Molecular Neurobiology

ISSN0893-7648/06/33(2): 133–153/$30.00

Homeostatic Regulation of Glutamate Release in Response to Depolarization

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Abstract

Proper nervous system function requires a balance between excitation and inhibition. Systems of homeostasis may have evolved in neurons to help maintain or restore balance between excitation and inhibition, presumably because excessive excitation can cause dysfunction and cell death. This article reviews evidence for homeostatic mechanisms within the hippocampus that lead to differential regulation of glutamate and γ-aminobutyric acid release in response to conditions of excess depolarization. We recently found differential effects on glutamate release at the level of action potential coupling to transmitter release, vesicular release probability, and vesicle availability. Such differential regulation may help to prevent excitotoxicity and runaway excitation.

Index Entries: Glutamate; GABA; depolarization; homeostasis; potassium; Na^+ channels; K^+ channels; action potential initiation; action potential propagation.

Introduction

In the central nervous system (CNS), glutamate and γ-aminobutyric acid (GABA) are major excitatory and inhibitory neurotransmitters, respectively. Balance between glutamate and GABA actions is critical for proper CNS function, partly because glutamate's actions can be double-edged. Excessive glutamate accumulation has been associated with neuronal damage in a myriad of neurological and neuropsychiatric disorders, including epilepsy, stroke, amyotrophic lateral sclerosis, bipolar disorder, Alzheimer's disease, Huntington's disease, and Parkinson's disease *(1,2)*. Therefore, it is not surprising that the nervous system might have endogenous mechanisms—both persistent and rapidly reversible—that help limit excessive excitation and toxicity *(3–5)*.

We, and others, have explored possible responses of the nervous system to excessive

Received May 23, 2005; Accepted August 4, 2005.
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excitation. Because synapses are highly malleable, it appears likely that glutamate and/or GABA synapses may be possible sites of homeostatic change. Strong evidence indicates that postsynaptic receptor function changes homeostatically, particularly in response to prolonged block of electrical activity *(6,7)*. We and others recently found that presynaptic changes are also important to synaptic homeostasis *(8–10)* when hippocampal synapses are challenged with depolarization. As expected from mechanisms that restore balance, these changes reduce glutamate output more than they change GABA output. Presynaptic changes specific to glutamate synapses may be somewhat surprising, because we usually think of presynaptic factors that control transmitter release (including machinery responsible for vesicle docking, priming, and Ca^{2+} sensing) as being more ubiquitous across transmitter phenotypes than postsynaptic receptor function. Nevertheless, there is emerging evidence for differences between glutamate and GABA release machinery that could be candidates to underlie selective homeostatic changes.

Homeostatic changes in postsynaptic or presynaptic function generally are persistent changes following a prolonged change in activity. We also recently found that glutamate and GABA release can be differentially regulated both rapidly and reversibly at the level of spike propagation in the axon *(11–13)*.The level at which this regulation occurred was again surprising to us because spike propagation was common to glutamate and GABA transmission. We have found that glutamate axons are susceptible to spike waveform changes and outright failure during depolarization—presumably accounting for observations of depressed glutamate synaptic transmission compared to GABA transmission—in response to excess depolarization. Unlike the persistent homeostatic changes, this form of modulation is completely and immediately reversible upon cessation of the excessive excitation. The molecular underpinnings of the effect remain unclear, but clues have been provided from recent work in other laboratories that examines differences in the intrinsic properties of principal neurons and interneurons.

This article reviews literature relevant to differential persistent and acute regulation of glutamate and GABA synapses. We include discussion of some of the cellular and molecular differences in the presynaptic cells that could conceivably underlie differential modulation. We conclude that at least within the hippocampus (a brain region particularly prone to excessive excitation), mechanisms exist to regulate glutamate vs GABA release differentially. Further work is needed to determine the molecular mechanisms of these forms of differential regulation and to determine the extent to which these mechanisms coincide with the glutamate/GABA cell type in other brain areas.

Persistent Homeostatic Change in Response to Prolonged Alterations in Activity

Activity is an important regulator of long-term synaptic strength in vivo. This section briefly reviews previous results that suggest synapses can respond with both post- and presynaptic changes that homeostatically restore balance between excitation and inhibition. We focus on our own recent finding that the presynaptic changes—notably, functional inactivation of glutamate terminals—are particularly prominent when hippocampal neurons are challenged with excessive depolarization.

Previous Studies of Synaptic Homeostasis

Much research has been performed on the persistent effects of altering electrical activity patterns in neurons. A common, simple approach to this question involves chronic electrical inhibition using Na^{+}-channel blockers, such as tetrodotoxin (TTX), or glutamate receptor antagonists. Imagine two plastic responses to chronic inhibition. In one scenario, homeostatic mechanisms may increase synaptic activity to counteract the excessive inhibition. In

another scenario, excess inhibition may cause a positive feedback cycle, further downregulating inactive synapses. Similarly, an increase in chronic activity may activate homeostatic mechanisms to keep synaptic excitation in check or may, through a Hebbian positive feedback mechanism, increase synaptic function further.

Consistent with the first hypothesis, neurons are believed to monitor their level of excitatory activity and to increase or decrease compensatory synaptic mechanisms as part of a homeostatic response that maintains neurons' overall firing rate (reviewed in ref. *14*). Specifically, neurons exhibit "disuse hypersensitivity" when deprived of neuronal activity for long periods of time *(15).* After activity blockade, the neuron responds with an increase in synaptic strength, perhaps compensating for the inactivity. Controversy exists regarding whether post- or presynaptic systems, or even both, are chiefly responsible for disuse hypersensitivity. Changes in intrinsic excitability of cells have also been observed as an adaptive response *(16),* but we confine our discussion to synaptic changes.

Some reports have suggested that an exclusively postsynaptic mechanism is responsible for the increase in synaptic strength following activity blockade in glutamatergic neurons. Support for postsynaptic homeostatic changes comes from three types of observations. First, chronic (≥48 h) treatment with TTX or ionotropic glutamate receptor blockers increases miniature excitatory postsynaptic current (mEPSC) amplitude but not frequency *(6,7).* Classically, this type of change is believed to reflect a postsynaptic alteration, such as an increase in either the number or the function of the postsynaptic glutamate receptors. Second, responses to exogenous glutamate or kainate (a weakly desensitizing α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA]/KA receptor agonist) are much greater in neurons exposed to activity blockade compared to untreated controls, which is consistent with a postsynaptic locus *(6,7).* Finally, chronic treatment with TTX or glutamate receptor blockers results in changes in the protein composition of the postsynaptic density, including increases in *N*-methyl-D-aspartate (NMDA) receptor subunits, structural proteins such as PSD-95, and signaling components such as CaMKII *(17).* Increased synaptic accumulation of AMPA receptors with activity blockade has also been reported *(18).*

As we have noted, such changes to the postsynaptic compartment of the glutamatergic synapse might be the logical target of selective (glutamate vs GABA) modulation, but some data also suggest an effect of activity blockade on presynaptic terminals. Studies using the styryl dye FM1-43 have shown that the number of vesicles released per action potential is increased upon activity blockade *(9).* A presynaptic change is further substantiated by the observation that activity blockade can result in an increase in mEPSC frequency but not in amplitude, suggesting a higher release probability or that more vesicles are available for release following activity blockade *(19–21).*

These presynaptic changes could result from morphological changes, such as an increase in the number of glutamate release sites and/or an increase in the size of individual synapses; evidence exists to support both of these possibilities. Immunocytochemistry has shown that activity blockade increases the number of clusters of the synaptic vesicle-associated protein synaptobrevin *(20);* electron microscopy showed that activity blockade increases both the number of docked vesicles and the size of the active zone *(9).* Postsynaptic density size also increases *(9),* suggesting that presynaptic and postsynaptic changes may, at times, work in concert.

The reasons for obtaining contrasting conclusions regarding the primary site of homeostasis in these studies are not fully understood. This is particularly true regarding the differences in observations regarding mEPSC frequency vs amplitude. Contributing factors may include differences in system (brain area or culture conditions), developmental stage examined *(22,23),* or the manner in which activity was blocked (postsynaptic

receptor blockade vs TTX treatment). Alternatively, the techniques used to examine synaptic signaling (electrophysiology, immunocytochemistry, or vital fluorescent dyes) may account for some discrepancies, emphasizing the need to assess changes with multiple methods.

Our Recent Studies of Homeostatic Responses to Depolarization

We found it striking that with a few exceptions, homeostasis has been evaluated by blocking electrical activity, although a plausible natural function of homeostasis is to prevent excessive activity and excitotoxicity. Although fewer studies have been conducted to examine the effects of chronic activity, evidence exists for what might be termed "overuse hyposensitivity." There is no consensus on whether the homeostatic change is pre- or postsynaptic. Treatment of cortical neurons with $[K^+]_o$ elevation up to 12 m*M*, which mimics enhanced electrical activity, results in a depression of mEPSC amplitude, with no change in either the kinetics or the frequency *(24)*. However, low-level activation of NMDA receptors in superior colliculus neurons results in a decrease in frequency, but not amplitude, of mEPSCs *(25)*.

Our own studies have indicated that chronic excitation leads to two separable alterations in presynaptic function *(8,26)*. We began our studies by investigating prolonged tonic depolarization of neurons, which has been shown to promote survival of neurons through the developmental period of natural cell death, which occurs just following synaptogenesis in many brain regions (reviewed in ref. *27*). In investigating the ability of chronic K^+ depolarization to promote the survival of cerebellar granule neurons and hippocampal neurons, we found that depolarization-reared neurons exhibited depressed Ca^{2+} currents, consistent with work in other neuronal systems *(28–30)*. Interestingly, this change appears to counteract survival-promoting Ca^{2+} influx *(26)*, although it is possible that depression of Ca^{2+} current is necessary to keep intracellular Ca^{2+} from becoming excitotoxic *(31)*. Neurons whose survival had been promoted by the addition of growth factors such as insulin-like growth factor-I did not show any change in Ca^{2+} current density. Furthermore, the effect on Ca^{2+} currents was specific, because other voltage-gated currents (Na^+, K^+) were unchanged *(26)*. Finally, when we dissected the various subtypes of Ca^{2+} channels, we found that current through multiple subtypes of HVA Ca^{2+} channels was depressed, including L, N, and P/Q types.

