Complex Response to Afferent Excitatory Bursts by Nucleus Accumbens Medium Spiny Projection Neurons

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The nucleus accumbens (NAc) of the ventral striatum is involved in attention, motivation, movement, learning, reward, and addiction. GABAergic medium spiny projection neurons that make up ~90% of the neuronal population are commonly driven by convergent bursts of afferent excitation. We monitored spiny projection neurons in mouse striatal slices while applying stimulus trains to mimic bursts of excitation. A stimulus train evoked a simple, short-lived postsynaptic response from CA1 hippocampal pyramidal neurons, but the train evoked a complex series of excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) from the NAc spiny projection neurons. As is commonly seen with projection neurons, the EPSC amplitudes initially displayed facilitation followed by depression, and that pattern was sensitive to the extracellular calcium concentration. In addition, there were two other novel observations. The spiny projection neurons responded to the stimulus train with a prolonged depolarization that was accompanied by a posttrain increase of spontaneous glutamatergic synaptic activity. Blocking AMPA/kainate glutamate receptors strongly inhibited the evoked EPSP/EPSCs, the posttrain spontaneous synaptic activity, and the prolonged depolarization. A potassium channel inhibitor increased and extended the prolonged postsynaptic depolarization, causing a long-lasting depolarized plateau potential. Our results indicate that burst-like activity along ventral striatal afferents is extended in time by additional spontaneous glutamate release that is integrated by the postsynaptic spiny projection neurons into a prolonged depolarization that may contribute to the depolarized “up state” observed in vivo.

INTRODUCTION

The nucleus accumbens (NAc) is involved in reward, attention, motivation, movement, and learning (Ikemoto and Panksepp 1999; Pennartz et al. 1994), and its dysfunction contributes to conditions such as drug addiction, Parkinson’s disease, and schizophrenia (Cserransky and Bardgett 1998; O’Donnell and Grace 1998; Spanagel and Weiss 1999). The NAc receives massive glutamatergic afferent innervation from the cerebral cortex, thalamus, hippocampus, and amygdala (Groenwegen et al. 1980; Kelley and Domesick 1982; Sessa et al. 1989; Kita and Kitai 1990). It is common for the convergent excitatory afferent activity to arrive in high-frequency bursts that drive medium spiny projection neurons more strongly than single action potentials. The information conveyed by a single action potential or by a burst can be qualitatively different (Calabresi et al. 1999; see review, Lisman 1997). The synaptic release of neurotransmitter and the concomitant postsynaptic response are influenced by the previous action potentials in the burst. Furthermore, the neurotransmitter concentration profile in the synaptic cleft varies depending on the stimulation intensity. These factors help to shape the overall postsynaptic response and thus influence the integration of information received by a single neuron.

In vivo intracellular recordings have shown that dorsal striatal medium spiny projection neurons undergo shifts in their membrane potential (O’Donnell and Grace 1995; Wilson and Groves 1981; Wilson and Kawaguchi 1996). The potential alternates between the resting “down state” and the “up state,” which is sustained briefly at a consistent depolarized value. A maintained barrage of afferent synaptic excitation is necessary to produce the depolarized up state (Calabresi et al. 1990; Wilson and Groves 1981). In striatal medium spiny projection neurons, the response to a single stimulus has been well characterized (Kombian and Malenka 1994; O’Donnell and Grace 1995; Pennartz and Kitai 1991; Pennartz et al. 1991), but the consequences arising from bursts of stimuli are not well characterized. Furthermore, the response to excitatory inputs is less well studied in the NAc of the ventral striatum. Therefore we whole cell clamped NAc spiny projection neurons and activated glutamatergic afferents with stimulus trains that were meant to simulate burst-like afferent inputs. Stimulus trains produced a complex response that arose from both the presynaptic glutamatergic afferents and from the postsynaptic spiny projection neurons. The results indicate that these spiny projection neurons and their afferents are exceptionally able to integrate bursts in a way not observed in other projection neurons, such as the hippocampal CA1 pyramidal neurons. Although the individual components of the response arise from previously known mechanisms, taken together the components contribute critical properties underlying the up state of spiny projection neurons. It is from the up state that medium spiny projection neurons send their brief efferent bursts that serve as the only output from the striatum.

