NMDA-Receptor-Dependent Synaptic Activation of Voltage-Dependent Calcium Channels in Basolateral Amygdala

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INTRODUCTION

Calcium influx into the postsynaptic membrane is considered a critical event in most cellular models of neuroplasticity. For N-methyl-D-aspartate (NMDA)-dependent long-term potentiation (LTP), it is commonly thought that Ca\(^{2+}\) influx through the channel associated with the NMDA receptor is a requirement for induction of LTP either through direct activation of Ca\(^{2+}\)-sensitive substrates and/or subsequent liberation of intracellular Ca\(^{2+}\) stores (Bliss and Collingridge 1993; Bliss and Lynch 1988; Madison et al. 1991). Consistent with this, high-resolution imaging studies showed that Ca\(^{2+}\) entry through NMDA receptors produced a local rise in Ca\(^{2+}\) at the dendritic spines of hippocampal pyramidal neurons (Müller and Connor 1991; Petrozzino et al. 1995).

However, other studies have localized voltage-dependent calcium channels (VDCCs) in the shafts and spines of dendrites of CA1 pyramidal neurons (Jaffe et al. 1994; Mills et al. 1994; Segal 1995), and Ca\(^{2+}\) influx through these channels during synaptic stimulation has been shown to be potentially greater than through ligand-gated channels (Miyakawa et al. 1992; Regehr and Tank 1992). Furthermore Ca\(^{2+}\) influx through VDCCs has been shown necessary for certain forms of NMDA-independent LTP (Aniksztejn and Ben-Ari 1991; Grover and Teyster 1990, 1995; Huber et al. 1995). In light of these findings, it is useful to address the relative abilities of NMDA- and non-NMDA-receptor-mediated synaptic potentials to activate VDCCs. This is especially relevant for those models addressing differences between NMDA-dependent and -independent forms of synaptic plasticity.

The amygdala is a group of related nuclei in the basal forebrain implicated in emotional learning such as fear and anxiety (e.g., Bechara et al. 1995; Fanselow and Kim 1994; Kapp et al. 1992). Both NMDA- and non-NMDA-receptor-mediated components contribute to excitatory neurotransmission in the amygdala (Gein and Chang 1992; Rainnie et al. 1991), whereas GABA\(_{A}\) and GABA\(_{B}\)-receptor-mediated components contribute to inhibitory neurotransmission in this brain area (Maren 1996). Similar to other regions of the brain, neurons in the amygdala have been shown to exhibit NMDA-receptor-dependent and -independent synaptic plasticity (Chapman et al. 1990; Gein et al. 1993; Huang and Kandel 1998; Li et al. 1998; Maren 1996; Shindou et al. 1993; Wang et al. 1997). Recent work using dissociated cells has shown that amygdaloid neurons have a variety of high- and low-threshold Ca\(^{2+}\) channels (Foehring and Scroggs 1994; Kaneda and Akaike 1989; Yu and Shinnick-Gallagher 1997).

In the present study, we observed an all-or-none depolarizing event evoked by low levels of synaptic activation in disinhibited pyramidal cells of basolateral amygdala. After determining that this event is mediated by VDCC activation, we found that the Ca\(^{2+}\) spike can be induced by NMDA-receptor-mediated EPSPs but not by AMPA-mediated EPSPs. In addition, this Ca\(^{2+}\) spike can be induced by AMPA EPSPs when K\(^{+}\) channels were blocked. These results provide for a mechanism of NMDA-receptor-dependent plasticity that is independent of Ca\(^{2+}\) influx through the NMDA receptor complex.

METHODS

Brain slice preparation

Brain slices containing the basolateral amygdaloid nucleus were prepared from 14- to 18-day-old male Sprague-Dawley rats. Under halothane anesthesia, the rats were decapitated and the brains were removed and chilled in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 25 NaHCO\(_3\), 25 dextrose, 3.3 KCl, 1 MgCl\(_2\), and 2 CaCl\(_2\). After hemisection, coronal brain slices (300-μm thick) were cut from each hemisphere using a vibrat-
ing tissue slicer. Slices were transferred to a holding chamber containing room-temperature ACSF bubbled with 95% O₂-5% CO₂ and were incubated for ≥1 h before being used in an experiment.

