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Glutamate-Mediated Extrasynaptic Inhibition: Direct Coupling of NMDA Receptors to Ca^{2+}-Activated K^+ Channels

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Summary

NMDA receptors (NMDARs) typically contribute to excitatory synaptic transmission in the CNS. While Ca^{2+} influx through NMDARs plays a critical role in synaptic plasticity, direct actions of NMDAR-mediated Ca^{2+} influx on neuronal excitability have not been well established. Here we show that Ca^{2+} influx through NMDARs is directly coupled to activation of BK-type Ca^{2+}-activated K^+ channels in outside-out membrane patches from rat olfactory bulb granule cells. Repetitive stimulation of glutamatergic synapses in olfactory bulb slices evokes a slow inhibitory postsynaptic current (IPSC) in granule cells that requires both NMDARs and BK channels. The slow IPSC is enhanced by glutamate uptake blockers, suggesting that extrasynaptic NMDARs underlie the response. These findings reveal a novel inhibitory action of extrasynaptic NMDARs in the brain.

Introduction

Glutamate evokes neuronal excitation through activation of three types of ionotropic receptors: α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid receptors (AMPARs), kainate receptors, and N-methyl-D-aspartate receptors (NMDARs). NMDARs are Ca^{2+}-permeable cation channels that depolarize neurons and contribute to excitatory synaptic transmission in the brain (McBain and Mayer, 1994). In addition, NMDAR-mediated Ca^{2+} influx is crucial for several forms of synaptic plasticity including long-term potentiation and depression (Malenka and Nicoll, 1993). It is unclear, however, if Ca^{2+} influx through NMDARs can directly alter neuronal excitability.

Ca^{2+}-activated K^+ channels play an important role in shaping neuronal function in a wide variety of central neurons (McManus, 1991; Sah, 1996). In particular, Ca^{2+}-activated K^+ channels underlie the fast and slow afterhyperpolarizations triggered by action potentials (Sah, 1996). Typically, voltage-gated Ca^{2+} channels are the primary Ca^{2+} source for activation of Ca^{2+}-activated K^+ channels (Lancaster and Nicoll, 1987; Gola and Crest, 1993; Marrion and Tavalin, 1998; Sah, 1996). However, it has been suggested that Ca^{2+} influx through NMDARs could open Ca^{2+}-activated K^+ channels in several systems. In hippocampal slices, glutamate-evoked membrane depolarization could be followed by a Ca^{2+}-dependent and K^+-mediated afterhyperpolarization (Nicoll and Alger, 1981). Similarly, NMDA application evoked a Ca^{2+}-dependent K^+ current in cultured hippocampal neurons (Zorumski et al., 1989). In lamprey spinal cord, NMDAR-driven activation of Ca^{2+}-activated K^+ channels has been proposed to play an important role in the membrane potential oscillations underlying locomotion (Wallen and Grillner, 1987; Hill et al., 1989; Grillner et al., 2001).

Although Ca^{2+}-activated K^+ channels represent a potential target for NMDAR-mediated Ca^{2+} influx, functional coupling of NMDARs and Ca^{2+}-activated K^+ channels has yet to be firmly established. To address this question, we studied the relationship between NMDARs and Ca^{2+}-activated K^+ channels in granule cells, local interneurons of the mammalian olfactory bulb.

Results

We first examined the effects of glutamate (100–200 μM) applied to granule cells in rat olfactory bulb slices. A puffer pipette was used to focally apply glutamate close to (within 50 μm) the cell bodies of granule cells. Brief application (40–100 ms) of glutamate to granule cells evoked an inward, excitatory current at hyperpolarized membrane potentials. At potentials more positive than −50 mV, this inward current was followed immediately by a slow outward current (Figure 1A). The nonlinear voltage dependence of the slow outward current was similar to that typically associated with NMDARs (McBain and Mayer, 1994). The outward current at −25 mV was abolished by 7-chlorokynurenate (7-Chlkyn, 50 μM; Figures 1B and 1C) and D-amino-5-phosphonovaleric acid (APV, 100 μM; data not shown), suggesting that NMDARs underlie the response.

A hyperpolarizing current driven by NMDARs was surprising given that NMDARs are ion channels nonselective for monovalent cations (McBain and Mayer, 1994). However, NMDARs are also highly permeable to Ca^{2+} (Mayer and Westbrook, 1987). Granule cell bodies express high levels of the high-conductance Ca^{2+}-activated K^+ channel *slo* (also termed maxi-K and BK channel) (Egan et al., 1993; Knaus et al., 1996). We next considered the possibility that the hyperpolarizing current was mediated by Ca^{2+} influx through NMDARs coupled to BK channel activation. Consistent with this hypothesis, bath application of low concentrations of the K^+ channel blocker tetraethylammonium (Hille, 1992) (TEA, 0.5–2 mM) reversibly inhibited the glutamate-evoked outward current (Figures 1B and 1C). Thapsigargin (10 μM) and ryanodine (25 μM) had no effect on the glutamate-evoked outward current (109% ± 20% of control, n = 6), suggesting that intracellular Ca^{2+} stores do not contribute to the response. However, application of a low Ca^{2+} external solution reduced markedly the outward current but did not block the initial excitatory component of the glutamate-evoked response at −25 mV (Figure 1D). The sensitivity of the outward current to low concentrations of TEA and requirement for extracellular Ca^{2+} suggested that Ca^{2+}-activated K^+ channels govern the response. Consistent with the immunola-