METHODS

Striatal and hippocampal brain slices were obtained from 14- to 21-day-old wild-type C57BL6 mice that were anesthetized with halothane before decapitation. Horizontal slices (250 μm) were prepared in ice-cold solution, placed in an incubator (for 30 min at 34°C), and continuously superfused with oxygenated solution containing (in mM) 124 NaCl, 3 KCl, 2 CaCl2, 8 MgCl2, 26 NaHCO3, 1.25 Na2HPO4, and 2.5 glucose. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
10 glucose (pH 7.4, 305 mosM). Recordings were made in the same solution except the MgCl₂ concentration was reduced to 1 mM, and the experiments were at room temperature. These methods were approved by the Center for Comparative Medicine at Baylor College of Medicine. Neurons in the NAc were visually identified by infrared video camera. Whole cell, patch-clamp recordings were performed using an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). Patch electrodes (3–5 MΩ) were filled with an internal solution containing (in mM) 130 K-glucurate, 4 KCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 7 phosphocreatine, and 0.3 GTP-Na (pH 7.2, 290 mosM). The series resistance was continuously monitored but was not compensated. Clampfit software (Axon Instruments) was used to generate the current and voltage pulses and to acquire the data. All potential values were corrected off-line for the liquid junction potential of 15 mV, estimated using the Clampex junction potential calculation tool (Axon Instruments). Compounds were applied (>5–10 min pre-equilibration) via the bathing solution continuously superfused at 5 ml/min, including D-2-amino-5-phosphovaleric acid (APV), 6-cyano-7-nitroquinoline-2,3-dione (CNQX), 4-aminopyridine (4-AP; all purchased from Sigma, St. Louis, MO), and tetrodotoxin (TTX; purchased from Alomone Labs, Jerusalem, Israel).

Synaptic currents were evoked locally by electrical stimuli delivered via a glass micropipette electrode placed in the NAc 200–300 μm from the recording electrode. The pipette electrode was filled with external solution, and the reference was painted silver on the outside tip of the electrode (see Ji et al. 2001). The tip diameter was typically 2–4 μm, and the stimulus intensities ranged from 20 to 100 μA with stimulus durations of 0.1–0.5 ms. The stimulus intensity was chosen to obtain reproducible, consistent synaptic responses (around half-maximal) without failures or spiking. The intensity was maintained constant throughout the recording period. The excitatory afferents stimulated in these experiments were not identified but were most likely a mixed set. Because the experimentally applied stimulus evoked synaptic current within the NAc simultaneously excites intrinsic and extrinsic fibers, this exact stimulus pattern is unlikely to arise in vivo. However, the experimental situation does serve to reveal synaptic mechanisms that may contribute to in vivo synaptic events. The peak amplitudes of evoked excitatory postsynaptic currents/potentials (EPSCs/EPSPs) were calculated from the baseline current just before each stimulus artifact. Because these measurements are influenced by the membrane potential under current-clamp conditions, the cell resting potential (V<sub>rest</sub>) was kept at a constant value by intracellular current injection throughout the recording session. Spontaneous synaptic events were detected and analyzed using Mini Analysis Program version 5.2.2 (Synaptosoft, Leonia, NJ). Clampex software (Axon Instruments) was used for exponential fitting of membrane currents or potentials. Data are presented as means ± SE. Data were analyzed statistically using Student’s t-test or ANOVA test, and significance was accepted at P < 0.05.