Because the chronic depolarization effect on Ca^{2+} current appeared to include those channel subtypes responsible for neurotransmitter release, we then examined whether the persistent depression of somatic Ca^{2+} influx extended to synaptic terminals. We examined recurrent (autaptic) synaptic signaling in solitary hippocampal micro-island neurons that had been exposed to elevated K^+ or control conditions. With a 16-h exposure to 30 m*M* of K^+ (which clamps the membrane potential near –20 mV), we found that both evoked EPSCs and inhibitory postsynaptic currents (IPSCs) were reduced in amplitude when cells were returned to normal medium. However, the degree of depression was significantly different between the two: IPSCs were only depressed by an average of 50%, whereas EPSCs were depressed by 87% (Fig. 1). With multiple-day exposure to K^+, IPSCs maintained a depression of 50%, but EPSCs could no longer be measured. This difference in sensitivity to K^+ treatment was the first indication that two distinct effects occurred: the first effect was common to both glutamatergic and GABAergic neurons, and the second effect was selective for glutamatergic synapses *(26)*. Evidence outlined below reinforces our suspicion that two separate mechanisms reduce transmitter release at glutamate synapses.

Our initial observation that chronic depolarization depressed Ca^{2+} currents in both cell types suggested to us that an alteration of the probability of vesicle release (p_r) might be a common effect to both glutamatergic and GABAergic cells. The probabilistic nature of

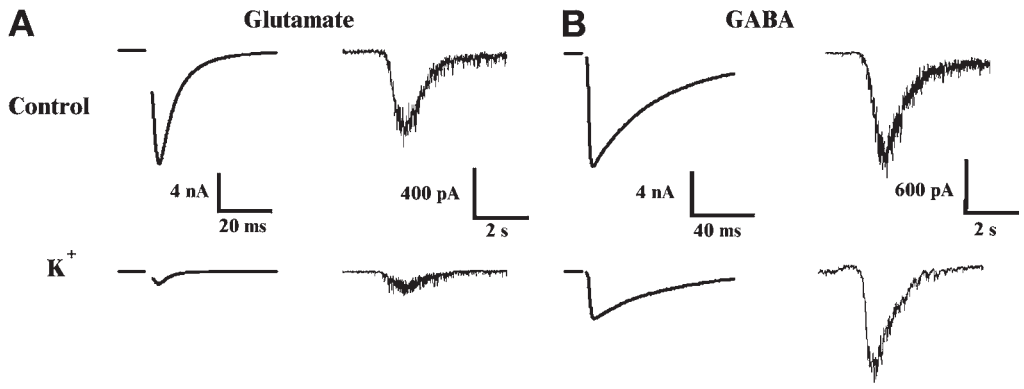

Fig. 1. Potassium depolarization depresses the readily releasable pool in glutamatergic, but not GABAergic, neurons. Representative traces of electrically evoked autaptic currents (left) and sucrose-evoked currents (right) in glutamatergic **(A)** or GABAergic **(B)** neurons after 16-h treatment with control medium or with medium containing 35 m*M* of K^+. Note that IPSCs were recorded with a high intracellular Cl^- concentration; therefore, currents are inward. (Reprinted from ref. *8* with permission from Elsevier.)

transmitter release can be understood according to the quantal hypothesis *(14)* and the binomial model of synaptic transmission *(32–34)*. In effect, the amplitude of the PSC is determined by the quantal size *(q)*, the number of release sites *(N)*, and the p_r. Generally, the size of an action potential-evoked postsynaptic current is proportional to the number of neurotransmitter-laden vesicles released from a presynaptic site, which, on average, is proportional to the product of N and p_r. Postsynaptic factors (receptor number, density, and sensitivity) and the amount of transmitter in each vesicle are also important influences on the evoked EPSC amplitude and affect q. Because the fusion of synaptic vesicles with the presynaptic plasma membrane is critically dependent on the presence of calcium ions, a decrease in Ca^{2+} influx at the synaptic terminal can decrease the likelihood that a vesicle exocytoses *(35,36)*.

To test whether the decrease in Ca^{2+} current we observed was responsible for the depression of IPSC amplitude after chronic depolarization, we recorded IPSCs in the presence of increasing amounts of extracellular Ca^{2+}. We reasoned that the increase in Ca^{2+} driving force should overcome the depression of Ca^{2+} current induced by the incubation in depolarizing conditions. Consistent with our hypothesis, the Ca^{2+} concentration response curve for IPSCs was significantly shifted to the right in depolarization-reared cells, with no difference in maximum response size. The EC_{50} value for K^+-treated cultures was 2.88 m*M*, compared to a value of 0.90 m*M* in control cultures. As further evidence for an effect on p_r, we also observed less IPSC paired-pulse depression (an assay of vesicle depletion) in depolarization-reared neurons *(26)*.

A decrease in paired-pulse depression was also observed in the more severely affected glutamatergic neurons exposed to elevated K^+ for 16 h, which is consistent with the idea that depressed Ca^{2+} currents altered p_r in both types of neurons. Unlike IPSCs, EPSCs depressed by depolarization could not be recovered by acutely increasing extracellular Ca^{2+} during the measurement of synaptic responses *(8)*. This suggested that additional mechanisms were responsible for the severe depression of glutamate transmission with tonic depolarization. We then questioned whether this selective effect on glutamatergic transmission had a pre- or postsynaptic locus.

Two pieces of evidence strongly suggested that postsynaptic targets were not altered in glutamatergic neurons following K^+ exposure. First, EPSCs mediated by AMPA and NMDA

receptors were depressed in parallel, excluding the possibility that AMPA receptors were withdrawn from the plasma membrane, as has been reported in other forms of synaptic plasticity *(37–39).* However, this evidence has caveats. Presynaptic depression may not always result in parallel decreases in the NMDA and AMPA components of the EPSC because of different affinities of the receptors for glutamate. Additionally, postsynaptic parallel changes in AMPA and NMDA currents have been reported *(40).* Second, we found that responses to exogenous application of kainate or NMDA were unchanged after K^+ treatment, suggesting that the overall postsynaptic density of ionotropic glutamate receptors was not altered.

Therefore, we began to test the idea that an additional presynaptic mechanism was responsible for the differential depression of glutamate release *(8).* mEPSC analysis revealed no change in postsynaptic charge transfer, but there was a significant decrease in the frequency of events, which is consistent with a presynaptic deficit. Additionally, the size of the readily releasable pool of vesicles (RRP), as measured using application of a hypertonic sucrose solution *(41),* was decreased after chronic K^+ exposure in glutamatergic, but not GABAergic, neurons (Fig. 1). This result is important because it clearly divorced the additional depression of glutamate transmission from the change in p_r associated with decreased Ca^{2+} current. Hypertonic sucrose causes Ca^{2+}-independent release of transmitter; hypertonicity bypasses Ca^{2+} influx and increased $[Ca^{2+}]_i$ *(41),* so depression of Ca^{2+} current cannot explain the depression of sucrose-evoked EPSCs. Depolarization rearing did not depress sucrose-evoked GABA transmission, which demonstrates that the RRP decrease is selective to glutamate transmission.

These observations provide classic evidence for a presynaptic change, but they depend on the presence of postsynaptic glutamate receptors as sensors of released transmitter. To eliminate this complication, we used recordings of synaptically stimulated glutamate transporters in astrocytes as an independent assay of glutamate release *(42–46).* A modest depolarization time of 4 h depressed astrocyte transporter responses to synaptically released glutamate by approx 50% *(8).*

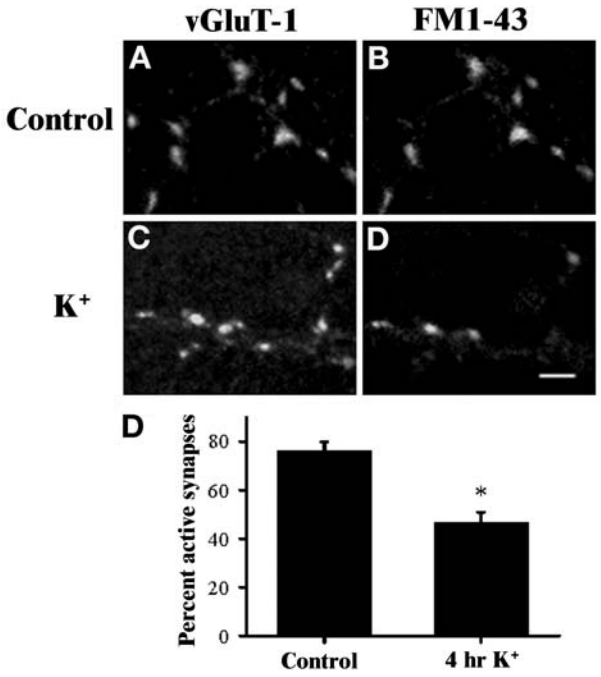

Fig. 2. The percentage of inactive synapses is increased by K^+ depolarization. **(A,B)** Control synapses labeled with FM1-43 to identify active synapses **(B)** and by *post hoc* vGluT-1 immunofluorescence **(A)** to identify glutamate synapses. **(C,D)** Similar panels from a culture treated for 4 h with 35 m*M* of K^+. Note the smaller fraction of active synapses (both vGluT-1 and FM1-43 positive) in the K^+-treated culture. Scale bar = 4 μm. **(E)** Summary of the percentage of active synapses from fields obtained by a naïve observer in five separate experiments ($p < 0.01$). (Reprinted from ref. *8* with permission from Elsevier.)