**Drugs**

The recording chamber was perfused with ACSF (2–3 ml/min) using a mechanical pump (Cole Palmer) and a mechanical valve was used for exchange of drug solutions. At this perfusion rate, fluid turnover in the recording chamber occurred in 20–30 s. All drugs were made as stock solutions and then diluted to final concentrations in ACSF. Final concentrations of drugs in ACSF were: dicrocilline (MK-801), 100 μM; 6-2-amino-5-phosphonovaleric acid (APV), 50 μM; bicuculline methiodide (BMI), 20 μM; tetrodotoxin (TTX), 0.5 μM; and 6,7-dinitroquinoxaline (DNQX), 20 μM. All stock solutions were made using distilled water except for DNQX, which was made with dimethyl sulfoxide (DMSO; final DMSO concentration in ACSF was never >0.2%). To avoid junction errors from BMI application, bicuculline methochloride (BMC, 20 μM) was substituted for BMI when blockade of GABA_A transmission was desired during ongoing recordings (i.e., to demonstrate “wash on” and “wash off” effects of bicuculline in Fig. 1A). BMC, BMI, APV, and DNQX were all obtained from Sigma (St. Louis). MK-801 was obtained from Research Biochemicals International (Natick, MA). TTX was obtained from Calbiochem (La Jolla, CA).

**Electrophysiological recordings**

For each experiment, slices were transferred to a submerged recording chamber mounted on an upright microscope (Zeiss Axioscope) equipped with infrared illumination and differential interference contrast optics. Temperature in the recording chamber was held at 30 ± 1°C throughout the recording period. For recording, individual pyramidal cells of the basolateral amygdala were visualized under high magnification. Whole cell patch-clamp electrodes were pulled from Corning 7740 glass (1.5 mm OD, WPI) using a Flaming-Brown micropipette puller (Sutter) and had input resistances of 2–4 MΩ. The standard electrode solution contained the following (in mM): 110 K-glucolate, 10 NaCl, 10 HEPES, 10 K_BAPTA, 4 QX314, and 2 MgATP. In some experiments, K-glucolate and K_BAPTA were replaced by Cs-glucolate (100 mM) and Cs_2BAPTA (10 mM). Voltage and current recordings were made using a Warner PC-501A patch-clamp amplifier. Synaptic stimulation was triggered by electrical stimulation applied through a monopolar tungsten electrode with a bath return placed in the medial basolateral nucleus adjacent to the external capsule. Stimuli were square wave current pulses (1–250 μA, 0.1 ms) delivered at 0.033 Hz. In some experiments, exogenous glutamate (500 μM) was pressure applied (10–15 psi, 10–100 ms) through a small diameter glass pipette placed within 100–200 μm of the recorded cell.

Although the disinhibited amygdala slice preparation often develops epileptiform bursts (Gean and Chang 1991), we attempted to minimize burst activity by restricting the recording to 30 min after superfusion of BMC or BMI. Slices showing spontaneous bursts (<10%) were discarded. The absence of significant epileptiform bursting in our preparation may have resulted also from the use of relatively thin slices (300 μm).

**Waveform analysis**

Signals were filtered (2 kHz) and amplified before being digitized (5 kHz) and saved to hard disk for off-line data analysis. To enhance the detection of the recruited Ca²⁺ spike during the rising phase of the evoked response, unaveraged raw waveforms were expanded and differentiated using data acquisition and analysis software (LabView, National Instruments). Ca²⁺ spikes imbedded in synaptic potentials were detected readily in differentiated records by the presence of a second slope peak at the onset of the Ca²⁺ spike. Differentiation of the raw waveform provided a sensitive measure for subtle changes in the onset kinetics of the evoked response (e.g., Stasheff et al. 1993).