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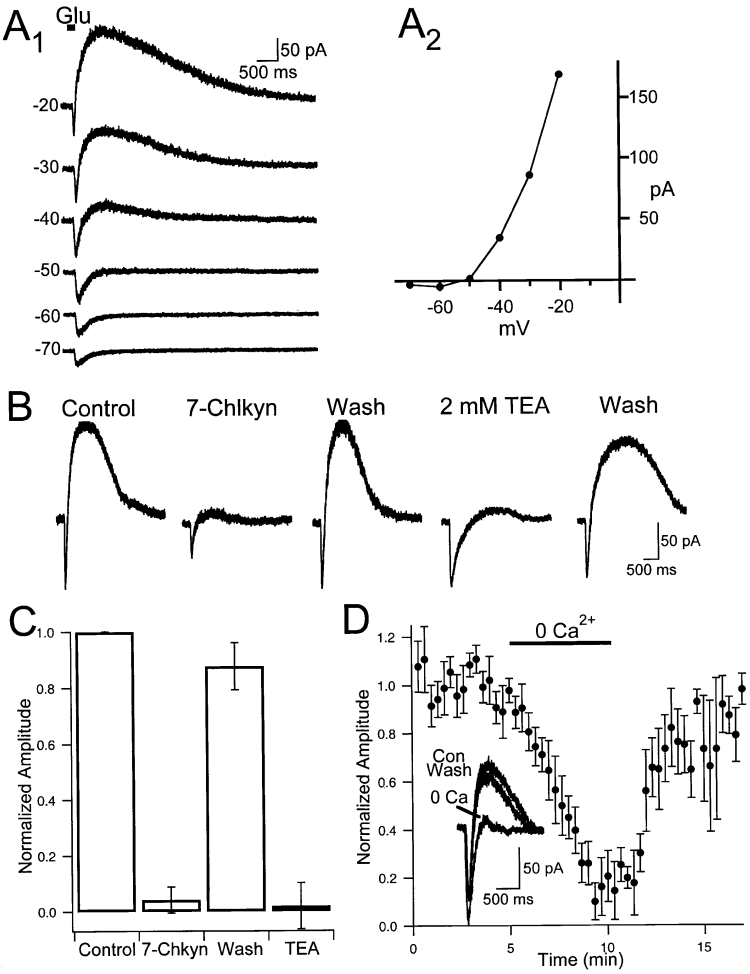

Figure 1. NMDARs Mediate an Inhibitory Current in Olfactory Bulb Granule Cells

(A_1) Glutamate (Glu, 40 ms, 100 μM) evokes an outward current at membrane potentials more depolarized than −50 mV in a granule cell.

(A_2) Current-voltage relationship of the late current shown in (A_1).

(B) 7-chlorokynurenate (7-Chlkyn, 50 μM) and TEA (2 mM) block the glutamate-evoked outward current.

(C) Summary of the actions of 7-Chlkyn (50 μM; n = 6) and TEA (1–2 mM; n = 6).

(D) Low extracellular Ca^{2+} blocks the glutamate-evoked outward current (n = 3). (Inset) Responses of a cell before (Con), during (0 Ca), and after (Wash) low Ca^{2+}.

Membrane potential (V_m) = −10 mV in (B), (C), and (D).

beling pattern of BK channels (Knaus et al., 1996), we found that glutamate generated the largest inhibitory currents when applied to the soma, while application greater than 100 μm from the soma in the dendritic region of the bulb (external plexiform layer) evoked conventional excitatory currents mediated by NMDARs (data not shown).

To confirm the nature of the channels governing the glutamate-evoked outward current, we studied the actions of the selective BK channel antagonists iberiotoxin (Galvez et al., 1990) and the nonpeptide paxilline (Sanchez and McManus, 1996). Bath application of iberiotoxin (150–250 nM) or paxilline (5–10 μM) abolished the outward current evoked by glutamate (Figures 2A and 2B) and revealed an underlying excitatory current that was abolished by NMDAR antagonists (Figure 2B).

Ca^{2+} entry through voltage-gated Ca^{2+} channels activates BK channels that contribute to action potential repolarization in central neurons (Lancaster and Nicoll, 1987; Sah, 1996). We next examined the role of voltage-gated Ca^{2+} channels in the glutamate-evoked outward current. We alternated a voltage step from −65 to 0 mV to activate Ca^{2+} channels with a brief application of glutamate at −20 mV (Figure 3A). Coapplication of nicardipine (20 μM), ω-conotoxin MVIIC (5–10 μM), and ω-conotoxin GVIA (2 μM) was used to block L-, N-, and P/Q-type Ca^{2+} channels (Randall and Tsien, 1995). The Ca^{2+} channel blockers reduced markedly the outward current evoked by the voltage step (Figure 3), indicating that voltage-gated Ca^{2+} channels can couple to Ca^{2+}-activated K^+ channels in granule cells. However, the Ca^{2+} channel antagonists failed to block the outward current generated by glutamate in the same cells (Figure 3). The glutamate-evoked inhibitory current was abolished by the subsequent application of low Ca^{2+} external solution (Figure 3B). These results indicate that voltage-gated Ca^{2+} channels do not contribute to the glutamate-evoked inhibitory current and suggest that calcium influx through excitatory NMDARs activates inhibitory BK channels in granule cells.