Finally, we examined presynaptic function optically using immunocytochemistry against synaptic vesicle proteins and using FM1-43 in live cells to identify active synapses (Fig. 2). We found no change in the number, density, or size of presynaptic terminals (as identified by synaptic vesicle markers). However, we found that the number of synaptic terminals capable of vesicle exo-/endocytosis was depressed by

an amount matching the EPSC depression (Fig. 2). Therefore, we proposed that the homeostatic change to glutamate synapses involves a binary functional inactivation of a subset of glutamate terminals *(8).* The inactivation results in an apparent decrease in the total number of vesicles immediately available for release (i.e., RRP) in response to both Ca^{2+}-dependent and -independent stimuli.

In summary, our extensive characterization of the homeostatic change at glutamate and GABA synapses in response to overactivity suggested primarily presynaptic changes over the time-course of exposures we examined. Surprisingly, perhaps, GABA transmission is depressed by an overall reduction in Ca^{2+} current density, which lowers p_r. Glutamate transmission is additionally affected by a decrease in the number of synapses that are capable of releasing neurotransmitter, resulting in depression of the total cellular RRP.

This binary inactivation of presynaptic terminals, specific to glutamate synapses, differs from several other reported mechanisms of synaptic homoeostasis. We have observed purely presynaptic adaptive changes, whereas others have observed purely postsynaptic change *(6,7,18,40).* Additionally, other groups have observed presynaptic adaptive changes; however, changes involved p_r changes or correlated changes in both p_r and RRP *(9,10),* rather than a binary functional change. Partial explanations for the discrepancy include the possibility that depolarization (our studies) elicits presynaptic changes, whereas hyperpolarization (many previous studies) yields postsynaptic changes; that different brain areas have different loci of synaptic change *(3);* or that the period of challenge (≤16 h in many of our experiments vs ≥48 h in many previous studies) may be important in the locus of change.

Molecular Candidates for Glutamate-Specific Presynaptic Changes

This selective presynaptic effect in glutamatergic neurons raises issues regarding what the molecular nature of this change might be—that is, because most of the proteins governing synaptic vesicle fusion and neurotransmitter release are identical between glutamatergic and GABAergic neurons, what proteins or signaling pathways could be the targets of chronic depolarization and result in the selective inactivation of glutamate synapses? Because the difference in GABA and glutamate synapses to depolarizing challenge is apparent with secretagogs that bypass Ca^{2+} influx, we are presumably interested in molecular candidates downstream of Ca^{2+} influx: buffers and sensors. One report suggested that the target-soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (tSNARE) protein synaptosome-associated protein 25 kDa (SNAP-25) is not expressed at GABAergic synapses but is replaced by its homolog SNAP-23 *(47).* Interestingly, the presence or absence of SNAP-25 alters Ca^{2+} influx in response to acute K^+ depolarization *(47).* Therefore, at face value, the difference in tSNAREs is likely more related to p_r effects, which we found did not differ between synaptic types.

At least three other protein families or signaling pathways show differential phenotypes in glutamatergic vs GABAergic neurons, although a specific role in depolarization-induced changes has not been specifically examined. The first of these is the brain-derived neurotropic factor (BDNF)/ tyrosine kinase (Trk)B pathway. Although BDNF is primarily a promoter of neuronal survival and differentiation, some recent attention has included its role in the modulation of synaptic signaling (reviewed in ref. *48*). BDNF both influences synaptogenesis by increasing synapse number *(49,50)* and regulates synapse maturation and/or stabilization once synapses have formed *(50–52).* This effect on maturation includes an increase in the number of docked vesicles *(50,53),* which likely corresponds with the physiologically defined RRP. This may occur through secretion of BDNF directly at the synaptic cleft, because BDNF has been localized to nerve terminals by immunocytochemistry *(54),* although dendritic release of BDNF has also been reported (reviewed in ref. *48*).

The primary difficulty with the BDNF/TrkB pathway as a potential target of the selective glutamate modulation by chronic depolarization is that BDNF has also been reported to potentiate GABAergic transmission (reviewed in ref. *55*). However, these results are not universally accepted, and others have reported that BDNF can depress electrically evoked IPSCs *(56)*. Overall, the effects of BDNF on inhibitory neurons have not been characterized as well as those on excitatory neurons, so this pathway remains an attractive potential candidate for persistent effects on glutamate synaptic function.

A second potential target for selective modulation of glutamate release is the Munc13 family of proteins. Munc13 proteins localize to the presynaptic terminal and regulate the function of the tSNARE syntaxin *(57)*. They are essential for the maturation of synaptic vesicles from a docked to a fusion-competent state, a process known as "priming" *(58,59)*. Therefore, Munc13 proteins help establish the functional RRP. Although lower organisms each contain only one Munc ortholog, the mammalian Munc13 family has three gene products, two of which (Munc13-1 and Munc13-2) are expressed in the hippocampus *(59,60)*. Interestingly, genetic deletion of Munc13-1 drastically depresses glutamatergic transmission, with a 90% reduction in both EPSC amplitude and the size of the functional RRP (as determined with hypertonic sucrose application) *(59)*. GABAergic transmission in the same cultures is unaffected, presumably because these synapses also express the Munc13-2 isoform (as does a very small percentage of glutamatergic synapses; refs. *59* and *60*). Therefore, selective modulation of Munc13-1 vs Munc13-2 could potentially alter glutamate, but not GABA, release.

The synapsin family is another protein family that has been demonstrated to have disparate roles in excitatory vs inhibitory neurons. Synapsins are the most abundant synaptic vesicle proteins, but their precise role in vesicle release has been elusive. This is true partly because the various roles assigned to synapsins were based on experiments in several systems to examine one or more of the three synapsin homologs. Until recently, no study had examined synaptic transmission in the absence of all three synapsin gene products. It was recently shown that the synapsins collectively regulate the size of the reserve pool of vesicles in glutamatergic terminals but directly regulate the size of the RRP (or the number of docked vesicles) in GABAergic terminals *(61)*. Although this makes synapsins an unlikely target for the modulation of glutamate release by chronic depolarization, it does provide another means by which these neurotransmitters can be selectively regulated. This example also indicates the possibility of different functions for ubiquitously expressed proteins.

As we learn more about the individual protein components of the synapse, the list of potential modulatory candidates is likely to grow. Calcineurin and dysbindin, which can each enhance glutamate release in simple assays of synaptic function, are other molecules that are currently attractive possibilities *(62,63)*, although their precise roles in other neurotransmitter systems are not completely clear. The signaling molecule CaMKIIα, although well-studied on the postsynaptic side of the cleft, is also believed to play an important role in glutamate release *(64,65)*. The presynaptic structural proteins β-neurexin, N-cadherin, and palladin are also interesting because they appear to localize specifically to excitatory synapses (refs. *66–68; see also* ref. *69*). Finally, vGluT, the transporter responsible for loading glutamate into synaptic vesicles represents an obvious example of selective expression at glutamatergic sites *(70,71)*. Although empty vesicles resulting from vesicular transporter modulation *(72,73)* would provide an attractive hypothesis to explain a differential depression of glutamate transmission, this mechanism is difficult to reconcile with the changes we have observed in FM1-43 cycling (Fig. 2).

As discussed earlier, the size of a PSC generated in response to an action potential is determined by q, N, and the p_r. Our own work illustrates that chronic depolarization selectively depresses the total available RRP (related to N). Many of the candidate molecules described earlier act at the level of the RRP or the total pool of vesicles. Because replenishment from reserve

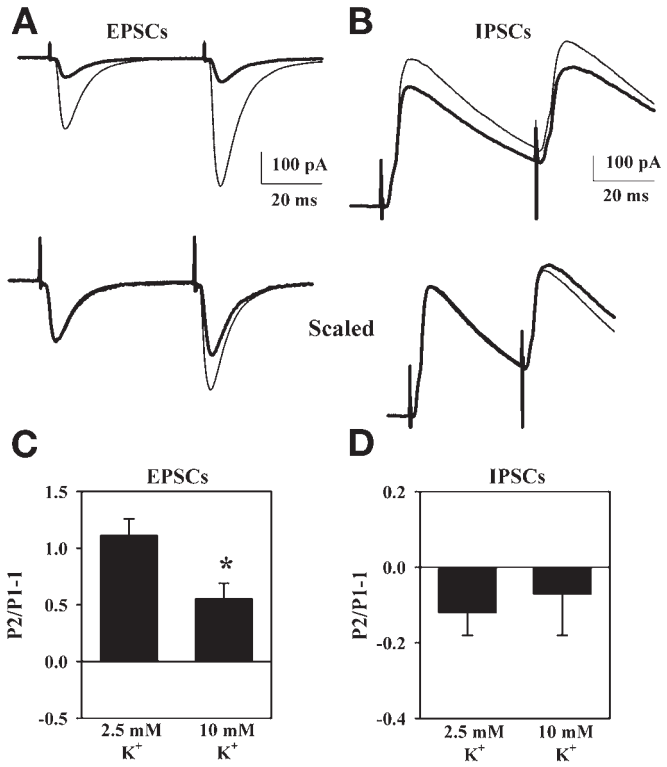

Fig. 3. Glutamate release is selectively reduced in the CA1 region of rat hippocampus during exposure to moderate rises in extracellular K^+ concentration ($[K^+]_o$). **(A,C)** EPSCs elicited by paired-pulse stimulation of the Schaffer collateral pathway (50-ms interval) exhibit paired-pulse facilitation in control conditions (2.5 m*M* of $[K^+]_o$, thin traces). Upon brief exposure to 10 m*M* of $[K^+]_o$ (< 5 min), both the amplitude of initial EPSCs and paired-pulse ratio were significantly depressed (bold traces). The lower traces in **(A)** are scaled by initial EPSC amplitude for paired-pulse ratio comparison. **(B,D)** Pharmacologically isolated IPSCs elicited by the same stimulation as EPSCs are not depressed in initial amplitude or paired-pulse ratio by 10 m*M* of $[K^+]_o$ exposure. Summary data for IPSCs are shown in (D). The summary data in (C) and (D) are expressed as P2/P1-1, such that negative values indicate paired-pulse depression and positive values indicate facilitation. (Data reproduced with permission from ref. *13*.)

vesicles and vesicle endocytosis ultimately supply the RRP, effects on these processes could also formally explain our results. Ultimately, the hallmark combination of diminished vesicle availability and specificity for glutamate synapses is our biggest clue toward discovery of the molecules involved in this form of synaptic plasticity.

