Network bursts in hippocampal microcultures are terminated by exhaustion of vesicle pools

Dror Cohen and Menahem Segal
Department of Neurobiology, The Weizmann Institute, Rehovot, Israel

Submitted 8 November 2010; accepted in final form 4 August 2011

Network bursts in hippocampal microcultures are terminated by exhaustion of vesicle pools. J Neurophysiol 106: 2314–2321, 2011. First published August 10, 2011; doi:10.1152/jn.00969.2010.—Synchronized network activity is an essential attribute of the brain. Yet the cellular mechanisms that determine the duration of network bursts are not fully understood. In the present study, synchronized network bursts were evoked by triggering an action potential in a single neuron in otherwise silent microcultures consisting of 4–30 hippocampal neurons. The evoked burst duration, ~2 s, depended on the recovery time after a previous burst. While interburst intervals of 35 s enabled full-length bursts, they were shortened by half at 5-s intervals. This reduction in burst duration could not be attributed to postsynaptic parameters such as glutamate receptor desensitization, accumulating afterhyperpolarization, inhibitory tone, or sodium channel inactivation. Reducing extracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_o$) relieved the effect of short intervals on burst duration, while depletion of synaptic vesicles with α-latrotoxin gradually eliminated network bursts. Finally, a transient exposure to high [K$^+$], slowed down the recovery time following a burst discharge. We conclude that the limiting factor regulating burst duration is most likely the depletion of presynaptic resources.

Synchronized network activity has been described in nearly every brain structure and has been associated with diverse functions of the network, including storage and transfer of memory (Fuster and Alexander 1971). Different synaptic and membrane properties contribute to the shaping of synchronized network bursts (Bacci et al. 1999; Cohen and Segal 2009). Yet the cellular properties that determine the duration of a network burst are still to be resolved. The importance of identifying the factors that limit network bursts is emphasized in some pathological states such as reading epilepsy where network activity related to the cognitive task spreads to other nonrelevant areas to evoke a seizure (Archer et al. 2003).

Emerging evidence suggests that the termination of network activity may depend on the combination of release probability and replenishment of readily releasable vesicle pools (Jones et al. 2007; Staley et al. 1998). Yet additional factors likely to delineate burst duration include strong transient inhibitory inputs sufficient to briefly clamp membrane potential and block firing (Bush and Sejnowski 1994), postsynaptic glutamate receptor desensitization (Arai and Lynch 1998), strong afterhyperpolarization (AHP) (Darbon et al. 2002), and sodium channel inactivation (Basarsky and French 1991).

Importantly, these mechanisms also predict a refractory period immediately after burst termination in which additional activation of the network should be smaller in magnitude or duration. Indeed, hippocampal neurons demonstrate an inverse correlation between spontaneous burst frequency and burst duration (Cohen et al. 2008). Yet identifying a causal relationship between burst frequency and duration requires controlling burst initiation so that bursts are ignited with identical stimuli on an otherwise quiet background.

Hippocampal neurons grown in microcultures offer an ideal experimental system for the investigation of burst duration, since they can generate network bursts in response to an action potential triggered in one member of the network (Lau and Bi 2005).

We investigated the termination of network bursts evoked by a single action potential in otherwise silent microcultures of rat hippocampal neurons. In these cultures the evoked burst duration depended on the recovery period from a previous burst. The reduction in burst duration after short interburst intervals was not due to glutamate receptor desensitization, strong AHP, or sodium channel inactivation. Depletion of synaptic vesicles with α-latrotoxin gradually suppressed network bursts. Reduced extracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_o$) relieved the priming effect of previous network activity on burst duration. We conclude that the limiting factor of ongoing network activity is most likely the availability of presynaptic pools of synaptic vesicles.