To examine the nature of the coupling between NMDARs and BK channels, we studied outside-out patches from granule cell somata. In low Mg^{2+} external solution, brief applications of glutamate (100 μM) evoked inward channel activity at −65 mV. Depolarization of the membrane to the NMDAR reversal potential (0 mV; Mayer and Westbrook, 1987) revealed glutamate-evoked outward channel openings (Figure 4A_1). The single channel amplitude of the glutamate-evoked currents at 0 mV averaged 6.5 ± 0.2 pA (n = 11; Figure 4A_2). Ensemble averages of recordings at −65 and 0 mV revealed similar kinetics of the glutamate-evoked re-

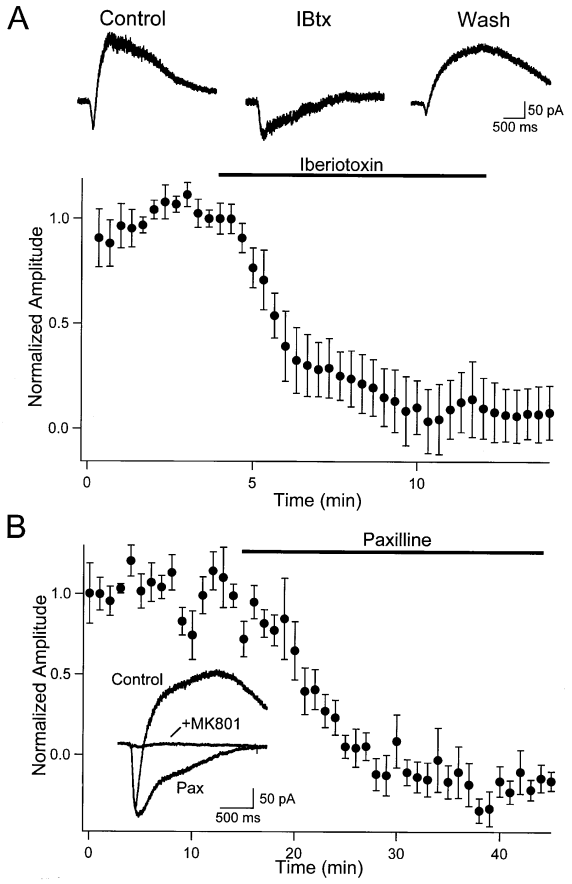

Figure 2. BK-Type Ca^{2+}-Activated K^+ Channels Underlie the Glutamate-Evoked Inhibitory Current

(A) Iberiotoxin (IBtx, 250 nM), blocks glutamate-evoked outward currents (n = 4, V_m = −10 mV). (Top) Response of one cell under control conditions, in IBtx, and following washout.

(B) Paxilline (5–10 μM) inhibits glutamate-evoked outward currents (n = 5). (Inset) Responses of a cell before (Control) and after paxilline (Pax). MK801 (20 μM) blocks the remaining NMDAR current.

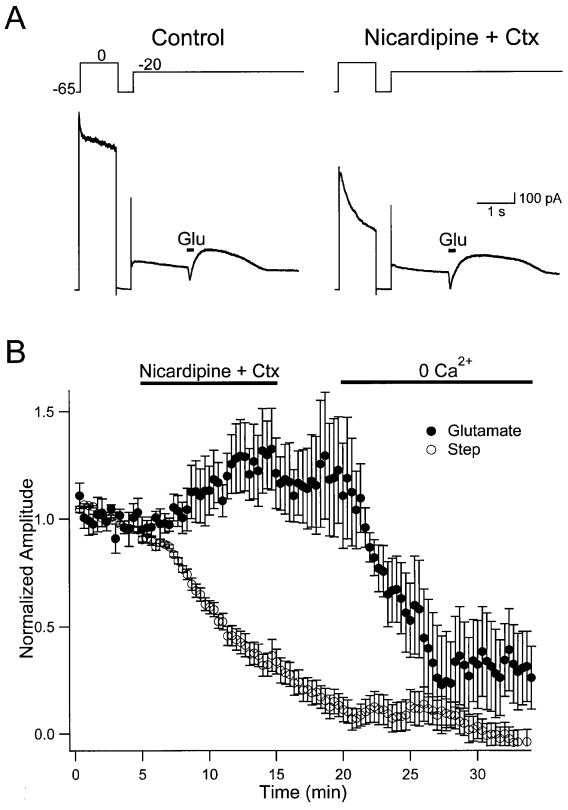

Figure 3. NMDAR-Mediated Activation of BK Channels Does Not Require Voltage-Gated Ca^{2+} Channels

(A) (Top) Voltage protocol for examining outward currents evoked by Ca^{2+} channels and glutamate application. (Bottom) Application of nicardipine (20 μM) and ω-conotoxins MVIIC (5 μM) and GVIA (2 μM) reduces the voltage step- but not the glutamate-evoked outward current in the same cell.

(B) Summary of responses to voltage steps (○) and glutamate (●) during application of Ca^{2+} channel blockers and the subsequent application of low Ca^{2+} aCSF (n = 5). Zero level of the voltage-step response is normalized to the outward current in low Ca^{2+} aCSF.

sponses at the two membrane potentials (Figure $4A_3$). We examined the rising phase of the currents by fitting them with a single exponential. The rising phases of the currents at −65 mV (19.4 ± 2.7 ms) and 0 mV (33.9 ± 10.7 ms) were not significantly different (p = 0.24, paired t test, n = 7). On average, the current at 0 mV began with a delay of 11.4 ± 5.4 ms compared to the response at −65 mV. However, the latencies of the currents at the two potentials were not significantly different (p = 0.08, n = 7). The similar kinetics of the responses suggest that the currents at both potentials are governed by a common mechanism. In agreement with this hypothesis, 7-Chlkyn (50 μM) blocked reversibly the channel activity at both −65 and 0 mV (n = 8; Figure 4B). This finding indicates that NMDARs are required for channel openings at both membrane potentials.