**RESULTS**

**Synaptic responses of medium spiny projection neurons to stimulus trains**

A stimulus train produced a burst of presynaptic action potentials that elicited a complex postsynaptic response from NAc spiny projection neurons (Figs. 1, A and B). Every stimulus evoked a fast depolarizing potential in current clamp, and the train was often followed by a prolonged slowly decaying depolarization (Fig. 1A, control). Compared with the resting membrane potential (V<sub>rest</sub> = −76 ± 1 mV; n = 22), the average amplitude of the prolonged depolarization was 6 ± 1 mV (n = 22) 100 ms after the last stimulus in the train. The prolonged depolarization returned to V<sub>rest</sub> with two time constants: 0.29 ± 0.06 and 3.9 ± 0.3 s. The prolonged depolarization was accompanied by increased spontaneous synaptic activity. The complex postsynaptic response was completely blocked by 0.5 μM tetrodotoxin (Fig. 1A, TTX) and recovered on washout (Fig. 1A, wash). Under voltage-clamp conditions, every stimulus train evoked an inward postsynaptic current, spontaneous posttrain synaptic activity, and a long-lasting depolarizing inward current (Fig. 1B, control). In 10 cells, we applied hyperpolarizing voltage steps (−10 mV, 100 ms) to measure the cell’s input resistance (R<sub>in</sub>) before and after a stimulus train. Spiny projection neurons had R<sub>in</sub> of 640 ± 40 MΩ under control conditions. There was a slight but not significant R<sub>in</sub> decrease by 20 ± 10% (P = 0.2) at 100 ms from the end of the train. Replacing extracellular Ca<sup>2+</sup> with the same concentration of Co<sup>2+</sup> abolished the response (Fig. 1B, Ca-free Co). The TTX and Co<sup>2+</sup> results indicate the synaptic origins of the spiny-neuron responses. When the same stimulus protocol was applied to the Schaffer collateral inputs onto hippocampal CA1 pyramidal neurons (n = 4), EPSCs were evoked; but no long posttrain depolarization or increased synaptic activity was seen (exemplified in Fig. 1C).

After the stimulus train, 85% of the spiny projection neurons responded with an increase in the frequency of spontaneous synaptic activity (2,000 ± 800% increase above baseline, n = 16; Figs. 1, A and B, and 2, A and B). The increased frequency...
of EPSCs declined back toward the baseline usually in ~10 s. Repeated trains (every 30 s) produced increases in spontaneous activity that were consistent and repeatable in their frequency and time course. Although the frequency of the posttrain EPSCs increased, the amplitude did not (Fig. 2, A and B): 12.6 ± 2 pA before and 13.1 ± 2 pA after the train (n = 4). This result suggested that the posttrain increased synaptic activity was presynaptic in origin, and that the increase in synaptic activity arose from asynchronous vesicular release that produced miniature EPSCs (mEPSCs) that do not require ongoing excitation of the afferent presynaptic terminal.

To investigate the nature of the EPSCs, we applied TTX (0.5 μM) to prevent spontaneous action potentials while we monitored the ongoing synaptic activity (Fig. 2C). In that case only mEPSCs are possible. Adding TTX while monitoring the spontaneous baseline EPSCs had no effect on the amplitude or frequency (Fig. 2, C and D): 0.92 ± 0.1 Hz in control and 0.93 ± 0.1 Hz in TTX (n = 4). These results show that the spontaneous baseline EPSCs recorded from spiny projection neurons are mEPSCs and not action-potential dependent. That conclusion, taken with the finding that the amplitude of the EPSCs does not change after the stimulus train, supports the conclusion that the increase in synaptic activity after a train arises mainly from asynchronous quantal release (mEPSCs).

Because we studied brain slices from young mice, we checked whether the increase of posttrain synaptic activity was due to the immaturity of the spiny projection neurons. By the third postnatal week, the input resistance and membrane time constant of spiny projection neurons are similar to adult neurons (Belleau and Warren 2000). There is, however, refinement of the dendritic arborization during the third week (Tepper et al. 1998). Therefore we divided the cells to create two groups of different ages comprising 14–16 and 19–21 days. The increase in the frequency of spontaneous synaptic activity was the same: 2,000 ± 900% (n = 7) and 1,800 ± 900% (n = 5), respectively. This result suggests that the poststimulus synaptic activity is not strongly dependent on the developmental stage of the neurons.

To verify that the predominant postsynaptic response was mediated by glutamate receptors (GluRs), we applied inhibitors. The N-methyl-D-aspartate (NMDA) receptor antagonist, APV (25 μM), had little effect (n = 5; Vm = −70 mV). As shown in Fig. 3A, APV only slightly altered the evoked EPSC amplitude (90 ± 8% of control; P = 0.4; n = 5) or the EPSC frequency (88 ± 9% of control; P = 0.4). APV did not significantly alter the posttrain increase in spontaneous synaptic activity (90 ± 10% of control; P = 0.5). Co-applied CNQX (20 μM) and APV (25 μM) prevented the evoked EPSCs and the posttrain synaptic activity and strongly reduced the slow inward current to 7 ± 2% (P = 0.004; n = 3). Comparing the example traces in Fig. 3B indicates the affect of CNQX/APV on the prolonged, depolarizing inward current. When CNQX was applied alone, we obtained similar results (n = 2). The nonselective metabotropic GluR antagonist, MCPG (1 mM), did not affect the posttrain depolarization recorded under current clamp (Vm = −75 mV) nor the posttrain slow inward current under voltage clamp (Vm = −70; n = 2; data not shown).

