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N-methyl-D-aspartate-evoked calcium uptake by kitten visual cortex maintained *in vitro*

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Summary. As a functional measure of NMDA receptor effectiveness in kitten striate cortex, the uptake of ^{45}Ca by visual cortical slices was measured after 2 minute bath applications of N-methyl-D-aspartate (NMDA). Significant Ca uptake occured in response to 12.5–100 μM NMDA in slices prepared from visual cortex of normal animals aged 28–48 days. Basal uptake (in the absence of NMDA) was increased and evoked uptake was decreased in visual cortical slices prepared from age-matched dark-reared animals. Four days of binocular deprivation in otherwise normally reared animals had no effect on basal uptake, but significantly lowered NMDA-evoked Ca uptake at agonist concentrations greater than 25 μM. These data suggest that even brief manipulations of sensory experience are sufficient to alter visual cortical calcium regulation.

Key words: Calcium uptake – NMDA receptor – Striate cortex – Deprivation – Kitten – Synoptic plasticity

Introduction

Neurons in the striate cortex of normal adult cats respond selectively to the visual presentation of oriented bars of light and most are activated by stimulation of either eye (Hubel and Wiesel 1962). Both of these properties depend on the visual environment experienced during a critical period of postnatal development (reviewed by Sherman and Spear 1982; Frégnac and Imbert 1984). For example, prolonged binocular deprivation during the critical period (BD) broadens or eliminates orientation selectivity, and binocular connections are modified after very brief periods of monocular deprivation (MD), strabismus, and reverse suture.

Bienenstock, Cooper and Munro (1982) have proposed a theoretical form of synaptic modification for the visual cortex that appears sufficient to account for these varied experimental results (reviewed by Bear *et al.* 1987). According to this theory, the synaptic efficacy of active geniculocortical inputs increases when the postsynaptic target is concurrently depolarized beyond a "modification threshold", θ_M. However, when the level of postsynaptic activity falls below θ_M, the strength of active synapses decreases. Importantly, the value of the modification threshold varies as a non-linear function of the average output of the postsynaptic neuron. This feature is important for model stability, and explains why the low level of postsynaptic activity caused by binocular deprivation does not drive the strengths of all cortical synapses to zero. The predictive success and simple basis of the model suggests that some types of visual cortical plasticity may be the result of a molecular mechanism which reflects the model's basic structure.

A growing body of evidence suggests that the activation of NMDA receptors and the entry of calcium postsynaptically may play a role in the mechanism for visual cortical plasticity. The NMDA receptor is a subtype of excitatory amino acid (EAA) receptor which has high affinity for the agonist N-methyl-D-aspartate (NMDA) and is selectively antagonized by 2-amino-5-phosphonovaleric acid, APV (Watkins and Evans 1981). This receptor is associated with a membrane channel that passes Ca^{2+} only when postsynaptic depolarization overcomes a voltage-dependent magnesium ion blockade (Nowak *et al.* 1984). A special role for this receptor in synaptic plasticity is suggested by several lines of evidence. Long-term potentiation (LTP) of synaptic effectiveness, normally resulting from tetanic electrical stimulation of excitatory afferents, cannot be induced when NMDA receptors are blocked in either the CA1 subfield of the hippocampus (Collingridge *et al.* 1983; Harris *et al.* 1984) or the visual cortex of rats (Artola and Singer 1987; Kimura *et al.* 1988) and kittens (Connors and Bear 1988). Likewise, modification of striate cortex by visual deprivation during the critical period is disrupted by chronic intracortical infusion of APV (Kleinschmidt *et al.* 1987; Gu *et al.* 1989; Bear *et al.* 1990). On the other hand, the application of N-methyl-D-aspartate to hippo-

