meyer 1997; Kato et al. 1999; Sandler and Barbara 1999), although only a subset of dendritic spines contain ER (Sabatini et al. 2002) and RyR (Sharp et al. 1993). On the other hand, CICR may not be involved in the Ca^{2+} transients evoked by back-propagating action potentials in dendrites (Markram et al. 1995) or some forms of somatic depolarization (Garaschuk et al. 1997). In these studies, CICR might not have been seen because a small increase in $[Ca^{2+}]_i$ stimulates Ca^{2+} accumulation into, rather than a release from, ER (Friel and Tsien 1991).

We observed that ryanodine reduced the rate of increase and peak amplitude of the depolarization-induced elevation of $[Ca^{2+}]_i$, suggesting that RyR-mediated CICR occurs as soon as Ca^{2+} enters the cell. This is in contrast to IP_3R-mediated Ca^{2+} release that, because it requires activation of G-protein–coupled receptors and subsequent production of IP_3, has a longer onset time than that of RyR-mediated CICR. Our data agree with the report of a rapid RyR-mediated elevation in $[Ca^{2+}]_i$ in sympathetic ganglion neurons (Friel and Tsien 1992). RyRs have fast activation time constants, ranging from 0.5 to 1 ms, regardless of the RyR isoform (Fill and Copello 2002). Hippocampal neurons express all three isoforms (Sharp et al. 1993), and thus prominent RyR-mediated responses are not unexpected in hippocampus.

Functional linkage between VGCC-mediated Ca^{2+} influx and RyR

Lenz et al. (1998) reported that, although VGCC-initiated Ca^{2+} influx was required to initiate DSI, all VGCCs were not equally effective in this role, despite producing equivalent Ca^{2+} currents; N- and L-type currents were much more effective than others. We now show that ER resident RyRs are critically involved in DSI. Our data thus suggest that RyRs are more closely associated with certain classes of VGCCs than others, and therefore that Ca influx through these VGCCs has the greatest influence in the initiation of DSI. This hypothesis is consistent with findings that N-type Ca^{2+} current is amplified by CICR (Sandler and Barbara 1999; Tully and Treistman 2004; Usachev and Thayer 1997) and that L-type channels may also couple functionally to neuronal RyRs (Chavis et al. 1996). The close apposition to the cell membrane of subsurface cisterns and extensions of RyR-bearing ER (Benedeczky et al. 1994; Berridge 1998) has suggested the existence of functional triads among RyR, N-type Ca^{2+} channels, and BK channels (Akita and Kuba 2000). It remains to be determined whether analogous triads are involved in eCB mobilization.

The likelihood that RyR is an essential Ca^{2+} source for DSI induction is consistent with a previous finding that EGTA and BAPTA were equally effective in blocking DSI (Lenz and Alger 1999). BAPTA is a fast Ca^{2+} buffer and can block Ca^{2+} diffusion beyond the nanodomains in the immediate vicinity of VGCCs. EGTA is a relatively slow buffer and allows Ca^{2+} diffusion beyond nanodomains but not much beyond microdo-

mains (Augustine et al. 2003). A spatial separation between RyRs and the associated VGCCs would allow BAPTA and EGTA to be equally effective in blocking DSI. RyRs are densely and ubiquitously distributed throughout hippocampal cell somata (Seymour-Laurent and Barish 1995). This probably juxtaposes some RyR channels, near the plasma membrane (Berridge 1998), with the VGCCs that are engaged in DSI induction. The RyRs may functionally couple to those VGCCs and act as Ca^{2+} sensors for the induction of DSI. Arrays of RyRs located on the ER deep in the cytosol may, by regenerative CICR, amplify the Ca^{2+} signals into the micromolar range required to induce DSI, without imposing tight spatial restrictions on the cellular availability of Ca^{2+}. Future work will be required to test for such functional arrangements between the subtypes of VGCCs and RyRs in hippocampal neurons.

$[Ca^{2+}]_i$ concentrations that have been reported to induce a half-maximal production of eCBs for retrograde signaling vary from ≤ 0.2 μM (Glitsch et al. 2000) to ≥ 3 μM (Brenowitz and Regehr 2003; Wang and Zucker 2001). This wide range of variability has not been fully explained. Ca^{2+} indicators could be influenced to some extent by temperature and by the concentrations of certain other substances likely to be found in the cytoplasm or introduced during the course of an experiment. Affinities, K_d values, of calcium indicator dyes reported in buffered solution do not necessarily match the K_d values when the same dyes are introduced in the cytosol of intact cells (Loughrey et al. 2003). Similar results were reported for the K_d values of fura-FF, fura-2, and mag-fura-5 (Brenowitz and Regehr 2003). In the present study, we used a low-affinity ratiometric indicator, fura-FF, for the measurement of depolarization-induced $[Ca^{2+}]_i$ signals, and a high-affinity ratiometic indicator, fura-2, for the measurement of caffeine-induced $[Ca^{2+}]_i$ signals, to avoid dye interference by caffeine. We found that a $[Ca^{2+}]_i$ of several micromolar was associated with 50% DSI in cultured hippocampal slices (Fig. 2*A*). Together these results suggest that many factors, both technical and biological, influence the estimates of $[Ca^{2+}]_i$ required for the induction of DSI.