Acute Depression of Glutamate Release

Several years ago, we were surprised to find that modestly depolarizing elevations of extracellular $[K^+]_o$, which delay recovery from Na^+-channel inactivation, selectively dampened glutamate release in cultured hippocampal neurons *(12)* as well as neurons in acutely prepared slices (ref. *13;* Fig. 3). Unlike responses of glutamate synapses to prolonged depolarization, these effects had a rapid onset and offset and were mimicked and enhanced by drugs that foster Na^+-channel inactivation *(11–13)*. Although the effects of these treatments on action potential waveform were relatively minor, the repercussions for glutamate release were not. In each of these conditions, glutamate release was selectively decreased compared to

GABA release. These results are consistent with observations that glutamate release is highly sensitive to variations in the waveform and reliability of propagating action potentials.

Multiple lines of evidence now suggest action potential initiation and propagation are much more variable than previously believed. Observations from somatodendritic recordings suggest that different expression patterns of voltage-gated ion channels endow principal glutamatergic neurons with different action potential firing patterns than interneurons within the hippocampus. If such differences extend to the axon, they could underlie differences in axonal properties and different sensitivities to drugs and membrane polarization. This section focuses on recently reported differences in channels underlying excitability of principal glutamatergic neurons vs GABAergic interneurons. It then reviews recently observed factors influencing spike propagation and fidelity in glutamate-releasing fibers of hippocampus. Finally, it examines work that has probed the influence of spike changes on transmitter output at presynaptic terminals.

Somatodendritic Na^+ and K^+ Channels: Regulation of Intrinsic Excitability and Spike Initiation

One of the most critical determinants of neuronal excitability is the expressed complement of voltage-gated Na^+ and K^+ channels. By understanding differences in channel properties in the somatodendritic compartment, which is much more experimentally accessible than the axon, we may be able to infer some difference in axonal behavior, which is our ultimate goal. Differential expression of voltage-gated channels results in distinct differences in firing patterns between principal glutamatergic neurons and other neuronal types *(74).* The A-type K^+ conductance (I_A) is an intriguing conductance that is at least partly responsible for the different firing patterns in different neuron types *(75).* Principal neurons in the hippocampus express high relative levels of Kv4.2 and 4.3 subunits that form fast-inactivating A-type K^+ channels *(76,77).* Conversely, these neurons express much lower levels of subunits Kv3.1 and 3.2, which are associated with fast, delayed rectifier currents (discussed later in this section; refs. *78* and *79*). Principal neurons expressing Kv 4.x members have a characteristically low maximum firing rate in response to somatic current injection (typically 20–50 Hz).

An interesting aspect of I_A function in pyramidal cells is the nonuniform distribution of I_A channels. Rapidly inactivating I_A currents are present in the soma and dendrites of CA1 pyramidal neurons *(80)* but are notably absent from the axon initial segment of cortical pyramidal neurons *(81).* Blockade of I_A currents in pyramidal neurons can result in dendritic hyperactivity *(82),* indicating that these currents contribute to signaling within the dendritic compartment. More general localization studies found Kv4.2 in pyramidal cell dendrites throughout the CNS, and Kv4.3 channels are apparently present in some classes of interneurons *(79,83).* In addition to endowing cells with a characteristic low maximal firing rate and participating in dendritic excitability, the inactivation properties of I_A conductances may contribute to activity-dependent spike broadening (further discussed later; ref. *84*). Overall, the expression of high levels of Kv4.x A-type K^+ channels in principal cells tends to permit phasic-spiking and limits tonic-spiking activity.

In direct contrast to the strong levels of the fast-inactivating I_A currents observed in glutamatergic neurons, many GABAergic interneurons express a fast, delayed rectifier-type, non-inactivating, voltage-gated K^+ conductances. These conductances arise from Kv3.x expression and endow a fast, tonic-spiking phenotype *(84,85).* One recent study using single-cell reverse transcriptase polymerase chain reaction found that fast-spiking basket cells in the dentate gyrus expressed high levels of Kv3.1 and 3.2 but lower levels of Kv4.2 and 4.3 than CA1 pyramidal cells *(78).* Because of the lack of inactivation during sustained activity, fast hyperpolarization is maintained even during repetitive spiking, resulting in higher maximal spiking frequency and resistance to

spike broadening *(84–86).* Interestingly, a model Kv3 conductance delivered to interneurons in the presence of Kv blockers (tetra-ethyl ammonium; 4-aminopyridine) rescues fast-spiking phenotypes and endows non-fast-spiking CA1 pyramidal cells with a fast-spiking phenotype *(84).* The high expression levels of fast, delayed rectifier K^+ conductances—specifically in GABAergic interneurons—may underlie some of the resistance of GABAergic synapses to acute manipulations, which have strong depressive effects on glutamate synapses.

Because voltage-gated K^+ channels are heteromers and contain combinations of Kv 1, 2, 3, and 4 family α- and β-subunits, participation of other Kv subunits in principal cell-firing properties is very likely. Knowledge of localization and function of various channel subunits has relied on pharmacology of questionable specificity and on correlation with levels of semiquantitative single-cell reverse transcriptase polymerase chain reaction *(78)* and immunohistochemistry *(79,83).* Loss- and gain-of-function experiments are complicated by heterogeneity of these channels not only in their specific subunit composition but also by their complex subcellular localization *(80,83).* Future studies employing transcriptional or functional knockdown (i.e., with small interfering RNA) will likely further clarify relative contributions of various Kvs in the spiking properties of principal neurons.

Although the molecular and biophysical variety of K^+ channels makes them particularly attractive candidates for promoting differences in excitability among cell types, recent data suggest that the complement of voltage-gated Na^+ channels expressed by principal neurons can also influence the pattern of activity in principal neurons vs interneurons. Many principal neurons express a "persistent" Na^+ current characterized by a prevalence of slow inactivation upon strong depolarization. This slow inactivation may normally promote spiking during fast, transient stimulation but potentially limits NaCh availability during repetitive spiking *(87,88)* and, therefore, could contribute to frequency-dependent changes in spike waveform.

Although studies are lacking in interneuron populations, it is interesting to note that many fast-spiking neurons express a "resurgent" Na^+ current linked to the $Na_V1.6$ α-subunit and β4-subunit of voltage-gated Na^+ channels *(89–92).* The resurgent Na^+ current provides additional membrane depolarization immediately following the hyperpolarizing phase of the action potential and is associated with a faster recovery from inactivation and greater availability of Na^+ channels, assisting in high-frequency spiking of cerebellar Purkinje neurons *(93).* Interestingly, principal glutamatergic neurons, such as CA3 pyramidal cells, lack this resurgent current *(91).* Although presence of resurgent currents is not exclusive to GABAergic neurons *(94,95),* notable absence of these currents in non-fast-spiking principal neurons may further influence the balance of excitation and inhibition in the CNS. Studies are needed to assess whether the resurgent currents influence other fast-spiking cells, such as the fast-spiking interneurons in the hippocampus and cortex.

In summary, a growing body of evidence suggests that intrinsic excitability differences exist between glutamatergic and GABAergic neurons. The specific complement of Na^+ and K^+ channels reviewed earlier broadly supports phasic-firing functions of principal cells and tonic-firing functions of interneurons. Although differences in maximal firing frequency certainly suggest that principal glutamatergic neurons have a lower maximum throughput capacity than GABAergic interneurons, the final output of synaptic terminals is determined by how these spikes propagate down the axon. Can the conductances found in the somatodendritic compartment lend insight into experimentally observed differences between principal neurons and interneurons regarding spike propagation?

The same conductances reviewed earlier are also important determinants of individual spike waveforms; therefore, if these conductances are expressed by the axon, they may play a large role in spike waveform and fidelity postinitiation. For example, Kv4.x-expressing neurons (principal neurons in the

case of the hippocampus) are associated with broader spikes during activity than those of Kv3.x fast-spiking neurons. At first glance, the broader spikes generated by Kv4.x neurons might be expected to promote more reliable propagation because a broader spike generates more charge influx (current) for propagating the spike. This is the opposite of our recent observations, which have suggested less secure firing of glutamate-releasing fibers than GABA-releasing fibers *(13)*. This raises the issue of whether the same conductances are expressed along the axon as in the somatodendritic compartment or whether other factors, such as branching patterns or passive membrane properties of the cells, might have a strong role in dictating axonal spike waveform and fidelity. Although direct assessment of channel densities and distribution in small unmyelinated axons remains difficult, the next section presents recent evidence for mechanisms that may alter spike waveform and fidelity in these axons.

Axonal Ion-Channel Expression and Morphology: Regulation of Propagating Spikes

After their generation through synaptic integration in the somatodendritic compartment, action potentials must successfully propagate down the axon to induce presynaptic vesicle fusion. However, the propagation of action potentials in glutamatergic neurons appears to be more complex than was once believed *(96)*. Current research indicates several postinitiation mechanisms of altering the propagated action potential and downstream transmitter release. Membrane conductances that alter axonal output by membrane hyperpolarization *(97–99)* as well as conductances that inhibit output by depolarization *(13,100,101)* have been reported.