The spontaneous synaptic events also were recorded in the presence of bicuculline (10 μM), which blocked GABAergic transmission in the NAc slice (n = 4; data not shown). Bicuculline did not strongly influence postsynaptic spontaneous synaptic activity (110 ± 10% of control; P > 0.05) or the posttrain slow inward current (108 ± 7% of control; P > 0.05). We conclude therefore that excitatory inputs onto medium spiny projection neurons are capable of producing the observed postsynaptic effects even in the presence of opposing inhibitory transmission.

**FIG. 2.** Stimulus trains induce an increase in the frequency of spontaneous EPSCs. A: continuous voltage-clamp recording (8-s duration) of EPSCs before and immediately after a train. Vm = −70 mV. B: plots of the average spontaneous EPSCs frequency and amplitude before (events sampled for 30 s) and immediately after (EPSCs sampled for 2 s) the stimulus train (n = 9 cells). The spontaneous EPSC frequency (P < 0.001), but not the amplitude, was significantly increased by the stimulus train. C: continuous recording (60-s duration) of EPSCs in control and TTX (0.5 μM) conditions. There was no stimulus train in this experiment. D: plots of the average spontaneous EPSCs frequency and amplitude in control and in TTX (n = 4 cells). There is no significant change in the EPSCs frequency or amplitude in TTX.
Evoked synaptic responses to the stimulus train are facilitated then depressed

A 10-Hz, 15-stimuli train caused facilitation followed by depression of the fast EPSPs/EPSCs (Fig. 4). The second to the fourth stimuli of the train usually evoked larger responses than the first one (facilitation). The later stimuli evoked smaller EPSPs/EPSCs than the first response to the train (depression). A typical averaged response is shown in Fig. 4A, and the average normalized response amplitude versus time is shown in Fig. 4B (under voltage clamp, n = 26 cells).

A train of stimulation commonly produces facilitation and depression of this kind. The literature indicates that this pattern in the synaptic responses is likely to arise from changes in the probability of transmitter release associated with presynaptic calcium signals (Stevens and Wang 1995). To assess that possibility, trains of stimuli were applied while varying the extracellular Ca\(^{2+}\) concentration, and depression of the EPSCs was quantified by calculating the ratio of the 2nd to the 1st EPSC amplitude in the train (A2/A1) and the ratio of the last EPSC amplitude in the train to the 1st EPSC amplitude (A15/A1). Both responses were from the same cell and are averages of 10 sequential traces recorded every 120 s. B: average plot of response amplitude vs. time for traces recorded under voltage-clamp conditions (n = 26 cells). The facilitation and depression were quantified by calculating the ratio of the 2nd to the 1st EPSC amplitude in the train (A2/A1) and the ratio of the last EPSC amplitude in the train to the 1st EPSC amplitude (A15/A1). The average A2/A1 and A15/A1 were 1.7 ± 0.2 and 0.58 ± 0.06, respectively, indicating that, despite the facilitation of the first few responses, evoked EPSCs were depressed at the end of stimulus train.

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FIG. 3. The response to a train of stimuli is reduced by inhibition of non-N-methyl-D-aspartate (NMDA) glutamate receptors. A: responses to repetitive stimulation under voltage clamp in control and in 20 μM 6-cyano-7-nitroquinolxalene-2,3-dione (CNQX) and 25 μM APV to inhibit non-NMDA and NMDA receptors, respectively, greatly reduced the evoked EPSCs, the posttrain increase in spontaneous EPSCs, and the prolonged inward current. Cells in A and B were held at −70 mV membrane potential and stimulated by trains of 15 stimuli delivered at 10 Hz. Inwardly directed evoked EPSCs are truncated. Traces are single trial examples.