There is no net driving force for NMDAR current at 0 mV (the reversal potential for the current in physiological solution), making it unlikely that the large amplitude single channel events reflect current flow directly through NMDAR channels. However, the driving force for Ca^{2+} influx is high even though there is no net current flow at the reversal potential (Mayer et al., 1987). The large outward single channel currents are consistent with the large conductance (~100 pS) of BK channels in physiological external solution (Chavis et al., 1998). To confirm the nature of the channels underlying the outward activity at 0 mV, we applied iberiotoxin to the external solution. Iberiotoxin (200 nM) blocked the glutamate-evoked channels at 0 mV but did not affect the inward channel openings mediated directly by NMDARs at −65 mV (Figure 5A, n = 5). After BK channels were inhibited, depolarization from 0 to +40 mV revealed glutamate-evoked NMDAR currents that were entirely blocked by APV or 7-Chlkyn (data not shown). Paxilline (5–10 μM) had identical effects on the glutamate-evoked channel events at −65 and 0 mV (n = 4; data not shown). Removing extracellular Ca^{2+} also abolished the outward channel openings at 0 mV (n = 4; Figure 5B). At the same time, the low Ca^{2+} external solution facilitated NMDAR current at −65 mV, consistent with the channel blocking action of divalent cations (Mayer and Westbrook, 1987). These

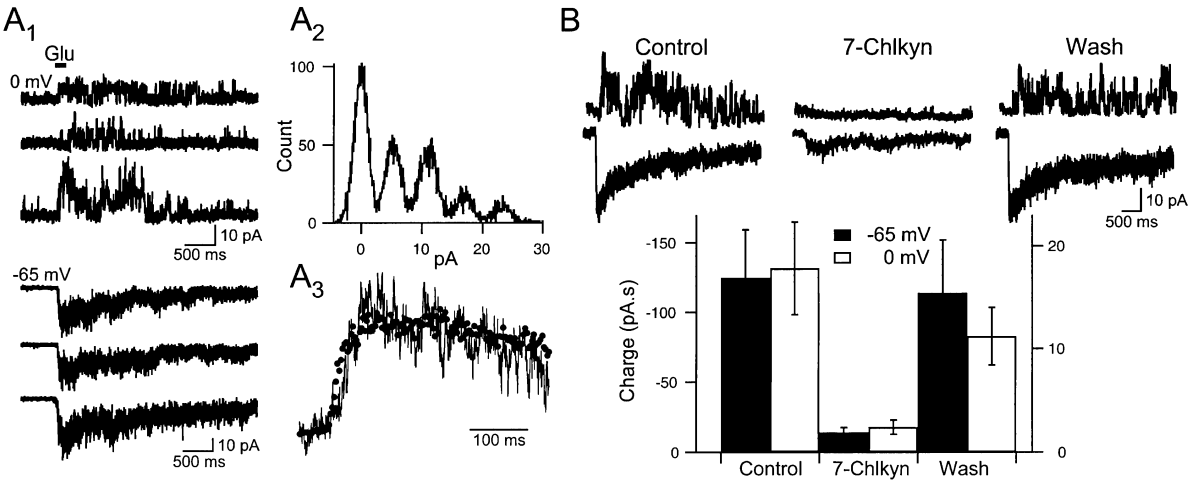

Figure 4. Glutamate Evokes Large Conductance, Outward Channel Events at the NMDAR Reversal Potential in Outside-Out Patches from Granule Cell Bodies

(A_1) Glutamate evokes channel activity at the NMDAR reversal potential (0 mV, top) and at −65mV (below).

(A_2) Histogram of the last trace at 0 mV in (A_1).

(A_3) Scaled averages of responses at −65 mV (dots) and 0 mV (line) show similar kinetics.

(B) 7-Chlkyn reversibly inhibits glutamate-evoked currents at –65 mV and 0 mV (n = 8). (Top) Sweeps at 0 mV (upper) and −65 mV (lower). The left axis represents charge at −65 mV and the right 0 mV.

results indicate that BK channels can be activated by calcium influx through NMDARs in somatic outside-out patches from granule cells.

BK channels require high levels (>1 μM) of intracellular Ca^{2+} for activation (McManus, 1991). To achieve such high local concentrations of Ca^{2+}, NMDARs must be in close proximity to BK channels (Naraghi and Neher, 1997). To determine the spatial domain of NMDAR-mediated Ca^{2+} influx, we studied the actions of calcium buffers on glutamate-evoked BK channel activation in outside-out patches. BK channel activation was unaffected when the relatively slow Ca^{2+} buffer EGTA (2–4 mM) was included in the patch pipette internal solution (Figure 5C). However, the fast buffer BAPTA (2 mM) abolished NMDAR-mediated activation of BK channels (Figure 5C). Both calcium buffers have a similar capacity for Ca^{2+}, but BAPTA has a much faster on-rate for Ca^{2+} binding (Adler et al., 1991; Naraghi and Neher, 1997). The simplest interpretation of these results is that NMDARs can be tightly coupled to BK channels within the somatic membrane of granule cells.

