NMDA-Dependent Currents in Granule Cells of the Dentate Gyrus Contribute to Induction but Not Permanence of Kindling

ÜMİT SAYIN,[1,4] PAUL RUTECKI,[1,3,4] AND THOMAS SUTULA[1–3]
[1]*Departments of Neurology and* [2]*Anatomy, and* [3]*The Neuroscience Training Program, University of Wisconsin, and the* [4]*William S. Middleton Veterans Administration Hospital, Madison, WI 53792*

Sayin, Ümit, Paul Rutecki, and Thomas Sutula. NMDA-dependent currents in granule cells of the dentate gyrus contribute to induction but not permanence of kindling. *J. Neurophysiol.* 81: 564–574, 1999. Single-electrode voltage-clamp techniques and bath application of the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovaleric acid (APV) were used to study the time course of seizure-induced alterations in NMDA-dependent synaptic currents in granule cells of the dentate gyrus in hippocampal slices from kindled and normal rats. In agreement with previous studies, granule cells from kindled rats examined within 1 wk after the last of 3 or 30–35 generalized tonic-clonic (class V) seizures demonstrated an increase in the NMDA receptor–dependent component of the perforant path–evoked synaptic current. Within 1 wk of the last kindled seizure, NMDA-dependent charge transfer underlying the perforant path–evoked current was increased by 63–111% at a holding potential of −30 mV. In contrast, the NMDA-dependent component of the perforant-evoked current in granule cells examined at 2.5–3 mo after the last of 3 or 90–120 class V seizures did not differ from age-matched controls. Because the seizure-induced increases in NMDA-dependent synaptic currents declined toward control values during a time course of 2.5–3 mo, increases in NMDA-dependent synaptic transmission cannot account for the permanent susceptibility to evoked and spontaneous seizures induced by kindling. The increase in NMDA receptor–dependent transmission was associated with the induction of kindling but was not responsible for the maintenance of the kindled state. The time course of alterations in NMDA-dependent synaptic current and the dependence of the progression of kindling and kindling-induced mossy fiber sprouting on repeated NMDA receptor activation are consistent with the possibility that the NMDA receptor is part of a transmembrane signaling pathway that induces long-term cellular alterations and circuit remodeling in response to repeated seizures, but is not required for permanent seizure susceptibility in circuitry altered by kindling.

INTRODUCTION

Seizures induce long-term cellular alterations in the hippocampus that include neuronal loss and mossy fiber sprouting in the dentate gyrus accompanied by alterations in the sequence of synaptic activation (Golarai and Sutula 1996b), in excitatory connectivity (Wuarin and Dudek 1996), evolving changes in inhibition (Buhl et al. 1996; Otis et al. 1994; Rutecki et al. 1996), increased susceptibility to recurrent seizures (kindling) (Goddard et al. 1969), and development of memory deficits in behavioral tasks that depend on the integrity of hippocampal pathways (Sutula et al. 1995). Recent studies have demonstrated that the induction of mossy fiber sprouting and the progression of kindling in response to repeated seizures are dependent on repeated activation of *N*-methyl-D-aspartate (NMDA) receptors (Sutula et al. 1996). Because NMDA receptors play a critical role in the induction of these seizure-induced cellular alterations in response to kindling, and NMDA-dependent gene expression may play a role in the development of long-term structural and functional alterations induced in hippocampal circuitry (Sprengel et al. 1998), it was of interest to examine in further detail how NMDA receptor–dependent synaptic transmission contributes to the induction and permanence of the kindled state.

NMDA and non-NMDA receptors play distinct roles in synaptic transmission and in the induction of a variety of forms of synaptic and circuit plasticity in the CNS. The NMDA receptor–gated channels typically contribute only minimally to synaptic transmission at membrane potentials in the range of −60 to −75 mV because of voltage-dependent block of the channel by Mg^{2+}. With depolarization of the membrane to −30 to −40 mV, the Mg^{2+} block is relieved, and NMDA-gated channels become permeable to Ca^{2+}, Na^{+}, and K^{+}, which results in a slowly developing, long-duration synaptic current. The influx of Ca^{2+} into the postsynaptic cell triggers a variety of second-messenger pathways, which can activate transcription factors such as c-fos, and may induce changes in gene expression that could play a role in the induction of long-term seizure-induced cellular alterations in the dentate gyrus (McNamara 1995). Repeated activation of the NMDA receptor during kindling plays a critical role in the progression of kindling and the induction of mossy fiber sprouting (Sutula et al. 1996).

As in many pathways of the CNS, fast synaptic transmission between axon terminals of the perforant path and the granule cells of the dentate gyrus is mediated primarily by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, but there is also a significant contribution by NMDA receptors under normal conditions (Keller et al. 1991; Lambert and Jones 1990). Previous studies have demonstrated that the initial high-frequency stimulation that induces kindled seizures increases the NMDA-dependent component of synaptic transmission in granule cells (Mody et al. 1988). The increase in NMDA-dependent synaptic transmission persists for at least 1 mo after stimulation (Mody et al. 1988), but it has not been clear whether the permanent increase in hippocampal excitability induced by kindling is due to a permanent increase in NMDA-dependent synaptic transmission, or might be caused by permanent seizure-induced cellular alterations such as neuronal loss or sprouting (Bengzon et al. 1997; Cavazos and

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TABLE 1. *Stimulus intensities and peak evoked currents at −70 mV*

	1–7 Days After 3 Class V Seizures	Age-Matched Normal Control	2.5–3 Months After 3 Class V Seizures	Age-Matched Normal Control	1–7 Days After 30–35 Class V Seizures	Age-Matched Normal Control	2.5–3 Months After 90–120 Class V Seizures	Age-Matched Normal Control
n	22	14	17	10	11	8	16	12
Stimulus intensity, μA	98.1 ± 15.8	114 ± 24.8	125.7 ± 31.8	180 ± 34	91.6 ± 18.8	211.6 ± 32.6	132 ± 41	84 ± 16.3
Peak current in nA at −70 mV	−1.7 ± 0.1	−1.25 ± 0.16	−1.58 ± 0.06	−1.06 ± 0.14	−1.04 ± 0.1	−0.92 ± 0.05	−1.2 ± 0.16	−0.82 ± 0.09

Values are means ± SE; *n* is number of cells. All differences not significant.

Sutula 1990; Cavazos et al. 1991, 1994; Sutula et al. 1988). The aim of this study was to address these possibilities by examining NMDA receptor–dependent currents in granule cells of the dentate gyrus during the induction of kindling, and at long intervals after the last evoked seizure in the fully kindled state. A preliminary report has been presented in abstract form (Sayin et al. 1996).

