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12. There were 12 male and 13 female monkeys. The degree of handedness measured before surgery formed a flat distribution from completely left-handed to almost completely right-handed; seven male and four female monkeys reached for food more often with their left hand, whereas the others were nominally right-handed. Seven monkeys had the left hemisphere retracted during surgery, five the right, and 13 had retraction of each side in successive operations. Analysis of variance indicated no reliable differences in lateralization associated with any of these variables. The order in which the different sets of stimuli were taught to the monkeys varied considerably, depending on the availability of subjects and training boxes.
13. The average number of discriminations learned was 14 (range 4 to 18). Most problems were taught as simultaneous two-choice discriminations in which the monkey was rewarded for pushing the correct one of two stimuli, one of which was projected on the upper of two screens and the other on the lower. The location of the correct stimulus on the two screens was randomized across trials. The rest of the problems were learned as successively presented two-choice discriminations, a procedure described in (*14*). The two methods gave similar results and are combined. Seventeen monkeys were also tested with six Go/No-Go (*15*) discriminations; again, no laterality was found.
14. One stimulus was presented on the upper of two screens. For stimuli designated correct, the upper screen should be pushed; for incorrect stimuli, the lower screen should be pushed.
15. In order to receive a food reward the monkey had to push the photograph of the positive face within 5 s, or withhold pushing the photograph of the negative face for 5 s. Both normal and left/right mirror image orientations of the photographs were used to control for lateral asymmetries inherent in naturalistic stimuli. Because laterality for discriminating monkeys and expressions did not differ significantly, the results are combined for this report.
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Contributions of Quisqualate and NMDA Receptors to the Induction and Expression of LTP

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The contributions of two subclasses of excitatory amino acid transmitter receptors to the induction and expression of long-term potentiation (LTP) were analyzed in hippocampal slices. The quisqualate/kainate receptor antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione) blocked 85% of the evoked field potential, leaving a small response that was sensitive to D-AP5 (D-2-amino-5-phosphonopentanoate), an *N*-methyl-D-aspartate (NMDA) receptor blocker. This residual D-AP5–sensitive response was of comparable size in control and previously potentiated inputs. High-frequency stimulation in the presence of DNQX did not result in the development of robust LTP. Washout of the drug, however, revealed the potentiation effect. Thus NMDA-mediated responses can induce, but are not greatly affected by, LTP; non-NMDA receptors, conversely, mediate responses that are not needed to elicit LTP but that are required for its expression.

SYNAPTIC RESPONSES IN THE HIPPOCAMPUS and other sites in the forebrain involve two types of excitatory amino acid receptors (*1*). Recent work suggests that LTP, a form of synaptic plasticity that may be involved in memory (*2*), changes those aspects of the response mediated by only one of these two receptor classes. Specifically, an antagonist of the NMDA receptor reduced the size of potentiated and control potentials in hippocampal slices by about the same absolute amount, in experiments with either single stimulation pulses in low Mg^{2+} media (*3*) or repetitive stimulation in normal medium (*4*) to elicit NMDA receptor–dependent responses. These results led to the curious conclusion that, although the currents initiated by the NMDA receptor are necessary for the induction of LTP, they are not themselves greatly influenced by the potentiation effect.

The introduction of drugs that selectively block non-NMDA excitatory amino acid transmitter receptors [for example, the quisqualate site (*5*)] allows for more direct tests of the idea that different classes of postsynaptic receptors contribute differentially to the induction and expression of LTP. Three experiments of this type are described here. First, we compared the effect of DNQX, a quisqualate/kainate receptor antagonist, on control and previously potentiated responses in hippocampal slices maintained in low-Mg^{2+} medium [reduction of extracellular Mg^{2+} attenuates the voltage-dependent blockade of the NMDA receptor ionophore (*6*)]. If NMDA-mediated currents are not affected by induction of LTP, then blocking the non-NMDA sites should eliminate the difference between control and potentiated responses. Second, we tested the prediction that high-frequency stimulation of the NMDA-mediated responses that remain after blockade of quisqualate receptors will not result in the development of a potentiation effect. Note, however, that this experiment could not distinguish between the absence of induction of LTP and the absence of its expression. Accordingly, we carried out a third study in which high-frequency stimulation was applied in the presence of DNQX to one of two pathways and then the drug was washed out of the slices. If potentiation can be triggered by repetitive stimulation of NMDA responses alone but is not expressed, then washing out the drug should reveal a difference between the two pathways.