Does synaptically released glutamate inhibit granule

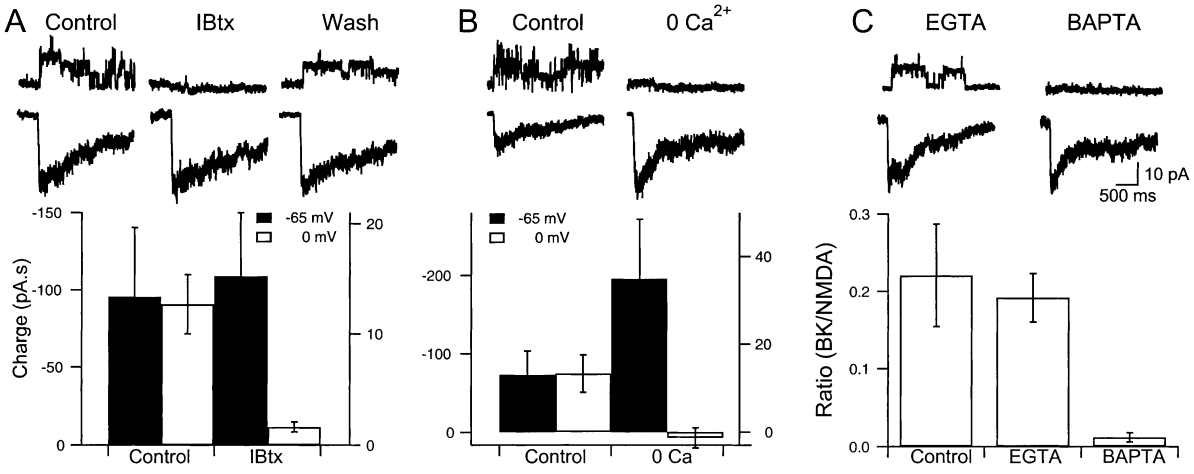

Figure 5. NMDARs Are Tightly Coupled to BK Channels in Outside-Out Patches

(A) Iberiotoxin (IBtx, 250 nM) reversibly blocks glutamate-evoked channels at 0 mV but not −65 mV (n = 5).

(B) Low Ca^{2+} aCSF facilitates the NMDAR response at –65 mV but abolishes BK current at 0 mV (n = 4).

(C) The ratio of BK to NMDAR channel activity is similar in patches recorded with low (0.2 mM, Control, n = 6) or high internal EGTA (2–4 mM, n = 6). Internal BAPTA (2 mM; n = 7) blocks coupling of NMDARs to BK channels. The left axes represents charge at −65 mV and the right 0 mV.