FIG. 4. Repetitive stimulation produces a facilitation and subsequent depression of evoked EPSPs/EPSCs. Increased spontaneous EPSC activity is not seen after the stimulus train in this figure because the traces are averages, which blend individual spontaneous EPSCs into the baseline. A: an averaged representative response to 15-stimuli train delivered at 10 Hz under current clamp (top; V\(_{\text{rest}}\) = −71 mV) and voltage clamp (bottom; V\(_{\text{hold}}\) = −70 mV). Both responses are from the same cell and are averages of 10 sequential traces recorded every 120 s. B: average plot of response amplitude vs. time for traces recorded under voltage-clamp conditions (n = 26 cells). The facilitation and depression were quantified by calculating the ratio of the 2nd to the 1st EPSC amplitude in the train (A2/A1) and the ratio of the last EPSC amplitude in the train to the 1st EPSC amplitude (A15/A1). The average A2/A1 and A15/A1 were 1.7 ± 0.2 and 0.58 ± 0.06, respectively, indicating that, despite the facilitation of the first few responses, evoked EPSCs were depressed at the end of stimulus train.

FIG. 5. The response to a train of stimuli is reduced by inhibition of non-N-methyl-D-aspartate (NMDA) glutamate receptors. A: responses to repetitive stimulation under voltage clamp in control and in 20 μM 6-cyano-7-nitroquinolxalene-2,3-dione (CNQX) and 25 μM APV to inhibit non-NMDA and NMDA receptors, respectively, greatly reduced the evoked EPSCs, the posttrain increase in spontaneous EPSCs, and the prolonged inward current. Cells in A and B were held at −70 mV membrane potential and stimulated by trains of 15 stimuli delivered at 10 Hz. Inwardly directed evoked EPSCs are truncated. Traces are single trial examples.

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A train of stimulation commonly produces facilitation and depression of this kind. The literature indicates that this pattern in the synaptic responses is likely to arise from changes in the probability of transmitter release associated with presynaptic calcium signals (Stevens and Wang 1995). To assess that possibility, trains of stimuli were applied while varying the extracellular Ca\(^{2+}\) concentration. The amplitudes of the first evoked EPSCs were decreased by 10 ± 20% in lowered Ca\(^{2+}\) (0.5 mM) and were increased by 140 ± 50% in elevated Ca\(^{2+}\) (4 mM). This amplitude change is indicated by the first EPSCs in the example traces of Fig. 5A. Within a 10-Hz train, facilitation of the evoked EPSCs was not observed in the high extracellular Ca\(^{2+}\) concentration, and depression of the EPSCs was enhanced as the train progressed (Fig. 5B, △, n = 6 cells). In contrast, lowering the extracellular Ca\(^{2+}\) caused much greater facilitation and converted depression at the later stimuli to facilitation (Fig. 5B, ○, n = 5 cells).

In another set of experiments, we inhibited potassium conductances with 4-aminopyridine (4-AP) to slow repolarization of the action potential reaching the presynaptic glutamate terminal. That effect should increase the Ca\(^{2+}\) entering the terminal during each stimulus. Consequently, 4-AP (10 and 100 μM) increased the first evoked EPSC amplitude by 2.5 ± 0.8 (n = 4) and by 4.5 ± 0.6 (n = 5), respectively. Figure 5C shows typical responses to the first two stimuli in control and 4-AP solutions. Facilitation was reduced and depression was enhanced by 10 μM 4-AP (Fig. 5D, ×, n = 4 cells). The higher dose of 4-AP (100 μM) prevented facilitation and increased depression (Fig. 5B, □, n = 5 cells).
Prolonged depolarization following the stimulus train

When a spiny projection neuron received a train of afferent stimulation, it responded with a prolonged depolarization or slow inward current, and the response required active synaptic input. As indicated by the affect of 4-AP (Fig. 6A), a potassium conductance influenced the posttrain, prolonged depolarization. A train of 15 stimuli at 10 Hz elicited a typical response in control solution with one action potential occurring on the peak of the third evoked EPSP (Fig. 6A, top). In 10 μM 4-AP, the stimulus train induced a larger prolonged depolarization, which grew steeper and decayed more slowly (Fig. 6A, middle). Spikes frequently appeared on the peak of the EPSPs. In 100 μM 4-AP, the prolonged depolarization grew larger, and the cell entered into a plateau-like depolarization (Fig. 6A, bottom). About 10 s after the stimulus train, the plateau depolarization recovered to the baseline. Furthermore, cells bathed in 100 μM 4-AP underwent spontaneous depolarizations at a frequency of one every 3–5 min. An example of a spontaneous depolarization is shown in Fig. 6C. Under voltage-clamp conditions (V_{hold} = −70 mV), treatment with 4-AP (10 and 100 μM) also caused a greater response (Fig. 6B). 4-AP increased the amplitude of the posttrain inward current and dramatically increased the spontaneous EPSC frequency.