Hippocampal slices (400 to 450 μm thick) were prepared from male Sprague-Dawley rats and maintained in an interface chamber under perfusion with a medium containing 124 m*M* NaCl, 3 m*M* KCl, 3 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 26 m*M* $NaHCO_3$, 1.25 m*M* KH_2PO_4, 10 m*M* glucose, and 2 m*M* L-ascorbate. The slices were incubated for 60 min in this medium before being switched to a medium containing only 20 to 100 μ*M* Mg^{2+}, a condition that substantially increases NMDA receptor–mediated potentials. After 1 hour of incubation, the flow was stopped, and the experiments were carried out in static conditions. Synaptic field potentials were recorded extracellularly in the stratum radiatum of CA1 and evoked by two independent groups of Schaffer-commissural afferents with two stimulating electrodes placed on either side of the recording pipette. The stimulation intensities were adjusted so that the electrodes evoked responses of similar sizes (between 2 and 3 mV) and, after 20 min of stable recordings, LTP was induced on one input by using short trains of high-frequency stimulation (ten bursts at 5 Hz com-

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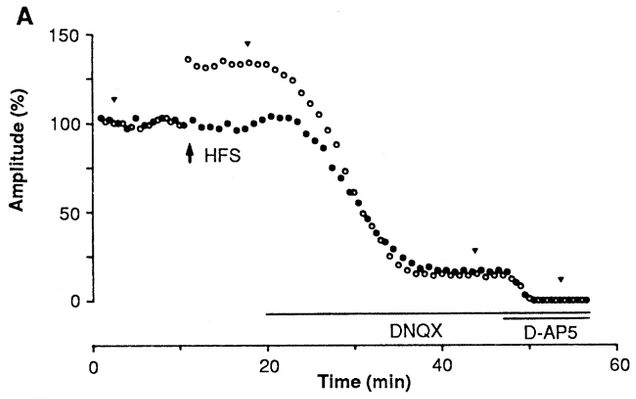

Fig. 1. Effect of DNQX on control and potentiated responses evoked in the presence of 50 μM magnesium. (**A**) Typical experiment illustrating the changes in EPSP amplitude produced by application of 10 μM DNQX on the control and potentiated responses evoked by two independent inputs within the same slice [●, control; ○, potentiated input; high-frequency stimulation (HFS)]. The responses left after DNQX application were completely blocked by 30 μM D-AP5. (**B**) Representative field EPSPs recorded in the same experiment at the times indicated by the triangles.

