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12. Although there might be slight differences in the propensity to form an S–S bond in the heterodimer and homodimers, the Gly-Gly linker should provide enough flexibility to minimize these differences [see (*9*)].
13. The inference of α helices can be made from CD spectra obtained in aqueous solution with much higher confidence than for other secondary structures; see R. W. Woody, in *The Peptides*, S. Udenfriend, J. Meienhofer, J. R. Hruby, Eds. (Academic Press, New York, 1985), vol. 7, pp. 15–114; Y.-H. Chen, J. T. Yang, K. H. Chau, *Biochemistry* **13**, 3350 (1974).
14. In the absence of GuHCl, S–S Fos-p1N homodimer, Jun-p1N homodimer, and Fos-p1N–Jun-p1N heterodimer have T_m values of 59°, 69°, and 71°C, respectively, that are independent of peptide concentration from ~0.7 to ~15 μ*M*. The melting transition is reversible for the Fos-p1N homodimer, but some hysteresis is observed with the Jun-p1N homodimer and the heterodimer. In the presence of 2*M* GuHCl (Fig. 2B), reversible thermal unfolding transitions are observed for all three species.
15. Preliminary results suggest that the isolated Jun leucine zipper peptide, without appended Cys-Gly-Gly residues, may not form a stable unique dimer. Thus, regions outside the leucine zipper may be important for homodimer formation by the intact Jun protein. Nevertheless, isolated Fos and Jun leucine zippers form a specific heterodimer even in the absence of an S–S bond (unpublished results): (i) An equimolar mixture of the Fos and Jun peptides has a CD spectrum that is not equivalent to the simple sum of the individual peptide spectra. Such a mixture exhibits concentration-dependent stability and is more stable than either peptide alone; and (ii) Sedimentation equilibrium experiments (4°C) indicate that an equimolar mixture of the Fos and Jun peptides exists as a single species with M_r = 9400 in the concentration range from 20 μ*M* to 0.25 m*M* (expected M_r for heterodimer = 9285). Note that any instability of the Jun homodimer not detected here would enhance heterodimer formation even further (see Fig. 2C).
16. It is likely that in the antiparallel orientation, S–S dimers associate to form higher order species in which individual helices can pair in a parallel manner (*9*).
17. Others have concluded that the leucine repeat regions in the Fos-Jun heterodimer are parallel, based on mutagenesis studies [(*3, 5*); see also W. H. Landschulz, P. F. Johnson, S. L. McKnight, *Science* **243**, 1681 (1989)].
18. In experiments with intact proteins, the Fos-Jun heterodimer is more stable than the Jun homodimer under conditions where the Fos homodimer is not detected (T. Smeal and M. Karin, personal communication), suggesting that the instability of the Fos homodimer also contributes to the specificity of heterodimer formation with the intact proteins. The tropomyosin heterodimer from *R. esculenta* appears to be favored thermodynamically in an analogous manner (S. S. Lehter, Y. Qian, S. Hvidt, personal communication).
19. The M_r for Fos-p1 is 9300 (constant over the concentration range from 40 μ*M* to 0.5 m*M*) as determined by sedimentation equilibrium studies at 4°C, demonstrating that it is dimeric. A Model E centrifuge was used at 52,000 rpm to make measurements in phosphate buffered saline (PBS: 0.15*M* NaCl, 10 m*M* phosphate, *p*H 7.0) as described in P. Gaceffa, C.-L. A. Wang, W. F. Stafford [*J. Biol. Chem.* **263**, 14196 (1988)].
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29. The peptides were mixed and incubated in either 2 m*M* HCl (control) or in redox buffer (250 μ*M* oxidized glutathione, 250 μ*M* reduced glutathione, 0.2*M* KCl, 0.1*M* tris, 1 m*M* EDTA, *p*H 8.7) for 1 hour at 23°C at a total reduced peptide concentration of 30 μ*M* and then analyzed by HPLC (an acetonitrile-H_2O gradient with segments of 0.1% and 0.2% acetonitrile increase per minute was used in the presence of 0.1% trifluoroacetic acid with an analytical Bio-Rad or Vydac C18 column). The redox conditions were chosen to minimize the amount of mixed disulfide (that is, between peptide and glutathione) and reduced peptide, while maximizing the amount of peptide S–S species. The redox experiments were repeated at 4° and 37°C with identical results.
30. CD spectra were recorded in a 1-mm pathlength cell at a total peptide concentration of 50 μ*M* in PBS. All peptide concentrations were determined by tyrosine absorbance (*11*). Thermal unfolding studies were performed in PBS with a 1-cm path length cell. An Aviv Model 60DS CD spectrophotometer with an HP Model 89100A Peltier temperature control unit was used.
31. We thank T. Alber, J. Hu, W. Lim, P. Matsudaira, C. Murre, T. Oas, D. Rio, R. Sauer, and J. Staley for helpful discussions; T. Curran, M. Karin, S. Lehrer, S. McKnight, R. Tjian, and I. Verma for sending us manuscripts before publication; and W. DeGrado and A. Frankel for helpful comments on the manuscript. Mass spectrometry analyses were performed at the MIT Mass Spectrometry Facility (supported by the NIH) or at M-Scan, Inc. (West Chester, PA). Supported by an NIH predoctoral fellowship (E.K.O.), and grants from the NIH (RR05711 to W.F.S.), Massachusetts Division of the American Cancer Society (P.S.K.), and the Lucille P. Markey Charitable Trust (P.S.K.).