Offprint requests to: M.F. Bear (address see above) campal slices *in vitro* can induce a form of synaptic potentiation that can last for 30 min (Collingridge *et al.* 1983; Kauer *et al.* 1988) or longer (Thibault *et al.* 1989). The idea that elevations in postsynaptic [Ca^{2+}] trigger the increase in synaptic strength is supported by the finding that intracellular injection of the Ca^{2+} chelator EGTA blocks the induction LTP in CA1 pyramidal cells (Lynch *et al.* 1983). Further, the intracellular release of Ca^{2+} from the photolabile calcium chelator nitr-5 produces a long-lasting potentiation of synaptic transmission that resembles LTP (Malenka *et al.* 1988). Taken together, these data indicate that the calcium conductance mediated by the NMDA receptor plays a special role in strengthening synaptic relationships in the cortex.

This molecular mechanism is consistent with the theory of Bienenstock *et al.* (1982) assuming that visual cortical NMDA receptors become sufficiently active to increase synaptic strength only when the postsynaptic target is depolarized beyond the modification threshold, θ_M (Bear *et al.* 1987; Bear 1988). The theory states that the value of θ_M varies as a non-linear function of the average output of the postsynaptic neuron. Does it follow that the effectiveness of NMDA receptor activation in triggering a synaptic modification is also a function of average cortical activity?

As a first step towards addressing this question, we have investigated the effects of visual deprivation on NMDA-stimulated ^{45}Ca uptake by slices of visual cortex maintained *in vitro*. Our results demonstrate that even brief periods of visual deprivation can significantly affect NMDA-stimulated calcium accumulation by visual cortical slices, It remains to be determined, however, if what we observe is a reflection of changes in the synaptic modification threshold.

Methods

Kittens used in this study are listed in Table 1. Most normally reared animals were purchased immediately post-weaning from Liberty Labs (Liberty Corner, NJ). Visual deprivation by lid suture was carried out under ketamine-xylazine anesthesia 4 days prior to

Table 1. Listed are each animal used in this study, the type of rearing (NR, normal rearing; DR, dark-rearing; BD, binocular deprivation; MD, monocular deprivation), the age when each animal was deprived and sacrificed (in days postnatal), and the calcium accumulated by slices of visual cortex after 2 minute incubations in 0–100 μM NMDA and 62.5 mM K. Each value for calcium accumulation is the mean of determinations on 4–8 slices

Animal	Rearing	Age when deprived	Age when sacrificed	Calcium accumulation (nmol per mg protein)					
				NMDA concentration (μM):					K
				0	12.5	25	50	100	
N1	NR		28	4.33		5.59			5.35
N2	NR		34	3.65	5.03	4.60	5.02	5.18	5.23
N3	NR		34	4.08	4.40	5.08	5.33	5.92	6.40
N4	NR		39	3.64	3.15	4.43	4.95	4.74	4.61
N5	NR		31	3.40	4.55	5.19	5.38	5.63	5.90
N6	NR		41	2.78	3.53	3.64	3.85	3.84	4.19
N7	NR		46	2.52	2.58	3.22	4.64	4.87	4.25
N8	NR		48	3.09	3.64	4.63	5.65	5.48	4.01
N9	NR		86	3.17	2.69	2.83	3.75	3.22	3.54
N10	NR		87	2.39	3.27	2.67	3.03	3.55	3.33
N11	NR		88	3.56	3.12	2.58	3.05	4.64	3.68
N12	NR		33	3.22	3.46	4.54	3.15	5.42	4.55
N13	NR		55	2.72		3.18			
N14	NR		56	2.92		4.05			
N15	NR		57	4.03		4.56			
D1	DR	7	32	3.59	3.88	4.01	3.91	4.66	4.93
D2	DR	7	33	4.33	4.93	5.42	5.04	6.05	5.72
D3	DR	7	40	4.49	4.76	4.56	5.48	5.16	5.50
D4	DR	7	43	4.21	4.12	4.54	4.04	3.76	4.38
D5	DR	7	32	4.88	4.84	5.15	5.55	6.72	4.92
D6	DR	7	46	3.49	4.18	4.80	4.74	3.89	4.57
B1	BD	38	42	2.45	2.85	3.93	3.43	3.90	3.28
B2	BD	41	45	3.00	3.53	2.50	2.68	3.91	4.17
B3	BD	44	48	4.09		5.41	5.89	5.75	5.74
B4	BD	28	32	3.20	4.16	4.22	3.96	4.36	3.72
B5	BD	30	34	2.64	3.09	3.57	3.14	3.41	3.21
B6	BD	30	34	2.34	3.41	2.69	3.44	3.19	3.93
M1	MD	37	41	2.48	4.03	3.87	5.46	5.63	
M2	MD	32	36	3.88	3.83	4.37	4.19	4.75	
M3	MD	36	40	3.07	3.55	4.78	4.29	4.70	
M4	MD	28	32	4.54	6.63	4.74	4.71	6.13	
M5	MD	35	39	4.41	3.67	4.50	4.95	4.77	