**RESULTS**

Whole cell current- and voltage-clamp recordings were taken from 74 pyramidal cells of the basolateral amygdala. At initial patch rupture, cells typically had a resting membrane potential of negative 60–70 mV [mean V_m = −62.3 ± 0.65 mV (mean ± SE); n = 24] and an input resistance of 150–250 Ω (mean R_k = 218.2 ± 10.75 Ω). In normal ACSF and with QX314 in the recording pipette to block sodium spikes, low-intensity stimulation applied to the basolateral nucleus evoked small EPSPs (average latency, 3.3 ms) previously demonstrated to be mediated by both NMDA and non-NMDA glutamatergic receptors (Calton et al. 1997; Gean and Chang 1992; Rainnie et al. 1991). After perfusion of bicuculline to block GABA_A-mediated inhibition, stimulation at the same intensity evoked a larger EPSP that recruited a slower onset, large-amplitude depolarizing potential resembling a Ca²⁺ spike (Fig. 1A). This putative Ca²⁺ spike occurred at an average latency of 19.69 ± 2.46 ms, had a distinct stimulus threshold, and varied little in amplitude with stimulus intensity (Fig. 1B). When raw waveforms were differentiated (Fig. 1C), the slope of the response during the rising phase clearly showed a second peak corresponding to the regenerative event.

![FIG. 1. Synaptically triggered excitatory postsynaptic potentials (EPSPs) recruit a putative Ca²⁺ spike in disinhibited basolateral amygdala. A: with QX314 in the recording electrode, stimulation (20 μA) in normal artificial cerebrospinal fluid (ACSF) evokes small mixed EPSPs. After bath application of bicuculline methochloride (BMC) to block GABA_A-mediated inhibition, the EPSPs recruit a slower onset depolarizing potential. Calibration bars: 200 ms and 20 mV. B: recruited potential occurs in an all or none fashion and has a distinct stimulus threshold. Calibration bars: 20 mV and 200 ms. C: raw traces from the waveform from B were expanded (top) and differentiated (bottom) to emphasize the recruitment of a Ca²⁺ spike at the higher stimulus intensity. Presence of a second slope peak in the differentiated record provides a marker for the occurrence of the Ca²⁺ spike in evoked responses. Calibration bars: 40 mV and 20 ms for raw waveforms; 4 V/s and 20 ms for differentiated waveforms. Stimulation intensities are shown below raw waveforms in the figure.](http://jn.physiology.org/DownloadedFromHttp://jn.physiology.org/DownloadedFromHttp://jn.physiology.org/)
Ionic mechanism of the recruited potential

Several experiments were conducted to verify that the recruited depolarizing potential is mediated by VDCCs. First, to demonstrate that this secondary depolarizing event occurs via a voltage-gated rather than a ligand-gated conductance, cells were alternately stimulated in current- and voltage-clamp recording modes (n = 4). Figure 2A shows traces from a typical experiment. As before, while recording under current-clamp conditions, EPSPs were evoked, and the EPSPs recruited the secondary depolarizing potential at higher stimulus intensities. In voltage-clamp mode, however, stimulation at similar intensities failed to elicit the secondary events, providing evidence that this secondary event is mediated through a voltage-gated conductance. Calibration bars: 20 mV and 100 ms. Stimulation intensities and glutamate application duration were shown to evoking EPSPs that recruited the secondary depolarizing potential at longer application duration (left). Bath application of Cd²⁺ (100 μM; right), a nonselective Ca²⁺ channel blocker, eliminated this secondary depolarizing potential without affecting the glutamate mediated depolarization. Calibration bars: 20 mV and 100 ms. Stimulation intensities and glutamate application duration were shown at the left of waveform in the A and B, respectively.

Relative abilities of NMDA EPSPs and non-NMDA EPSPs to recruit Ca²⁺ spikes

Given that excitatory neurotransmission in pyramidal cells of the basolateral amygdala is mediated by both NMDA and non-NMDA receptors (Gean and Chang 1992; Rainnie et al. 1991), we sought to determine if both forms of excitatory transmission were equally effective at recruiting the voltage-dependent Ca²⁺ spikes.