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NMDA Antagonists Differentiate Epileptogenesis from Seizure Expression in an in Vitro Model

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In an electrographic model of seizures in the hippocampal slice, both of the *N*-methyl-D-aspartate (NMDA) antagonists 2-amino-5-phosphonovaleric acid and 5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801) prevented the progressive development of seizures but did not block previously induced seizures. Thus, a process dependent on the NMDA receptor–ionophore complex establishes a long-lasting, seizure-prone state; thereafter the seizures depend on non-NMDA receptor–ionophore mechanisms. This suggests that there is an important distinction between epileptogenesis and seizure expression and between antiepileptogenic and anticonvulsant pharmacological agents.

TO BETTER UNDERSTAND AND TREAT human epilepsy, it is important to understand not only the expression of individual seizures but the development of a lasting seizure-prone state, that is, epileptogenesis. In epilepsy, excessive electrical activity in one region of the brain can induce

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healthy neural networks in another region to develop epileptic activity (*1, 2*). Researchers in our laboratory have used an in vitro hippocampal slice model in which repeated electrical stimulation produces spontaneous and triggered bursts of population spikes; others have studied very similar systems (*3, 4*). Like many models of epilepsy, all these systems exhibit a short-duration (100-ms) spontaneous burst, which is a probable correlate of the in vivo interictal spike (IIS). However, clinical treatment of epilepsy focuses on preventing the occurrence of seizures rather than interictal events. We have now shown that repeated electrical stimulation of hippocampal slices from rats 22 to 32 days of age produces electrical afterdischarges, lasting 30 to 60 s, that resemble the electrical activity recorded during in vivo seizures (*5*). These electrographic seizures (EGSs) develop gradually (Fig. 1A). They display tonic and clonic-like phases as well as all-or-none triggering about a narrow stimulus threshold (*6*). Once induced, they may be evoked for the life of the preparation, even after extended stimulus-free periods (Fig. 1B) (7).

To study the role of NMDA receptors in the induction of EGSs, we bath-applied the NMDA antagonist D-2-amino-5-phosphonovaleric acid (D-APV) at concentrations of 20 μM ($n = 4$) or 50 μM ($n = 6$) for 10 to 15 min before the first stimulus train. Under these conditions, six to ten trains typically elicited only minimal afterdischarges and there was little progression in their duration or pattern (Fig. 2A) (*8*). D-APV also prevents the induction of spontaneous interictal bursts (*9*). After a 10-min washout period, repeated stimulus trains induced interictal bursts and EGSs in seven of eleven slices (Fig. 2B) (*10*); thus, the temporary blockade of NMDA receptors did not compromise the capacity of these slices to develop EGSs or interictal bursts in the standard artificial cerebrospinal fluid (ACSF).