METHODS

Kindling procedures

Male Sprague Dawley rats (250–300 g) were anesthetized with a combination of ketamine (80 mg/kg im) and xylazine (10 mg/kg im) and were stereotaxically implanted with chronic electrodes for stimulation and recording in the olfactory bulb (9 mm anterior, 1.2 mm lateral, 1.8 mm ventral to bregma), amygdala (1.5 mm posterior, 4.2 mm lateral, 8.8 mm ventral to bregma), or angular bundle (8.1 mm posterior, 4.4 mm lateral, and 3.5 mm ventral to bregma). The electrodes were fixed to the skull with screws and dental acrylic. After a 2-wk recovery period following electrode placement, the unrestrained awake animals in the kindling group received twice daily kindling stimulation (5 days per week) with a 1-s train of 62-Hz biphasic constant-current 1.0-ms square-wave pulses. The stimulation was delivered at the lowest intensity that evoked an afterdischarge (AD) according to standard procedures (Cavazos et al. 1994). The electroencephalogram and AD were recorded from the bipolar electrode, which could be switched to the stimulator by a digital circuit for the delivery of the kindling stimulation. The evoked behavioral seizures were classified according to standard criteria (Sutula and Steward, 1986). Rats were studied within 1 wk of 3 or 30–35 evoked class V seizures, or at 2.5–3 mo after 3 or >90 evoked generalized tonic clonic (class V) seizures.

Preparation of hippocampal slices

The rats were anesthetized with pentobarbital sodium (60 mg/kg ip), and the brain was rapidly removed and transferred to iced normal saline. The hippocampus was dissected and cut in the transverse plane at a thickness of 400–450 μm using a McIllwain tissue chopper. The slices were maintained in interface chamber superfused with oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (ACSF) at 32–36°C. The ACSF composition (in mM) was 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose. The slices were allowed to equilibrate in ACSF for 2 h before recording. Recordings were obtained in 10 μM bicuculline methiodide (Sigma) to block γ-aminobutyric acid-A ($GABA_A$) receptor–mediated inhibition.

Stimulation and recording procedures

Intracellular recordings in granule cells of the dentate gyrus were evoked by stimulation of perforant path fibers in the outer molecular layer of the dentate gyrus in hippocampal slices from kindled rats and age-matched controls. The recordings were obtained using glass microelectrodes (impedances of 25–40 MΩ) filled with cesium acetate to block outward K^+ currents and QX-314 to block action-potential generation. The outer molecular layer was stimulated every 20 s by a stainless steel bipolar electrode that delivered constant-current pulses of 0.05 ms duration at a range of intensities from 40 to 200 μA. Recordings were obtained with an Axoclamp 2B amplifier in the single-electrode voltage-clamp mode at a switching frequency of 3–5 kHz. A separate oscilloscope was used to adjust capacitance compensation and sampling rate. Synaptic currents were recorded at holding potentials from −70 to +30 mV in 10-mV steps in response to a perforant path stimulus. The stimulus intensity was adjusted to evoke a synaptic current of at least 0.5 nA with an apparent monoexponential decay at a holding potential of −70 mV. Recordings that demonstrated voltage-dependent contaminants such as regenerative currents indicated by escape voltage transients, failure to reverse near 0 mV, or clear polysynaptic components were excluded. The evoked intracellular potentials or currents were amplified and displayed on an oscilloscope. The evoked currents were recorded, stored, and analyzed using a DIGIDATA 1200 AD converter (Axon Instruments) and PCLAMP 6.02. Clampex 6.02 was used for stimulus generation and data collection. Clampfit 6.02 was used for analysis of 10–90% rise time constants, decay time constants, and signal analysis.

Data analysis and statistics

Voltage-clamp recordings were made from 44 granule cells in normal rats and 66 granule cells in kindled rats (Table 1). Because not every cell was subjected to the same sequence of measurements and bathing conditions, the number of cells for specific observations are provided separately in the text and figures. Charge transfer was measured as the integrated area under the curve of evoked synaptic current from the stimulus to 100 ms, when the inward current had typically decayed nearly to baseline (see Fig. 3 for additional details of the calculation). Differences in current amplitude, charge transfer, and current-voltage (*I-V*) relationships were evaluated for statistical significance with the Student's *t*-test when the data were normally distributed, or the Mann-Whitney rank sign test. When multiple comparisons were required, one-way analysis of variance (ANOVA) followed by Dunnett's test or the Newman-Keuls test were used post hoc for paired comparisons.

RESULTS

Synaptic currents recorded from granule cells in the dentate gyrus (DGCs) of hippocampal slices obtained from kindled rats within 1–7 days after 3 or 30–35 class V seizures ($n = 33$), and at 2.5–3.0 mo after 3 or 90–120 class V seizures ($n = 33$), were compared with currents in age-matched, normal controls ($n = 44$, see Table 1). There were no significant differences in

TABLE 2. *Kinetic features and reversal potentials of synaptic currents*

	10–90% Rise Time at −70 mV, ms	Decay Tau at −70 mV, ms	10–90% Rise Time at −30 mV, ms	Decay Tau 1 at −30 mV, ms	Decay Tau 2 at −30 mV, ms	Reversal Potential, mV
Normal	2.7 ± 0.1	4 ± 0.28	3.5 ± 0.15	6 ± 0.8	30 ± 2.76	1.1 ± 0.56
Acute kindled	2.48 ± 0.08	4.56 ± 0.3	4.09 ± 0.24*	6.2 ± 0.86	32.3 ± 2.8	1.6 ± 0.84
2–3 mo after the last seizure	2.67 ± 0.09	4.57 ± 0.34	4.02 ± 0.33	6.08 ± 1.04	26.4 ± 1.85	1.34 ± 0.9

Values are means ± SE. * Versus normal, $P = 0.035$, Student's *t*-test. All other differences across groups not significant, analysis of variance.

stimulus intensities required to evoke currents in kindled and control groups, but there was a trend toward slightly lower stimulation intensities required to evoke currents in the granule cells from kindled rats as compared with age-matched controls (Table 1).

Perforant path–evoked currents in granule cells from normal rats

In DGCs from normal rats at a holding potential of −70 mV, inward current was evoked by a perforant path stimulus at a latency of 1–2 ms. The rise time and decay time of the evoked inward current at holding potentials from −70 to 0 mV (see Table 2) were comparable with previously published studies for granule cells from rats (Keller et al. 1991) and resected human dentate gyrus (Isokawa et al. 1997). The duration of the inward current was longer at holding potentials positive to −50 mV (see Fig. 1*A*). The reversal potential of the evoked inward current was 1.1 ± 0.56 (SE) mV and did not differ from kindled groups (see Table 2). In agreement with previous studies (Isokawa et al. 1997; Keller et al. 1991; Lambert and Jones 1990), a component of the inward current evoked by perforant path stimulation was blocked by 50 μM d-2-amino-5-phosphovalerate (APV) and was therefore NMDA receptor dependent (Fig. 1*A*).

I-V plots and calculation of charge transfer before and after bath application of 50 μM APV were used to characterize the timing and voltage dependence of the NMDA component of the perforant path–evoked inward current in DGCs from normal rats. For the granule cell from the normal rat illustrated in Fig. 1*A*, the *I-V* relationship measured at 20 ms after the stimulus (Fig. 1*B*) and evoked charge transfer (Fig. 1*C*) demonstrated an APV-sensitive inward current recorded at holding potentials of −40 to −30 mV. Synaptic charge transfer was calculated before and after application of 50 μM APV (see Fig. 3*B* for additional details of the calculation). The APV-sensitive component of evoked synaptic charge transfer increased at holding potentials positive to −50 mV, reached a maximum at −30 to −20 mV, and was not detected at 0 mV (Fig. 1*C*).