sacrifice. Kittens to be dark-reared were taken from a breeding colony operated by the Brown University Animal Care Facility. Mother and kittens were transfered from the colony within 7 days of birth (before the time of natural eye opening) to a standard breeding cage in a darkroom. Care for these animals was provided in accordance with procedures approved by the Brown University Institutional Animal Care and Use Committee.

Prior to sacrifice, each kitten was pre-anesthetized with a small i.m. dose of a ketamine/xylazine mixture (20 and 2.5 mg per kg, respectively) followed by an intravenous or intraperitoneal dose of sodium pentobarbitol (10 mg per kg and upwards as needed to maintain deep surgical anesthesia). Ketamine was used to induce anesthesia because it could be administered easily to kittens in the darkroom. Although there is good evidence that ketamine blocks NMDA receptor channels (Anis *et al.* 1983; Thompson *et al.* 1985; MacDonald *et al.* 1987), we are confident that this did not compromise our experiments. First, the dose was small enough that its anesthetic effects would have worn off prior to the removal of blocks of visual cortex. Second, slices of area 17 were washed in a large volume of artificial cerebrospinal fluid before incubation with NMDA (see below). Finally, pilot studies indicated that anesthesia with sodium pentobarbitol alone did not alter the results of the calcium uptake assay. Because all animals were treated identically, comparisons between groups remain valid.

The anesthetized kitten was placed in a stereotaxic head-holder and a large craniotomy was performed to expose the dorsal surface of the cerebrum. The dura overlying visual cortex was excised and the animal decaptitated. The brain surface was irrigated with cold oxygenated artificial cerebrospinal fluid (ACSF; 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM d-glucose, 20 mM HEPES buffer, pH 7.4) as blocks of visual cortex were gently removed. These blocks, illustrated schematically in Fig. 1A, consisted mostly of area 17. The blocks were cut in the coronal plane into 400 μm slices using a McIlwain tissue chopper and the slices were gently separated in a petri dish containing ACSF at 4° C. Slices were allowed to equilibrate for 45 min in a chamber containing continuously oxygenated ACSF at room temperature. Slices within ~1.5 mm of the anterior and posterior block incisions were discarded.

After equilibration, cortical slices were transfered into twelve plexiglass cylinders with nylon mesh bottoms which rested in twelve wells containing oxygenated room temperature ACSF (schematically illustrated in Fig. 1B). Each cylinder held 4 slices. ACSF in the wells was continually bubbled with warmed humidified oxygen from small tubes within the cylinders. Slices were exposed to preincubation, incubation, and postincubation media by transfering the cylinders between sets of wells containing the different media (Fig. 1C). Slices in each cylinder were exposed to a unique set of preincubation and incubation solutions, and thus constituted one experimental condition. Most conditions were run in duplicate within each experiment (i.e., 8 slices per condition).