To assess its anticonvulsant potential against established EGSs, we bath-applied D-APV after EGSs had been induced and had remained stable for at least three stimulus trains in standard ACSF; D-APV [20 μM ($n = 5$), 50 μM ($n = 8$), or 100 μM ($n = 5$)] failed to substantially alter the tonic-clonic pattern or the total duration of EGSs (Fig. 3). Individual population bursts within an EGS were often shorter than in standard ACSF, but this did not affect the overall pattern and duration of EGSs. The occurrence of interictal bursts is little altered by D-APV (*9*).

Thus, although D-APV prevented the induction of EGSs by stimulus trains, it did not suppress them once they were established. There are several explanations for this loss of sensitivity: (i) increased release of endogenous glutamate or aspartate may have overwhelmed the competitive antagonism of D-APV; (ii) the affinity of the NMDA receptor for D-APV may have been reduced; (iii) the NMDA receptor–coupled channel may have become uncoupled from the NMDA receptor, such that the channel was activated by some other pathway; or (iv) the NMDA channel may not have been necessary for the maintenance of EGSs.

To distinguish among these possibilities, we repeated the above experiments with the noncompetitive NMDA channel blocker 5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801) (*11*). In the first set of experiments, MK-801 (10 μM) was applied for at least 90 min before stimulation to allow use-dependent block to take place (*12*). This concentration effectively blocks long-term potentiation (LTP), as well as the NMDA receptor–mediated component of synaptic transmission in low-Mg^{2+} media in the hippocampal slice (*12*). Although there was no suppression of single stimulus–evoked fields, MK-801 effectively prevented any afterdischarges during a series of ten stimulus trains ($n = 5$). As with D-APV, no interictal bursts developed.

On the other hand, as with D-APV, when EGSs were first induced in standard ACSF, the subsequent application of MK-801 (10 μM) for 30 to 127 min failed to significantly alter the course of the established EGSs or interictal bursts. Individual bursts within an EGS were affected as with D-APV (*13*). Thus, our results with MK-801 are consistent with the interpretation that the NMDA channel itself is not required to maintain EGS expression. Although any of the first three changes in NMDA receptor effectiveness proposed above (increased glutamate

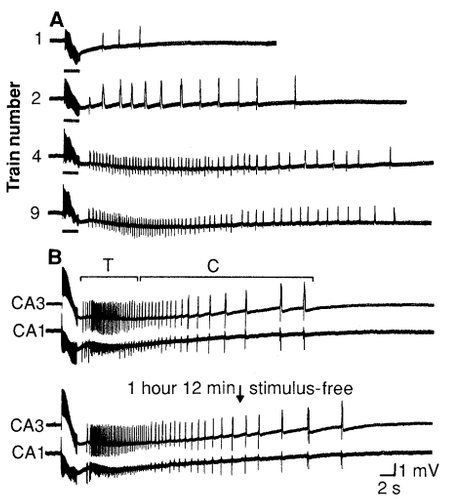

Fig. 1. Induction of electrographic seizures. (**A**) Field recordings from area CA3 of the hippocampal slice show the progressive enhancement of afterdischarges that follows successive stimulus trains (bars) (*26*). After two to ten trains, these develop into a stereotypical pattern characterized by a constant duration and two distinct phases and are then designated as electrographic seizures (EGSs). (**B**) Recordings from another slice (upper traces) show that seizures were concurrent in CA3 and CA1 and illustrate more clearly the tonic (T) phase of rapidly firing population spikes and the clonic-like (C) phase of less frequent and sometimes clustered population bursts. (Lower traces) After more than an hour (72 min) without additional stimuli, a similar seizure was elicited.