The prolonged depolarization seen in current clamp was strongly reduced by introducing the Ca^{2+} chelator, bis-(o-aminophenoxy)N,N,N',N'-tetraacetic acid (BAPTA, 30 mM), into the patch pipette to reduce the postsynaptic rise of intracellular Ca^{2+}. Figure 7A shows three responses to repetitive stimulation recorded under current clamp taken 2, 4, and 10 min after the rupture of the seal. The prolonged depolarization decreased with time, indicating the finite rate of BAPTA entry.
into the spiny projection neuron from the patch pipette \((n = 6)\) cells. We measured the area under the curve of the prolonged depolarization (in a 3-s interval) 10 min after the seal rupture in control cells and in cells recorded with BAPTA in the pipette. The area was significantly smaller in cells loaded by BAPTA than in control cells (2.5 ± 0.5 mV s⁻¹ in 7 BAPTA-loaded cells vs. 9 ± 2 mV in 9 control cells; \(P < 0.05\); Fig. 7B). Moreover, prolonged depolarization declined faster in BAPTA loaded cells (decay fitted with 2 exponentials with \(\tau_{fast}\) and \(\tau_{slow}\) 0.12 ± 0.02 and 1.7 ± 0.3 s, respectively) than in control cells (\(\tau_{fast}\) and \(\tau_{slow}\) 0.29 ± 0.06 and 4 ± 2 s, respectively).

The local stimulation applied in the NAc likely excites dopaminergic afferents and cholinergic interneurons. We tested whether modulation of dopamine or acetylcholine receptors could alter the generation of the prolonged depolarization. The following agents did not significantly affect the prolonged depolarization (data not shown): atropine (2 \(\mu M\)), methyllycaconitine (10 nM), DHβE (5 \(\mu M\)), sulpiride (5 \(\mu M\)), or dopamine (20 \(\mu M\); +10 \(\mu M\) sodium metabisulphite).

**DISCUSSION**

In vivo some medium spiny projection neurons spend relatively long periods in a silent, resting down state that is occasionally interrupted by brief depolarizations to an up state (Wilson 1993; Wilson and Kawaguchi 1996). Those projection neurons fire efferent action potentials that serve as the output from the striatum only from the up state. That depolarized state is achieved by a maintained barrage of convergent afferent excitation arising from the cerebral cortex and thalamus, but in particular, afferent excitation from the hippocampus is necessary to induce the switch to the up state (Calabresi et al. 1990; O’Donnell and Grace 1995; Wilson and Kawaguchi 1996). We examined the impact of stimulus trains to model the convergent afferent bursts of activity that are known to excite striatal projection neurons. Although the response of medium spiny projection neurons to a single stimulus has been characterized previously (Kombian and Malenka 1994; O’Donnell and Grace 1995; Pennartz and Kitai 1991; Pennartz et al. 1991), less is known about the influence of afferent bursts (Calabresi et al. 1999).

In this study, we examined the response of NAc medium spiny projection neurons to a well-defined train of excitation using the in vitro slice preparation. We found that the afferents to the projection neurons are able to provide prolonged excitation that extends beyond the stimulus train. The extended excitation is expressed as a large increase in the frequency of spontaneous glutamate release even after the stimulus train has stopped. The evidence suggests that this glutamate release is primarily arising from asynchronous quantal release (i.e., mEPSCs). Simultaneous with the enhanced afferent glutamate release, the postsynaptic spiny projection neuron enters a prolonged depolarized state that amplifies the incoming excitation. In vivo and under certain experimental conditions in vitro that depolarization may achieve a plateau that is the up state (O’Donnell and Grace 1995; Plenz and Kitai 1998; Vergara et al. 2003).

In summary, the stimulus train evoked EPSCs in the NAc projection neurons that displayed facilitation and later depression, a feature shared by other CNS synapses. The medium spiny projection neurons were unusual in their ability to integrate afferent excitation in two ways. First, there was a dramatic posttrain increase in spontaneous EPSCs. Second, the stimulation produced a prolonged posttrain depolarization.