The calcium uptake assay was a modification of the procedure published by Ichida *et al.* (1982). Slices were first warmed to 35° C during a 5-min preincubation. The preincubation was also used in some experiments to expose slices to 100 μM D,L–APV. Preincubation was followed by incubation in ACSF solutions containing approximately 2.5 μCi $^{45}CaCl_2$ and either 0, 12.5, 25, 50 or 100 μM NMDA. In addition, one condition, lacking NMDA and containing a high concentration of K^+ (62.5 mM KCl, 60 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM D-glucose, 20 mM HEPES buffer), was used to monitor depolarization-induced calcium uptake. Pilot studies indicated that both NMDA- and K^+-stimulated calcium uptake by slices were linear beyond 4 min of incubation. In the experiments described here, a 2 min incubation was used. Incubation was terminated by a 10-s wash in a 4° C postincubation solution containing La^{3+} (120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM $LaCl_3$, 10 mM d-glucose, 20 mM HEPES buffer, pH 7.4), followed by a 30-min postincubation in a fresh bath of the same solution (Drapeau and Blaustein 1983; Retz and Coyle 1984). Slices were removed from the postincubation medium by vacuum filtration and sonicated in 1.0 ml deionized distilled water. The ^{45}Ca content of each slice was determined by scintillation counting in