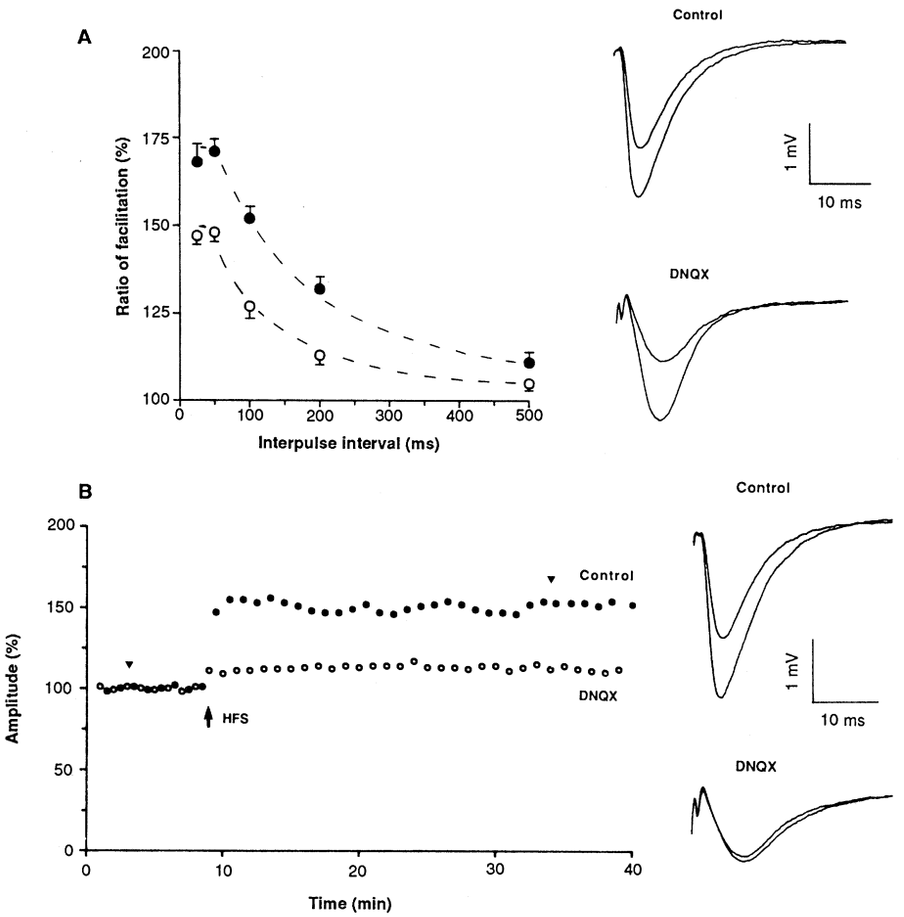

Fig. 2. Effect of DNQX on responses to repetitive stimulation. (**A**) Degree of paired-pulse facilitation expressed as a function of the interpulse interval and measured on the initial slope of responses recorded before (○) and after (●) application of 10 μM DNQX. Each point represents the mean ± SEM of five separate experiments. Representative EPSPs recorded before and after DNQX application and elicited with a 50-ms interpulse interval are illustrated superimposed on the right. (**B**) Effect of high-frequency stimulation (HFS) applied to one input before (●) and to the other after (○) application of 10 μM DNQX. The representative EPSPs recorded on the two pathways, at the times indicated by the triangles, are illustrated on the right.

posed of four pulses at 100 Hz). The pulse duration was doubled during the high-frequency stimulation to satisfy the cooperativity requirement for LTP induction (7).

Incubation of hippocampal slices in the presence of DNQX results in a complete blockade of synaptic responses. However, when Mg^{2+} is omitted or greatly reduced in the perfusing medium, then a significant component of the response remains insensitive to the drug even after a long period of incubation. This component is completely blocked by a subsequent application of 20 to 50 μM D-AP5 (Fig. 1). In 12 experiments, the amplitude of the D-AP5–sensitive response remaining after application of 10 to 25 μM DNQX corresponded to 15.5% ± 1.2% (mean ± SEM) of the amplitude of the field potential before drug application. This represents slightly less than the D-AP5–sensitive component of a synaptic response evoked by single-pulse stimulation in low Mg^{2+} conditions, but without blockade of the quisqualate type of glutamate receptors (*8*). The reason for this small difference is probably related to the different degree of postsynaptic depolarization produced in the two conditions, a factor that is expected to affect currents through NMDA receptors. Alternatively, DNQX could have a slight antagonist action on NMDA receptors (*9*). The NMDA responses recorded after DNQX application usually had a slower time course than the original field potentials (Fig. 1, B and C, and Fig. 2).