METHODS

 Cultures. Animal handling was done in accordance with protocols reviewed and approved by the Institutional Animal Care and Use Committee of the Weizmann Institute and in accordance with Israeli national guidelines on animal care. Cultures were prepared as detailed previously (Bekkers and Stevens 1991; Papa et al. 1995). Briefly, Wistar rats were decapitated on embryonic day 19, and their brains were removed and placed in a chilled (4°C), oxygenated Leibovitz L15 medium (GIBCO, Paisley, UK) enriched with 0.6% glucose and gentamicin (20 μg/ml; Sigma, St. Louis, MO). Hippocampal tissue was mechanically dissociated and passed to the plating medium consisting of 5% heat-inactivated horse serum (GIBCO), 5% fetal calf serum, and B-27 (1 μl/1 ml; GIBCO) prepared in minimum essential medium (MEM) Earle salts (GIBCO), enriched with 0.6% glucose, gentamicin (20 μg/ml), and 2 mM GlutaMAX (enriched MEM). Dissociated neurons, diluted to 5–7 × 10$^4$ cells/ml, were plated on agarose-covered glass coverslips sprayed with fine droplets (150–700 μm in diameter) of substrate solution containing rat tail collagen (BD Biosciences) at 1 mg/ml and poly(β-lysine) (Sigma) at 0.3 mg/ml. Twenty-four hours after plating, coverslips were inverted just above a hippocampal glial feeder layer that had been grown on a 24-well plate for 2 wk prior to the plating of the neurons. Cells were left to grow in the incubator at 37°C and 5% CO$_2$ for 7 days, at which time the medium was changed to 10% horse serum in enriched MEM plus a mixture of 5′-fluoro-2-deoxyuridine and uridine (20 μg and 50 μg/ml, respectively).
respectively; Sigma) to block glial proliferation. Three days later, the medium was replaced by 10% horse serum in enriched MEM supplemented with B-27 (1 μl/ml; Gibco). Thirteen- to eighteen-day-old cultures were transferred to a recording chamber heated to 35°C and superfused with standard recording medium [containing (in mM) 10 HEPES, 4 KCl, 2 CaCl₂, 1 MgCl₂, 139 NaCl, and 10 d-glucose, adjusted with sucrose to an osmolality of 340 mosM and with NaOH to a pH of 7.4].

**Electrophysiology.** Neurons were recorded with patch pipettes containing (in mM) 136 K-gluconate, 10 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 0.3 Na-GTP, 1 Mg-ATP, and 5 phosphocreatine, pH 7.2 (with a resistance of 5–8 MΩ). Biocytin (0.4%) was added to the pipette solution to visualize the patched neurons. Bursts were evoked either by injecting 5-ms depolarizing current pulses sufficient to evoke a single action potential in one neuron in current-clamp mode or by a 5-ms depolarization to 0 mV in voltage-clamp mode. Network burst duration was defined as the time from the onset of the evoked action potential generating the burst to the time point when the falling phase of the polysynaptic current (PSC) crossed the threshold (at half of the averaged PSC amplitude) with no additional threshold crossing in the following 500 ms. To investigate the dependence of the evoked bursts on prolonged activation of voltage-gated channels in the triggered neuron, cells were hyperpolarized to −100 mV immediately after the 5-ms depolarization to 0 mV. To differentiate between inhibitory PSCs (IPSCs) and excitatory PSCs (EPSCs), cells were clamped at −35 mV immediately after the 5-ms depolarization to 0 mV. Excitatory synaptic currents were blocked with the glutamate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM). Continuous release of vesicles from presynaptic terminals was induced by α-latrotoxin (0.07 nM). Drugs were added to the slowly superfusing medium. To investigate the effects of a massive depletion of transmitter from presynaptic terminals, 30 mM K+ was locally administered by a 30-s pressure application through a patch pipette.

Signals were amplified with a Multiclamp700B amplifier and recorded with Clampex 9.2 software (Axon Instruments, Union City, CA). The electrophysiological data were subjected to a 600-Hz low-pass filter and analyzed off-line with Clampfit-9. Spontaneous IPSCs (sIPSCs) were analyzed off-line with Mini-Analysis software (Synapsoft, Decatur, GA) with a detection threshold of 10 pA. IPSCs were blocked by the glutamate receptor antagonist DNQX (10 μM; Fig. 1, E and F), which did not affect the single spike evoked by the depolarization, demonstrating the dependence of the evoked burst on synaptic currents. The evoked bursts overcame a hyperpolarizing step to −100 mV immediately after the first spike (Fig. 1G), suggesting that evoked action potential triggered a network reverberation that was no longer dependent on the initially activated neuron.