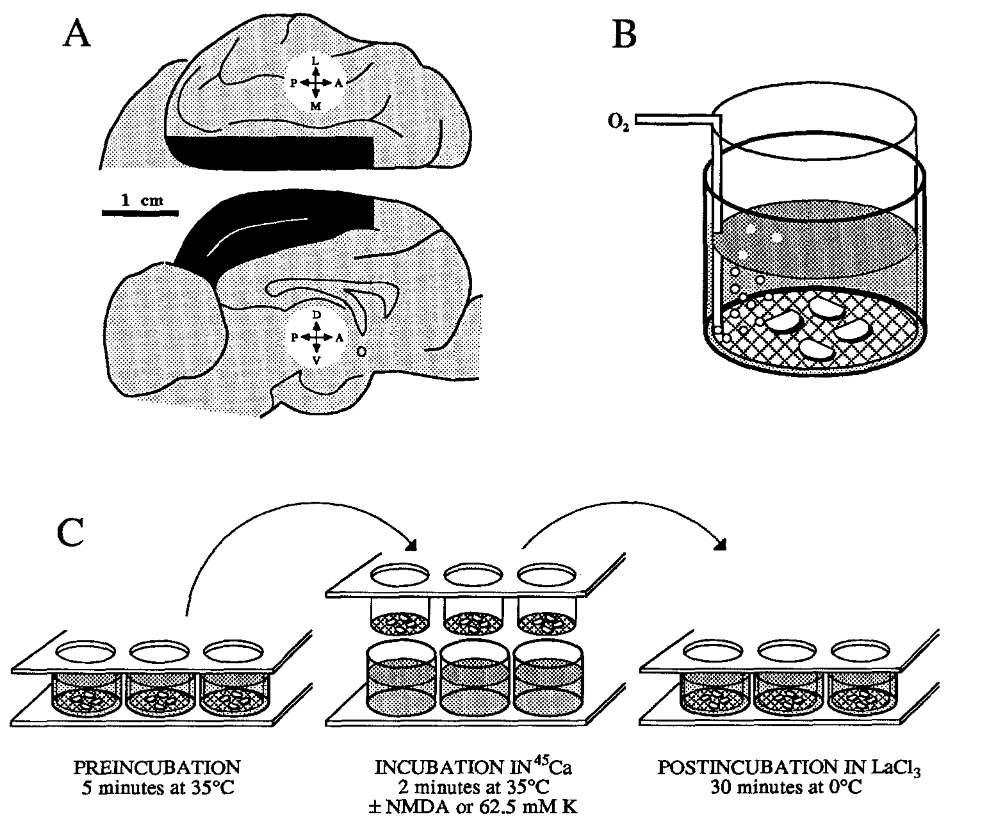

Fig. 1. A Dissection of visual cortical tissue blocks. *Top:* dorsal view of the left hemisphere to illustrate the region of cortex dissected to prepare slices (black region). *Bottom:* medial aspect of a midsagittal view of a cat brain to illustrate the tissue dissected to prepare visual cortical slices (black region). The sagittal incision intersects the splenial sulcus and isolates a block of cortex containing primarily area 17. This block is subsequently cut in the coronal plane into 400 μm thick slices. **B** Schematic illustration of the slice incubation apparatus. Slices rested on the nylon mesh bottoms of plexiglass cylinders that fit snugly into wells containing the various incubation media. Tubing within cylinder walls supplied uninterrupted oxygen during all phases of incubation. Dissolved oxygen within the wells was greater than 85% saturation at 20° C. **C** The slices were exposed to different media simply by transferring the cylinders from well to well

comparison to aliquots of incubation media, which served as standards. Protein content of each slice was determined by the method of Bradford (1976). The calcium accumulation by each slice was expressed in nmoles per mg protein.

Individual slice ^{45}Ca content values were averaged to arrive at a single mean calcium accumulation for each condition in the experiment (conditions run in duplicate were expressed as the single mean of 8 slices). At least 5 separate experiments were performed, and tissue calcium accumulation for each condition was defined as the mean ± SEM of the individual experimental means for that condition. Calcium content values were compared across NMDA treatments, animal ages, and rearing histories by t-test, regression, and ANOVA, where appropriate. The confidence level for significance in all tests was $p < 0.05$.

Results

Evoked increases in calcium accumulation were measured in slices derived from 9 normally reared (NR) animals, aged 28 to 48 days. Slices were subjected to 2 minute incubations in media containing micromolar NMDA and 62.5 mM K^+, and the results are shown in table 1. NMDA incubation caused a dose-dependent increase in slice calcium accumulation from control levels of 3.41 ± 0.19 nmol/mg to a maximum of 5.14 ± 0.23 nmol/mg with 100 μM NMDA. The NMDA-evoked increase, illustrated in Fig. 2A, was significant (1-way

