Stimulation of Phosphoinositide Turnover by Excitatory Amino Acids

Pharmacology, Development, and Role in Visual Cortical Plasticity[a]

MARK F. BEAR AND SERENA M. DUDEK

Center for Neural Science
Brown University
Providence, Rhode Island 02912

INTRODUCTION

It is a widely held belief that the modification of brain, especially neocortex, by sensory experience is a likely basis for memory in mammals. One model system in which such experience-dependent changes can be studied is the ascending visual pathway in the cat. In this system, the frontally placed retinae give rise to two independent streams of sensory information that are combined at the level of the striate cortex (area 17) to form a single percept of visual space. Wiesel and Hubel demonstrated 25 years ago that the binocular connections in cat visual cortex can be dramatically altered by simple forms of sensory deprivation, such as the temporary closure of one eyelid (monocular deprivation; MD) or the misalignment of the two eyes (strabismus).[1] The modification of binocular connections by sensory experience does not occur throughout life, however; it is restricted to a "critical period" of early postnatal development.[2] The sensitivity of cat visual cortex to brief (7-14 day) MD develops at about 3 weeks of age and disappears by 3 months of age.[3] This is a period of rapid head and body growth in which plasticity of binocular connections evidently is normally necessary to maintain proper retinal correspondence.

We divide the problem of visual cortical plasticity into three parts. *First,* what controls the onset and duration of the critical period? The answer to this question is unknown at present, but some interesting possibilities include specific patterns of gene expression[4,5] that may be under hormonal control.[6] *Second,* within the critical period, what factors enable synaptic modification to proceed? This question is prompted by the observation that many experience-dependent modifications of visual cortex seem to require that animals attend to visual stimuli and use vision to guide behavior.[7] The best candidates for "enabling factors" are the neuromodulators acetylcholine and norepinephrine that are released in visual cortex by fibers arising from neurons in the basal forebrain and brain stem.[8] *Third,* when modifications are allowed to occur during the critical period, what controls their direction and magnitude? This is the part of the problem where we believe that excitatory amino acid (EAA) receptors play a central role.[9-13]

Consider a single cortical neuron receiving input (via the lateral geniculate nucleus) from homotypic points on the two retinae. When patterns of retinal activity in the

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two eyes occur in register, then the binocular connections in cortex consolidate and strengthen during the critical period. However, when the patterns of retinal activity are brought out of register, either by MD or strabismus, then the binocular connections are disrupted: effective inputs are retained and ineffective inputs are lost, where "effectiveness" is determined by whether an input drives the cortical neuron beyond some threshold. These theoretical considerations have led naturally to the hypothesis that excitatory synapses in visual cortex are consolidated during development when their activity consistently correlates with target depolarization beyond a critical level.[14–16] What mechanism can support this form of "Hebbian" modification?

One strong candidate is the *N*-methyl-D-aspartate (NMDA) receptor mechanism. NMDA receptors are thought to coexist postsynaptically with other types of EAA receptors.[17,18] Together, both NMDA- and non-NMDA-type EAA receptors mediate excitatory synaptic transmission in the kitten striate cortex.[19] The ionic conductances activated by non-NMDA receptors at any instant depend only on input activity, and are independent of the postsynaptic membrane potential. However, the ionic channels linked to NMDA receptors are blocked with Mg^{2+} at the resting potential, and become effective only upon membrane depolarization.[20,21] Another distinctive feature of the NMDA receptor channel is that it will conduct calcium ions.[22,23] Hence, the passage of Ca^{2+} through the NMDA channel could specifically signal when pre- and postsynaptic elements are concurrently active. This property led naturally to the hypothesis that synaptic consolidation in the visual cortex occurs when the NMDA receptor-mediated Ca^{2+} flux exceeds some critical level.[9]

As the other papers in this volume attest, this hypothesis is supported by work on a wide range of model systems. Besides the pioneering studies on long-term potentiation (LTP) in adult hippocampus,[24,25] work on the retinotectal projection in goldfish,[26] and on both the retino- and isthmotectal projections in frogs,[27–30] indicates that NMDA receptor activation is an essential step in the strengthening of coactive synapses. This hypothesis is also supported by several lines of research in the visual cortex. For example, it is now well documented that a form of LTP can be elicited in visual cortex and that this depends on NMDA receptor activation.[31–34] Furthermore, blockade of visual cortical NMDA receptors *in vivo* disrupts the physiological[13,35,36] and anatomical[37] consequences of visual deprivation during the critical period. Thus, there appears to be strong support for the idea that NMDA receptor mechanisms subserve a Hebbian form of synaptic modification in many locations in the vertebrate brain, including the cat visual cortex.