Perforant path–evoked currents in granule cells from kindled rats

DGCs from kindled rats ($n = 22$) studied within 1 wk after the last of three generalized tonic clonic (class V) seizures evoked by kindling stimulation of the olfactory bulb demonstrated larger inward synaptic current evoked by perforant path stimulation compared with DGCs from normal rats ($n = 14$, compare Figs. 1*A* and 2*A*). These results confirmed previous observations that kindling induced by stimulation of a variety of limbic sites, including the amygdala and hippocampal commissures (Kohr et al. 1993; Kohr and Mody 1994; Mody et al. 1988), increases the NMDA-dependent component of synaptic transmission in DGCs. Except for an increase in the 10–90% rise time of the inward current evoked at −30 mV in granule cells from kindled rats studied at 1–7 days after the last seizure, there were no significant differences in rise times or decay times between kindled and control groups (see Table 2).

The *I-V* plot at 20 ms for the DGC from a kindled rat in Fig. 2*A* demonstrated a prominent APV-dependent region of negative slope conductance between −50 and −10 mV compared with the DGC from a normal rat (compare Figs. 1*B* and 2*B*). Calculation of evoked charge transfer also revealed a prominent APV-sensitive, voltage-dependent increase in charge transfer between −40 and −10 mV (compare Figs. 1*C* and 2*C*). These results were consistent with an increase in the NMDA-dependent component of the inward current evoked in DGCs from kindled rats and confirmed previous observations (Mody et al. 1988). Within 1 wk after the last of 30–35 class V seizures in DGCs ($n = 11$) from kindled rats, there were also significant increases in the amplitude and duration of the inward synaptic currents and the NMDA-dependent component of the inward current evoked at 20 ms and a holding potential of −30 mV compared with DGCs from normal rats ($n = 8$, data not shown).

Because the NMDA receptor–dependent current evoked in granule cells by perforant path stimulation has a long duration and is voltage dependent, the ratio of the inward current at 20 ms to peak inward current at a holding potential of −30 mV has been used as a measure of the NMDA component of the inward evoked current (Keller et al. 1991). The ratio of the inward current at 20 ms to peak inward current at a holding potential of −30 mV was also used in this study to compare seizure-induced alterations in NMDA-dependent synaptic currents in normal and kindled rats (Fig. 3*A*). This measure was compared with the NMDA receptor–dependent charge transfer in DGCs from kindled and normal rats (Fig. 3, *B* and *C*). The area under the inward synaptic current waveform from the time of stimulus to 100 ms was measured before and after application of 50 μM APV, and the calculated difference was the NMDA-dependent charge transfer (Fig. 3*B*). In DGCs from both normal and kindled rats, there was a significant correlation between the ratio of the inward current at 20 ms to the peak inward current and the NMDA receptor–dependent charge transfer at a holding potential of −30 mV ($r = 0.686$, $P < 0.0001$, Fig. 3*C*). There were also linear relationships between the ratio of inward current at 20 ms to peak inward current (expressed as a percent) and the NMDA-dependent charge transfer in granule cells from normal rats ($r = 0.425$, $P < 0.05$, ○, Fig. 3*C*) and kindled rats ($r = 0.8$, $P < 0.0001$, ●, Fig. 3*C*).