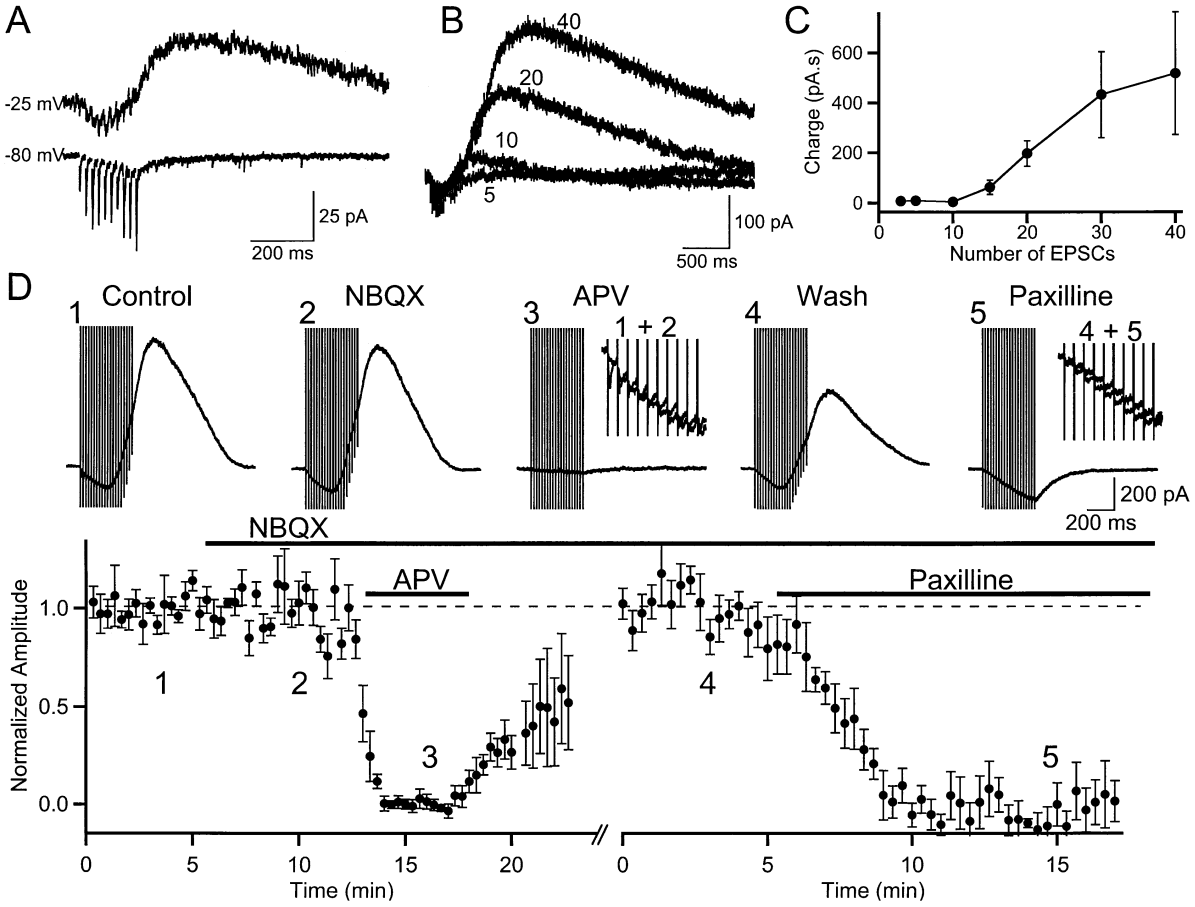

Figure 6. Synaptically Released Glutamate Evokes a Slow Inhibitory Postsynaptic Current (IPSC) in Granule Cells

(A) The response of a cell to a 50 Hz stimulus train reveals fast AMPAR EPSCs at −80 mV and a slow outward component following a small depolarizing current at −25 mV.

(B) In a representative cell (V_m = −20 mV) the amplitude of the slow IPSC increases with the number of stimuli.

(C) The amplitude of the slow IPSC as a function of stimulus number (n = 3).

(D) The slow IPSC is unaffected by NBQX (20 μM) but blocked by APV (100 μM). Following washout of APV, the slow IPSC is also abolished by paxilline (5–10 μM). Sweeps are from one cell at the times indicated on the summary plot. (Insets) The first 10 EPSCs during the train on an expanded scale before and after NBQX (1 + 2) and paxilline (4 + 5). Stimulus artifacts were digitally subtracted in (A) and (B).

cells via the coupling of NMDARs to BK channels? To address this question, we stimulated mitral cell axons that make glutamatergic synapses onto the dendritic spines of granule cells in the granule cell layer (Shepherd and Greer, 1998). Brief trains of stimuli (50 Hz, 10–40 pulses) evoked excitatory postsynaptic currents (EPSCs) at hyperpolarized potentials (−60 to −80 mV, Figure 6A) that were abolished by the AMPAR antagonist 1,2,3,4-tetrahydro-6-2,3-dioxobenzo{f}quinoxaline-7-sulfonamide (NBQX, 20 μM) and APV (100 μM; data not shown). Depolarization revealed a slow outward current in response to synaptic stimulation in granule cells (Figure 6A). Although single stimuli reliably evoked EPSCs, the slow IPSC required repetitive synaptic stimulation (Figures 6B and 6C). This suggests that the slow IPSC has a sharp dependence on the amount of glutamate released in the slice. The slow IPSC was insensitive to NBQX but abolished by APV or 7-Chlkyn (n = 6; Figure 6D). Similar to the responses to exogenously applied glutamate, paxilline (5–10 μM) blocked the slow IPSC without affecting the NMDAR EPSC (Figure 6D).

The strong dependence of the slow IPSC on the number of stimuli suggested that high concentrations of glutamate were required to generate the inhibitory response. Indeed, the lack of a slow IPSC in response to one or a few stimuli that evoke EPSCs mediated by AMPA and NMDARs also suggests that NMDARs are not coupled to BK channels on spine synapses. One possibility is that NMDARs coupled to BK channels occur at extrasynaptic locations. To address this possibility, we examined cells in which briefer trains of stimuli evoked clear NMDAR EPSCs but no slow IPSC. Under these conditions, application of the glutamate uptake blocker L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC, 200 μM) enhanced the late component of the NMDAR EPSC and produced a slow IPSC that was abolished by the subsequent application of iberiotoxin (200 nM, n = 4; Figure 7). Similar results were obtained by lowering the temperature of the bathing solution (25°C) to reduce the activity of glutamate transporters (Asztely et al., 1997) (n = 3; data not shown). The dependence of the slow IPSC on prolonged glutamate release and its regu-

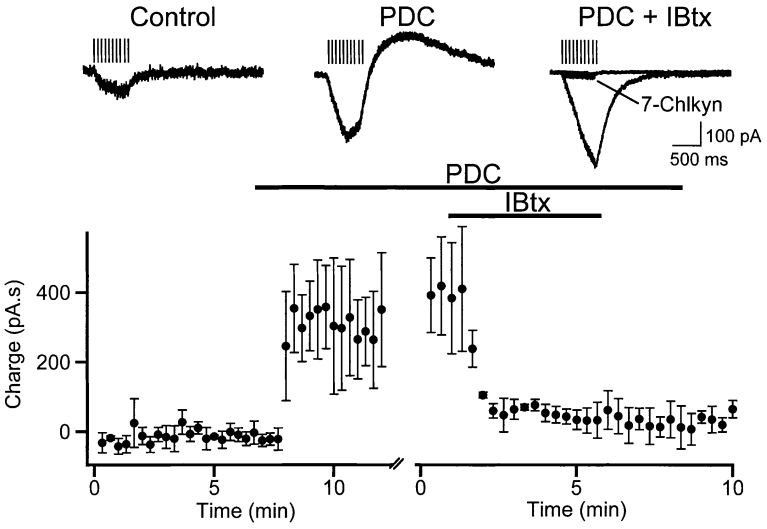

Figure 7. Blocking Glutamate Uptake Enhances the Slow IPSC

Top traces show responses to 30 stimuli that evoked a small NMDAR EPSC at −20 mV (Control) in a granule cell. Bath application of the glutamate uptake blocker PDC (200 μM) enhanced the initial excitatory NMDAR EPSC and produced a slow IPSC (PDC). The slow IPSC in the presence of PDC is blocked by iberiotoxin (PDC + IBtx, 250 nM), and the remaining EPSC is abolished by 7-Chlkyn (50 μM). Summary plot reflects results of four cells. Stimulus artifacts were digitally subtracted.

lation by glutamate transporters suggests that glutamate spillover activates NMDARs coupled to BK channels at an extrasynaptic location.