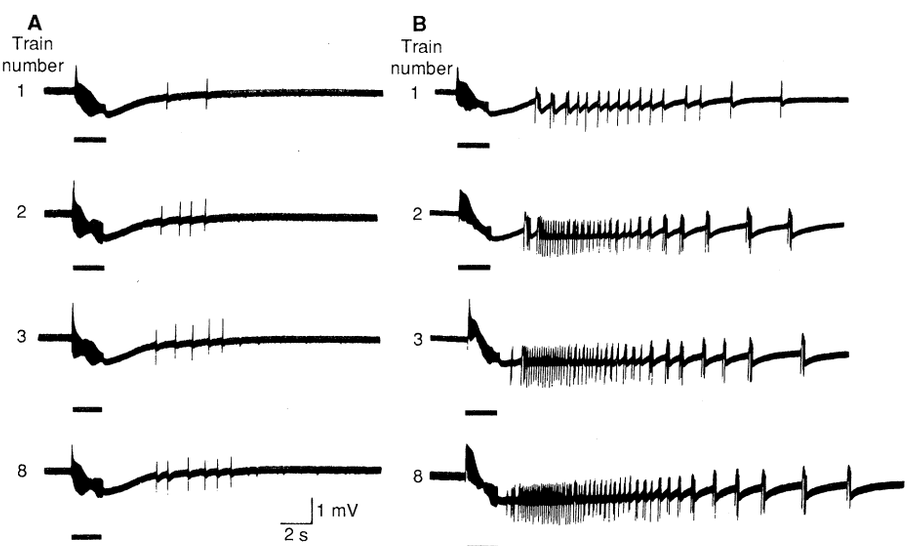

Fig. 2. D-APV prevents EGS induction. (**A**) When slices were incubated with the competitive NMDA receptor antagonist D-APV (50 μM), repeated stimulus trains induced little enhancement of afterdischarges (no significant difference in the number of discharges after the first and last stimulus trains, at 20 μM ($n = 4$, $P > 0.5$) or 50 μM ($n = 6$; $0.2 < P < 0.4$, paired t test). (**B**) After the washout of D-APV with ACSF, the same slice showed the typical development of EGSs. Bar indicates stimulus train in (A) and (B).

or aspartate release, decreased affinity, or uncoupling) may have accompanied epileptogenesis, such changes must have made little difference to EGS expression, because EGSs continued to occur despite direct blockade of the channels to which these receptors are coupled.

Therefore, our results indicate that NMDA channels are necessary for the induction of EGSs but not for the maintenance of the seizure-prone state (*14*). It is conceivable that the induction process caused a permanent change in the NMDA channel (for example, a conformational change) such that (i) MK-801 no longer effectively blocked the channel and that simultaneously (ii) D-APV no longer effectively blocked the associated receptor, or the channel became coupled to a non–NMDA receptor (for example, the kainate-quisqualate receptor). However, it seems unlikely that both of these processes would occur together.

It seems more likely that NMDA channels induce the seizure-prone state by activating or inactivating a separate system for seizure expression that is then no longer dependent on NMDA channels. Indeed, there is evidence that NMDA receptor activation via electrical stimulation can effect several lasting changes in inhibition and excitation (*15*). On the other hand, we and other investigators (*4, 16*) have failed to demonstrate a consistent reduction in evoked inhibitory postsynaptic potentials (IPSPs) in the CA3 area of the hippocampus with the stimulus parameters used in this study. Furthermore, we have also examined the responses to individual stimulus pulses within stimulus trains and have found little change between those in the first stimulus train and those that trigger stable EGSs. Thus, all trains activated the cell population to a similar extent.