The effects of DNQX on control and potentiated responses were measured in 11 experiments by using two responses elicited within the same slice that were initially of comparable size. High-frequency stimulation produced a 39% ± 4% increase in the slope and a 30% ± 2% increase in the amplitude of one of the responses. Application of DNQX reduced the size of the two responses, but to a greater degree on the potentiated than control excitatory postsynaptic potentials (EPSPs); thus, the NMDA response remaining after drug application was very similar if not smaller on the potentiated as compared to the control input (Fig. 1). In a slice-by-slice comparison, the amplitude of the NMDA response, expressed for each pathway as percent of the amplitude of baseline EPSPs (before high-frequency stimulation or drug application) was on the average slightly smaller on the potentiated than on the control input of the same slice (13.6% ± 1.3% versus 15.5% ± 1.2%). It appears therefore that DNQX eliminated the difference between pathways produced by LTP.

A possible explanation for this result would be that currents through NMDA receptors are maximal in control stimulation

and thus could not become larger with LTP. We tested this idea by using paired pulses to transiently augment transmitter release and response amplitudes. Paired-pulse facilitation was still observed in the presence of DNQX even when responses were increased to levels from before drug administration by adjusting the stimulation intensity (Fig. 2A). In fact, the degree of facilitation was greater after DNQX application than before, an effect observed at all interpulse intervals tested. It is unlikely therefore that currents through NMDA receptors were saturated in control pathways.

The above experiments suggested that the changes associated with LTP are mediated by quisqualate receptors with little contribution from the NMDA receptors. As a further test of this, we examined the effect of high-frequency stimulation applied to NMDA responses that remain after applying DNQX. Afferent stimulation with high-frequency bursts of the type that induce robust LTP in absence of DNQX did not produce similar changes in the size of the residual NMDA responses (Fig. 2B). In seven experiments in which responses were set to a comparable size in the two conditions, the mean degree of potentiation recorded in presence of 10 μM DNQX was only 12% ± 4%, a result that contrasts with the 52% ± 6% ($P < 0.01$; Mann-Whitney U test) increase in EPSP amplitude produced by high-frequency stimulation of different inputs before DNQX application.

The absence of a marked potentiation in the presence of DNQX could be interpreted in two ways: either high-frequency stimulation of NMDA responses failed to induce LTP or NMDA responses did not express the potentiation effect. To distinguish between these possibilities, we carried out six experiments in which we compared the rate of recovery during washout of DNQX of the responses evoked on two pathways, one of which had received high-frequency stimulation in presence of the drug. If LTP had been induced but was not expressed by NMDA responses, then washing out the quisqualate receptor antagonist should reveal the potentiation effect. As a second test we compared the effect of applying high-frequency trains on the two pathways after washout of the drug. The results of a typical experiment are illustrated in Fig. 3, A through C, and a summary of six experiments is shown in Fig. 3, D and E. As indicated, washout of DNQX (*10*) resulted in a larger increase in size of the responses recorded on the input that had received high-frequency stimulation in the presence of the drug. The difference in the slopes of the washout curves for the two inputs was highly significant ($P < 0.01$). Also high-frequency stimulation applied on both inputs after washout of DNQX resulted in a far greater degree of LTP on the control than on the previously stimulated input (Fig. 3, C and E). The difference was clearly significant ($P < 0.01$) for both amplitude and slope measurements. It appears therefore that LTP can be induced in the presence of DNQX, but is not expressed by NMDA responses.

By using a selective antagonist of the quisqualate type of glutamate receptors, we have shown that induction of LTP does not affect field EPSPs dependent on NMDA receptors and that LTP can be induced but not manifested by high-frequency stimulation of NMDA receptor–mediated responses. These results point to two conclusions. First, LTP induction does not appear to require the participation of quisqualate receptors. This observation strengthens the idea that NMDA receptor–mediated currents initiate the potentiation effect (*1, 11*). Second, our results suggest that the responses dependent on activation of quisqualate but not NMDA receptors exhibit a marked potentiation effect. The selective properties indicated by this pattern of results restricts