A second form of modification, however, appears to be required to explain visual cortical plasticity. Recall that, according to our reasoning above, a theoretical consequence of input activity that *fails* to correlate with postsynaptic depolarization is a weakening of synaptic efficacy.[16] Indeed, there is direct evidence that retinal activity promotes synaptic weakening in striate cortex under conditions where cortical neurons are unable to respond normally.[13,38] What mechanism can support this form of use-dependent synaptic depression?

Clearly, activation of non-NMDA receptors can signal input activity regardless of the level of postsynaptic depolarization. This line of reasoning has led us to investigate further the consequences of activating non-NMDA receptors in the visual cortex, with the specific aim of identifying a possible mechanism of synaptic weakening. We have become interested in a type of non-NMDA receptor, which we call the Q_2 receptor, that is linked, probably via a G protein, to the enzyme phospholipase C (PLC). PLC catalyzes the hydrolysis of membrane phosphoinositides to form diacylglycerol (DAG) and inositol trisphosphate (IP_3), both of which serve as intracellular second messengers (FIG. 1). In this paper, we discuss our current understanding of the pharmacology and development of the Q_2 receptor in the neocortex. In addition, we present preliminary

evidence suggesting that activation of this receptor may be required for some forms of experience-dependent modification in kitten visual cortex.

PHARMACOLOGICAL CHARACTERIZATION OF THE Q_2 RECEPTOR

The ability of certain EAAs to stimulate the hydrolysis of phosphoinositides was first demonstrated in cultures of striatal neurons.[39] Subsequently, EAA-stimulated phosphoinositide (PI) turnover was shown to occur in a number of tissues, including hippocampal slices, cerebellar granule cells in culture, cortical synaptoneurosomes, and frog oocytes injected with rat brain mRNA.[40–49] Although differences between systems exist, there is general agreement that PI turnover in most locations is potently stimulated by quisqualate, ibotenate, glutamate, and *trans*-1-amino-1,3-cyclopentane dicarboxylic acid (*trans*-ACPD). Sensitivities to other EAAs have been reported, but this evidently depends on the source of tissue. For example, NMDA stimulates PI turnover in striatal and cerebellar neurons in culture.[39,42]

To investigate the relative effectiveness of different EAAs in neocortex, we assayed PI turnover in synaptoneurosomes prepared from rat and cat.[11,48] In both tissues we found ibotenate, quisqualate, and glutamate to be effective agonists. We also found that NMDA was ineffective in stimulating PI turnover (FIG. 2), and that the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5) did not reduce glutamate-stimulated PI turnover. Thus, in neocortex, PI turnover is stimulated by EAAs specifically at a *non*-NMDA receptor site. Work using hippocampus[40] and frog oocytes injected with rat brain mRNA[43] has led to a similar conclusion.

It was originally proposed that two different EAA receptors stimulate PI turnover in neurons: a receptor that prefers ibotenate, and one that prefers quisqualate.[50] Evidence leading to this hypothesis is that ibotenate is most effective in stimulating PI turnover in hippocampus, whereas quisqualate is the more potent agonist in striatum, cerebellum, and rat whole forebrain. Schoepp and Johnson,[45] however, have demonstrated in hippocampal slices that maximal stimulation of PI hydrolysis by ibotenate and quisqualate

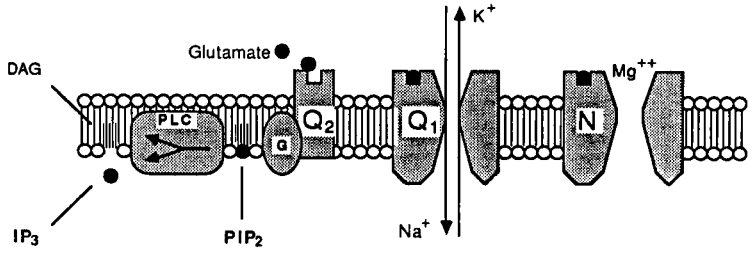

FIGURE 1. A hypothetical postsynaptic response to glutamate. Upon release as a neurotransmitter, glutamate binds to the *N*-methyl-D-aspartate receptor (N), which is blocked by magnesium at resting membrane potentials, and to a quisqualate receptor associated with an ion channel (Q_1). Additionally, glutamate may bind to a second type of quisqualate receptor linked to phosphoinositide metabolism (Q_2). Upon the binding of glutamate to the Q_2 receptor, phospholipase C (PLC) is activated via a G protein (G); PLC breaks down phosphatidyl inositol-4,5-bisphosphate (PIP_2) into second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3).