**Facilitation and depression of evoked synaptic currents**

In response to the stimulus train, the amplitude of the evoked EPSCs was initially facilitated and later depressed. That response to a stimulus train is common and is widely accepted to arise from the change in the probability of glutamate release (Craeger et al. 1980; Debanne et al. 1996; Dobrunz and Stevens 1997; Magleby 1987; Murthy et al. 1997; Zucker 1989). A presynaptic mechanistic interpretation leads to the general expectation that synapses with a low release probability exhibit facilitation, and those with a high release probability usually exhibit depression. The facilitation is thought to arise from \(Ca^{2+}\) accumulation in the presynaptic terminal, which increases the probability of vesicular release (Augustine et al. 1994; Delaney and Tank 1994; Radcliffe and Dani 1998; Stevens and Wang 1995; Tank et al. 1995; Zucker 1993). The later stimuli cause depression mainly because there is some depletion of the vesicular pool that is released by action potentials (Zucker 1989).

Our results are consistent with a presynaptic interpretation because lowering of extracellular \(Ca^{2+}\) enhanced facilitation. In low \(Ca^{2+}\), the probability of release was lower, and the accumulation of \(Ca^{2+}\) in the presynaptic terminal had a greater facilitating effect on release. High external \(Ca^{2+}\) or 4-AP decreased facilitation because the initial release probability was high and the accumulation of \(Ca^{2+}\) with repeated stimuli was not necessary. Further support for the presynaptic nature of the facilitation/depression pattern of evoked EPSCs is that the amplitude of the spontaneous EPSCs was the same immediately before and after the stimulus train. At the end of the train, the evoked EPSCs were strongly depressed, but the spontaneous EPSCs were unchanged in amplitude. A postsynaptic mechanism for depression, such as shunting by a decreased input resistance or glutamate receptor desensitization (Jones and Westbrook 1996; Otis et al. 1996), would have resulted in a decrease in the amplitude of spontaneous EPSCs immediately after the train. A potential role for the posttrain spontaneous EPSCs, discussed in the following text, requires that they maintain an effective amplitude.

**Increased frequency of spontaneous EPSCs associated with the stimulus train**

After the stimulus train, the frequency of spontaneous EPSCs increased dramatically, by a factor of \(~20\) for a few seconds. Our finding that the baseline, spontaneous EPSCs were not influenced by TTX indicated they did not arise from action potentials, supporting the conclusion that the EPSCs arose from asynchronous vesicular glutamate release (mEPSCs). Several types of synapses have previously shown a smaller increase in asynchronous quantal release for roughly seconds (Barrett and Stevens 1972; Cohen and Van der Kloot 1986; Geppert et al. 1994; Goda and Stevens 1994; Mennerick and Zorumski 1995; Rahamimoff and Yaari 1973; Zengel and Magleby 1981; Zucker and Lara-Estrella 1983). Previous evidence has indicated that asynchronous release arose from...
elevated presynaptic Ca\(^{2+}\) because it was produced using Ca\(^{2+}\) ionophores or via sustained high-frequency stimulation (Delaney and Tank 1994; Ravin et al. 1997). Furthermore, treatment with membrane-permeable Ca\(^{2+}\) chelators decreased this type of release (Atluri and Regehr 1998; Cummings et al. 1996; Tang et al. 2000). This asynchronous release uses a pathway that is distinct from that used during the rapid release evoked by action potentials (Geppert et al. 1994; Goda and Stevens 1994). In hippocampal cultures from mutant mice lacking synaptotagmin I, only the asynchronous form of neurotransmitter release was observed (Geppert et al. 1994). Thus it is not surprising that we found evoked release diminished at the end of the stimulus train, but the asynchronous quantal release was greatly enhanced in frequency and the amplitude was undiminished.

In our recordings from NAc medium spiny projection neurons, the excitatory afferents gave potent and long-lasting bursts of posttrain mEPSCs. The posttrain mEPSCs (seen under current clamp) occurred in the same time bursts of posttrain mEPSCs. The posttrain mEPSCs (seen under current clamp) occurred in the same time period and were influenced by the same treatments. For example, 4-AP greatly enhanced both the posttrain EPSCs and the depolarization (Fig. 6). The exceptional strength of the asynchronous release after an afferent train would contribute to the maintenance of the prolonged, posttrain depolarization. Therefore it is key for the prolonged depolarization that the spontaneous mEPSCs have a sufficient (and undiminished) amplitude. To our knowledge, no such increase in spontaneous activity after repetitive afferent simulation has been detected in vivo (e.g., O’Donnell and Grace 1995). However, the in vivo experiments have been done using intracellular sharp electrodes that filter the signal and give a higher recording noise compared with patch-clamp recordings. We can only say with certainty that high fidelity patch-clamp recordings in striatal brain slices reveal a significant posttrain effect.