In hippocampal pyramidal cells, action potentials initiated in temporal isolation from other spikes propagate with a high degree of reliability *(13,102–104)*. However, axonal spikes can be made to fail by hyperpolarization, which presumably relieves inactivation of I_A channels *(97)*. When Kv blockers are included in the intracellular pipet to inhibit I_A, spike conduction following hyperpolarization is increased *(97)*. Under physiological conditions, hyperpolarization may also activate hyperpolarization-activated cation currents, or H currents *(98)*, which act to repolarize the membrane. Activation of hyperpolarizing K^+ conductances (i.e., I_A currents, BK-type Ca^{2+}-dependent K^+ channels; refs. *105* and *106*) may directly oppose spike propagation by counteracting Na^+ currents and reducing overall charge carried by spikes. However, hyperpolarization would relieve inactivation of voltage-gated Na^+ channels, which are also important in determining axonal spike propagation *(13)*. Notably, the I_A currents implicated in spike failures *(97)* also limit somatodendritic excitability in pyramidal neurons (as discussed in the previous section). Although I_A currents are clearly important for determining aspects of spike waveform from somatic recordings, the inaccessibility and small size of the axons studied has prevented direct observation of these channels on axonal membranes.

Because of the intrinsic differences between GABA- and glutamate-releasing neurons noted in the previous section, it seems possible that GABAergic and glutamatergic axons differ in their capacity for propagating action potentials. Whereas differences between GABA and glutamatergic axons have not been demonstrated for the hyperpolarization-block discussed earlier in this section, depolarization by $[K^+]_o$ and sustained activity affect glutamate release more than GABA release in the hippocampus *(8,12)*. Similar glutamate-selective effects are observed when Na^+-channel inactivation is enhanced pharmacologically and when Na^+ currents are reduced via low, subsaturating concentrations of TTX *(11)*. These results indicate that depolarization acts, at least partly, by reducing Na^+ currents during the action potential. When hippocampal pyramidal neurons are depolarized by moderate increases in $[K^+]_o$, axonal spike reliability decreases *(13,101)*. The effects on propagating spikes include both depression

of the propagating spike waveform and outright failures, which are attributed to accumulated Na^+-channel inactivation *(13).* Unfortunately, these studies, which relied on extracellular stimulation methods, did not distinguish initiation deficits (i.e., increased threshold, increased refractoriness) from propagation deficits.

To test action potential propagation with direct control (and verification) of spike initiation, several groups have turned to paired somatic/axonal recordings. Recent studies have found that sustained depolarization at the soma can induce severe waveform changes to propagating action potentials. Our recent results, utilizing paired recordings from CA3 pyramidal somas and their unmyelinated axons up to 800 μm away, suggest that axons conduct spikes with little decrement during brief (<2 sec), repetitive spiking under baseline conditions, despite strong waveform changes observed at the soma *(107).* On the other hand, a sustained envelope of depolarization during epileptiform events contributes to strong axonal changes. Such prolonged activity eventually results in outright axonal failure, particularly during bursting activity in pyramidal neurons (ref. *107;* Fig. 4).

Interestingly, Purkinje cell principal neurons of the cerebellum (which are GABAergic projection neurons and possess myelinated axons) exhibit similar axonal changes during prolonged activity *(108,109).* The techniques allowing these extracellular measurements from axons to occur simultaneously with somatic intracellular recording have only recently been developed, and the mechanisms and generality of these results remain to be tested. As mentioned previously, depolarization likely causes axonal propagation changes through a complex combination of activation and inactivation of voltage-gated conductances. We predict that when subjected to similar prolonged activity, interneurons of the hippocampus would not show the same changes in axonal propagation that principal cells exhibit.

The morphology of axons certainly plays a role in propagation. Branch points and axonal

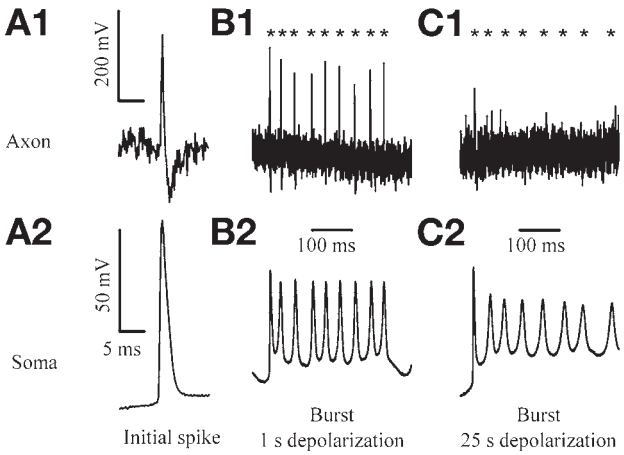

Fig. 4. Rat CA3 pyramidal cell axons fail to propagate high-frequency spikes during depolarizing pathophysiological discharges. **(A)** Simultaneous intracellular recordings from the soma (A2) and extracellular recording from the axon (A1) enable observation of propagating spikes in individual axons with explicit verification of action potential initiation (via the somatic recording). In depolarizing $[K^+]_o$ (8 m*M*) conditions, initial action potentials elicited by short (1s) intracellular current injections at the soma propagated with a high degree of reliability (A). Many CA3 pyramidal neurons responded to this 1 s of depolarization with a regenerative depolarization that persisted for approx 25 s after cessation of intracellular current injection. **(B)** Early in these epileptiform events, high-frequency burst-spiking observed at the soma (B2) resulted in reliable spike propagation to the axon (B1). **(C)** Later in these epileptiform events (approx 25 s), similar burs-spiking in the soma (C2) failed to result in detectable spikes in the axon (C1). (*See also* ref. *105.*)

varicosities have long been hypothesized to influence propagation (boosting) or failure (branch-point failure). In the hippocampus, glutamatergic axons have extensive branching patterns *(110).* Interneurons vary tremendously in their patterns of axonal branching *(111)* and, therefore, might predict variability in axonal fidelity among interneuron subtypes. Although branch points in larger diameter axons have been closely studied for their role in propagation *(112,113),* the inaccessibility of

small-diameter fibers in the CNS has prevented direct study of the influence of branchpoints on hippocampal action potential propagation.

Varicosities have also been proposed as sites of conduction failure *(114).* CA3 pyramidal cells give rise to axons that form many thousands of *en passant*-type synapses. These swellings may influence the propagation of action potentials down the axon by both the change in passive properties of the axon and the differential expression of ion channels in these presynaptic elements *(115,116).* The majority of interneurons do not make *en passant* synapses *(111).* If spike propagation is compromised by the presence of large numbers of varicosities, then glutamatergic axons may be more susceptible to propagation failure than GABAergic interneuron axons. However, one intriguing study argued against the finding that varicosities directly enhance propagation failures. Increased voltage-gated Na^+-channel density is observed at mossy fiber terminals; this is a specialized *en passant* synapse *(115).* It is evident that more studies are needed to investigate the contribution of axonal morphology to propagation reliability in small diameter axons.

Propagation failure in glutamatergic fibers represents the maximal inhibition of downstream transmission. However, waveform changes may occur in the absence of an all-or-none failure *(107).* The consequences of such waveform changes on propagation down the axon are likely to be quite complex, and more studies are needed to investigate the effects of Na^+ and K^+ channels on spike propagation with proper control of spike initiation. In cases where spike waveform is significantly altered but propagation is maintained, transmitter output at the presynaptic terminal may still be strongly affected.

In summary, it is clear that propagating action potentials in glutamatergic axons are subject to regulation via waveform modulation and all-or-none failure. Glutamatergic axons in the CNS appear to be quite sensitive to alteration of Na^+ and K^+ conductances, indicating that action potential propagation in these axons can be extremely fragile under certain conditions. Techniques likely to increase our understanding of spike propagation are being developed and refined. Single-axon recording, paired soma/axon recording, and other techniques will likely lead us to greater understanding of spike propagation in CNS axons and may be used to study factors responsible for fidelity differences in the axons of different cell types.

Mechanisms of Acute Glutamate Release Modulation at the Presynaptic Terminal

After an action potential has propagated down the axon and reached the presynaptic terminal, several mechanisms may participate in selective altering of the glutamate output. Modulation of Ca^{2+} influx is a common final pathway for many synaptic modulators. For example, activation of G protein-coupled receptors may selectively decrease Ca^{2+} influx at some glutamate terminals *(117–119).* Additionally, differences in Ca^{2+}-channel subtype *(120,121),* Ca^{2+}-channel density near release sites *(122),* Ca^{2+} buffering *(111,123),* or Ca^{2+} sensitivity of transmitter release could participate in selective, acute alterations of transmitter release at different terminals. In addition to these mechanisms of directly modulating presynaptic Ca^{2+}, we have found that waveform alterations in hippocampal glutamatergic fibers are likely to reach synaptic terminals and may account for at least some of the depression of glutamate release *(11,13).* Therefore, it is instructive to review recent studies regarding how changes in the waveform of the presynaptic action potential at model glutamate synapses influence subsequent Ca^{2+} influx and transmitter release.

The waveform of action potentials as they invade the terminal has been shown to determine the magnitude and kinetics of Ca^{2+} transients *(124,125).* Therefore, modulating the waveform of propagating action potentials modulates Ca^{2+} entry. Most studies of the influence of spike width on glutamatergic transmission have shown that spike broadening is

associated with increases in Ca^{2+} entry and transmitter release *(126–129).* In some cases, the increases in Ca^{2+} entry during spike broadening did not enhance transmitter release during sustained activity *(125).* Other specialized conductances, such as BK and SK Ca^{2+}-activated K^+ channels, have been localized to glutamatergic terminals and may counteract spike broadening and increased Ca^{2+} influx during sustained activity *(106,130,131).* Additionally, Ca^{2+} influx during sustained activity can be limited by extracellular Ca^{2+} depletion *(132)* or Ca^{2+}-channel inactivation *(133).*

Notably, spike broadening in these studies is produced by blockade of voltage-gated K^+ conductances, resulting in widening by prolonging the repolarization phase of the spikes. In light of the depressive synaptic effects of Na^+-channel block, which affect action potential waveform on the rising phase as well, the specific spike waveform change (i.e., broadening on rising or falling phase) may determine the impact on transmitter release at terminals. Our most recent work suggests that one of the most prominent effects of sustained depolarization and activity is a decrease in the rising phase of the action potential *(13,107).* It will be interesting to see whether this particular mechanism of broadening has different effects on transmitter release than broadening by K^+-channel inhibitors such as 4-AP. It appears likely that if rise time slowing is severe enough to depress spike amplitude, overall Ca^{2+} influx may be depressed and transmission may be dampened.