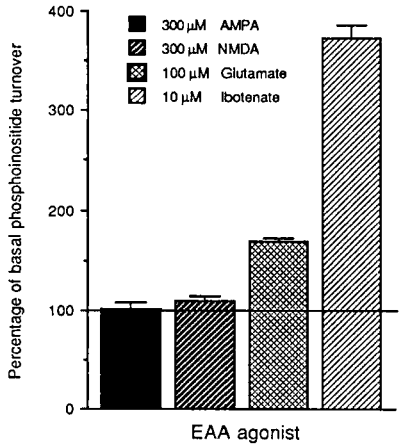

FIGURE 2. The effects of different excitatory amino acids on [^{3}H]inositol phosphate accumulation in synaptoneurosomes prepared from 5-week-old kittens. Glutamate and ibotenate stimulate PI turnover while *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) are without effect. Results are means ± SEMs from at least three experiments. Methods in reference 11.

is not additive, suggesting that they act at a single site (in contrast, stimulation by quisqualate and acetylcholine is additive, confirming that these act at separate sites). Similarly, Desai and Conn[51] have presented evidence that quisqualate and ibotenate both act as partial agonists at the same site stimulated by *trans*-ACPD. This conclusion is also supported by work by Sugiyama *et al.*[43] in the oocyte preparation. It thus seems reasonable to conclude that in cortex PI turnover is stimulated by a single type of non-NMDA EAA receptor.

The pharmacological profile of EAA-stimulated PI turnover in hippocampus and neocortex suggests that the quisqualate (Q) receptor is specifically involved. However, the selective Q agonist α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) does not stimulate PI turnover (FIG. 2), and the Q antagonists 6-cyano-7-nitroquinoxaline (CNQX) and kynurenate do not block quisqualate-stimulated PI turnover. For this reason, we divide Q receptors into two classes, Q_1 and Q_2 (FIG. 1). Q_1 receptors are those sensitive to AMPA, CNQX, and kynurenate; activation of these receptors leads to an ionic conductance. Q_2 receptors are those sensitive to *trans*-ACPD; activation of these receptors leads to PI hydrolysis. It should be noted that other nomenclatures have been proposed: Q_p or IBO_p receptor,[50] metabotropic glutamate receptor (mGluR or Glu_BR),[52] and the ACPD receptor.[53] We favor Q_2 because of its simplicity (and because we thought of it).

It has been shown that quisqualate and glutamate potently stimulate PI turnover in cultured astrocytes.[54,55] How do we know what fraction of the EAA-stimulated PI turnover we measure in cortex is neuronal in origin? Palmer *et al.*[56] have shown in rat hippocampal slices that NMDA inhibits quisqualate-stimulated PI turnover in a calcium-dependent fashion. These data suggest that the bulk of the EAA-stimulated PI turnover measured in hippocampus occurs in neurons because astrocytes do not express NMDA receptors.[57] It should be noted that Baudry *et al.*[58] found that NMDA also inhibits carbachol-, histamine-, and depolarization-induced stimulation of PI turnover in hippocampal slices. We have performed similar experiments in visual cortical slices, and also find that NMDA can completely block quisqualate stimulation of PI turnover (FIG. 3). The inhibition of PI turnover by NMDA in slices may be solely due to excitotoxicity, but the interesting possibility remains that this may be a physiologically relevant regulatory mechanism.[56] In any case, these data support the idea that Q_2 receptors are expressed on visual cortical neurons.

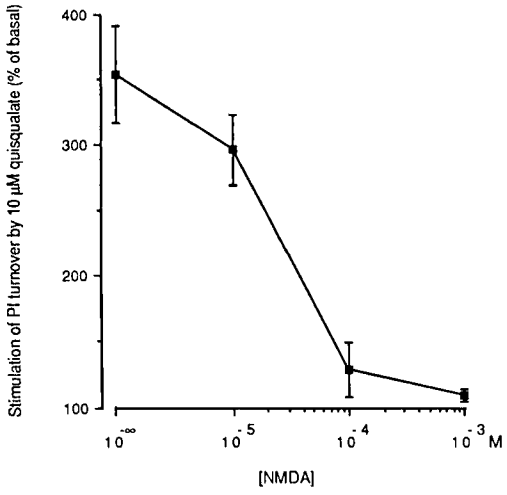

FIGURE 3. Inhibition of quisqualate-stimulated PI hydrolysis by NMDA in slices of kitten visual cortex. Methods are described in reference 56. Results are from four experiments and represent means ± SEMs.