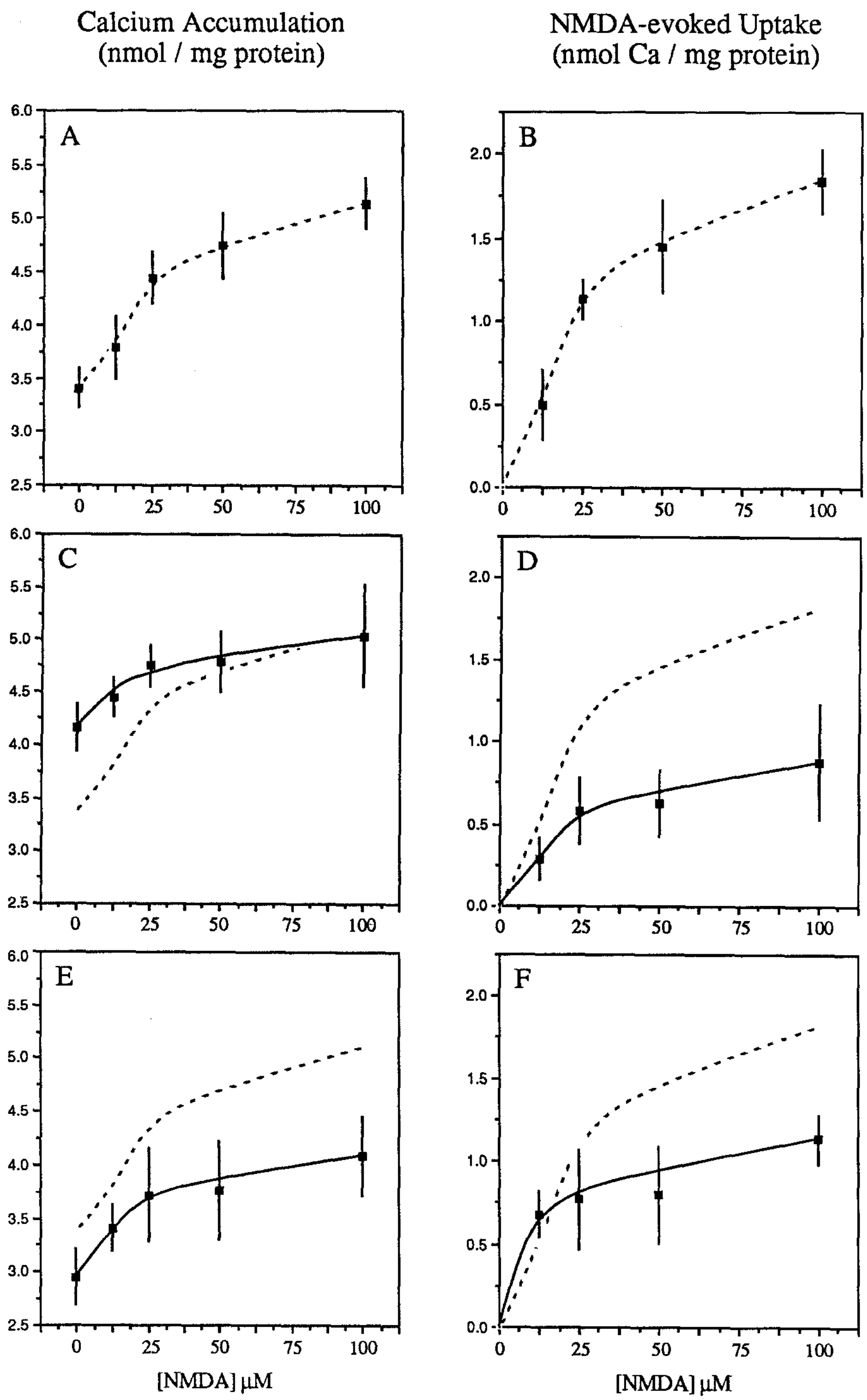

Fig. 2. A, C, E Accumulation of calcium (after 2-min incubations) by slices from age-matched animals that were raised normally (**A**), reared in complete darkness (**C**) or briefly binocularly deprived by lid suture (**E**). Points are means (±SEM) of values listed in Table 1. **B, D, F** NMDA-evoked uptake of calcium by slices of the same three experimental groups. Uptake is defined as calcium accumulation of slices in a treated condition less the basal (unstimulated) accumulation. Uptake values are calculated for each experiment; points in the graphs represent the means (±SEM) of these values. To facilitate comparisons between groups, the accumulation and uptake curves for slices from normal animals are replotted in **C–F** (dashed lines)

ANOVA, $p < 0.001$). 62.5 mM K^+ treatment also significantly increased calcium accumulation over control levels (correlated nondirectional t-test, $p < 0.001$). To assess more accurately the response to NMDA and K^+ treatment, the results of each experiment were recalculated by subtracting mean basal (unstimulated) calcium accumulation from the mean calcium accumulation of each treated condition. The resulting data represent NMDA- and K^+-evoked calcium *uptake* over control levels (Figs. 2B, 3). These data show that NMDA treatment elicited significant calcium uptake (1-way ANOVA, $p < 0.002$).

To determine what part of this uptake occurred through an NMDA receptor-dependent mechanism, slices from 5 normal animals were preincubated with 100 μM D,L–APV and then incubated with 100 μM D,L–APV and 25 μM NMDA. The results were compared to APV-free conditions in the same experiments. These experiments showed that APV treatment significantly reduced the calcium uptake evoked by 25 μM NMDA. Untreated slices accumulated 1.24 ± 0.20 nmol/mg during incubation with 25 μM NMDA, while APV-treated slices accumulated only 0.34 ± 0.32 nmol/mg. This 70% reduction, shown in Fig. 4, was statistically significant (correlated directional t test, $p < 0.025$). In contrast, neither basal calcium content nor K^+-stimulated calcium uptake were significantly reduced by APV treatment.