We next examined granule cells using current-clamp recording. Patch electrodes contained QX-314 (5 mM) to block Na^+-dependent action potentials and cells were depolarized to −40 mV to inactivate Ca^{2+} channels. Under these conditions, trains of stimuli (40–60 pulses at 50 Hz) evoked a depolarizing EPSP followed by an IPSP (Figure 8A). Bath application of paxilline (10 μM) abolished the IPSP, confirming that it was mediated by BK channels (n = 4).

Discussion

NMDARs mediate slow excitatory synaptic transmission throughout the CNS. Here we have described a novel inhibitory action of NMDARs in olfactory bulb interneurons. Ca^{2+} influx through NMDARs generates hyperpolarizing currents in granule cells by activating BK-type Ca^{2+}-activated K^+ channels. NMDARs are tightly coupled to BK channels in outside-out membrane patches from granule cell bodies. Furthermore, synaptically released glutamate mediates a slow IPSC governed by NMDARs and BK channels. The regulation of the slow

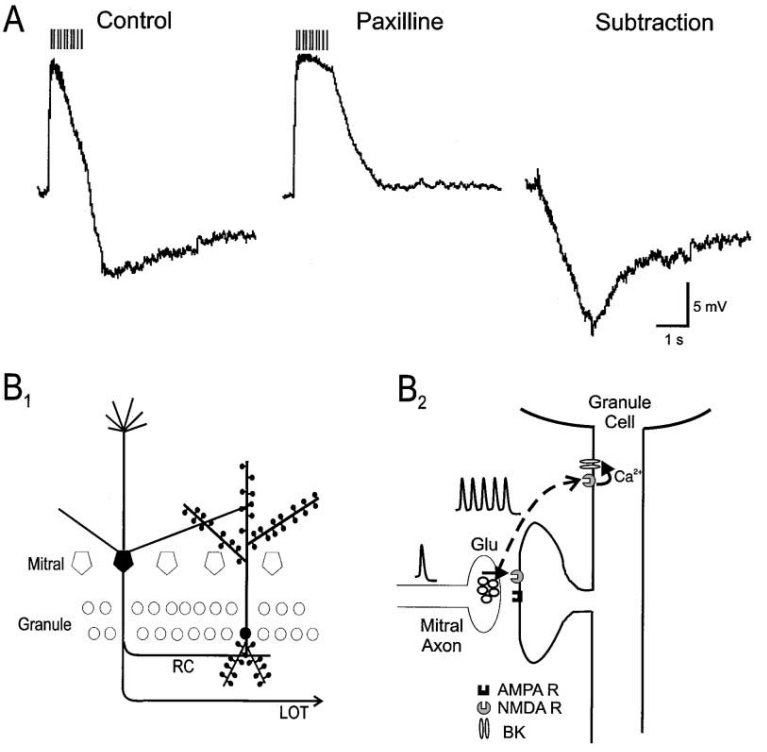

Figure 8. BK Channels Underlie an Inhibitory Postsynaptic Potential (IPSP) in Granule Cells

(A) A train of stimuli (60 pulses, 50 Hz) evokes a depolarization followed by a hyperpolarization (Control). Paxilline (10 μM) abolishes the IPSP without affecting the initial depolarization (Paxilline). Digital subtraction of the traces reveals the isolated BK channel-mediated IPSP (Subtraction). Stimulus artifacts are blanked and shown schematically above the traces.

(B_1) Schematic diagram of mitral and granule cells in a bulb slice. Mitral cell axons project out of the bulb via the lateral olfactory tract (LOT) and form recurrent collateral (RC) synapses in the granule cell layer.

(B_2) Diagram of synaptic and extrasynaptic actions of glutamate released from mitral cell axons onto the basal dendrites of granule cells. Dashed line represents "spillover" of transmitter during repetitive activation of mitral axons.

IPSC by glutamate uptake mechanisms suggests that the coupling of NMDARs and BK channels occurs at an extrasynaptic location (Figure 8B).

We have shown that exogenously applied glutamate evokes a hyperpolarizing current when applied to the somata of granule cells in olfactory bulb slices. The hyperpolarization required both NMDARs and BK channels. The NMDAR-mediated hyperpolarization did not require voltage-gated Ca^{2+} channels or release of Ca^{2+} from intracellular stores, suggesting a tight coupling to BK channels. While glutamate is the major excitatory transmitter in the brain, recent studies have revealed inhibitory actions of the transmitter mediated by metabotropic glutamate receptors (mGluRs). mGluRs govern inhibitory responses via activation of G protein-coupled inwardly rectifying $K+$ channels in the rat cerebellum (Knoflach and Kemp, 1998) and forebrain of songbirds (Dutar et al., 2000). mGluRs have also been found to inhibit central neurons via activation of Ca^{2+}-activated K^+ channels (Chavis et al., 1998; Fiorillo and Williams, 1998; Holmes et al., 1996). However, this action of mGluRs requires second messenger mobilization of Ca^{2+} from intracellular stores and may also involve L-type Ca^{2+} channels (Fagni et al., 2000). In contrast to mGluR-mediated inhibition, NMDAR-mediated inhibition in the olfactory bulb is much less complex and simply requires tight coupling of NMDAR Ca^{2+} influx to BK-type Ca^{2+}-activated K^+ channels.