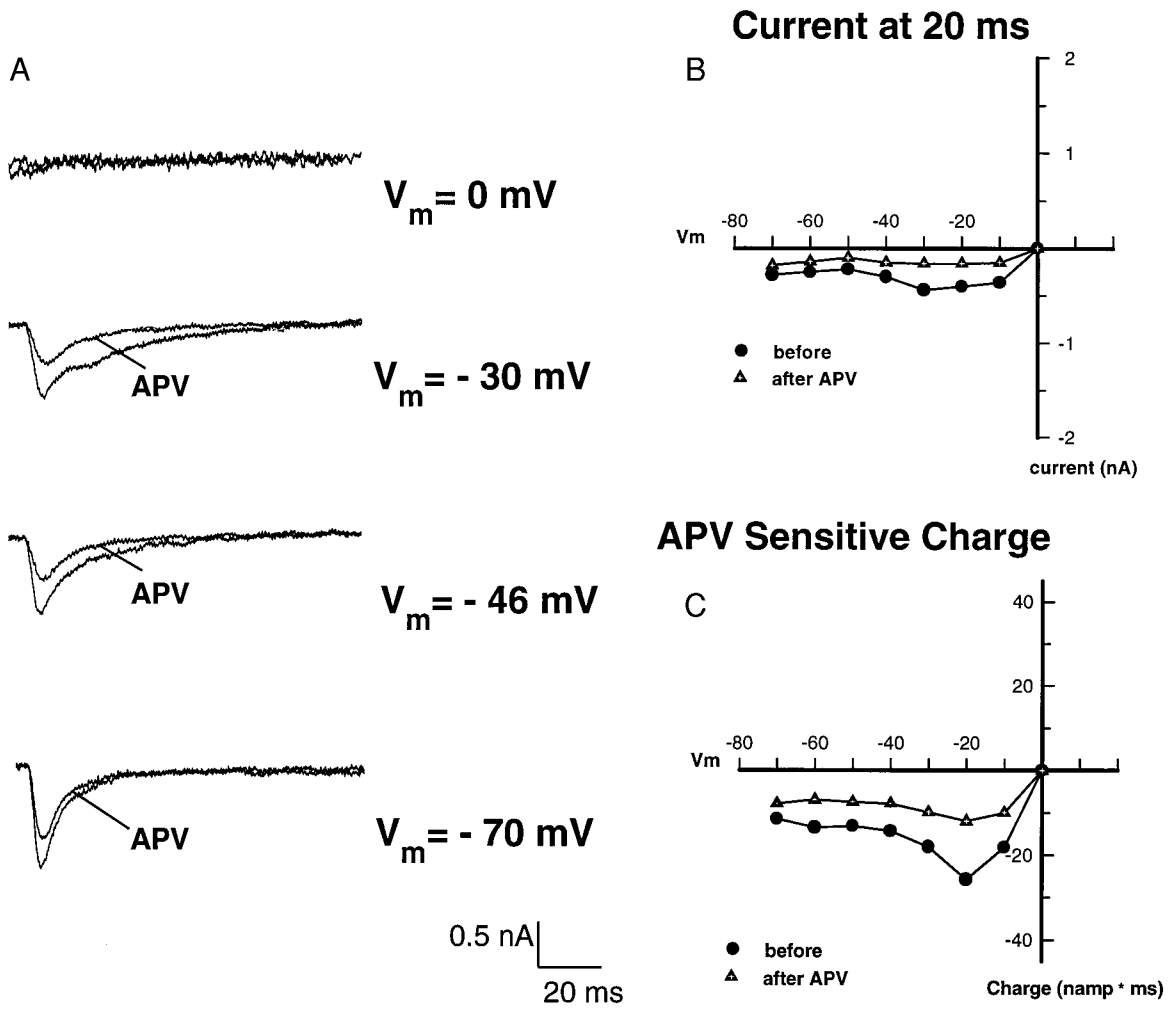

FIG. 1. Synaptic currents evoked by perforant path stimulation in granule cells from normal rats. *A*: representative example of synaptic currents evoked in a granule cell by perforant path stimulation in a hippocampal slice from a normal rat. In this cell, the holding membrane potential (V_m) was varied in steps from −70 to 0 mV. In granule cells from normal rats, the duration of the inward current was longer at holding potentials positive to −50 mV. Bath application of the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovaleric acid (APV; 50 μM) reduced the amplitude and duration of the inward current and demonstrated that a substantial portion of the inward current at holding potentials positive to −50 mV was NMDA receptor dependent. *B*: current-voltage (*I-V*) plot at 20 ms after perforant path stimulation derived from the evoked currents in *A* demonstrates a small region of negative slope conductance at a holding potentials of −40 to −10 mV. *C*: to further evaluate the magnitude of the NMDA component of the synaptic current, synaptic charge transfer was calculated before and after bath application of 50 μM APV. Charge transfer was calculated by measuring the area under the curve of inward current from the onset of the stimulus to 100 ms (see METHODS and Fig. 3*B* for details of calculation). Charge transfer curves for the currents illustrated in *A* demonstrated an increase in the APV-sensitive charge transfer at a holding potential of −30 mV, which reached a maximum at −20 mV.

There was a significant increase in the ratio of the inward current at 20 ms to peak inward current at a holding potential of −30 mV within 1–7 days after the last of 3 or 30–35 class V seizures compared with controls ($n = 33$, $P < 0.001$, Fig. 4, *A* and *B*). Significant increases in the ratio of the inward current at 20 ms to peak inward current at a holding potential of +20 mV were also observed within 1 wk after the last of 3 class V seizures ($P < 0.05$, Fig. 4*A*) and 30–35 class V seizures ($P < 0.001$, Fig. 4*B*). Significant increases in NMDA-dependent charge transfer were observed in DGCs at holding potentials of −40, −30, and −20 mV within 1 wk after the last of 3 or 30–35 class V seizures ($n = 14$) compared with DGCs from normal controls ($n = 12$, Fig. 4, *C* and *D*).

Perforant path–evoked currents in granule cells at 2.5–3.0 mo after kindled seizures

The increase in the NMDA-dependent component of the inward current of granule cells from kindled rats was not permanent. The amplitude, duration, voltage sensitivity, and APV sensitivity of the inward currents evoked in granule cells by perforant path stimulation in hippocampal slices obtained at 2.5–3 mo after the last of 3 or 90–120 class V seizures did not differ from granule cells in age-matched normal rats (Fig. 5, *A* and *B*). This effect was not dependent on the site of kindling stimulation and was observed in rats that received kindling stimulation delivered to the olfactory bulb ($n = 11$), perforant path ($n = 4$), and amygdala ($n = 1$). There were no significant differences in the ratio of the inward current at 20 ms to peak

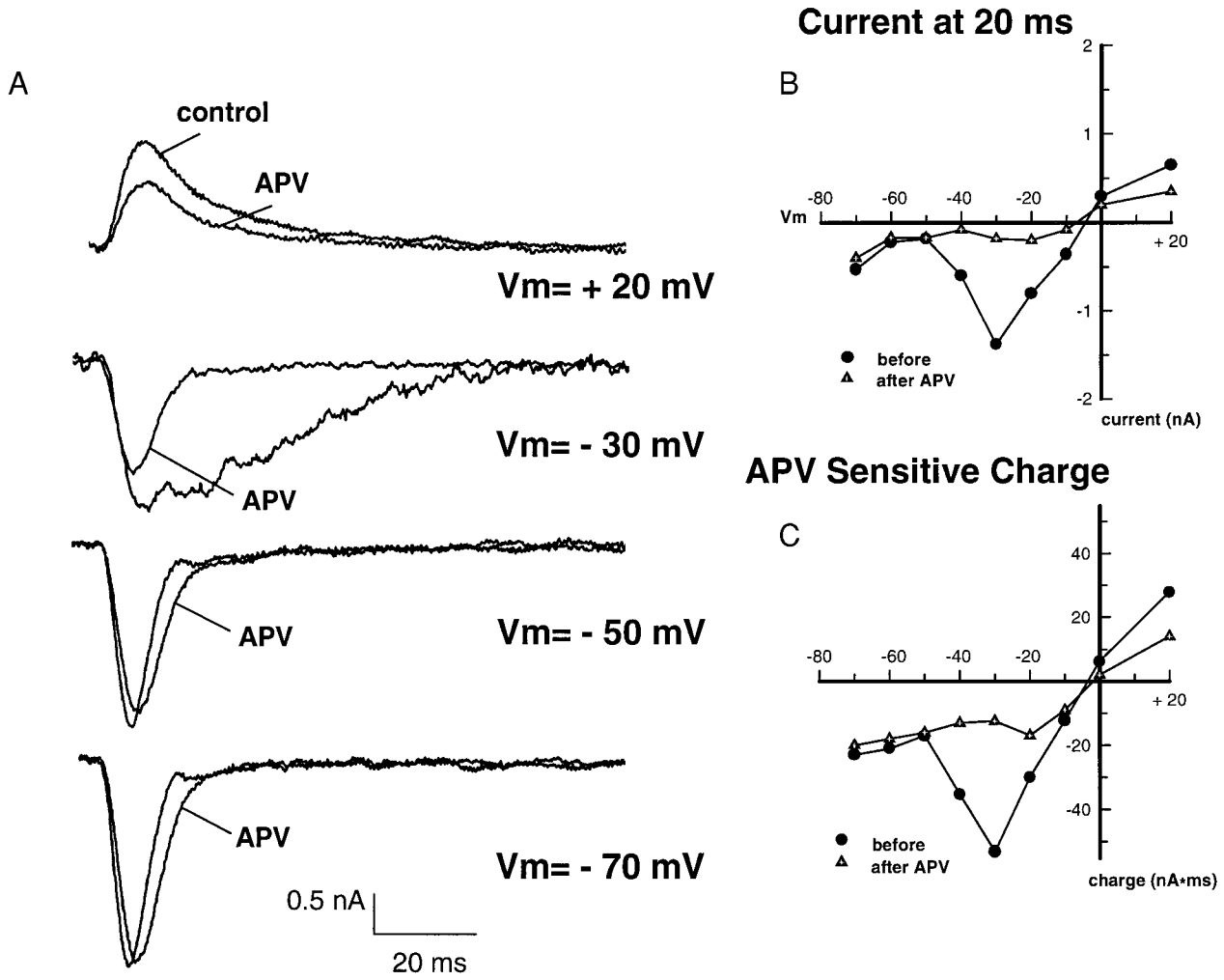

FIG. 2. Synaptic currents evoked by perforant path stimulation in granule cells from kindled rats. *A*: representative example of synaptic currents evoked in a granule cell by perforant path stimulation in a hippocampal slice from a kindled rat within 1 wk after the last of 3 generalized tonic clonic (class V) seizures. In this cell, the holding membrane potential (V_m) was varied in steps from −70 to +20 mV. At a holding potential of −30 mV, there was an increase in the amplitude of the perforant path–evoked synaptic current measured at 20 ms in granule cells from kindled rats (compare with Fig. 1*A*), and also a pronounced increase in the duration of the inward current. *B*: *I-V* plot at 20 ms after perforant path stimulation derived from the evoked currents in *A* demonstrates a pronounced region of negative slope conductance from a holding potential of about −50 mV, which reaches a maximum at about −30 mV (compare with Fig. 1*B*). The region of negative slope conductance was consistently more prominent in granule cells from kindled rats compared with normal controls. *C*: charge transfer calculated for the currents illustrated in *A* demonstrated an increase in the APV-sensitive charge transfer at holding potentials positive to −50 mV. There was a prominent region of APV-sensitive charge transfer in granule cells from kindled rats studied at 1–7 days after the last evoked seizure (compare with Fig. 1*C*).