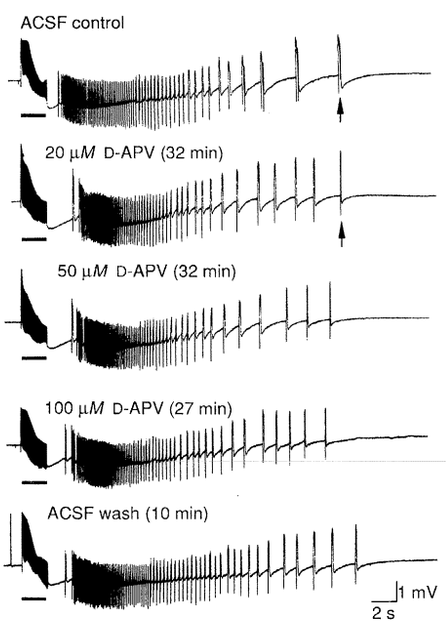

Fig. 3. D-APV does not block established EGSs. Stable EGSs were first induced in the standard medium, ACSF (top trace). The subsequent application of D-APV (20, 50, or 100 μ*M*) failed to suppress them (no significant change in discharge number at any concentration; $P > 0.01$, paired t test), despite the fact that individual discharges were of shorter duration (compare at arrows). No significant change in seizure pattern was noted after washout of D-APV (bottom trace). Bars indicate stimulus train.

Both D-APV and MK-801 decreased the duration of individual bursts within EGSs, which is consistent with models of interictal activity (*9, 17, 18*). Such findings indicate that there is an NMDA receptor–dependent component to these bursts. Indeed, other investigators have found an additional long-latency, NMDA receptor–dependent component to synaptic transmission in dentate granule cells after in vivo kindling (*19*). However, because the reduction of burst duration by NMDA antagonists did not alter the pattern of EGSs, the ability of the neuronal population to fire extended bursts must not be critical for the processes that initiate and terminate the tonic and clonic-like phases of synchronous firing in EGSs.

NMDA antagonists suppress seizures in several models of epilepsy (*18, 20, 21*), although the degree of suppression varies considerably (*22*). As a whole, these are models in which seizures are elicited by a single transient treatment or result from a condition that exists a priori. Seizure expression in a gradually acquired epileptic state may be less dependent on NMDA channel–mediated processes. Rather, it may be the development of such a seizure-prone state that relies critically on NMDA channel activation.

In fact, in kindling (an in vivo model of seizures in which an epileptic state develops gradually) NMDA antagonists significantly retard the development of the kindled state; however, they fail to suppress previously kindled seizures in nontoxic doses (*21, 23*). These results are consistent with our in vitro findings.

The gradual development of EGSs is reminiscent of learning. In various models of learning, including visual system plasticity, spatial learning, and LTP, NMDA receptor activation is essential for the acquisition of the learned state (*24*). This may correspond to the seizure-prone state in our model. More definitive elucidation of the mechanisms underlying the acquisition of this state may prove as difficult as it has been for LTP.

We have demonstrated pharmacological differentiation between the processes of epileptogenesis and seizure expression, indicating that the mechanisms underlying these two processes are also separable. This distinction has been only recently recognized (*1, 9, 25*). Our model provides an opportunity to study these separate processes in detail, in vitro. Furthermore, our results suggest that the anticonvulsant effects of NMDA antagonists may be restricted to certain forms of epilepsy, and it may be more accurate to view them as antiepileptogenic agents.

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26. Transverse hippocampal slices (625 μm) were prepared from male Sprague-Dawley rats 22 to 32 days old and perfused at 31° to 33°C in a submerged recording chamber. ACSF was bubbled with a mixture of 95% O_2 and 5% CO_2 and contained 120 m*M* NaCl, 3.3 m*M* KCl, 1.23 m*M* NaH_2PO_4, 25 m*M* $NaHCO_3$, 0.9 m*M* $MgSO_4$, 1.3 m*M* $CaCl_2$ (Mg^{2+} and Ca^{2+} in accordance with values measured in normal rat CSF [G. G. Somjen, *J. Neurophysiol.* **44**, 617 (1980); J. G. Chutkow and S. Meyers, *Neurology* **18**, 963 (1968)]), and 10 m*M* dextrose. D-APV (Cambridge Research Biochemical, Cambridge, England) and MK-801 were dissolved in this ACSF and bath-applied. Extracellular field recordings were made from stratum pyramidale of area CA1 and CA3 with glass microelectrodes containing 2*M* or 0.15*M* NaCl (1 to 10 megohms). Stimulus trains (60 Hz, 2 s, twice the intensity of the single pulse that evoked the maximum orthodromic population spike in CA3) were delivered every 10 min to stratum radiatum of CA3 via monopolar tungsten electrodes. Between the first stimulus train and the establishment of stable EGSs (that is, at least three consecutive EGSs of constant pattern and duration), the number of individual discharges following a stimulus train increased by 21.1 ± 4.0 (mean ± SEM, paired *t* test, $P < 0.001$; $n = 20$). Although robust EGSs could be induced in slices from 22- to 32-day-old rats, minimal afterdischarges were elicited in those from more mature rats and virtually no afterdischarges in those from adult rats (older than 4 months).
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A Novel mRNA of the A4 Amyloid Precursor Gene Coding for a Possibly Secreted Protein