Certainly, the elucidation of the function of EAA-stimulated PI turnover depends on the development of potent and selective antagonists of the Q_2 receptor. Several compounds with antagonist-like properties have been described, including 2-amino-4-phosphonobutyrate (AP4), 2-amino-3-phosphonopropionate (AP3), and L-serine-*O*-phosphate (LSOP). Nicoletti *et al.*[41] have shown that both AP4 and LSOP inhibit stimulation of PI turnover by ibotenate in adult rat hippocampal slices. These compounds, however, are not selective for the Q_2 receptor.[46] Moreover, AP4 and LSOP actually *stimulate* PI turnover in hippocampal slices from neonatal rats. The use of AP3 as a Q_2 inhibitor will be discussed in detail below, when we consider the involvement of these receptors in visual cortical plasticity.

DEVELOPMENT OF THE Q_2 RECEPTOR

One of the most striking features of EAA-mediated PI turnover is its dependence on the age of the animal. First to describe the developmental profile of the Q_2 receptor was Nicoletti *et al.*[41] Using rat hippocampal slices, they showed that stimulation of PI hydrolysis by ibotenate and glutamate is at least 3 times greater in neonates than in adults. A similar developmental profile was found using rat neocortical synaptoneurosomes.[48] We found that glutamate-stimulated PI turnover is maximal at 1 week of age, when it is approximately 5 times the adult value (FIGS. 4A & 5A). By 5 weeks of age, however, EAA-stimulated PI turnover declines to the low adult level. It is interesting to note that Q_2 receptors appear to develop postnatally, as glutamate was found to be ineffective in stimulating PI turnover in newborn rat neocortex. Thus, the increase in EAA-stimulated PI turnover occurs coincident with the period of most rapid synaptogenesis in rat neocortex.[59] These observations have led to speculation that EAA-stimulated PI turnover plays a special role in cortical development and plasticity.[41,48]

As was discussed earlier, modification of binocular connections in visual cortex by sensory experience is also restricted to a critical period of early postnatal development.

In kittens, sensitivity to lid closure begins at about 3 weeks of age, peaks at 5 weeks, and disappears between 12 and 16 weeks.[3] We were, of course, keenly interested in how well the developmental profile for EAA-stimulated PI turnover maps onto this profile of the critical period. A synaptoneurosome preparation of kitten area 17 was used to investigate this possibility.[11]

As illustrated in FIGURE 4B, the developmental differences in glutamate-stimulated PI turnover in kitten striate cortex were similar to those observed in rat neocortex; stimulation in the neonate was greater than 3 times the adult value. The more potent agonist ibotenate was used to stimulate PI turnover in the striate cortex at different postnatal ages, and the results are shown in FIGURE 5B. The postnatal changes in ibotenate-stimulated PI turnover show a remarkable correlation with the development of ocular dominance plasticity in kitten striate cortex. Thus, when the striate cortex is most sensitive to sensory deprivation, it is also most sensitive to stimulation of PI hydrolysis by certain excitatory amino acids.

It is interesting to note that other receptors linked to PI turnover, such as the M_1 acetylcholine receptor and the α_1 norepinephrine receptor, do not share this same developmental profile. For example, in rat hippocampus, Nicoletti *et al.*[41] found that norepinephrine does not potently stimulate PI turnover until *after* 3 weeks of age. In kitten striate cortex, the muscarinic agonist carbachol stimulates PI hydrolysis at all ages, indicating that the transient increase in stimulated PI turnover during the critical period is relatively specific to a mechanism linked to the Q_2 receptor.

The normal development of Q_2 receptors in striate cortex evidently requires visual experience. We found that the transient rise in ibotenate-stimulated PI turnover at 5 weeks of age did not occur in the striate cortex of dark-reared kittens. In contrast, stimulation by carbachol was unaffected by visual deprivation. These findings further support the idea that EAA-stimulated PI turnover may play a specific role in develop-