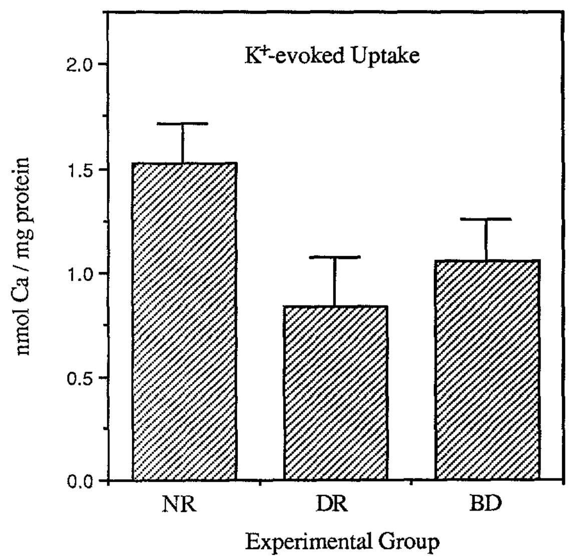

Fig. 3. Calcium uptake evoked by 62.5 mM K^+ in slices from age-matched animals that were raised normally (NR), reared in complete darkness (DR) or briefly binocularly deprived (BD). The reduction in K^+-evoked uptake in slices from DR animals is significant ($p < 0.05$)

Detailed analysis of these experimental results suggested that stimulated calcium uptake declined in slices from animals of increasing age. To examine this possibility further, calcium uptake was measured in slices obtained from 6 additional kittens, aged 55 to 88 days. Only control and 25 μM NMDA conditions were run in 86–88 day old animals (Table 1). Slices from these additional animals showed markedly lower NMDA-stimulated increases in calcium content than did those from the 28 to 48 day old animals (2-way ANOVA, $p < 0.001$). In addition, slices from old animals showed significantly reduced K^+- stimulated increases in Ca content (noncorrelated t-test, $p < 0.02$). Subsequent analysis showed that 25 μM NMDA-stimulated uptake was inversely correlated with increasing animal age ($R = 0.84$, $p < 0.001$; Fig. 5). K^+-stimulated uptake also significantly declined with increasing age ($R = 0.70$, $p < 0.02$); however, basal calcium content remained constant ($R = 0.36$).

To assess the effects of visual experience on NMDA-evoked calcium uptake, slices derived from 6 dark-reared (DR) animals were incubated under conditions identical to those described for normal slices. The DR animals were age-matched to the NR group (mean ages were 37.7 and 37.1 days, respectively). The calcium accumulation of NMDA and K^+-treated slices from DR animals (Table 1), was not significantly different from that of NR slices. However, basal (unstimulated) Ca accumulation

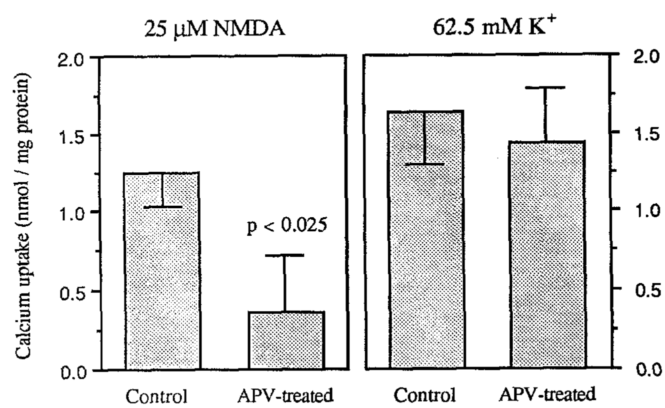

Fig. 4. LEFT: Effect of APV treatment on calcium uptake evoked by 25 μM NMDA. Control slices were incubated in 25 μM NMDA alone; APV-treated slices were preincubated in 100 μM D,L–APV for 5 min and then incubated in both 25 μM NMDA and 100 μM D,L–APV. Bars represent means (± SEM) of 5 experiments on slices from normally reared animals (N5, N6, N7, N8, N12). APV reduction is significant ($p < 0.025$). RIGHT: Effect of APV treatment on K^+-stimulated calcium uptake in the same animals