**Results.** Hippocampal neurons plated on small permissive islands can grow and form small-scale (4–30 neurons) networks. The dendrites and axons were also confined within the poly(d-lysine) droplet, as demonstrated both in phase microscopy and after a cell was filled with biocytin (Fig. 1, A and B). The few cases in which axons escaped to neighboring islands were excluded from the analysis. The majority of the cultures (56 of 82 in 4 different preparations) were not active spontaneously under the conditions tested. However, these same silent networks displayed a synchronized burst, encompassing the entire neuronal population within each island, after a single action potential evoked in one neuron (Fig. 1, C and D). These bursts were blocked by the glutamate receptor antagonist DNQX (10 μM; Fig. 1, E and F), which did not affect the single spike evoked by the depolarization, demonstrating the dependence of the evoked burst on synaptic currents. The evoked bursts overcame a hyperpolarizing step to −100 mV immediately after the first spike (Fig. 1G), suggesting that evoked action potential triggered a network reverberation that was no longer dependent on the initially activated neuron.

Previous work in our lab has demonstrated an inverse correlation between burst frequency and burst duration in spontaneously active large-scale neuronal networks (Cohen et al. 2008). We took advantage of the fact that most microcultures were not active spontaneously to study the refractory period after an evoked burst discharge. Figure 2A demonstrates bursts that were generated by a single action potential evoked alternately at 5- and 35-s intervals, illustrating a reduction in burst duration when they are generated at short intervals after a priming burst. In extreme cases the action potential evoked after a 5-s interval failed to generate a network burst (Fig. 2B). Bursts evoked after a 5-s interval showed a 56% (P < 0.001) reduction in duration compared with bursts evoked after 35 s (Fig. 2C). While a 35-s interval was sufficient for full recovery, as can be seen from the ratio of consecutive bursts (Fig. 2D), the ratio between consecutive bursts evoked after 5- and 35-s intervals, respectively, was 0.46 (P < 0.001).

The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.

To investigate the role of synaptic inhibition in burst termination, we first investigated the contribution of inhibitory neurons to the expression of spontaneous network bursts. After spontaneous activity was recorded, microcultures were subjected to immunostaining for glutamic acid decarboxylase (GAD), the enzyme marker for inhibitory neurons. Figure 3A presents a microculture in which 5 of 15 neurons were clearly GAD, the enzyme marker for inhibitory neurons. This culture is presented in Fig. 3A. The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.

To investigate the role of synaptic inhibition in burst termination, we first investigated the contribution of inhibitory neurons to the expression of spontaneous network bursts. After spontaneous activity was recorded, microcultures were subjected to immunostaining for glutamic acid decarboxylase (GAD), the enzyme marker for inhibitory neurons. Figure 3A presents a microculture in which 5 of 15 neurons were clearly GAD, the enzyme marker for inhibitory neurons. This culture is presented in Fig. 3A. The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.

To investigate the role of synaptic inhibition in burst termination, we first investigated the contribution of inhibitory neurons to the expression of spontaneous network bursts. After spontaneous activity was recorded, microcultures were subjected to immunostaining for glutamic acid decarboxylase (GAD), the enzyme marker for inhibitory neurons. Figure 3A presents a microculture in which 5 of 15 neurons were clearly GAD, the enzyme marker for inhibitory neurons. This culture is presented in Fig. 3A. The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.

To investigate the role of synaptic inhibition in burst termination, we first investigated the contribution of inhibitory neurons to the expression of spontaneous network bursts. After spontaneous activity was recorded, microcultures were subjected to immunostaining for glutamic acid decarboxylase (GAD), the enzyme marker for inhibitory neurons. Figure 3A presents a microculture in which 5 of 15 neurons were clearly GAD, the enzyme marker for inhibitory neurons. This culture is presented in Fig. 3A. The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.

To investigate the role of synaptic inhibition in burst termination, we first investigated the contribution of inhibitory neurons to the expression of spontaneous network bursts. After spontaneous activity was recorded, microcultures were subjected to immunostaining for glutamic acid decarboxylase (GAD), the enzyme marker for inhibitory neurons. Figure 3A presents a microculture in which 5 of 15 neurons were clearly GAD, the enzyme marker for inhibitory neurons. This culture is presented in Fig. 3A. The slow refractoriness of network bursts can result from either post- or presynaptic factors. We examined which of these factors can underlie the network refractoriness in the following experiments.
Neurons were voltage clamped at −35 mV immediately after burst generation to differentiate between the inward-going EPSC and the outward-going IPSC (Fig. 3I). Both IPSCs and EPSCs were detected throughout the burst, suggesting that the termination of the burst did not result from increased inhibitory drive at the burst end. Also, similar burst durations were recorded when GABA<sub>α</sub> receptors were blocked with gabazine (20 μM; Fig. 3, G–K), further suggesting that GABA does not regulate burst duration or refractoriness in this system.