inward current at a holding potential of −30 or +20 mV in granule cells from kindled rats examined at 2.5–3 mo after the last of 3 or 90–120 class V seizures (Fig. 6, *A* and *B*). This effect was not dependent on the site of kindling, as the ratio of inward current at 20 ms to peak inward current in hippocampal slices obtained from rats kindled in the olfactory bulb (62.5 ± 9.9%) or the perforant path (59.8 ± 7.3%) did not differ from the ratio in age-matched controls (66.8 ± 4.5%, not significant). There was also no difference in NMDA-dependent charge transfer at 2.5–3 mo after 3 or 90–120 class V seizures compared with DGCs from age-matched normal controls (Fig. 6, *C* and *D*).

DISCUSSION

Single-electrode voltage-clamp techniques and bath application of the NMDA antagonist APV were used to evaluate the time course of seizure-induced alterations in the perforant path–evoked synaptic current in granule cells of the dentate gyrus from kindled rats. The results confirmed the following observations from previous studies: *1*) the presence of an NMDA-dependent component in the synaptic current evoked by perforant path stimulation in the granule cells of normal rats (Keller et al. 1991; Lambert and Jones 1990), and *2*) an increase in the NMDA-dependent component of the synaptic current evoked by a perforant path stimulus in granule cells from rats kindled by olfactory bulb stimulation, as observed previously in granule cells from kindled rats that received amygdala and hippocampal commissural stimulation (Kohr et al. 1993; Kohr and Mody 1994; Mody et al. 1988). In addition, the experiments provided new quantitative details about seizure-induced increases in the NMDA-dependent component of synaptic currents at perforant path–granule cell synapses, and

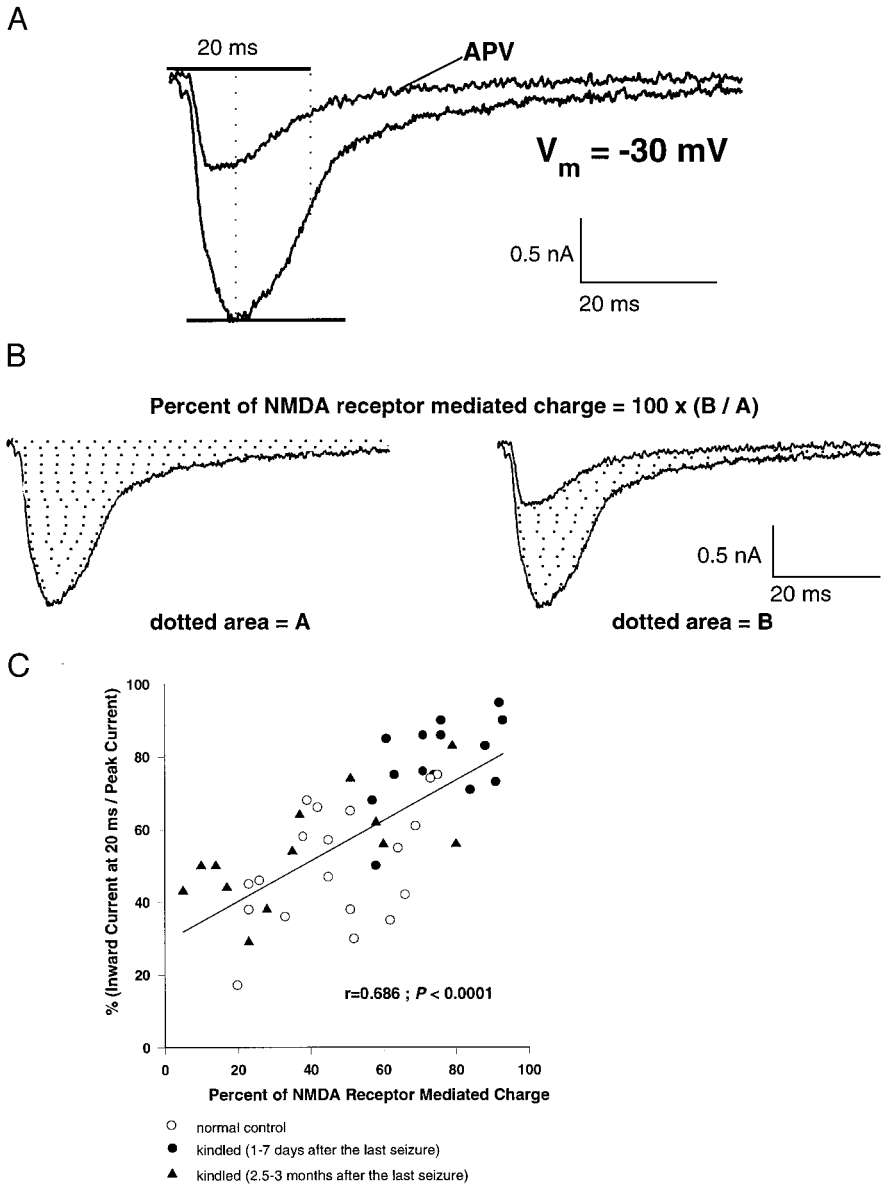

FIG. 3. Analysis of evoked synaptic currents. Because the NMDA receptor–dependent current evoked in granule cells by perforant path stimulation has a long duration and is voltage dependent, the ratio of the inward current at 20 ms to peak inward current at a holding potential of −30 mV was used as a measure of the NMDA component of the inward current. *A*: inward current evoked in a granule cell by perforant path stimulation before and after bath application of 50 μM APV in a hippocampal slice from a kindled rat at 7 days after the last of 3 class V seizures. The baseline is indicated by the top solid horizontal line. The peak current before bath application of APV is indicated by the left vertical dotted line that extends from the top to the bottom solid horizontal line. The current at a latency of 20 ms after the stimulus is indicated by the right dotted vertical line that extends from the baseline to the current trace in the absence of APV. *B*: example of the method for calculation of the percent of NMDA-dependent synaptic charge. The area under the curve of inward current before bath application of 50 μM APV is indicated by the dotted region in the *left trace*. The dotted area in the *right trace* is the area after subtracting the area under the current trace following bath application of APV. The ratio of 100 × (B/A) is the NMDA-dependent charge transfer. *C*: there was a linear relationship between the ratio of inward current at 20 ms to peak inward current (expressed as a percent) and the NMDA-dependent charge transfer in both normal and kindled rats ($r = 0.686$, $P < 0.0001$ for the combined groups of normal and kindled granule cells). There was a linear relationship between the ratio of inward current at 20 ms to peak inward current (expressed as a percent) and the NMDA-dependent charge transfer in granule cells from normal rats (○, $r = 0.425$, $P = 0.034$). There was also a linear relationship between inward current at 20 ms to peak inward current (expressed as a percent) and the NMDA-dependent charge transfer in granule cells from kindled rats ($r = 0.81$, $P = 0.0001$). ●, granule cells from kindled rats studied at 1–7 days after the last seizure; ▲, granule cells studied at 2.5–3.0 mo after the last seizure. These observations demonstrated that the ratio of the inward current at 20 ms to peak inward current is a reliable measure of NMDA-dependent synaptic charge transfer in granule cells from both normal and kindled rats.