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The gene, encoding the A4 peptide found in the amyloid core of senile plaques isolated from the cerebral cortex of patients with Alzheimer's disease, produces at least three precursors that resemble cell surface receptors. A clone isolated from a human brain complementary DNA library contained the structural sequence for an A4 amyloid peptide precursor with a serine protease inhibitor domain in which 208 amino acids at the carboxyl terminal are replaced by 20 amino acids derived from nucleotide sequences with homology to the Alu repeat family. This protein devoid of the transmembrane domain most likely represents a secreted form of the A4 amyloid peptide precursor.

ALZHEIMER'S DISEASE IS A DEGENerative disorder characterized by neuronal loss and brain lesions such as senile plaques and neurofibrillary tangles (*1–4*). Senile plaques contain an amyloid core from which a 4.2-kD peptide was isolated as a major constituent (*5*). This peptide called the A4 peptide is identical to the β-amyloid protein found in the vascular amyloid deposits (*6*). From the amino acid sequence, several groups have synthesized oligonucleotides to isolate A4 amyloid peptide precursor (APP) cDNAs that hybridize with a 3.2- to 3.4-kb mRNA doublet expressed in the normal brain and other tissues (*7–10*).

Additional studies have shown that the gene encoding the A4 APP produces at least three different mRNAs referred to as APP_{695}, APP_{751}, and APP_{770} (*11–13*). APP_{751} and $_{770}$ are identical to the previously described APP_{695} (*7*), except for nucleotide inserts coding for a protease inhibitor domain. The three mRNAs appear to arise by alternative splicing of a single gene. In addition to the 3.2- to 3.4-kb mRNA doublet, a 2-kb RNA is also found in Northern blot analysis. We report that this mRNA can encode a secreted form of the A4 APP that is devoid of the A4 peptide and the transmembrane domain.

A 1.4-kb cDNA fragment (*14*) was used for the screening of λgt11 cDNA libraries constructed from the cerebral cortex or the cerebellum of a 54-year-old individual with Alzheimer's disease. Of several hybridizing clones, one clone from each library showed a nucleotide sequence that diverged at position 1630 from the sequence described by Kang *et al.* (*7*). The clone isolated from the cerebral cortex library was shown by DNA sequence analysis to contain a serine protease inhibitor domain at position 865 (Fig. 1). Nucleotide sequence homology was analyzed by the ALIGN program (IntelliGenetics). From position 1630 to position 1900, alignment of the sequence with the human consensus Alu repeat unit showed 70% homology. The 3′ end of the cDNA molecule, which is similar to the consensus Alu sequence, encodes 20 amino acids that lack both the A4 peptide and the hydrophobic carboxyl terminus corresponding to the transmembrane domain of the receptor. The previous potential N-glycosylation site at positions 496 to 498 of the Kang sequence (*7*) is replaced by another one at positions 551 to 553 of the new clone reported here.

The 1.4-kb cDNA probe used for the screening detected several RNAs in Northern blot analysis. In addition to the strong 3.2- to 3.4-kb bands, a band was present at 2 kb (Fig. 2B). The same band was recognized by another cDNA probe from the divergent sequence (Fig. 2A). The existence of the novel mRNA was confirmed by enzymatic amplification of the cDNA region where divergence occurred. Whereas no am-

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