First, NMDA EPSPs were isolated by bath application of non-NMDA-receptor blocker, DNQX (Fig. 3A). Although the addition of DNQX resulted in a shift to the right in the input/output plot, isolated NMDA EPSPs nevertheless evoked the Ca²⁺ spike at higher stimulus intensities in all cells tested (n = 16, Fig. 3B). In contrast, non-NMDA EPSPs isolated by bath perfusion of a competitive NMDA-receptor antagonist APV (50 μM) failed to recruit the Ca²⁺ spike in 9 of 11 cells, even when stimulus intensity was increased by a factor of 10 (Fig. 4, A and C).

We considered the possibility that the two cells showing Ca²⁺ spikes in the presence of APV may have done so because APV is a competitive NMDA-receptor blocker and thus subject

![Figure 2](image-url)

**Figure 2.** Recruited potential is mediated by voltage-dependent calcium channels (VDCCs). A: in the presence of bicuculline methiodide (BMI), synaptic response under current-clamp mode (EPSPs, left) evoked the recruited potential at higher stimulus intensities. In the same cell, synaptic response under voltage-clamp mode (EPSCs, right) failed to evoke the recruited event, suggesting that the event is mediated through a voltage-gated conductance. Calibration bars: 20 mV and 100 ms for current-clamp traces; 215 pA and 50 ms for voltage-clamp traces. B: exogenous glutamate (300 mM), applied by picospritzer in the presence of TTX, evoked depolarizing response comparable to EPSPs that recruited the secondary depolarizing potential at longer application duration (left). Bath application of Cd²⁺ (100 μM; right), a nonselective Ca²⁺ channel blocker, eliminated this secondary depolarizing potential without affecting the glutamate mediated depolarization. Calibration bars: 20 mV and 100 ms. Stimulation intensities and glutamate application duration were shown at the left of waveform in the A and B, respectively.

![Figure 3](image-url)

**Figure 3.** Isolated N-methyl-D-aspartate (NMDA) EPSPs are effective at recruiting the Ca²⁺ spike. A: in the presence of BMI alone (left), the Ca²⁺ spike is elicited at relatively low stimulus intensities. Isolated non-NMDA EPSPs by 6,7-dinitroquinoxaline (DNQX) evoked the Ca²⁺ spike at higher stimulation intensity than the mixed EPSPs (right). Top traces: raw waveforms; bottom traces: differentiated waveforms. Numbers to the left of waveforms are stimulation intensities. Calibration bars: 10 mV and 100 ms for raw waveforms; 2.5 V/s and 100 ms for differentiated waveforms. B: peak EPSP amplitude plotted as a function of stimulus intensity. Large increases in amplitude (● and ▲) indicate recruitment of Ca²⁺ spikes in the presence of BMI alone (●) or both of BMI and DNQX (▲).
Large increase in amplitude (2) indicates the recruitment of the Ca\textsuperscript{2+} shown. This result showed that Ca\textsuperscript{2+} intensities tested. Washout of APV (right) resulted in the return of the synap- 

C

Effect of elongation of non-NMDA EPSPs on Ca\textsuperscript{2+} spikes

Previous studies have reported that non-NMDA EPSPs, although usually larger in peak amplitude, tend to be shorter in duration than NMDA EPSPs due to rapid desensitization (Kiskin et al. 1986; Trussell and Fischback 1989). In our preparation, the mean half time to decay for non-NMDA EPSPs was 68.9 ms compared with 96.2 ms for mixed EPSPs (although this more rapid decay appeared to be accounted for in part by the greater peak depolarization with increasing stimulus intensity). Therefore we sought to determine if an elongated non-NMDA EPSP could elicit a Ca\textsuperscript{2+} spike. First, we studied the effect of cyclothiazide, a drug that elongates non-NMDA EPSPs by inhibiting desensitization of the AMPA receptor (Yamada and Tang 1993). After evoking a maximal non-NMDA EPSPs in the presence of APV or MK801, some cells were perfused with cyclothiazide. Cyclothiazide had no effect on peak amplitude of non-NMDA EPSPs but increased the mean duration (214 ± 21 ms) and half time to decay (101 ms), resulting in an average increase in non-NMDA EPSP area of 100.2 ± 22.6% (Fig. 6). The prolonged non-NMDA EPSPs still failed to evoke the Ca\textsuperscript{2+} spike in seven of the eight cells tested (Fig. 6).