We recently reported yet another level of acute control of glutamate release in response to depolarizing stimuli *(134).* This mechanism apparently reflects a change in p_r rather than persistent changes to the RRP or changes to spike propagation in glutamate axons *(134).* We found that up to 80% of available vesicles at glutamate synapses in cultures of hippocampal neurons expressed strong reluctance to exocytose in response to 20-Hz action potential trains. The reluctant vesicles could be "coaxed" into fusing by driving more Ca^{2+} into presynaptic terminals. Similar phenomena of reluctance have been noted at other glutamate synapses *(135–137)* and may be associated with Ca^{2+}-channel inactivation during stimulus trains *(133).* GABA synapses in hippocampal cultures apparently do not express the same reluctance *(134).* Therefore, there are multiple mechanisms by which neurons can exercise acute differential control over glutamate vs GABA release.

Conclusions: Acute Response to Depolarization

We have presented some of the factors likely to account for relative differences in the sensitivity of glutamate vs GABA release to agents that alter action potential conduction. The distinction between the responses of glutamate terminals vs GABA terminals is admittedly oversimplified. Even within the hippocampus, there are at least 16 different interneuron classes, which likely possess different channel compositions, passive membrane properties, and transmitter release properties *(99,111).* This suggests that variability can be found among subclasses of neurons in the properties of action potential propagation and coupling to transmitter release. Similarly, the precise rules governing action potential coupling at a calyceal glutamate synapse in the brain stem are unlikely to be identical to those governing release at small *en passant* synapses within *stratum radiatum* of hippocampus. Nevertheless, differential sensitivity to action potential modulators is found in both hippocampal culture *(11,12)* and slices *(13),* suggesting some generality. Certainly, the generality of the differences between glutamate and GABA signaling reviewed in this article is experimentally testable.

Overall Conclusions

In summary, this article has focused on recent work from our own laboratory to suggest presynaptic mechanisms for the persistent and acute depression of glutamate release

when neurons are challenged with excessive excitation. The work fits into a broader context of research on homeostatic mechanisms regulating neuronal excitability. Although it is not surprising that neurons are capable of homeostatically regulating their communication, we have been surprised that the level of regulation has occurred through mechanisms (i.e., presynaptic vesicle release and action potential conduction) that one might not expect *a priori.* It is clear that excessive activity also elicits changes counterproductive to homeostasis, such as axonal sprouting of glutamate fibers *(138),* loss of inhibitory input *(139,140),* excitatory actions of GABA *(141),* and reverse uptake of glutamate *(142).* Future therapeutic strategies for disorders of excessive activity may exploit and maximize acute and persistent homeostatic mechanisms such as those described here and may minimize positive-feedback changes that would exacerbate damage.

References

1. Zorumski C. F. and Olney J. W. (1993) Excitotoxic neuronal damage and neuropsychiatric disorders. *Pharmacol. Ther.* **59,** 145–162.
2. Mattson M. P. (2003) Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. *Neuromol. Med.* **3,** 65–94.
3. Burrone J. and Murthy V. N. (2003) Synaptic gain control and homeostasis. *Curr. Opin. Neurobiol.* **13,** 560–567.
4. Turrigiano G. G. (1999) Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* **22,** 221–227.
5. Malva J. O., Silva A. P., and Cunha R. A. (2003) Presynaptic modulation controlling neuronal excitability and epileptogenesis: role of kainate, adenosine and neuropeptide Y receptors. *Neurochem. Res.* **28,** 1501–1515.
6. O'Brien R. J., Kamboj S., Ehlers M. D., Rosen K. R., Fischbach G. D., and Huganir R. L. (1998) Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **21,** 1067–1078.
7. Turrigiano G. G., Leslie K. R., Desai N. S., Rutherford L. C., and Nelson S. B. (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391,** 892–896.
8. Moulder K. L., Meeks J. P., Shute A. A., Hamilton C. K., de Erausquin G., and Mennerick S. (2004) Plastic elimination of functional glutamate release sites by depolarization. *Neuron* **42,** 423–435.
9. Murthy V. N., Schikorski T., Stevens C. F., and Zhu Y. (2001) Inactivity produces increases in neurotransmitter release and synapse size. *Neuron* **32,** 673–682.
10. Piedras-Renteria E. S., Pyle J. L., Diehn M., et al. (2004) Presynaptic homeostasis at CNS nerve terminals compensates for lack of a key Ca2+ entry pathway. *Proc. Natl. Acad. Sci. USA* **101,** 3609–3614.
11. Prakriya M. and Mennerick S. (2000) Selective depression of low-release probability excitatory synapses by sodium channel blockers. *Neuron* **26,** 671–682.
12. He Y., Zorumski C. F., and Mennerick S. (2002) Contribution of presynaptic Na(+) channel inactivation to paired-pulse synaptic depression in cultured hippocampal neurons. *J. Neurophysiol.* **87,** 925–936.
13. Meeks J. P. and Mennerick S. (2004) Selective effects of potassium elevations on glutamate signaling and action potential conduction in hippocampus. *J. Neurosci.* **24,** 197–206.
14. Bear M. F. (1995) Mechanism for a sliding synaptic modification threshold. *Neuron* **15,** 1–4.
15. Sharpless S. K. (1975) Supersensitivity-like phenomena in the central nervous system. *Fed. Proc.* **34,** 1990–1997.
16. Desai N. S., Rutherford L. C., and Turrigiano G. G. (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nat. Neurosci.* **2,** 515–520.
17. Ehlers M. D. (2003) Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci.* **6,** 231–242.
18. Wierenga C. J., Ibata K., and Turrigiano G. G. (2005) Postsynaptic expression of homeostatic plasticity at neocortical synapses. *J. Neurosci.* **25,** 2895–2905.
19. Bacci A., Coco S., Pravettoni E., et al. (2001) Chronic blockade of glutamate receptors enhances presynaptic release and downregulates the interaction between synaptophysin-synaptobrevin- vesicle-associated membrane protein 2. *J. Neurosci.* **21,** 6588–6596.
20. Luthi A., Schwyzer L., Mateos J. M., Gahwiler B. H., and McKinney R. A. (2001) NMDA recep-

tor activation limits the number of synaptic connections during hippocampal development. *Nat. Neurosci.* **4,** 1102–1107.
21. Thiagarajan T. C., Piedras-Renteria E. S., and Tsien R. W. (2002) alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron* **36,** 1103–1114.
22. Burrone J., O'Byrne M., and Murthy V. N. (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* **420,** 414–418.
23. Kirov S. A., Goddard C. A., and Harris K. M. (2004) Age-dependence in the homeostatic upregulation of hippocampal dendritic spine number during blocked synaptic transmission. *Neuropharmacology* **47,** 640–648.
24. Leslie K. R., Nelson S. B., and Turrigiano G. G. (2001) Postsynaptic depolarization scales quantal amplitude in cortical pyramidal neurons. *J. Neurosci.* **21,** RC170.
25. Shi J., Aamodt S. M., Townsend M., and Constantine-Paton M. (2001) Developmental depression of glutamate neurotransmission by chronic low- level activation of NMDA receptors. *J. Neurosci.* **21,** 6233–6244.
26. Moulder K. L., Cormier R. J., Shute A. A., Zorumski C. F., and Mennerick S. (2003) Homeostatic effects of depolarization on Ca2+ influx, synaptic signaling, and survival. *J. Neurosci.* **23,** 1825–1831.
27. Franklin J. L. and Johnson E. M. Jr. (1992) Suppression of programmed neuronal death by sustained elevation of cytoplasmic calcium. *Trends Neurosci.* **15,** 501–508.
28. Franklin J. L., Fickbohm D. J., and Willard A. L. (1992) Long-term regulation of neuronal calcium currents by prolonged changes of membrane potential. *J. Neurosci.* **12,** 1726–1735.
29. Murrell R. D. and Tolkovsky A. M. (1993) Role of voltage-gated Ca2+ channels and intracellular Ca2+ in rat sympathetic neuron survival and function promoted by high K+ and cyclic AMP in the presence or absence of NGF. *Eur. J. Neurosci.* **5,** 1261–1272.
30. Li M., Jia M., Fields R. D., and Nelson P. G. (1996) Modulation of calcium currents by electrical activity. *J. Neurophysiol.* **76,** 2595–2607.
31. Choi D. W. (1995) Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci.* **18,** 58–60.
32. Johnson E. W. and Wernig A. (1971) The binomial nature of transmitter release at the crayfish neuromuscular junction. *J. Physiol. (Lond.)* **218,** 757–767.
33. Zucker R. S. (1973) Changes in the statistics of transmitter release during facilitation. *J. Physiol. (Lond.)* **229,** 787–810.
34. Quastel D. M. (1997) The binomial model in fluctuation analysis of quantal neurotransmitter release. *Biophys. J.* **72,** 728–753.
35. Zucker R. S. (1989) Short-term synaptic plasticity. *Annu. Rev. Neurosci.* **12,** 13–31.
36. Zucker R. S. (1999) Calcium- and activity-dependent synaptic plasticity. *Curr. Opin. Neurobiol.* **9,** 305–313.
37. Isaac J. T. R., Crair M. C., Nicoll R. A., and Malenka R. C. (1997) Silent synapses during development of thalamocortical inputs. *Neuron* **18,** 269–280.
38. Liao D., Hessler N. A., and Malinow R. (1995) Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375,** 400–404.
39. Atwood H. L. and Wojtowicz J. M. (1999) Silent synapses in neural plasticity: current evidence. *Learn. Mem.* **6,** 542–571.
40. Watt A. J., van Rossum M. C., MacLeod K. M., Nelson S. B., and Turrigiano G. G. (2000) Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* **26,** 659–670.
41. Rosenmund C. and Stevens C. F. (1996) Definition of the readily releasable pool of vesicles at hippocampal synapses. *Neuron* **16,** 1197–1207.
42. Mennerick S. and Zorumski C. F. (1994) Glial contributions to excitatory neurotransmission in cultured hippocampal cells. *Nature* **368,** 59–62.
43. Mennerick S. and Zorumski C. F. (1995) Paired-pulse modulation of fast excitatory synaptic currents in microcultures of rat hippocampal neurons. *J. Physiol. (Lond.)* **488,** 85–101.
44. Diamond J. S., Bergles D. E., and Jahr C. E. (1998) Glutamate release monitored with astrocyte transporter currents during LTP. *Neuron* **21,** 425–433.
45. Linden D. J. (1997) Long-term potentiation of glial synaptic currents in cerebellar culture. *Neuron* **18,** 983–994.
46. Luscher C., Malenka R. C., and Nicoll R. A. (1998) Monitoring glutamate release during LTP with glial transporter currents. *Neuron* **21,** 435–441.
47. Verderio C., Pozzi D., Pravettoni E., et al. (2004) SNAP-25 modulation of calcium dynamics underlies differences in GABAergic and