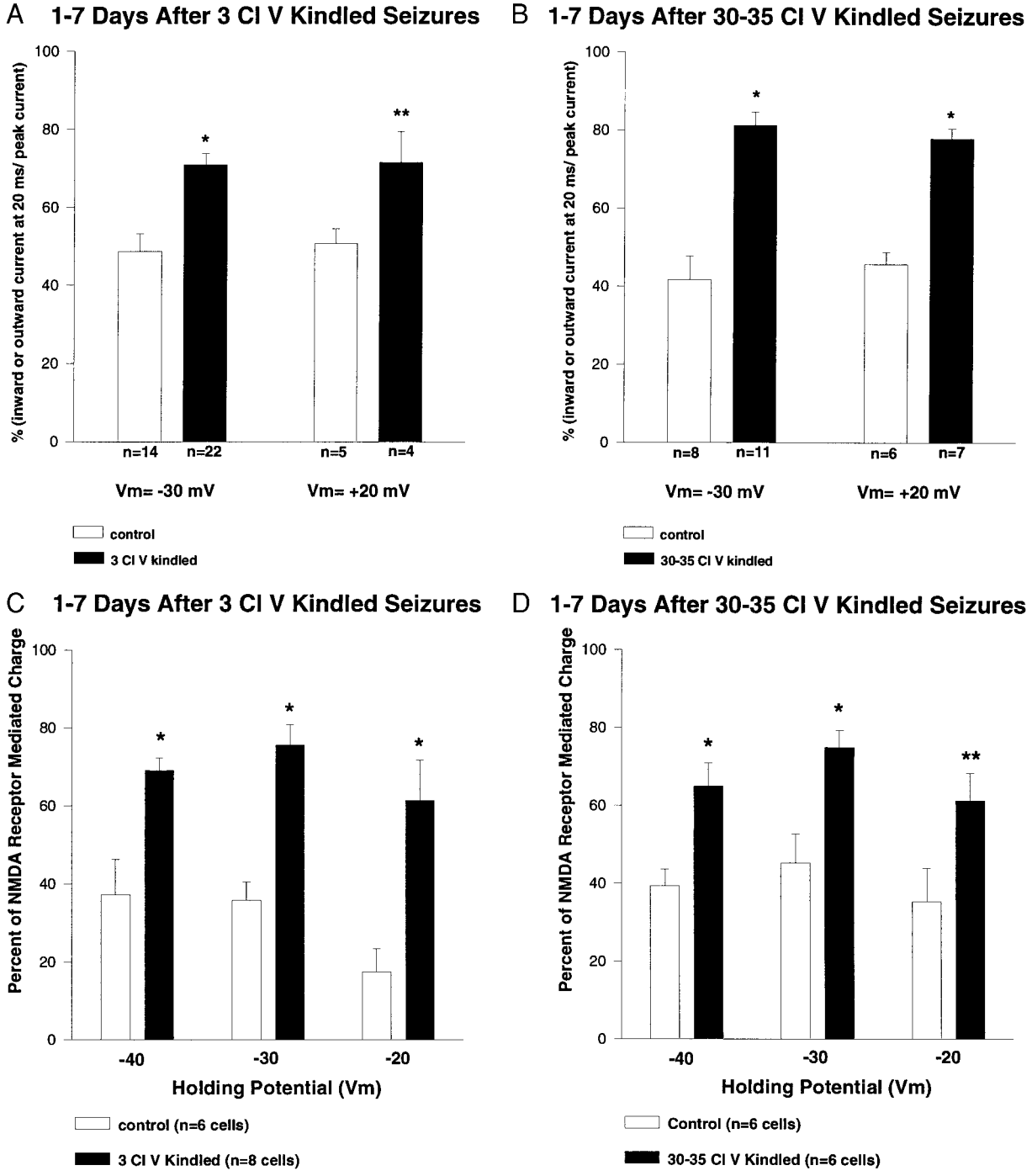

FIG. 4. Increase in NMDA-dependent evoked synaptic current in granule cells of kindled rats. *A*: ratio of the inward current at 20 ms to the peak current at a holding potential (V_m) of −30 mV, a measure of the NMDA component of the evoked synaptic current, was increased in granule cells from kindled rats examined within 1 wk after the last of 3 class V seizures compared with age-matched controls ($P < 0.001$). There was also an increase in the ratio of the outward current at 20 ms to peak outward current at a holding potential of +20 mV within 1 wk after the last of 3 class V seizures ($P < 0.05$). *B*: there was an increase in the ratio of the inward current at 20 ms to the peak current in granule cells from rats studied within 1 wk after 30–35 class V kindled seizures at a holding potential of −30 mV ($P < 0.001$). There was also an increase in the ratio of the outward current at 20 ms to peak outward current at a holding potential of +20 mV within 1 wk after the last of 30–35 class V seizures ($P < 0.001$). *C*: granule cells at holding potentials of −40, −30, and −20 mV from kindled rats examined within 1–7 days after the last of 3 class V kindled seizures demonstrated significant increases in the percent of NMDA receptor–dependent charge transfer calculated by measuring the area under the curve of inward current from the stimulus to 100 ms before and after bath application of 50 μM APV (see METHODS and Fig. 4 for details of calculation). *D*: increases in the percent of NMDA-dependent charge transfer at holding potentials of −40, −30, and −20 mV were also noted in granule cells from rats examined within 1–7 days after 30–35 class V seizures.