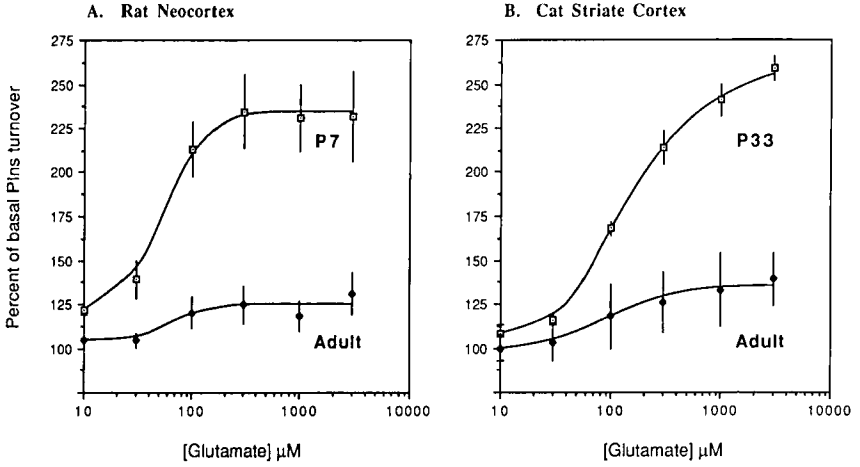

FIGURE 4. Stimulation of PI turnover by glutamate. Accumulation of [^{3}H]inositol phosphate was measured in synaptoneurosomes prepared from **(A)** rat neocortex and **(B)** cat striate cortex. In both cases, glutamate was more effective in young animals (postnatal day 7 in the rat, and 33 in the cat) than in adults. Results are means ± SEMs from at least three experiments. Data in **A** redrawn from reference 48; data in **B**, from reference 11, Table 1.

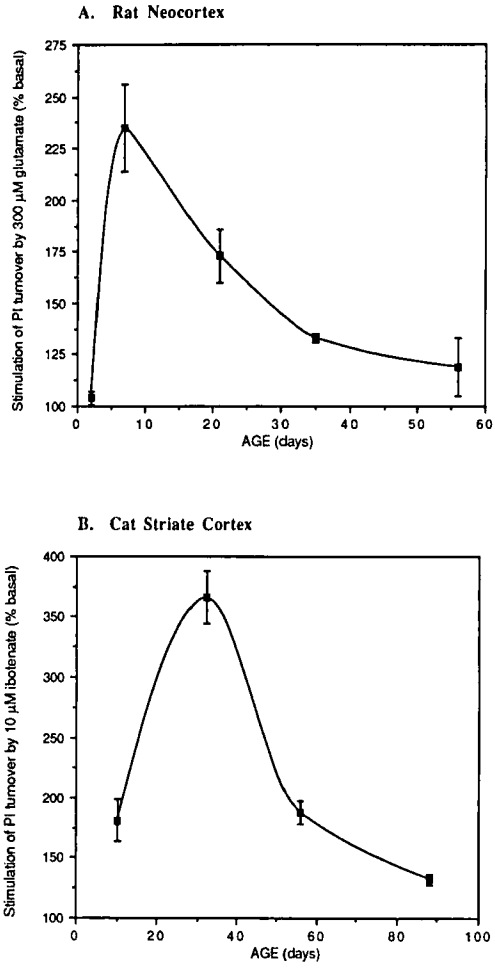

FIGURE 5. Age dependence of EAA-stimulated PI turnover. [^{3}H]Inositol phosphate was measured in synaptoneurosomes prepared from **(A)** rat neocortex and **(B)** cat striate cortex. PI turnover was stimulated with 300 μM glutamate in the rat and 10 μM ibotenate in the cat. Results are means ± SEMs from at least three experiments. Data in **A** redrawn from reference 48; data in **B**, from reference 11.

mental plasticity because dark rearing is known to postpone the onset of the critical period.[60]

Q_2 RECEPTORS AND OCULAR DOMINANCE PLASTICITY

The evidence discussed in the preceding section suggests that at the same ages that retinal activity drives synaptic modification in kitten striate cortex, excitatory synaptic activity also stimulates a specific second-messenger cascade in visual cortical neurons. These data are therefore consistent with our hypothesis that Q_2 receptors play a central role in the activity-dependent modification of striate cortex during the critical period. Direct tests of this hypothesis have been hampered, however, by the lack of a potent and selective antagonist.

One compound that has shown some promise as an inhibitor of EAA-stimulated PI turnover is 2-amino-3-phosphonopropionate (AP3). Using hippocampal minces prepared from adult rats, Schoepp and Johnson[46] found that D,L-AP3 inhibits PI turnover stimulated by 100 μM ibotenate with an IC_{50} of approximately 120 μM. It is of interest to note that AP3 had long been considered inert at EAA receptors, which is not surprising, as AP3 has no effect on the specific binding of ^{3}H-labeled ligands to the other EAA receptor types (kainate,[61] AMPA,[62] *N*-acetylaspartylglutamate,[46] various NMDA receptor ligands[63]). Thus, it appears that the action of AP3 is selective for the Q_2 receptor.