Early in development when neurons undergo dynamic cytodifferentiation and synaptogenesis, RyR3 and RyR1 mRNA levels are high in CA1, but decline after the first 2 wk postnatal. RyR2, on the other hand, increases postnatally, and remains high in the adult, being primarily expressed in somatodendritic compartments (Mori et al. 2000; Sukhareva et al. 2002). We found a difference in the ability of ryanodine to inhibit DSI in the immature hippocampus compared with adult hippocampus (Fig. 5*G*). The RyR dependency of DSI induction was greater in organotypic cultured slices and immature acute slices than in adult acute slices, in which a reduced degree of DSI could still be seen in the presence of ryanodine. Because CB1R shows the adult pattern of expression at the perinatal period (Morozov and Freund 2003), this difference suggested that, later in development, a RyR-independent mechanism of DSI induction may develop. A previous observation by Lenz et al. (1998) that neither CPA nor dantrolene affected DSI in acute adult hippocampal slices suggests this possibility, although the high-Ca^{2+} buffering used in that study (2 mM BAPTA plus 0.2 mM Ca^{2+} in the pipette) might have obscured the CICR component of DSI. Additionally, dantrolene is a skeletal-muscle relaxant that effectively blocks RyR1 and RyR3, but not RyR2 (Zhao et al. 2001). Because RyR2 is a major isoform in CA1 pyramidal cells in adults, the lack of sensitivity of RyR2 to dantrolene might also contribute to the discrepancy.

In conclusion, we report for the first time that RyR-mediated CICR plays a major role in the induction of DSI in the hippocampal CA1 pyramidal cells. CICR is the major determinant of DSI in young cells, and its contribution is still significant, although decreased, in adults. Numerous drugs and other agents affect CICR. Evidence is rapidly developing that eCBs are involved in a wide range of crucial roles in the brain. In establishing that CICR is a central component of eCB-mediated retrograde signaling, our work suggests that a fuller understanding of CICR in the hippocampal CA1 neurons will yield valuable insights into the mechanisms of Ca^{2+}-dependent mobilization of eCBs.

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Volume 89, April 2003

Pages 1748–1760: Schäfer SS, Berkelmann B, and Dadfar F. "Magnitude of Oscillations in the Response of Ia Muscle Spindle Endings Under a Static γ Stimulation of Increasing Frequency" (http://jn.physiology.org/cgi/content/full/89/4/1748; doi:10.1152/jn.00952.2001). During production, the DOI number was misrepresented in this article. The correct DOI number is presented here: doi: 10.1152/jn.00952.2001.

Volume 94, July 2005

Pages 119–135: Frechette ES, Sher A, Grivich MI, Petrusca D, Litke AM, and Chichilnisky EJ. "Fidelity of the Ensemble Code for Visual Motion in Primate Retina" (doi:10.1152/jn.01175.2004; http://jn.physiology.org/cgi/content/full/94/1/119). In the METHODS (in the first paragraph of *Stimuli*), the mean light intensity was misrepresented: "intensity 9,200 (8,700; 7,100)" should read "intensity 4,300 (4,200; 2,400)."

Volume 95, February 2006

Pages 862–881: Larsson J, Landy MS, and Heeger DJ, "Orientation-Selective Adaptation to First- and Second-Order Patterns in Human Visual Cortex" (doi:10.1152/jn.00668.2005; http://jn.physiology.org/cgi/content/full95/2/862). During production, the URL to direct readers to Supplemental Data was misrepresented. This has been corrected and replaced in the on-line version of the final published article (http://jn.physiology.org/cgi/content/full/00668.2005/DC1). Therefore the on-line version now deviates from the print journal with regard to this correction.

Volume 95, March 2006

Pages 1380–1396: Jiang W, Jiang H, and Stein BE. "Neonatal Cortical Ablation Disrupts Multisensory Development in Superior Colliculus" (doi:10.1152/jn.00880.2005; http://jn.physiology.org/cgi/content/full/95/3/1380). Important information was inadvertently omitted from the legends of Figs. 2 and 4. This information is presented here. Figure 2: lesion designations at *bottom right* ("LS Lesion" and "AES & LS Lesion") should read "rLS Lesion" and "AES & rLS lesion," respectively. All lesions are shown on the right hemisphere for illustrative purposes. Figure 4: all receptive fields are plotted on the right hemifield for illustrative purposes.