Changes in intrinsic properties were examined in current-clamped neurons. The action potentials eliciting network bursts after the different recovery periods were not significantly different (Fig. 4A). Accordingly, action potential thresholds (Fig. 4B) and durations (Fig. 4D) were similar, while action potential amplitudes were slightly but significantly reduced by 1.2 mV (<i>P</i> < 0.001; Fig. 4C) after shorter (5 s) recovery intervals. Examination of the second and last action potentials during 35-s bursts revealed no difference in these parameters.
Membrane potentials were hyperpolarized by are generated in shorter intervals. Burst durations at 35-s and 5-s intervals, demonstrating a reduction in burst duration when the bursts each generated with a single action potential evoked alternately at 5-s interval: burst durations at 35-s and 5-s intervals. C: ratio of burst durations between a burst generated at a 35-s interval and the previous 35-s interval burst, demonstrating full recovery of burst duration after a 35-s interval, compared with the ratio of a burst generated at a 5-s interval and the previous 35-s interval burst. \( *P < 0.05 \).

(datum not shown). Membrane potentials were hyperpolarized by 1.6 mV \( (P < 0.018) \) after the priming of full-blown burst (Fig. 4E). However, membrane conductances measured just before the two evoked action potentials were similar (Fig. 4F). Thus the slow refractoriness of burst discharge is not likely to be explained by a change in either postsynaptic potassium or sodium conductances.

To examine the likelihood that a postsynaptic receptor desensitization underlies the reduction in burst duration after short recovery, we analyzed sEPSCs recorded immediately prior to and after a burst. Figure 4G presents an evoked burst recorded in voltage-clamp mode. The sEPSCs recorded just before and immediately after this burst are presented in Fig. 4, H and I, respectively. Figure 4J demonstrates similar-amplitude sEPSCs before and after a burst. The duration and decay rate of the sEPSCs also were not different between the groups (paired comparisons, data not shown). We found a slight tendency toward an increase in the frequency of sEPSCs immediately after a burst \( (P < 0.107, \text{paired } t\text{-test}; \text{Fig. 4J}) \) that may be accounted for by an increase in residual calcium in presynaptic terminals following a burst. Taken together, these results rule out postsynaptic receptor properties as playing a significant role in burst termination.

We then examined the possible involvement of presynaptic factors in determining burst duration. Initially, \( \alpha \)-latrotoxin \( (0.07 \text{ nM}) \), known to induce the release of vesicles from presynaptic terminals via both calcium-dependent and -independent mechanisms (Deak et al. 2009), was slowly infused onto the neurons in the culture (Fig. 5A). Shortly afterward, the otherwise silent culture began to discharge prolonged bursts (Fig. 5B). However, with a longer exposure to the drug, the prolonged bursts were gradually reduced in duration to be substituted by large EPSCs (Fig. 5C). Before the treatment, a single action potential could trigger a burst (Fig. 5D). However, after the prolonged exposure to \( \alpha \)-latrotoxin the same stimulus could no longer ignite a burst (Fig. 5E). These results suggest that after a massive release of synaptic vesicles the network cannot sustain bursting, even though the cells do produce random EPSCs.

We then examined the role of \( [\text{Ca}^{2+}]_o \), in the refractory period after a burst discharge. Figure 5F presents the reduction in burst duration after a short recovery interval at normal divalent cation concentrations \( (2 \text{ mM Ca}^{2+}, 1 \text{ mM Mg}^{2+}) \). When the normal divalent cation concentrations were replaced by \( 1 \text{ mM Ca}^{2+} \) and \( 2 \text{ mM Mg}^{2+} \) (Fig. 5G), the same neuron discharged bursts of similar durations after both short and long recovery intervals. Figure 5H illustrates that the duration of the first burst (following 35-s recovery) was reduced by 56% \( (P < 0.033) \) after introduction of the low-calcium medium, but not the duration of the second burst (following 5-s recovery). The ratio between the second burst and the first burst shifted from 0.49 in the normal recording medium to 0.9 \( (P < 0.005) \) in the low-calcium medium. The AHPs measured immediately after the bursts in the two media were similar (data not shown), suggesting that the reduced calcium concentration did not affect the slow refractoriness by modulating the AHP. These results suggest that when the release of vesicles during the first burst is reduced, because of the reduced calcium influx to presynaptic terminals, the effect of the first burst on the duration of the second burst is dramatically reduced.