There are several indications, however, that the mechanism of AP3 action is complex. First, the effectiveness of AP3 as an inhibitor is age dependent. Using hippocampus from 1-week-old rats, Schoepp and Johnson[47] found that AP3 inhibited ibotenate-stimulated PI turnover with an IC_{50} of $\sim$370 μM, more than 3 times the adult value. Second, AP3 itself has been shown to stimulate PI turnover, weakly in comparison with quisqualate and ibotenate, but nonetheless significantly ($\sim$200% of basal with 1 mM AP3; Schoepp and Johnson[47]). Finally, the inhibitory effects of AP3 seem to vary with the potency and concentration of the EAA agonist used to stimulate PI turnover.[48,51] These considerations have led to the hypothesis that AP3 inhibits EAA-stimulated PI turnover by acting as a "partial agonist" at the Q_2 receptor.[46,47,51]

Our own experiments using AP3 to antagonize EAA-stimulated PI turnover in visual cortex have yielded mixed results. In synaptoneurosomes, we find that AP3 inhibits PI turnover stimulated by 10 μM ibotenate with an apparent IC_{50} of about 80 μM (FIG. 6). Using slices of visual cortex, however, we find that the stimulation of PI turnover by 10 μM quisqualate is not inhibited by AP3 in concentrations up to 1 mM[64]; in fact, the stimulation of PI turnover by quisqualate and AP3 appears to be additive. There are a number of possible explanations for our results (differences in the preparation of the tissue, for example). They are consistent, however, with the hypothesis that AP3 is a partial agonist where, by definition, the inhibitory effect depends on the concentration and efficacy of the "pure" agonist used.[65]

These caveats notwithstanding, at the present time AP3 remains the most selective pharmacological tool available to test the hypothesis that EAA-stimulated PI turnover plays a specific role in visual cortical plasticity. Therefore, we performed a series of

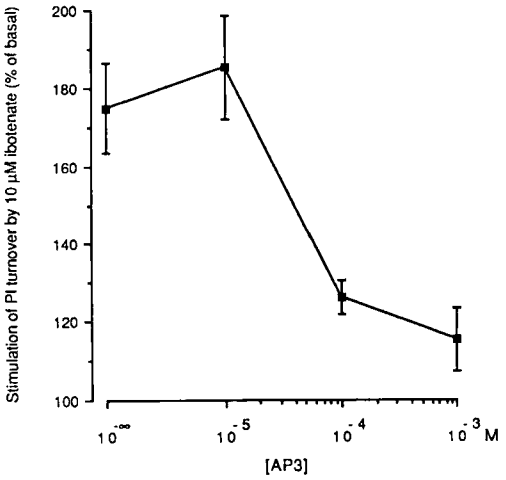

FIGURE 6. 2-Amino-3-phosphonopropionate (AP3) inhibition of ibotenate-stimulated PI turnover. AP3 inhibits stimulation of PI turnover by 10 μM ibotenate in synaptoneurosomes from kitten striate cortex (kittens aged 5-7 weeks). Results are from five experiments and are expressed as means $\pm$ SEMs.

experiments to see if the chronic infusion of AP3 into the visual cortex would interfere with the synaptic modifications that are elicited by brief MD during the critical period. In our initial experiments, kittens were reared normally to approximately 8 weeks of age, at which time one eyelid was sutured closed (TABLE 1). At the same time, minipumps were implanted that could deliver solutions directly to the visual cortex at a rate of 1 μl/hr. AP3 (50 mM) dissolved in sterile Ringer was infused into the hemispheres contralateral to the deprived eye; Ringer alone was infused into hemispheres ipsilateral to the deprived eye. Then, 7-8 days later, the animals were prepared for the physiological assessment of cortical binocularity according to routine procedures.[13]

Electrophysiological recordings from the Ringer-treated (control) hemispheres confirmed that the period of MD was sufficient to cause an ocular dominance shift; the large majority of visual cortical neurons responded preferentially to stimulation of the eye that had been open. In striking contrast, most neurons responded vigorously through both eyes in the contralateral, AP3-treated hemispheres (FIG. 7). These experiments suggest that AP3 treatment can block the effects of MD in the visual cortex contralateral to the deprived eye in 2-month-old kittens. Preliminary work, however, suggests that AP3 treatment is less effective under conditions where the effects of MD are more severe, for example, in the hemispheres ipsilateral to the deprived eye in younger kittens. Although more work needs to be done, our impression is that the effectiveness of AP3 in blocking the ocular dominance shift after MD varies as a function of age. This perhaps is not surprising considering the age dependency of AP3's inhibition of EAA-stimulated PI turnover.[47,48]