glutamatergic responsiveness to depolarization. *Neuron* **41,** 599–610.
48. Poo M. M. (2001) Neurotrophins as synaptic modulators. *Nat. Rev. Neurosci.* **2,** 24–32.
49. Vicario-Abejon C., Collin C., McKay R. D., and Segal M. (1998) Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J. Neurosci.* **18,** 7256–7271.
50. Tyler W. J. and Pozzo-Miller L. D. (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *J. Neurosci.* **21,** 4249–4258.
51. Bolton M. M., Lo D. C., and Sherwood N. T. (2000) Long-term regulation of excitatory and inhibitory synaptic transmission in hippocampal cultures by brain-derived neurotrophic factor. *Prog. Brain Res.* **128,** 203–218.
52. Tartaglia N., Du J., Tyler W. J., Neale E., Pozzo-Miller L., and Lu B. (2001) Protein synthesis-dependent and -independent regulation of hippocampal synapses by brain-derived neurotrophic factor. *J. Biol. Chem.* **276,** 37,585–37,593.
53. Collin C., Vicario-Abejon C., Rubio M. E., Wenthold R. J., McKay R. D., and Segal M. (2001) Neurotrophins act at presynaptic terminals to activate synapses among cultured hippocampal neurons. *Eur. J. Neurosci.* **13,** 1273–1282.
54. Altar C. A., Cai N., Bliven T., et al. (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389,** 856–860.
55. Vicario-Abejon C., Owens D., McKay R., and Segal M. (2002) Role of neurotrophins in central synapse formation and stabilization. *Nat. Rev. Neurosci.* **3,** 965–974.
56. Tanaka T., Saito H., and Matsuki N. (1997) Inhibition of GABA(A) synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J. Neurosci.* **17,** 2959–2966.
57. Betz A., Okamoto M., Benseler F., and Brose N. (1997) Direct interaction of the rat unc-13 homologue Munc13-1 with the N terminus of syntaxin. *J. Biol. Chem.* **272,** 2520–2526.
58. Aravamudan B., Fergestad T., Davis W. S., Rodesch C. K., and Broadie K. (1999) Drosophila UNC-13 is essential for synaptic transmission. *Nat. Neurosci.* **2,** 965–971.
59. Augustin I., Rosenmund C., Sudhof T. C., and Brose N. (1999) Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature* **400,** 457–461.
60. Varoqueaux F., Sigler A., Rhee J. S., et al. (2002) Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proc. Natl. Acad. Sci. USA* **99,** 9037–9042.
61. Gitler D., Takagishi Y., Feng J., et al. (2004) Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *J. Neurosci.* **24,** 11,368–11,380.
62. Yakel J. L. (1997) Calcineurin regulation of synaptic function: from ion channels to transmitter release and gene transcription. *Trends Pharmacol. Sci.* **18,** 124–134.
63. Numakawa T., Yagasaki Y., Ishimoto T., et al. (2004) Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. *Hum. Mol. Genet.* **13,** 2699–2708.
64. Hinds H. L., Goussakov I., Nakazawa K., Tonegawa S., and Bolshakov V. Y. (2003) Essential function of alpha-calcium/calmodulin-dependent protein kinase II in neurotransmitter release at a glutamatergic central synapse. *Proc. Natl. Acad. Sci. USA* **100,** 4275–4280.
65. Ninan I. and Arancio O. (2004) Presynaptic CaMKII is necessary for synaptic plasticity in cultured hippocampal neurons. *Neuron* **42,** 129–141.
66. Benson D. L. and Tanaka H. (1998) N-cadherin redistribution during synaptogenesis in hippocampal neurons. *J. Neurosci.* **18,** 6892–6904.
67. Song J. Y., Ichtchenko K., Sudhof T. C., and Brose N. (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc. Natl. Acad. Sci. USA* **96,** 1100–1105.
68. Hwang S. J., Pagliardini S., Boukhelifa M., et al. (2001) Palladin is expressed preferentially in excitatory terminals in the rat central nervous system. *J. Comp. Neurol.* **436,** 211–224.
69. Graf E. R., Zhang X., Jin S. X., Linhoff M. W., and Craig A. M. (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell* **119,** 1013–1026.
70. Fremeau R. T. Jr., Troyer M. D., Pahner I., et al. (2001) The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron* **31,** 247–260.
71. Bellocchio E. E., Reimer R. J., Fremeau R. T. Jr., and Edwards R. H. (2000) Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* **289,** 957–960.
72. Daniels R. W., Collins C. A., Gelfand M. V., et al. (2004) Increased expression of the Drosophila

vesicular glutamate transporter leads to excess glutamate release and a compensatory decrease in quantal content. *J. Neurosci.* **24,** 10,466–10,474.
73. Wojcik S. M., Rhee J. S., Herzog E., et al. (2004) An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proc. Natl. Acad. Sci. USA* **101,** 7158–7163.
74. Connors B. W. and Gutnick M. J. (1990) Intrinsic firing patterns of diverse neocortical neurons. *Trends Neurosci.* **13,** 99–104.
75. Song W. J. (2002) Genes responsible for native depolarization-activated K+ currents in neurons. *Neurosci. Res.* **42,** 7–14.
76. Malin S. A. and Nerbonne J. M. (2000) Elimination of the fast transient in superior cervical ganglion neurons with expression of KV4.2W362F: molecular dissection of IA. *J. Neurosci.* **20,** 5191–5199.
77. Shibata R., Nakahira K., Shibasaki K., Wakazono Y., Imoto K., and Ikenaka K. (2000) A-type K+ current mediated by the Kv4 channel regulates the generation of action potential in developing cerebellar granule cells. *J. Neurosci.* **20,** 4145–4155.
78. Martina M., Schultz J. H., Ehmke H., Monyer H., and Jonas P. (1998) Functional and molecular differences between voltage-gated K+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. *J. Neurosci.* **18,** 8111–8125.
79. Serodio P. and Rudy B. (1998) Differential expression of Kv4 K+ channel subunits mediating subthreshold transient K+ (A-type) currents in rat brain. *J. Neurophysiol.* **79,** 1081–1091.
80. Hoffman D. A., Magee J. C., Colbert C. M., and Johnston D. (1997) K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* **387,** 869–875.
81. Colbert C. M. and Pan E. (2002) Ion channel properties underlying axonal action potential initiation in pyramidal neurons. *Nat. Neurosci.* **5,** 533–538.
82. Frick A., Magee J., Koester H. J., Migliore M., and Johnston D. (2003) Normalization of Ca2+ signals by small oblique dendrites of CA1 pyramidal neurons. *J. Neurosci.* **23,** 3243–3250.
83. Rhodes K. J., Carroll K. I., Sung M. A., et al. (2004) KChIPs and Kv4 alpha subunits as integral components of A-type potassium channels in mammalian brain. *J. Neurosci.* **24,** 7903–7915.
84. Lien C. C. and Jonas P. (2003) Kv3 potassium conductance is necessary and kinetically optimized for high-frequency action potential generation in hippocampal interneurons. *J. Neurosci.* **23,** 2058–2068.
85. Rudy B., Chow A., Lau D., et al. (1999) Contributions of Kv3 channels to neuronal excitability. *Ann. NY Acad. Sci.* **868,** 304–343.
86. Lien C. C., Martina M., Schultz J. H., Ehmke H., and Jonas P. (2002) Gating, modulation and subunit composition of voltage-gated K(+) channels in dendritic inhibitory interneurones of rat hippocampus. *J. Physiol. (Lond.)* **538,** 405–419.
87. Magistretti J. and Alonso A. (1999) Slow voltage-dependent inactivation of a sustained sodium current in stellate cells of rat entorhinal cortex layer II. *Ann. NY Acad. Sci.* **868,** 84–87.
88. Agrawal N., Hamam B. N., Magistretti J., Alonso A., and Ragsdale D. S. (2001) Persistent sodium channel activity mediates subthreshold membrane potential oscillations and low-threshold spikes in rat entorhinal cortex layer V neurons. *Neuroscience* **102,** 53–64.
89. Burgess D. L., Kohrman D. C., Galt J., et al. (1995) Mutation of a new sodium channel gene, Scn8a, in the mouse mutant 'motor endplate disease'. *Nat. Genet.* **10,** 461–465.
90. Kohrman D. C., Smith M. R., Goldin A. L., Harris J., and Meisler M. H. (1996) A missense mutation in the sodium channel Scn8a is responsible for cerebellar ataxia in the mouse mutant jolting. *J. Neurosci.* **16,** 5993–5999.
91. Raman I. M. and Bean B. P. (1997) Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. *J. Neurosci.* **17,** 4517–4526.
92. Grieco T. M., Malhotra J. D., Chen C., Isom L. L., and Raman I. M. (2005) Open-channel block by the cytoplasmic tail of sodium channel beta4 as a mechanism for resurgent sodium current. *Neuron* **45,** 233–244.
93. Raman I. M. and Bean B. P. (2001) Inactivation and recovery of sodium currents in cerebellar Purkinje neurons: evidence for two mechanisms. *Biophys. J.* **80,** 729–737.
94. Afshari F. S., Ptak K., Khaliq Z. M., et al. (2004) Resurgent Na currents in four classes of neurons of the cerebellum. *J. Neurophysiol.* **92,** 2831–2843.
95. Do M. T. and Bean B. P. (2004) Sodium currents in subthalamic nucleus neurons from Nav1.6-null mice. *J. Neurophysiol.* **92,** 726–733.
96. Debanne D. (2004) Information processing in the axon. *Nat. Rev. Neurosci.* **5,** 304–316.