Second, trains of stimulation were employed to elongate non-NMDA EPSPs. In the absence of NMDA-receptor block-ade, Ca\textsuperscript{2+} spikes could be elicited reliably by either single

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to competitive displacement under conditions of increased glutamate concentrations in the synapse after high-intensity stimulation. Therefore we also studied the ability of non-NMDA EPSPs isolated by the noncompetitive NMDA antagonist MK-801 to generate the Ca\textsuperscript{2+} spike (Fig. 4B). After perfusion of MK-801 and 3 min of conditioning stimulus (0.1 Hz at subthreshold intensity) to activate the use-dependent block of NMDA receptors, the non-NMDA EPSPs failed to recruit Ca\textsuperscript{2+} spikes at maximal stimulus intensities in all cells tested (n = 7). The conditioning stimulation alone failed to eliminate the Ca\textsuperscript{2+} spikes in control cells (n = 2; data not shown). This result showed that Ca\textsuperscript{2+} spikes mediated by

Figure 5 shows findings from a typical experiment examining NMDA EPSPs at different holding potentials in Mg\textsuperscript{2+}-free ACSF. In all cells tested (n = 8), there was a clearly discernible membrane voltage at which the evoked amplitudes increased with further depolarization due to recruitment of the Ca\textsuperscript{2+} spike. In contrast, after isolating non-NMDA EPSPs by the perfusion of APV (n = 6, Fig. 4B) or MK801 (n = 7, data not shown), the amplitude of the response varied linearly with holding voltage in all cells tested with larger-amplitude responses occurring at more hyperpolarized holding potentials. This result provided further evidence that the Ca\textsuperscript{2+} spike can be elicited by isolated NMDA EPSPs but not by the isolated non-NMDA EPSPs. We found that maximal intensity non-NMDA-receptor-mediated EPSPs that failed to generate Ca\textsuperscript{2+} spikes were much larger in peak amplitude when measured at the cell body than mixed EPSPs that were threshold for evoking a spike (48.5 ± 1.65 vs. 20.2 ± 1.8 mV, respectively).

Figure 4. Isolated Non-NMDA EPSPs are ineffective at recruiting the Ca\textsuperscript{2+} spike. A: in the presence of BMI alone (left), low-intensity stimulation reliably evoked the Ca\textsuperscript{2+} spike. Bath application of d-2-amino-5-phosphonovaleric acid (APV; middle) eliminated recruitment of the Ca\textsuperscript{2+} spike at all stimulus intensities tested. Washout of APV (right) resulted in the return of the synap- 

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stimulation pulses or by a stimulus train (4 pulses at a frequency of 100 Hz; Fig. 7). As expected, the stimulus intensity required to elicit the spike was less for the train stimuli, providing evidence that a stimulus train is more effective at depolarizing the cell than a single stimulus. After perfusion of APV (50 μM), stimulus trains elicited the Ca²⁺ spike in five of five neurons tested (Fig. 7). Again, this might be because APV is a competitive NMDA-receptor blocker, and thus subject to competitive displacement under conditions of high glutamate concentrations in the synapse. Therefore we also used train stimulation in the presence of the noncompetitive antagonist, MK801. In this case, although the duration of the postsynaptic depolarization was greatly prolonged relative to single stimuli, stimulus trains failed to elicit the Ca²⁺ spike in six of seven neurons tested (Fig. 7).

Third, we examined the possibility that K⁺ channels may reduce the duration of non-NMDA EPSPs, preventing them from evoking the Ca²⁺ spike. Such regulation of EPSPs by K⁺ channels has been demonstrated in the hippocampus (Hoffman et al. 1997). To block K⁺ channels and otherwise eliminate the voltage-dependent K⁺ conductance, K⁺ in the internal electrode solution was replaced by Cs⁺ (see METHODS). Under these conditions, single non-NMDA EPSPs (n = 5) were consistently able to activate the Ca²⁺ spike even at relatively low stimulus intensity (Fig. 8). As seen in Fig. 8, the blockade of K⁺ channels with Cs⁺ allowed the generation of Ca²⁺ spikes by non-NMDA EPSPs. The elongated time course of the Ca²⁺ spikes reflects the removal of voltage dampening normally provided by K⁺ efflux through voltage-dependent K⁺ channels.