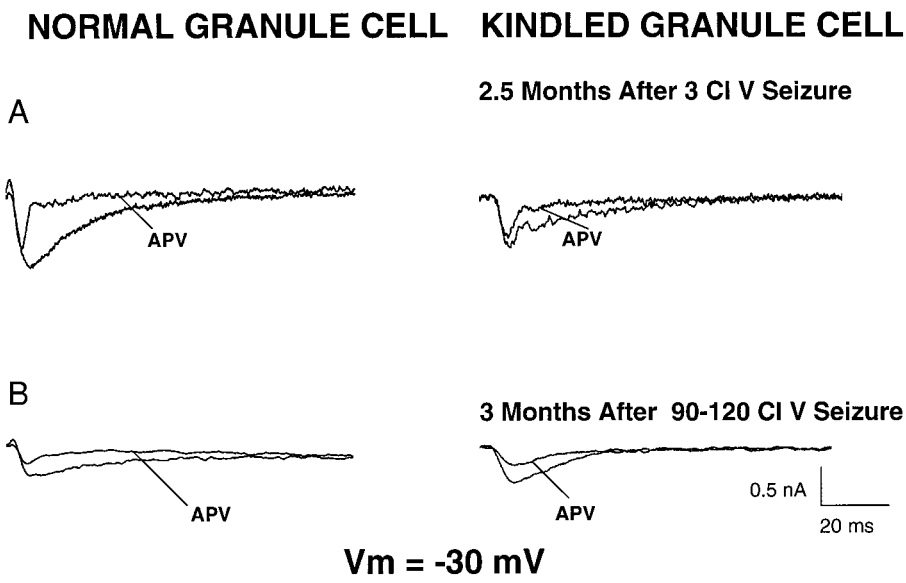

FIG. 5. Synaptic currents evoked by perforant path stimulation in granule cells from kindled rats at 2.5–3.0 mo after the last evoked seizure. *A*: representative examples of synaptic currents evoked by perforant-path stimulation in a granule cell at a holding potential of −30 mV from an age-matched normal rat and a kindled rat that was examined at 2.5 mo after the last of 3 class V kindled seizures. The APV-dependent component of the inward current was comparable with an age-matched normal control (compare with Figs. 1 and 2). *B*: representative examples of evoked synaptic currents in a granule cell from an age-matched normal rat and a kindled rat examined at 3 mo after the last of >90 class V kindled seizures. The APV-dependent component of the inward current was comparable with an age-matched normal control.

revealed that the kindling-induced increase in the NMDA-dependent component of the evoked synaptic current, which was detected as long as 4–6 wk after the last seizure in previous studies (Kohr et al. 1993; Kohr and Mody 1994; Mody et al. 1988), was no longer apparent at 2.5–3 mo after the last kindled seizure. At this interval after the last evoked seizure, the NMDA-dependent component of the current evoked in granule cells by a perforant path stimulus in slices from rats kindled by stimulation of the olfactory bulb and perforant path was indistinguishable from age-matched controls.

Although it might be of interest to consider the possibility that the initial increase and evolving alterations in the NMDA-dependent component of the evoked granule cell current varied as function of the site of kindling stimulation, this study was not specifically designed to address this question. Our results and previous studies (Kohr et al. 1993; Kohr and Mody 1994; Mody et al. 1988), however, clearly demonstrate that the NMDA-dependent current evoked in granule cells by activation of the perforant path is increased by kindling of a variety of sites and is not limited only to kindling of the direct monosynaptic input from the entorhinal cortex. These results confirm long-standing observations that the changes induced by kindling of the perforant path are transynaptic in the hippocampus and are not restricted to the pathway of stimulation (Goddard et al. 1969; Messenheimer et al. 1979). These previous observations and our results are also consistent with other studies which demonstrated that afferent input to the rodent hippocampal formation is bilaterally propagated in the dentate gyrus (Golarai and Sutula 1996), and that the initial ADs are likewise bilaterally propagated in limbic pathways (Stringer and Lothman 1992). Our results furthermore provided no evidence suggesting that the evolving alteration in the NMDA-dependent current, which decreased and was comparable with controls at 2.5–3 mo after the last seizures, was dependent on the site of stimulation. Because the NMDA-dependent current at 2.5–3 mo after the last seizure evoked by kindling of the perforant path did not differ from age-matched controls or from rats that received kindling stimulation of the olfactory bulb, it seems unlikely that there are significant differences in the persistence of seizure-induced alterations in the NMDA-dependent current in rats on the basis of kindling of the monosynaptic perforant pathway or polysynaptic pathways to granule cells from the olfactory bulb or amygdala.

The time course and APV sensitivity of the seizure-induced alterations in the perforant path–evoked synaptic current suggest that NMDA-dependent synaptic transmission in granule cells could contribute to abnormal hippocampal excitability in the early stages of kindling, but not at long intervals after the last evoked seizure. Furthermore, previous studies have demonstrated that repeated activation of the NMDA receptor plays a critical role in the induction of mossy fiber sprouting and the progression of kindling (Sutula et al. 1996). These observations implicate the NMDA receptor in the induction of kindling and the long-term structural and functional alterations in the dentate gyrus associated with repeated kindled seizures, but also demonstrate that increases in NMDA receptor–dependent synaptic transmission in granule cells cannot account for the permanence of the kindling effect.

NMDA-dependent synaptic currents in granule cells of normal rats

The synaptic current evoked in granule cells by perforant path stimulation in normal rats displayed voltage dependence, time course, and APV sensitivity that were consistent with NMDA receptor–dependent synaptic transmission, as demonstrated in previous current-clamp and voltage-clamp studies (Keller et al. 1991; Lambert and Jones 1990). The cell bodies of granule cells are compact, but it is unlikely that adequate space clamp can be achieved by single-electrode voltage-clamp or other whole cell clamp methods throughout the extent of the granule cell dendrites. Because the enhanced APV-sensitive component of the synaptic current was apparent not only at a holding potential of −30 mV but was also observed at +20 mV, it is unlikely that the inward current can be explained by a Ca^{2+} current or other dendritic voltage-dependent currents.