It should be noted that in cases where AP3 treatment blocks the ocular dominance shift, it does not appear to disrupt other receptive field properties such as orientation and direction selectivity. This result contrasts with the effects of chronic NMDA receptor blockade, where most affected neurons lack normal selectivity and responsiveness.[13] This observation leads us to believe that AP3 is probably not exerting its effects on visual cortical plasticity by blocking NMDA receptors.[b] Of course, the interpretation of our results using AP3 is still complicated by all the uncertainties concerning the action of AP3 on the Q_2 receptor. There is clearly a need for a pure antagonist of EAA-stimulated PI turnover. Nonetheless, our results are consistent with the hypothesis that the Q_2 receptor is centrally involved in the experience-dependent modification of visual cortex during the critical period.

Assuming that this hypothesis is in fact correct, then what is the specific contribution of the Q_2 receptor to the mechanisms of visual cortical plasticity? Theoretical analysis led us to propose that Q_2 activation might play a specific role in the process of activity-dependent synaptic weakening.[10-12] Evidence in support of this hypothesis has been obtained recently by Stanton and colleagues in slices of hippocampus. They find that activation of excitatory afferents coincident with strong hyperpolarization of the postsynaptic target neuron leads to a lasting depression of synaptic effectiveness.[66] And, induction of this form of long-term depression, or "LTD," evidently is blocked by application of AP3.[67]

Of course it is possible that Q_2-mediated PI turnover plays an entirely different role in the mechanisms of synaptic plasticity than the one we propose here. For example, Q_2-stimulated PI turnover might lead to the release of intracellular Ca^{2+} and thus

[b] This also raises a question of theoretical interest. Is the absence of AP3 effects on selectivity to be expected if the Q_2 receptor specifically mediates use-dependent decreases in synaptic strength? Although this question requires further study, post hoc reasoning suggests that the blockade of a synaptic weakening signal would not necessarily have an effect on receptive field properties that had been established previously.

TABLE 1. Effects of AP3 on Ocular Dominance Plasticity

Animal	Age at Implant/MD (days)	Age at Recording (days)	Hemisphere	Treatment	Open Eye Dominance[a]	Binocularity[b]	N^c
SE1	53	60	L	Ringer	0.74	0.36	27
			R	AP3	0.44	0.65	33
SE4	58	66	L	Ringer	0.89	0.19	38
			R	AP3	0.28	0.76	37
UO4	57	64	L	Ringer	0.96	0.08	27
			R	AP3	0.19	0.78	34

[a] Open eye dominance is the number of cells in ocular dominance group 5 plus 0.5 times the number of cells in group 4 divided by the total number of classifiable cells (group 5, by convention, is the monocular group dominated by the open eye). A value of 1.0 would represent a complete ocular dominance shift to the open eye.

[b] Binocularity is defined as the total number of cells in OD groups 2 to 4 divided by the total number of classifiable cells and represents the proportion of cells that are binocular.

[c] *N* refers to the number of units recorded in each hemisphere.