**Discussion**

We have demonstrated that in the presence of GABA_A-receptor blockade, synaptic activation will evoke large depolarizing spikes mediated by VDCCs in pyramidal cells of the basolateral amygdala. In addition, in this model system, NMDA-receptor-mediated synaptic transmission is required for activating these voltage-dependent Ca²⁺ spikes. We considered the possibility that non-NMDA EPSPs failed to activate the Ca²⁺ spike because of insufficient postsynaptic depolarization. However, this seems not to be the case. Large-amplitude non-NMDA EPSPs, which greatly exceeded NMDA EPSPs capable of eliciting the Ca²⁺ spikes, failed to activate the VDCCs. Considering that non-NMDA EPSPs, although usually larger in peak amplitude, tend to be shorter in duration than NMDA EPSPs due to rapid desensitization (Kiskin et al. 1986; Trussell and Fischback 1989), the duration of non-NMDA-receptor EPSPs were increased by application of cyclothiazide or trains of stimulation. These manipulations still failed to recruit the Ca²⁺ spike. This finding suggests that there are other mechanisms for preferential activation of VDCCs by NMDA receptors. These mechanisms may play a critical role in the neuroplasticity underlying learning and memory in the amygdala (McKernan and Shinnick-Gallagher 1997). However, because we obtained brain slices from animals at an age when developmental modifications are occurring in both glutamate receptors (Kew et al. 1998; Vallano 1998) and VDCCs (McEvoy et al. 1998), these mechanisms also might reflect a developmental phenomenon.

A possible explanation for the present findings is that the origin of the spike is electronically distant from the soma, and there is a difference between the characteristics of the distally occurring EPSPs and EPSPs measured at the soma. EPSPs measured at the soma reflect the summation of many distal synaptic inputs and do not necessarily mirror EPSPs seen at single synaptic sites. These distally occurring non-NMDA EPSPs could be of insufficient duration to evoke the Ca²⁺ spikes not only due to the fast dissociation rate and the greater
desensitization of non-NMDA receptors but also due to activation of K⁺ channels. Whereas unitary synaptic events mediated by NMDA receptors can last for several hundred milliseconds, non-NMDA receptor-mediated currents are much briefer, typically less than a few milliseconds (Jahr and Lester 1992; Lester et al. 1990; Randall et al. 1990). Given our mean observed latency for the evoked Ca²⁺ spikes by NMDA-receptor-mediated transmission of 19.7 ms, it is likely that our distal non-NMDA EPSPs evoked by single stimulus pulses were too brief to activate the observed Ca²⁺ spike. Additionally, the rapid activation of K⁺ channels enables them to be activated by non-NMDA EPSPs, thus reducing the duration and amplitude of EPSPs. Such regulation of EPSPs by K⁺ channels has been demonstrated in the hippocampus (Hoffman et al. 1997). The blockade of K⁺ channels would be expected to prolong unitary depolarizations by preventing the repolarization of the membrane by voltage-dependent K⁺ currents and by increasing input resistance, thereby increasing the decay constant of the depolarization. This mechanism is supported by our observation that non-NMDA EPSPs could elicit the Ca²⁺ spike under the blockade of K⁺ channel with Cs⁺. Although QX-314 has been reported to block some K⁺ channels, it was clear that in our preparation this effect was insignificant in