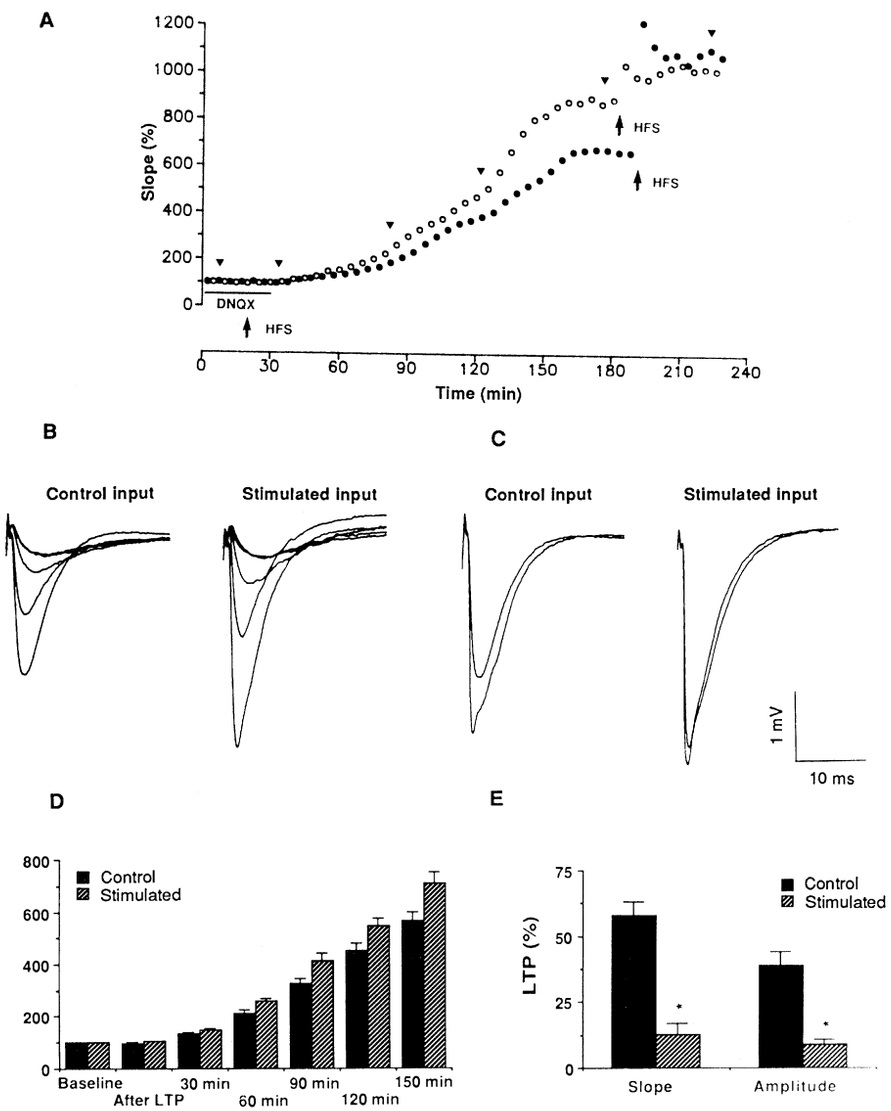

Fig. 3. Effect of DNQX washout on responses that received high-frequency stimulation in the presence of the drug. (**A**) Typical experiment illustrating the recovery in EPSP slope measured during washout of 10 μM DNQX on the responses evoked on a control (●) and previously stimulated (○) pathway. High-frequency stimulation (HFS) was applied as indicated by the arrows. (**B**) Representative EPSPs recorded during washout of DNQX in the experiment illustrated in (A) at the times indicated by the triangles. (**C**) EPSPs recorded before and 20 min after the high-frequency trains applied after washout of DNQX in the same experiment. (**D**) Summary of the changes in EPSP slope measured in six experiments on control and stimulated inputs during DNQX washout. The difference in the slopes of the washout curves for the two inputs (control, 3.22% ± 0.20% per minute; potentiated, 4.30% ± 0.23% per minute; $n = 6$) was highly significant ($P < 0.01$). (**E**) Effect of high-frequency stimulation applied in the same experiment on the two pathways after washout of DNQX.

the number of cellular events that could be responsible for the expression of the potentiation effect. Specifically, it would seem that the differential contribution of the two receptor types to LTP is incompatible with the idea that the potentiation effect results from an increase in transmitter release by a fixed population of terminals (*12*), since this would presumably affect both types of receptors. So it is noteworthy that NMDA-mediated responses exhibit robust paired-pulse facilitation, a transient presynaptic form of plasticity; moreover, binding studies suggest that NMDA receptors are at least as numerous as quisqualate receptors in area CA1 of hippocampus (*13*) and in postsynaptic densities (*14*).