A long-lasting (>1 min) depression of synaptic vesicles exocytosis was reported by Garcia-Perez et al. following exhaustive use of excitatory hippocampal synapses (Garcia-Perez et al. 2008). This prolonged synaptic depression, termed "supply rate depression," is attributed to a reduction in the rate of vesicles replenishment in addition to the canonical depletion of the ready releasable pool (RRP). We investigated the effects of extensive synaptic transmission on evoked network bursts (Fig. 6A) by locally applying the recording medium supplemented with 30 mM K+ for 30 s (Fig. 6B). In most cases \((4 \text{ of } 7)\), bursts were absent after a 33-s recovery from the treatment (Fig. 6C). Bursts demonstrated a 70% recovery 6 min after the treatment \( (1 \text{st burst } P < 0.03, \text{ 2nd burst } P < 0.02, \text{ paired } t\text{-test}; \text{Fig. 6, D–F}) \). The ratio between the two bursts was preserved after this recovery (data not shown). Similar 85% and 78% reductions in the duration of the first and second bursts, respectively, after 33 s of recovery was demonstrated when 20 \( \mu \text{M DNQX} \) and 50 \( \mu \text{M 2-amino-5-phosphonovaleric acid (APV)} \) were added to the locally applied medium, suggesting that the prolonged reduction in burst duration is not a result of a postsynaptic receptor mechanism (Fig. 6, E and F). A shorter, 2-s application of the high-potassium medium did not reduce the duration of the bursts (Fig. 6, E and F),
suggesting that such network burst attenuation takes place only after extensive network activity as suggested previously (Garcia-Perez et al. 2008). Thus microcultures expressed prolonged reduction in burst duration following extensive synaptic activity, in agreement with the "supply rate depression" hypothesis.

**DISCUSSION**

In the present study we investigated the possible factors determining ongoing network bursts in microcultures of hippocampal neurons is most likely determined by the availability of synaptic vesicles.

**Fig. 3.** The minor role of synaptic inhibition in terminating bursts in small-scale networks. A: immunostaining for NeuN (red) and GAD (green, marked as yellow when colocalized with NeuN) illustrates a culture that contains 5 inhibitory neurons in a total of 15 cells. B: calcium imaging of the spontaneous activity of the culture presented in A. C: microculture containing 8 glutamic acid decarboxylase (GAD)-negative neurons. D: spontaneous activity of the culture presented in C, displaying activity patterns similar to those seen in B. E: lack of correlation between % of inhibitory neurons and number of bursts recorded over 2 min. F: lack of correlation between % of inhibitory neurons and burst duration. G: current-clamp recording of evoked bursts when the neuron was held at −60 mV before gabazine. H: the neuron recorded in G in current-clamp mode held at −60 mV after 20 μM gabazine application. I: the neuron recorded in G in voltage-clamp mode held at −35 mV immediately after spike generation. J: the neuron recorded in G in voltage-clamp mode held at −35 mV immediately after spike generation after gabazine administration. K: burst durations before and after gabazine. *P < 0.05.

**Fig. 4.** The reduction in burst duration could not be accounted for by an accumulating afterhyperpolarization (AHP), sodium channel inactivation, or receptor desensitization. A: the evoked action potentials generating the 1st and 2nd bursts presented in Fig. 2A had similar properties (threshold, rise time, duration) after short and long recovery periods.

To conclude, burst duration in microcultures of hippocampal neurons is most likely determined by the availability of synaptic vesicles.
hippocampal neurons. We demonstrate a long refractory period after a burst discharge and propose that this is due to depletion of neurotransmitters from presynaptic terminals.

The effect of past activity on burst termination could not be explained by strong AHP or inactivation of voltage-gated sodium channels, as similar membrane properties were measured following either short (5 s) or long (35 s) recovery intervals. Blocking GABAA receptors did not induce a significant increase in burst durations, ruling out inhibition as a limiting mechanism of ongoing network bursts. Receptor desensitization was also ruled out as a possible factor as the sEPSCs measured just prior to and immediately after a network burst were similar in amplitude. In contrast, the depletion of synaptic vesicles produced by the application of α-latrotoxin diminished the expression of evoked network bursts. When the release of vesicles during bursts was reduced, because of reduced calcium influx to presynaptic terminals, the effect of shorter recovery periods on burst duration was relieved. These results suggest that the exhaustion of presynaptic resources dictates burst duration.