![FIG. 6. High-intensity non-NMDA EPSPs that fail to recruit Ca²⁺ spikes depolarize the cell body more than mixed EPSPs that recruit Ca²⁺ spikes. A: threshold EPSPs (---) and Ca²⁺ spikes (–—) were evoked in the presence of BMI. B: after perfusion of MK801 to isolate non-NMDA EPSPs in the same cell, quadrupling the stimulus intensity produced cell body depolarization larger than that seen in threshold, mixed EPSPs without recruiting the Ca²⁺ spike (··· is threshold mixed EPSP from above, ⋯⋯ is higher intensity stimulation in presence of MK801). Application of 200 μM cyclothiazide, a drug that inhibits desensitization of the AMPA receptor, further increased the amount of non-NMDA depolarization without recruiting the Ca²⁺ spike (—). C: hyperpolarizing the cell to −90 mV resulted in an increase in normal and cyclothiazide enhanced non-NMDA EPSP amplitudes, providing evidence that the EPSPs did not recruit a Ca²⁺ spike. Left: raw waveforms; right: (traces marked by arrows) are differentiated waveforms. Calibration bars: 15 mV and 200 ms for raw waveforms; 7 V/s and 200 ms for differentiated waveforms.](http://jn.physiology.org/)

![FIG. 7. High-frequency stimulus trains elicit Ca²⁺ spikes in the presence of competitive NMDA-receptor blocker, APV, but not in the presence of non- competitive NMDA-receptor blocker, MK801. Top: in the absence of APV, the Ca²⁺ spike was evoked reliably by either single stimulus pulses (left) or a train of 4 stimuli delivered 10 ms apart (right). Middle: after perfusion of APV to isolate non-NMDA EPSPs, single stimulus pulses (left) failed to elicit Ca²⁺ spikes but train stimulation (right) elicited the Ca²⁺ spikes. Bottom: after perfusion of MK801 to isolated non-NMDA EPSPs, both single pulse (left) and train stimulation (right) failed to elicit the Ca²⁺ spikes. Top traces: raw waveforms; bottom traces: differentiated waveforms. Numbers to the left of waveforms are stimulus intensities. Calibration bars: 20 mV and 100 ms for raw waveforms; 10 V/s and 100 ms for differentiated waveforms.](http://jn.physiology.org/)

![FIG. 8. Non-NMDA EPSPs elicit Ca²⁺ spikes under conditions of potassium channel blockade. Left: Ca²⁺ spikes elicited in presence of BMI using Cs⁺ in the internal electrode solution. Cesium resulted in marked prolongation of the decay of the synaptic potential and Ca²⁺ spike. Right: in presence of APV (50 μM), non-NMDA EPSPs evoked by single stimulation elicited Ca²⁺ spike. Calibration bars: 30 mV and 500 ms.](http://jn.physiology.org/)
comparison to the effect of Cs\(^+\). In addition, we were able to elicit an analogous Ca\(^{2+}\)-mediated phenomenon in the absence of QX-314, using instead superfused TTX and exogenously applied glutamate.

Colocalization of NMDA receptors (but not non-NMDA receptors) with certain types of VDCCs also might provide a mechanism for preferential activation of VDCCs by NMDA transmission. A recent immunolabeling study examining the subcellular locations of glutamate receptor subunits in lateral and basolateral amygdala found a different distribution pattern between NMDA and non-NMDA-receptor subunits (Farb et al. 1995). Whereas most (~75%) of the labeled dendritic NMDA-receptor subunits were found in spines, the majority (~59%) of labeled non-NMDA-receptor subunits were localized to the shaft. Although this distribution pattern does not preclude the possibility of colocalization of NMDA and non-NMDA receptors within single spines as has been shown in the hippocampus (Bekkers and Stevens 1989), these findings suggest that the relative contribution of NMDA and non-NMDA receptors in excitatory transmission is different across different regions of the dendritic membrane in these cells. As of yet, no studies have examined the dendritic localization of VDCCs in cells of the amygdala. However, given recent evidence of active VDCCs in dendritic spines of other cells (Jaffe et al. 1994; Mills et al. 1994; Segal 1995), it is plausible that NMDA receptors tightly colocalize with VDCCs in dendritic spines, allowing only local NMDA EPSPs to activate the VDCCs. Alternatively, non-NMDA receptors might colocalize tightly with potassium channels, keeping prolonged depolarization from activating VDCCs.