We found that NMDARs and BK channels are colocalized in outside-out membrane patches from the cell bodies of granule cells. Furthermore, while the fast Ca^{2+} chelator BAPTA prevented NMDAR-mediated Ca^{2+} influx from activating BK channels, the slow buffer EGTA was without effect. These results indicate that Ca^{2+} influx through NMDARs activates BK channels within a small spatial domain. Glutamate always activated more NMDARs than BK channels in individual patches, suggesting that there is not a one-to-one relationship in their functional coupling. One possibility is that only a fraction of NMDARs on the granule cell soma can activate BK channels. Alternatively, Ca^{2+} influx through several neighboring NMDARs may be required to generate sufficiently high concentrations of Ca^{2+} to activate BK channels. Although we cannot distinguish between these two possibilities, the BAPTA sensitivity of BK channel activation suggests that such a Ca^{2+} microdomain (Naraghi and Neher, 1997) must be highly localized (on the order of nanometers).

In addition to inhibitory responses to exogenously applied glutamate, we have also shown a slow IPSC mediated by NMDARs and BK channels in granule cells. Collateral branches of mitral cell axons synapse onto the basal dendritic spines of granule cells and represent the most likely source of synaptically released glutamate (Shepherd and Greer, 1998); however, we cannot exclude a contribution of glutamatergic fibers from other bulb neurons in these experiments.

The slow IPSC mediated by NMDARs was only invoked during repetitive activation of glutamatergic nerve endings. The lack of a slow IPSC in response to single stimuli indicates that synaptic NMDARs are not functionally coupled to BK channels. Furthermore, the slow IPSC was greatly facilitated by blockade of active glutamate uptake. Together, these findings suggest that the slow IPSC occurs at a site that is distant from synaptic contacts. Thus, the slow IPSC is mediated by extrasynaptic NMDARs in close proximity to BK channels. Glutamate spillover can mediate excitatory transmission between mitral cell dendrites (Isaacson, 1999), and the slow IPSC in granule cells suggests that glutamate spillover from mitral cell axons also governs inhibition of granule cells. Granule cell somata show high levels of BK channel expression (Knaus et al., 1996) and represent the most likely target for glutamate spillover from nearby dendritic spines.

Our results suggest that glutamate spillover from synapses on granule cells occurs during high-frequency activity when elevated concentrations of transmitter overwhelm active transport. Thus, the temporal dynamics of transmitter release can alter the relative degree of excitation or inhibition mediated by NMDARs. This temporal dependence may be of particular importance in the olfactory bulb, where oscillatory activity has been suggested to contribute to odor coding (Freeman, 1983; Laurent, 1999). Given the widespread expression of both NMDARs (Petralia et al., 1994) and BK channels (Knaus et al., 1996) in central neurons, the degree of coupling of the two channel types may play an important role in shaping the actions of glutamate in diverse brain regions.

Experimental Procedures

Horizontal slices (300 μm) of the olfactory bulb were prepared from 14- to 28-day-old Sprague-Dawley rats and viewed under differential contrast optics (BX50, Olympus). Slices were superfused with an artificial cerebrospinal fluid (aCSF) containing (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose, and 0.1 picrotoxin, which was equilibrated with 95% O_2/5% CO_2. All experiments except those examining synaptic responses were performed in the presence of tetrodotoxin (TTX, 1 μM). Mg^{2+} was omitted from the aCSF used for outside-out patch experiments and cytochrome C (0.5 mg/ml) was added to the aCSF in experiments using conotoxins. Whole-cell recordings were performed at 31°C–33°C, and outside-out patch experiments were performed at room temperature. Glutamate was applied locally to granule cells and outside-out patches via a puffer pipette (Picospritzer, Parker). Synaptic responses were evoked via a bipolar stimulating electrode placed in the granule cell layer.

Patch electrodes (3–5 MΩ) contained (in mM): 125 $KMeSO_4$, 4 KCl, 10 HEPES, 10 Phosphocreatine, 3 MgATP, and 0.5 NaGTP. For whole-cell recordings, EGTA was 0.2–2 mM. In some outside-out patch experiments, EGTA was replaced by BAPTA (2–4 mM). Series resistance, which was always $<$10 MΩ, was routinely compensated by $>$90%. Voltage-clamp responses were recorded with an Axopatch-200B (Axon Instruments, Foster City, CA). All responses were filtered at 2 KHz and digitized at 5 KHz (ITC-18; Instrutech, Mineola, NY). Data were collected and analyzed using Axograph (Axon Instruments) and IGOR Pro (Wavemetrics, Lake Oswego, OR). The amplitudes of glutamate- and synaptically evoked currents were quantified by integrating the response during the 2–4 s following stimulation. Unless indicated otherwise, all traces represent the average of five to ten trials. Summary data are expressed as mean $\pm$ SEM.

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