Volume 95, January 2006

Pages 379–400: Victor JD, Mechler F, Repucci MA, Purpura KP, and Sharpee T. "Responses of V1 Neurons to Two-Dimensional Hermite Functions" (doi:10.1152/jn.00498.2005;http://jn.physiology.org/cgi/content/full/95/1/379).As a result of a normalization error, the values of the filters *L* (but not *E*) plotted in Figs. 3, 5, and 6 are twice as large as they should be. Consequently, cells described as "under-rectified" (Figs. 3, *A* and *B*; 5, *A* and *B*; and 6*A*) should have been described as "half-wave rectifying," consistent with the illustrated poststimulus histograms. Correction of this error shifts values of the index I_{sym} (quoted in the text and plotted on the abscissa of Fig. 11) toward 1, i.e., the "complex" end of the simple versus complex spectrum. The correct value of the index and the published value are related by

$$I_{corrected} = (3 + 5I_{published})/(5 + 3I_{published})$$

Correction of this error also alters the values of $I_{c\text{-}p}$ by decreasing the weighting of contributions from the *L*-component, although these changes are slight (Fig. 9). The error does not affect the index I_{shape}, which quantifies the difference between the responses to Cartesian and polar stimuli and thus does not alter any of the conclusions of the paper. Corrected Figs. 9 and 11, with legends, are presented here.

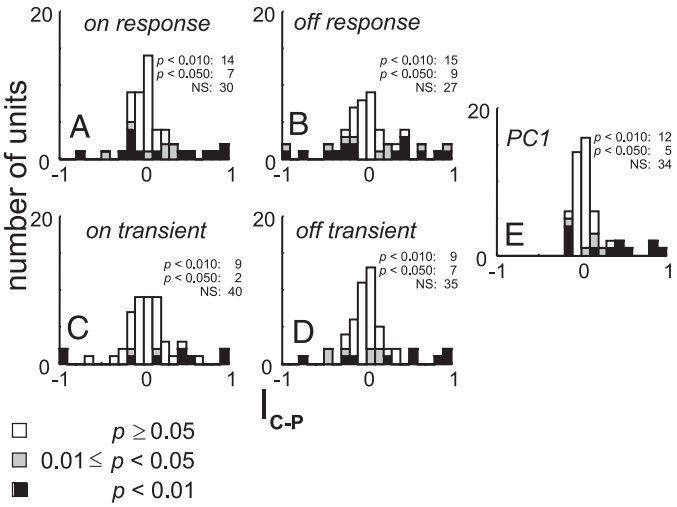

FIG. 9. Distribution of relative responsiveness to Cartesian and polar stimuli, $I_{c\text{-}p}$ (*Eq. 11*). Values >0 indicate larger responses to Cartesian stimuli; values <0 indicate larger responses to polar stimuli. Significance levels are calculated by jackknife and are shown as in Fig. 7. Each panel contains calculations based on a different response measure.

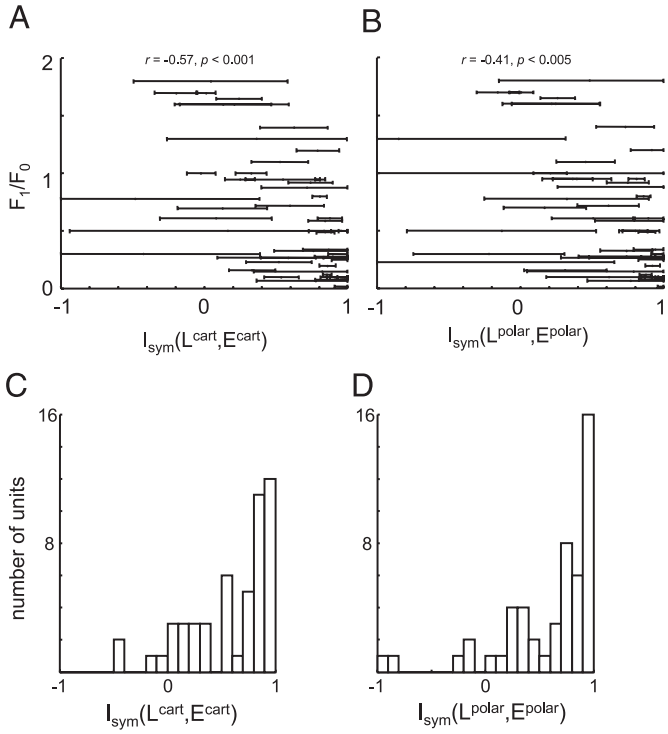

FIG. 11. Relationships of indexes of overall nonlinearity $I_{sym}(L, E)$ (*Eq. 5*) determined from Cartesian (*A*) and polar (*B*) responses to the F_1/F_0 ratio used to classify cells as simple and complex. For both Cartesian and polar measurements, units with I_{sym} close to 1 tended to have small values ("complex") of the F_1/F_0 ratio. *C* and *D*: distribution of these indexes across the population. Distributions for Cartesian and polar responses are similar.