Prolonged posttrain depolarization

Although a single evoked response decayed rapidly to the prestimulus baseline, a stimulus train produced a prolonged depolarization or a slow inward current. It does not seem that the slow inward current arose from the summation of the slow, NMDA component of the EPSCs because the NMDA receptor antagonist, APV, did not strongly block this inward current under voltage-clamp conditions. In fact, in 1 mM Mg\(^{2+}\) while under voltage clamp ($V_{\text{hold}} = -70$ mV), there is little activation of NMDA receptors. Thus the slow inward current was not mediated mainly by NMDA receptors. However, NMDA receptors might help to maintain the prolonged depolarization (Kita 1996; Vergara et al. 2003) because the Mg\(^{2+}\) block is removed by depolarization, and more NMDA receptors are activated at more positive membrane potentials. Due to their slower kinetics, NMDA receptors should participate in maintaining the depolarization under current clamp conditions or in vivo. Because large amounts of glutamate could be released during repetitive stimulation, glutamate might remain longer in the synaptic cleft and even escape into the extrasynaptic space (Barbour et al. 1994). We evaluated the possibility that mGluRs were activated due to spillover of glutamate, but a nonselective mGluR antagonist did not reduce the prolonged depolarization. The local stimulus train also released acetylcholine and dopamine, but those neurotransmitters did not seem to participate strongly in the generation of the prolonged depolarization.

Perfusion of BAPTA into the postsynaptic cell strongly reduced the prolonged depolarization, indicating the importance of a postsynaptic Ca\(^{2+}\) signal for this phenomenon. Furthermore, apparently postsynaptic K\(^{+}\) conductances effectively controlled the extent of the prolonged depolarization. After inhibition of K\(^{+}\) conductances by 4-AP, the prolonged depolarization became larger and eventually arose spontaneously without exogenous stimulation. Synaptic currents depolarize the medium spiny neuron dendrites enough to activate voltage-dependent Ca\(^{2+}\) channels (Akopian and Walsh 2002). Moreover, Ca\(^{2+}\) conductance is located in dendritic Ca\(^{2+}\) transients during depolarized up states (Kerr and Plenz 2002). Thus the Ca\(^{2+}\) signal is properly timed, and based on our BAPTA results, it contributed to the production of the posttrain inward current underlying the prolonged depolarization.

Medium spiny neuron response to burst-like synaptic activity

The posttrain depolarization seen under current clamp occurred during the enhanced spontaneous EPSCs (likely mEPSCs) seen under voltage clamp. Neither the posttrain burst of mEPSCs nor the prolonged depolarization was significantly strong after applying the stimulus train to the Schaffer collateral afferents onto CA1 hippocampal pyramidal neurons. A comparison of the CA1 and medium spiny neurons exemplifies the unusual characteristics of the response observed in the NAc. Our results suggest the following interpretation. There are multiple excitatory afferents converging onto each medium spiny projection neuron (Groenewegen et al. 1980; Kelley and Domesick 1982; Kita and Kita 1990; Sesack et al. 1989). Bursts of activity along these excitatory afferents onto spiny projection neurons produce heightened asynchronous glutamate release and an additional calcium-influenced conductance immediately following a burst. The mEPSCs arising from many afferents will tend to sum and maintain their convergent excitation over the time of the asynchronous release. The electrical properties of the medium spiny neuron also are able to integrate the afferent excitation to achieve the prolonged depolarization. Potassium conductances limit the depolarization and are particularly important for the maintenance of the plateau potential (Wilson and Kawaguchi 1996). Thus the posttrain spontaneous mEPSCs maintain the excitation even from slightly mismatched afferent activity, and the postsynaptic spiny projection neuron integrates that overlapping excitation to achieve the prolonged depolarization. In vivo that prolonged depolarization may contribute to the up state from which the spiny neurons send efferent activity that serves as the output from the striatum.

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