97. Debanne D., Guerineau N. C., Gahwiler B. H., and Thompson S. M. (1997) Action potential propagation gated by an IA-like K+ conductance in hippocampus. *Nature* **389,** 286–289.
98. Soleng A. F., Chiu K., and Raastad M. (2003) Unmyelinated axons in the rat hippocampus hyperpolarize and activate an H current when spike frequency exceeds 1 Hz. *J. Physiol. (Lond.)* **552,** 459–470.
99. Somogyi P. and Klausberger T. (2005) Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J. Physiol. (Lond.)* **562,** 9–26.
100. Malenka R. C., Kocsis J. D., Ransom B. R., and Waxman S. G. (1981) Modulation of parallel fiber excitability by postsynaptically mediated changes in extracellular potassium. *Science* **214,** 339–341.
101. Poolos N. P., Mauk M. D., and Kocsis J. D. (1987) Activity-evoked increases in extracellular potassium modulate presynaptic excitability in the CA1 region of the hippocampus. *J. Neurophysiol.* **58,** 404–416.
102. Mackenzie P. J. and Murphy T. H. (1998) High safety factor for action potential conduction along axons but not dendrites of cultured hippocampal and cortical neurons. *J. Neurophysiol.* **80,** 2089–2101.
103. Cox C. L., Denk W., Tank D. W., and Svoboda K. (2000) Action potentials reliably invade axonal arbors of rat neocortical neurons. *Proc. Natl. Acad. Sci. USA* **97,** 9724–9728.
104. Raastad M. and Shepherd G. (2003) Single-axon action potentials in the rat hippocampal cortex. *J. Physiol. (Lond.)* **548,** 745–752.
105. Bielefeldt K. and Jackson M. B. (1993) A calcium-activated potassium channel causes frequency-dependent action-potential failures in a mammalian nerve terminal. *J. Neurophysiol.* **70,** 284–298.
106. Hu H., Shao L. R., Chavoshy S., et al. (2001) Presynaptic Ca2+-activated K+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J. Neurosci.* **21,** 9585–9597.
107. Meeks J. P., Jiang X., and Mennerick S. (2005) Action potential fidelity during normal and epileptiform activity in paired soma/axon recordings from rat hippocampus. *J. Physiol. (Lond.),* **566,** 425–441.
108. Khaliq Z. M. and Raman I. M. (2005) Axonal propagation of simple and complex spikes in cerebellar Purkinje neurons. *J. Neurosci.* **25,** 454–463.
109. Monsivais P., Clark B. A., Roth A., and Hausser M. (2005) Determinants of action potential propagation in cerebellar Purkinje cell axons. *J. Neurosci.* **25,** 464–72.
110. Ishizuka N., Weber J., and Amaral D. G. (1990) Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J. Comp. Neurol.* **295,** 580–623.
111. Freund T. F. and Buzsaki G. (1996) Interneurons of the hippocampus. *Hippocampus* **6,** 347–470.
112. Grossman Y., Parnas I., and Spira M. E. (1979) Differential conduction block in branches of a bifurcating axon. *J. Physiol. (Lond.)* **295,** 283–305.
113. Stoney S. D. Jr. (1990) Limitations on impulse conduction at the branch point of afferent axons in frog dorsal root ganglion. *Exp. Brain Res.* **80,** 512–524.
114. Luscher H. R. and Shiner J. S. (1990) Computation of action potential propagation and presynaptic bouton activation in terminal arborizations of different geometries. *Biophys. J.* **58,** 1377–1388.
115. Engel D. and Jonas P. (2005) Presynaptic action potential amplification by voltage-gated Na+ channels in hippocampal mossy fiber boutons. *Neuron* **45,** 405–417.
116. Leao R. M., Kushmerick C., Pinaud R., et al. (2005) Presynaptic Na+ channels: locus, development, and recovery from inactivation at a high-fidelity synapse. *J. Neurosci.* **25,** 3724–3738.
117. Yoon K. W. and Rothman S. M. (1991) Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. *J. Neurosci.* **11,** 1375–1380.
118. Moore K. A., Nicoll R. A., and Schmitz D. (2003) Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. *Proc. Natl. Acad. Sci. USA* **100,** 14,397–14,402.
119. Kimura M., Saitoh N., and Takahashi T. (2003) Adenosine A(1) receptor-mediated presynaptic inhibition at the calyx of Held of immature rats. *J. Physiol. (Lond.)* **553,** 415–426.
120. Wilson R. I., Kunos G., and Nicoll R. A. (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron* **31,** 453–462.
121. Lei S. and McBain C. J. (2003) GABA B receptor modulation of excitatory and inhibitory synaptic transmission onto rat CA3 hippocampal interneurons. *J. Physiol. (Lond.)* **546,** 439–453.

122. Rozov A., Burnashev N., Sakmann B., and Neher E. (2001) Transmitter release modulation by intracellular Ca2+ buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. *J. Physiol. (Lond.)* **531,** 807–826.
123. Felmy F. and Schneggenburger R. (2004) Developmental expression of the Ca2+-binding proteins calretinin and parvalbumin at the calyx of held of rats and mice. *Eur. J. Neurosci.* **20,** 1473–1482.
124. Helmchen F., Borst J. G. G., and Sakmann B. (1997) Calcium dynamics associated with a single action potential in a CNS presynaptic terminal. *Biophys. J.* **72,** 1458–1471.
125. Borst J. G. and Sakmann B. (1998) Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. *J. Physiol. (Lond.)* **506,** 143–157.
126. Wheeler D. B., Randall A., and Tsien R. W. (1996) Changes in action potential duration alter reliance of excitatory synaptic transmission on multiple types of Ca2+ channels in rat hippocampus. *J. Neurosci.* **16,** 2226–2237.
127. Sabatini B. L. and Regehr W. G. (1997) Control of neurotransmitter release by presynaptic waveform at the granule cell to Purkinje cell synapse. *J. Neurosci.* **17,** 3425–3435.
128. Qian J. and Saggau P. (1999) Modulation of transmitter release by action potential duration at the hippocampal CA3-CA1 synapse. *J. Neurophysiol.* **81,** 288–298.
129. Geiger J. R. and Jonas P. (2000) Dynamic control of presynaptic Ca(2+) inflow by fast-inactivating K(+) channels in hippocampal mossy fiber boutons. *Neuron* **28,** 927–939.
130. Obermair G. J., Kaufmann W. A., Knaus H. G., and Flucher B. E. (2003) The small conductance Ca2+-activated K+ channel SK3 is localized in nerve terminals of excitatory synapses of cultured mouse hippocampal neurons. *Eur. J. Neurosci.* **17,** 721–731.
131. Raffaelli G., Saviane C., Mohajerani M. H., Pedarzani P., and Cherubini E. (2004) BK potassium channels control transmitter release at CA3-CA3 synapses in the rat hippocampus. *J. Physiol. (Lond.)* **557,** 147–157.
132. Rusakov D. A. and Fine A. (2003) Extracellular Ca2+ depletion contributes to fast activity-dependent modulation of synaptic transmission in the brain. *Neuron* **37,** 287–297.
133. Xu J. and Wu L. G. (2005) The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron* **46,** 633–645.
134. Moulder K. L. and Mennerick S. (2005) Reluctant vesicles contribute to the total readily releasable pool in glutamatergic hippocampal neurons. *J. Neurosci.* **25,** 3842–3850.
135. Wu L. G. and Borst J. G. (1999) The reduced release probability of releasable vesicles during recovery from short-term synaptic depression. *Neuron* **23,** 821–832.
136. Schneggenburger R., Meyer A. C., and Neher E. (1999) Released fraction and total size of a pool of immediately available transmitter quanta at a calyx synapse. *Neuron* **23,** 399–409.
137. Schneggenburger R., Sakaba T., and Neher E. (2002) Vesicle pools and short-term synaptic depression: lessons from a large synapse. *Trends Neurosci.* **25,** 206–212.
138. Esclapez M., Hirsch J. C., Ben-Ari Y., and Bernard C. (1999) Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J. Comp. Neurol.* **408,** 449–460.
139. Hsu M. and Buzsaki G. (1993) Vulnerability of mossy fiber targets in the rat hippocampus to forebrain ischemia. *J. Neurosci.* **13,** 3964–3979.
140. Cossart R., Dinocourt C., Hirsch J. C., et al. (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat. Neurosci.* **4,** 52–62.
141. Korn S. J., Giacchino J. L., Chamberlin N. L., and Dingledine R. (1987) Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *J. Neurophysiol.* **57,** 325–340.
142. Rossi D. J., Oshima T., and Attwell D. (2000) Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* **403,** 316–321.