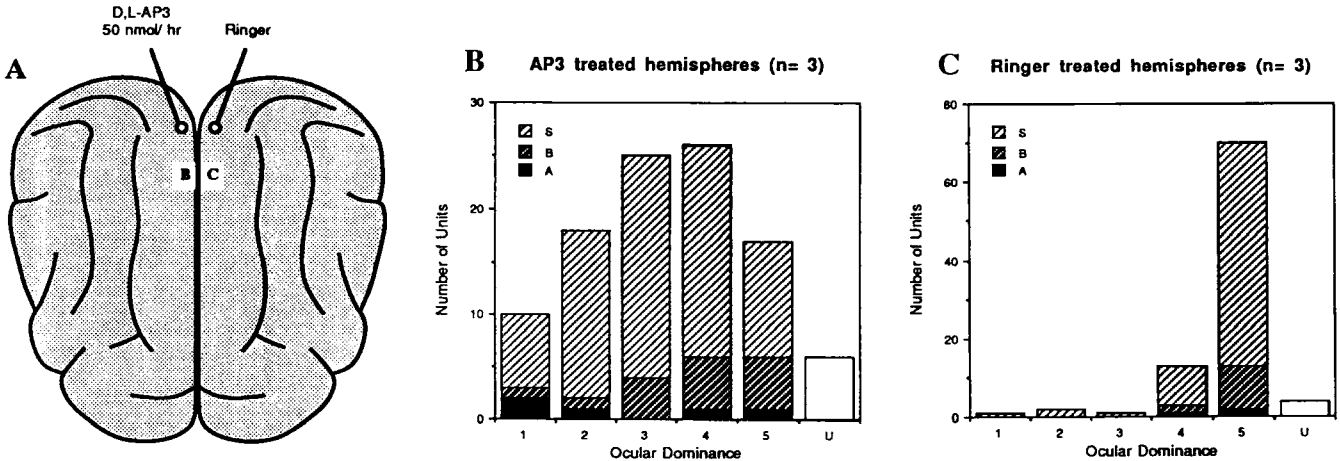

FIGURE 7. Effect of AP3 on ocular dominance plasticity. (**A**) Dorsal view of a kitten brain to illustrate the experimental design. Miniature osmotic pumps (ALZA 2001) were used to deliver either 50 mM AP3 dissolved in Ringer, or Ringer alone, into striate cortex at a rate of 1 μl/hr via chronically implanted stainless steel canulae fasioned from 27-gauge hypodermic needles. At the same time, the left eyelid was sutured closed. Then, seven to eight days later, recordings were made 3-6 mm anterior to the infusion canulae. Based on previous measurements,[13] we estimate that extracellular [AP3] is ~200 μM in the sample zone (marked "B" in **A**). Control recordings indicated that neurons in the sample zone retained visual responses during AP3 infusion. (**B** & **C**) Ocular dominance (OD) and selectivity histograms compiled from recordings in the AP3-or Ringer-treated cortices in three experiments (see TABLE 1). Multi-unit and single-unit recordings were sampled every 100 μ along multiple tangential electrode tracks, and were classified for OD and orientation selectivity. Neurons assigned to OD categories 1 and 5 were activated by either the left or the right eyes, respectively, but not both. Cells assigned to OD category 3 were activated equally strongly by stimulation of either eye. Neurons in OD categories 2 and 4 were binocular, but their responses were clearly dominated by either the left or right eyes, respectively. Neurons for which no receptive field could be identified were recorded as unclassifiable (U). Filled bars represent the number of cells within each OD group that were aspecific (A) for stimulus orientation; darkly hatched bars, cells that showed a weak bias (B) for stimulus orientation; and lightly hatched bars, neurons that were selective (S) for orientation.

substitute for NMDA-receptor-mediated Ca^{2+} signals. Alternatively, Q_2 receptor activation may simply mimic the effects of other modulatory inputs that are known to stimulate PI turnover. For example, acetylcholine stimulates PI turnover in cortical neurons, and both ACh and the Q_2 agonist *trans*-ACPD decrease a Ca^{2+}-activated potassium conductance in pyramidal cell dendrites.[68] Thus, it is possible that Q_2 receptors affect synaptic plasticity by modulating postsynaptic excitability. On the other hand, because the neuron is so highly compartmentalized, it may not be correct to assume that a consequence of PI turnover at one location is equivalent to that at another. If Q_2 receptors are in fact located beneath modifiable excitatory synapses, it is the PI turnover occurring in dendritic spines that is of special interest. Here, either the diacylglycerol or inositol trisphosphate arms of this biochemical pathway could selectively trigger decreases in synaptic strength.

SUMMARY AND CONCLUSIONS

Theoretical analysis suggests that in the visual cortex during early postnatal development, afferent activity can yield either an increase or a decrease in synaptic strength depending on the pattern of EAA receptor activation in cortical neurons. This motivated us to study the mechanism of EAA-stimulated phosphoinositide turnover in visual cortex. Available evidence suggests that PI hydrolysis is stimulated by EAAs primarily at a single receptor site (Q_2 receptor), and that this site is distinct from both the traditional quisqualate (Q_1) receptor and the NMDA receptor. NMDA does, however, inhibit EAA-stimulated PI turnover in visual cortex, confirming that the Q_2 receptor is on visual cortical neurons (as opposed to glia). We find that Q_2 receptors in the neocortex are expressed transiently during postnatal development. The developmental time-course of EAA-stimulated PI turnover correlates precisely with the critical period when synaptic modifications are most readily elicited in visual cortex by changes in sensory experience. The compound AP3 can inhibit EAA-stimulated PI turnover, probably by acting as a partial Q_2 agonist, and under some circumstances AP3 evidently can interfere with experience-dependent synaptic modifications.

Increases in synaptic strength in visual cortex, as elsewhere, have been linked specifically to activation of NMDA receptors. We propose that decreases in synaptic strength may be specifically related to activation of the Q_2 receptor. Further tests of this hypothesis will require the development of selective and potent antagonists.

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