A dependence of burst length on the time interval after previous bursts was reported in CA3 neurons in a slice preparation (Staley et al. 1998). However, in that experiment network bursts were not identical in size and duration as they discharged spontaneously by the elevation of extracellular potassium. Here we have recapitulated this observation by having bursts ignited by a single action potential triggering the same set of synapses.

Anatomical and electrophysiological estimations of RRRPs in hippocampal neurons are in the order of 1–15 vesicles (Schikorski and Stevens 1997; Stevens and Tsujimoto 1995). Exhausted active zones are assumed to refill within 10 s (Stevens and Tsujimoto 1995). In accordance with these estimations, we have found full recovery of burst duration following a 35-s interval, while a 5-s recovery did enable burst discharges, but these were shortened by half. Therefore, the causal relationship between the recovery interval and burst duration is only valid at a time window needed for the replenishment of glutamate vesicles, <30 s after a previous burst.

The spontaneous release of synaptic vesicles is mediated primarily by Ca2+-dependent mechanisms (Xu et al. 2009) and correlated with calcium transients in presynaptic terminals (Emptage et al. 2001). As sEPSCs undergo depression during repetitive stimulations because of the depletion of presynaptic vesicles, calcium concentration is built up in presynaptic terminals to increase asynchronous release (Hagler and Goda 2001) as well as sEPSC frequency (Cummings et al. 1996). In the present study the sEPSC recorded before and after the evoked bursts have averaged amplitudes...
similar to those of miniature EPSCs (Cohen and Segal 2009), suggesting that they reflect the spontaneous release of single vesicles. In agreement, we do not find a reduction in the frequency of such EPSCs after evoked bursts, but rather a tendency toward an increase in spontaneous release frequency, suggesting that the depletion of presynaptic vesicles is balanced by the increased probability of spontaneous release due to residual calcium.

In their model, Volman et al. suggested that small networks’ reverberatory activity is maintained by enhanced asynchronous transmitter release from presynaptic terminals depending on residual presynaptic calcium (Volman et al. 2007). The suppression of burst discharges by α-latrotoxin further demonstrates the dependence of network bursts on presynaptic vesicle pools. This toxin induces synaptic vesicle exocytosis both in calcium-dependent mechanism, in which it most likely acts as a Ca\(^{2+}\) ionophore (Khvotchev and Sudhof 2000; Van Renterghem et al. 2000), and in calcium-independent mechanism, in which it most likely operates directly on the transmitter release machinery (Deak et al. 2009). The combination of both pathways leads to the depletion of presynaptic vesicle pools after prolonged treatments (Tzeng et al. 1978). The large EPSCs recorded immediately following α-latrotoxin, which reflect the synchronous release from several terminals, have reached threshold to evoke network bursts. However, as network activity persisted with not enough time to recuperate, and while presynaptic vesicle pools were gradually emptied by the ongoing treatment of the drug, the bursts were gradually reduced in duration and finally aborted. After the prolonged α-latrotoxin treatment the evoked action potentials could no longer generate network bursts, although large EPSCs could still be recorded. Thus the network cannot sustain reverberations when the vesicle pools are not sufficient for more then one threshold-passing synchronous EPSC, a situation in which spontaneous release of single vesicles is still not limited.

An extensive synaptic transmission that maintains the RRP in a nearly empty steady state induces a slowly recovering synaptic depression termed “supply rate depression” that is attributed to a reduction in the refill kinetics of the RRP (Garcia-Perez et al. 2008). The slow recovery of burst duration following high-K\(^+\) treatment is in agreement with the timescale of supply rate depression and further supports the notion that burst duration is dependent on the availability of synaptic vesicles.

Different presynaptic proteins regulate synaptic vesicle pool size and refill kinetics (Gitler et al. 2004; Toonen et al. 2006). Some of these genetic manipulations produce seizures (Rosalh et al. 1995). The investigation of burst termination and recovery described here can be further exploited to better understand the contribution of different proteins to both normal and abnormal neuronal network activity.

ACKNOWLEDGMENTS

We thank Efrat Biton and Dr. Daniel Gitler for help with the cultures.

GRANTS

This work was supported by a grant from the Israel Science Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
REFERENCES