On the other hand, several postsynaptic mechanisms could account for the selective changes produced by LTP. A first possibility would be a modification of the biophysical properties of the quisqualate, but not NMDA, receptor-ionophore complex, such as changes in the major conductance state of the channel (*15*). Other explanations are suggested by electron microscopic experiments that show that LTP is correlated with a change in the morphology of dendritic spines and, perhaps related to this, an increase in the number of synaptic profiles located on dendritic shafts and of spines with very short necks (*16*). Formation of new synapses lacking NMDA receptors (*17*) would account for the pattern of results described here, while changes in the shape (and thus biophysics) of existing spines might also produce the observed effects by differentially affecting the fast rising (non–NMDA receptor–mediated) versus more slowly developing (NMDA receptor–mediated) synaptic responses. Some models of dendritic spines suggest that changes in spine neck parameters could have effects of this type (*18*).

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Molecular Cloning of Two Types of GAP Complementary DNA from Human Placenta

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The *ras* p21 GTPase-activating protein (GAP) was purified from human placental tissue. Internal amino acid sequence was obtained from this 120,000-dalton protein and, by means of this sequence, two types of complementary DNA clones were isolated and characterized. One type encoded GAP with a predicted molecular mass of 116,000 daltons and 96% identity with bovine GAP. The messenger RNA of this GAP was detected in human lung, brain, liver, leukocytes, and placenta. The second type appeared to be generated by a differential splicing mechanism and encoded a novel form of GAP with a predicted molecular mass of 100,400 daltons. This protein lacks the hydrophobic amino terminus characteristic of the larger species, but retains GAP activity. The messenger RNA of this type was abundantly expressed in placenta and in several human cell lines, but not in adult tissues.

THE *ras* P21 GTPASE-ACTIVATING protein (GAP) was first identified as a cytoplasmic factor from *Xenopus* oocytes that greatly stimulated GTPase activity of normal N-*ras* p21 without affecting oncogenic mutants (*1*). Presence of this factor explained how normal p21 proteins could exist in their inactive, GDP-bound states in vivo, while oncogenic mutants remain GTP-bound and thus fully active. Subsequently, it was shown that extracts from many, if not all, types of higher eukaryotic cells contain GAP activity, and that GAP interacts with *ras* p21 proteins at a site that is essential for *ras* action (*2, 3*). This site, the putative effector binding site, corresponds to amino acids 32 to 40 of mammalian p21 proteins; deletions or certain point mutations in this region prevent transformation of cells by v–H-*ras* and prevent activation of *Saccharomyces cerevisiae* adenylate cyclase in vitro (*4*). Since GAP interacts at this site, it is likely that GAP itself is an effector of *ras* action, and that elucidation of other biochemical properties of GAP may shed light on the role of *ras* oncogenes in cellular transformation.

Recently, Gibbs and co-workers purified GAP from bovine brain and confirmed that this purified protein, which has an apparent size of 125,000 daltons, was able to stimulate GTPase activity of normal H-*ras* p21 without affecting GTPase activity of position 12 mutants (*5*). By means of the sequence obtained from this purified protein, a cDNA clone encoding bovine GAP was obtained (*6*). Furthermore, it was shown that oncogenic forms of *ras* p21 in their GTP-bound forms bind to GAP; this observation strongly supports the suggestion that GAP is an effector of *ras* action. In this paper, we describe the purification of GAP from human placenta and the molecular cloning of cDNA encoding this protein. In addition, we describe a second form of GAP cDNA that could encode GAP with an altered NH_2-terminus; we also demonstrate

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