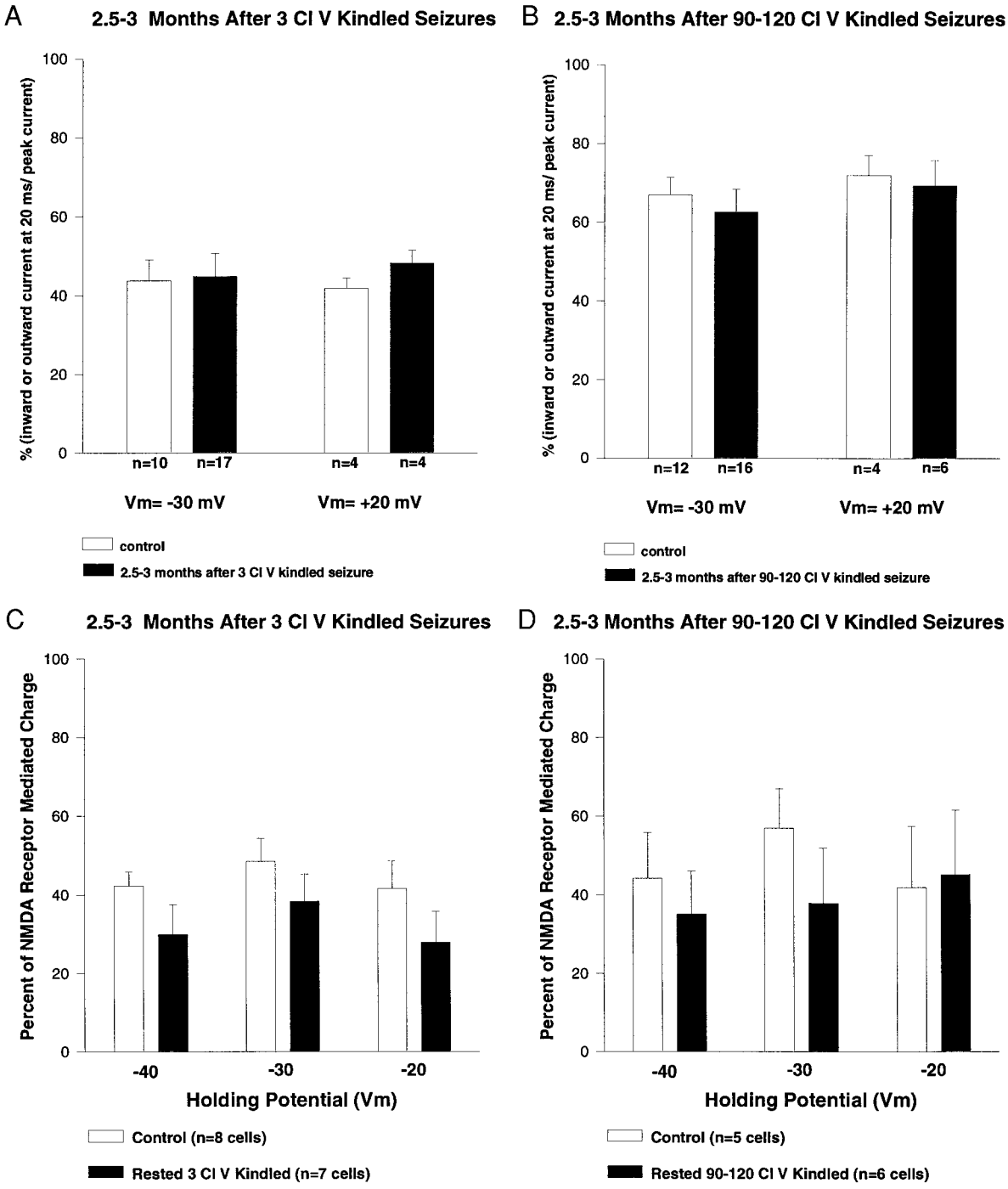

FIG. 6. NMDA-dependent synaptic current at 2.5–3 mo after the last evoked seizure was comparable with age-matched normal controls. The ratio of the inward current at 20 ms to the peak current at a holding potential −30 mV in granule cells from kindled rats examined at 2.5–3.0 mo after the last of 3 class V kindled seizures (*A*) or the last of 90–120 class V kindled seizures (*B*) did not differ from age-matched normal controls. The ratio of the outward current at 20 ms to the peak outward current at a holding potential +20 mV in granule cells from kindled rats examined at 2.5–3.0 mo after the last of 3 class V kindled seizures (*A*) or 90–120 class V kindled seizures (*B*) did not differ from age-matched normal controls. NMDA-dependent charge transfer at 2.5–3.0 mo after the last evoked seizure in granule cells from kindled rats was comparable with age-matched normal controls. There were no significant differences in NMDA receptor–dependent charge transfer at holding potentials of −40, −30, and −20 mV measured at 2.5–3 mo after the last of 3 class V kindled seizures (*C*) or the last of 90–120 class V kindled seizures (*D*).

Despite the possibility of some contribution of voltage-dependent calcium currents in dendrites to the evoked inward currents, the results of this study and previous whole cell voltage-clamp recordings are consistent with the presence of a substantial NMDA-dependent component of perforant path synaptic transmission in granule cells of normal rats, which confirms earlier studies (Keller et al. 1991; Lambert and Jones 1990).

Time course of increases in NMDA-dependent synaptic currents induced by kindling

The present experiments also confirmed previous observations that kindled seizures increase NMDA receptor–dependent synaptic transmission in granule cells of the dentate gyrus (Mody et al. 1988). The increase is rapidly detected in granule cells after kindling stimulation and is accompanied by increasing sensitivity to iontophoresis of NMDA, by alteration of activation/inactivation kinetics of calcium currents, and by changes in the properties of NMDA receptor–gated channels including alterations in Mg^{2+} sensitivity (Kohr et al. 1993; Kohr and Mody 1994; Mody et al. 1988). These altered properties may reflect underlying molecular alterations or changes in subunit composition of the NMDA receptor channel complex (Kraus et al. 1994, 1996). Previous studies have detected alterations in NMDA receptor channel properties at 24–72 h after the last seizure. In this study, increases in the voltage-dependent NMDA-dependent currents were also observed within 1–7 days after the last seizure. Seizures rapidly induce alterations in single channel properties of the NMDA receptor (Kohr et al. 1993; Kohr and Mody 1994), which persist for as long as 4–6 wk after kindled seizures. At 2.5–3 mo after the last of either 3 or 90–120 class V seizures, perforant path–evoked synaptic currents in granule cells, and the NMDA-dependent component of these currents, could not be distinguished from age-matched normal controls. Repeated seizures evoked by kindling stimulation induce permanent increases in susceptibility to seizures, but the increase in the NMDA component of synaptic transmission induced in granule cells by kindling is clearly not permanent.

Role of the NMDA receptor in seizure-induced plasticity

The increase in NMDA-dependent synaptic current evoked in granule cells of kindled rats by perforant path stimulation is not permanent, but recent studies have provided evidence that the NMDA receptor plays a critical role in the induction of permanent structural and functional alterations induced by repeated kindled seizures. Administration of the NMDA antagonist MK801 before each kindling stimulation, which prolongs stimulation-induced seizures relative to untreated controls and increases the duration and number of electrographic seizures required to evoke class V seizures, not only prevents the behavioral progression of kindling, but also prevents the development and progression of seizure-induced mossy fiber sprouting (Sutula et al. 1996). The intracellular domain of the NR2 subunit of the NMDA receptor also plays a role in the progression of kindling and mossy fiber sprouting (Sprengel et al. 1998). The NMDA receptor has also been implicated in the induction of mossy fiber sprouting after seizures evoked by kainic acid (Cantallops and Routtenberg 1996; McNamara and Routtenberg 1995). These observations suggest that the NMDA receptor could be a critical link in a signal transduction pathway that translates acute effects of seizures into long-term structural and functional alterations in hippocampal circuitry.

The present results are consistent with the possibility that repeated activation of the NMDA receptor during seizures increases NMDA-dependent synaptic transmission, and initiates a series of molecular and cellular alterations that initially contribute to enhanced excitability in hippocampal pathways and eventually induce long-term structural and functional alterations in the dentate gyrus. In support of this possibility, NMDA receptor antagonists such as MK801 have pronounced effects on the progression of kindling and epileptogenesis in chronic experimental models of epilepsy, but are for the most part weak anticonvulsants against generalized seizures or fully kindled class V seizures epilepsy (Loscher and Honack 1991; McNamara 1988; Yoshida et al. 1997).

These experiments provided evidence that increases in NMDA-dependent synaptic transmission are distinct from the long-term effects of repeated NMDA receptor activation on structural and functional alterations in the circuitry of the dentate gyrus. Although anticonvulsant potency of NMDA antagonists is relatively weak, NMDA receptor antagonism may be potentially valuable as treatment for the long-term effects of seizures because of the effects on the induction of sprouting and other kindling-induced cellular processes that may reduce seizure susceptibility (Sutula et al. 1996). For this therapeutic purpose, development of NMDA antagonists with acceptable toxicity profiles and weak anticonvulsant activity (Loscher 1998) may still be worthwhile to prevent the undesirable long-term consequences of repeated seizures, which include increases in susceptibility to seizures and memory dysfunction. Brief administration of drugs targeting the NMDA receptor or signal transduction pathways and genes activated by the NMDA receptor may be useful to modify induction processes that lead to long-term cellular alterations in hippocampal circuitry, epilepsy, and memory disturbances.

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Address for reprint requests: T. Sutula, Dept. of Neurology, H6/570, University of Wisconsin, Madison, WI 53792.

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