In spite of our inability to recruit the Ca\(^{2+}\) spike by isolated non-NMDA EPSPs, non-NMDA glutamate receptor antagonists clearly increased the threshold stimulus intensity required to recruit the Ca\(^{2+}\) spike (see Fig. 3). This may be because the stimulation pathway is polysynaptic, whereas a major component of excitatory neurotransmission in the amygdala is accomplished via non-NMDA receptors (Gean and Chang 1992; Rannie et al. 1991). Also, non-NMDA-receptor-mediated EPSPs enhance NMDA-receptor-mediated EPSPs by providing sufficient depolarization to relieve the Mg\(^{2+}\) block of the NMDA receptor. An interesting observation is that calcium spikes were evoked by trains of stimulation in the presence of APV but not in the presence of MK801. There are reports that LTP in the basolateral complex of the amygdala was blocked partially by a moderate concentration (50 \(\mu M\)) of APV, whereas it was fully blocked by higher concentration (100 \(\mu M\)) of APV (Huang and Kandel 1998; Maren 1995). Together with these reports, our result suggest that the action of APV on the NMDA receptor is subject to competitive displacement under conditions of high glutamate concentrations in the synapse.

Preferential activation of these channels by NMDA EPSPs is important because these Ca\(^{2+}\) currents may contribute to NMDA-dependent plasticity under normal conditions even though the Ca\(^{2+}\) spikes observed in this study may occur only in hyperexcitable states such as under blockade of GABA\(_A\) receptors. It remains to be determined whether this Ca\(^{2+}\) current might be evoked locally in the postsynaptic membrane during LTP-inducing tetanic stimulation.

In addition, because we recorded whole cell events at the soma, we cannot be certain whether non-NMDA EPSPs activate VDCCs locally in distal dendrites that simply fail to propagate to the soma or whether they might occur too temporally or spatially disparate to be integrated into a somatic spike. However, we think it is most likely that the whole cell events we recorded accurately represent local events in the distal dendrites. Studies of hippocampal LTP show that non-NMDA-dependent LTP can be elicited that is VDCC-dependent and typically requires blockade of K\(^+\) channels (Huang and Malenka 1993; Petrozzino and Connor 1994). This suggests that Ca\(^{2+}\) influx through dendritic VDCCs is sufficient to induce synaptic potentiation but that in the presence of NMDA-receptor blockade, even tetanic stimulation fails to activate these VDCCs unless K\(^+\) channels are blocked. Moreover, the observation that Ca\(^{2+}\) spikes can be triggered by non-NMDA EPSPs under these conditions might explain why certain types of NMDA-independent, but VDCC-dependent, cellular events induced by K\(^+\) channel blockers require non-NMDA EPSPs (Aniksztejn and Ben-Ari 1991; Johnston et al. 1992).

In conclusion, we have found that NMDA-receptor-mediated neurotransmission preferentially activates VDCCs in pyramidal cells of the basolateral amygdala. This finding supports the possibility that NMDA-receptor-dependent processes that require the influx of Ca\(^{2+}\), such as neuroplasticity, could be mediated by activation of VDCCs in addition to Ca\(^{2+}\) influx through the NMDA-receptor complex. Although anatomic data suggest the possibility of subcellular colocalization of NMDA receptors and VDCCs in these cells, this remains to be verified. Our data show that simply prolonging the non-NMDA-receptor-mediated EPSP depolarization with stimulus trains or cyclothiazide is insufficient to activate these spikes. This suggests that the duration of the unitary non-NMDA-receptor-mediated EPSPs in the distal dendrites may be too brief to activate distally located VDCCs in these cells. Previous studies have indicated a critical role for K\(^+\) channels in regulating these dendritic Ca\(^{2+}\) conductances (Hoffman et al. 1997). These studies, along with our data, suggest that increased input resistance in the dendrites and blockade of K\(^+\) channel-mediated repolarization are the necessary conditions for activation of VDCCs in the absence of NMDA-receptor stimulation. This model is supported by our finding that prolonging non-NMDA-receptor-mediated depolarizations by K\(^+\) channel blockade enables the triggering of the Ca\(^{2+}\) spikes.

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