Volume 95, March 2006

Pages 1571–1587: Vingerhoets RAA, Medendorp WP, and Van Gisbergen JAM. "Time Course and Magnitude of Illusory Translation Perception During Off-Vertical Axis Rotation" (doi: 10.1152/jn.00613.2005; http://jn.physiology.org/cgi/content/full/95/3/1571). During production, *Eq. 1* was misrepresented. This equation as been corrected and replaced in the online version of the final published article. Therefore the online version now deviates from the print journal with regard to this correction.

Pages 1843–1852: Neusch C, Papadopoulos N, Müller M, Maletzki I, Winter SM, Hirrlinger J, Handschuh M, Bähr M, Richter DW, Kirchhoff F, and Hülsmann S. "Lack of the Kir4.1 Channel Subunit Abolishes K^+ Buffering Properties of Astrocytes in the Ventral Respiratory Group: Impact on Extracellular K^+ Regulation" (doi:10.1152/jn.00996.2005; http://jn.physiology.org/cgi/content/full/95/3/1843). C. Neusch and N. Papadopoulos contributed equally to this article. A statement affirming this fact appeared in the first-published version at Articles in PresS (http://jn.physiology.org/cgi/reprint/00996.2005v1); however, the statement was inadvertently left out of the final-published version.

Pages 1966–1975: Stark E, Drori R, and Abeles M. "Partial Cross-Correlation Analysis Resolves Ambiguity in the Encoding of Multiple Movement Features" (doi:10.1152/jn.00981.2005; http://jn.physiology.org/cgi/content/full/95/3/1966). During production, *Eqs. 6*, *8*, and *15–17* were incorrectly cited within the text. These in-text citations have been corrected and replaced in the online version of the final published article. Therefore the online version now deviates from the print journal with regard to these corrections.

Volume 95, April 2006

Pages 2314–2325: Wicher D, Agricola H-J, Söhler S, Gundel M, Heinemann SH, Wollweber L, Stengl M, and Derst C. "Differential Receptor Activation by Cockroach Adipokinetic Hormones Produces Differential Effects on Ion Currents, Neuronal Activity, and Locomotion" (doi:10.1152/jn.01007.2005; http://jn.physiology.org/cgi/content/full/95/4/2314). During production, the URL to direct readers to Supplemental Data was misrepresented. This has been corrected and replaced in the on-line version of the final published article (http://jn.physiology.org/cgi/content/full/01007.2005/DC1). Therefore the on-line version now deviates from the print journal with regard to this correction.

Volume 95, May 2006

Pages 2947–2950: Rittenhouse CD, Siegler BA, Voelker CC, Shouval HZ, Paradiso MA, and Bear MF. "Stimulus for Rapid Ocular Dominance Plasticity in Visual Cortex" (doi:10.1152/jn.01328.2005; http://jn.physiology.org/cgi/content/full/95/5/2947). In the author line, the middle initial of Courtney Voelker was misrepresented. Corrected name is presented here: Courtney C. Voelker.

Pages 3001–3011: Isokawa M and Alger BE. "Ryanodine Receptor Regulates Endogenous Cannabinoid Mobilization in the Hippocampus" (doi:10.1152/jn.00975.2005; http://jn.physiology.org/cgi/content/full/95/5/3001). Twice in the article (in the third paragraph of the INTRODUCTION and in the second paragraph of the DISCUSSION), the citation for "Straiker and MacKie 2005" should have been presented as "Straiker and Mackie 2005," as listed in the REFERENCES.

Volume 95, June 2006

Pages 3948–3954: Ziburkus J, Cressman JR, Barreto E, and Schiff SJ. "Interneuron and Pyramidal Cell Interplay During In Vitro Seizure-Like Events " (doi:10.1152/jn.01378.2005; http://jn.physiology.org/cgi/content/full/95/6/3948). During production, the URL to direct readers to Supplemental Data was misrepresented. This has been corrected and replaced in the on-line version of the final published article (http://jn.physiology.org/cgi/content/full/01378.2005/DC1). Therefore the on-line version now deviates from the print journal with regard to this correction.