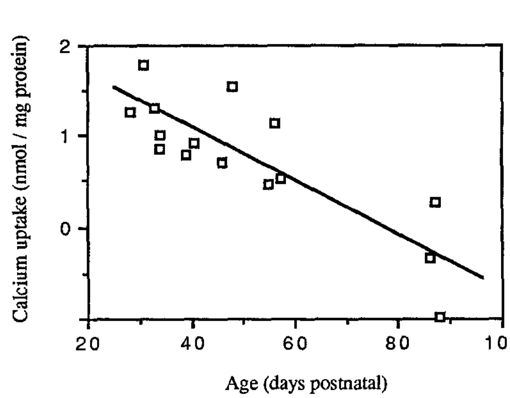

Fig. 5. Age-related decline in 25 μM NMDA-evoked Ca^{2+} accumulation. Each point represents 25 μM NMDA-evoked calcium uptake over control levels in a single experiment using slices from normal animals. The decline is significant ($p < 0.001$) and approximates the illustrated line with $R = 0.84$. K^+-evoked uptake also declined with age in these animals; however, basal accumulation did not

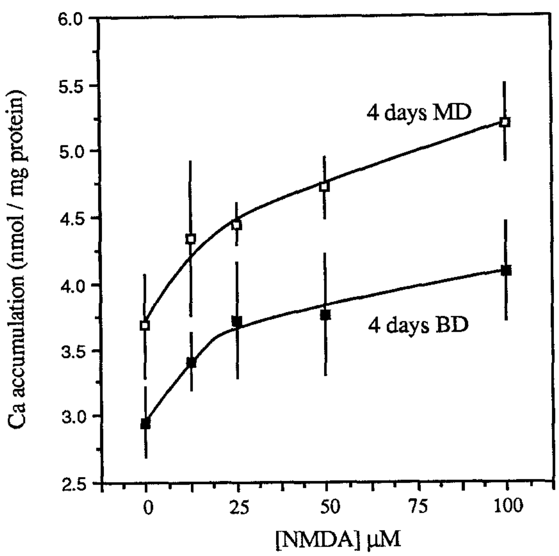

Fig. 6. Comparison of calcium accumulation by slices derived from monocularly deprived (open boxes) and BD (filled boxes) animals. MD and BD animals were deprived for 4 days. The difference between visual cortical slices from MD and BD kittens is significant ($p < 0.001$)

was significantly elevated in the slices from DR animals (noncorrelated t-test, $p < 0.01$; Fig. 2C). As a result, NMDA-evoked calcium *uptake* was significantly lower in visual cortical slices from DR animals than in slices from NR animals (2-way ANOVA, $p < 0.001$; Fig. 2D). K^+-evoked uptake was also significantly decreased (noncorrelated t-test, $p < 0.05$; Fig. 3).

Slices derived from animals binocularly deprived (BD) by lid suture for 4 days (mean age 39.2 days) were also subjected to the standard incubation conditions (Table 1). NMDA-treated slices from BD animals showed significantly lower calcium accumulation than did slices from NR animals (2-way ANOVA, $p < 0.001$; Fig. 2E). The K^+ treated slices from BD animals showed slightly lower Ca accumulation than did those from NR animals, but this difference did not achieve statistical significance (noncorrelated t-test, $p < 0.10$). Slices from monocularly deprived (MD) animals (mean age 37.6 days) were also incubated in micromolar NMDA (but not in 62.5 mM K^+; see Table 1). Calcium accumulation by these slices was not significantly different from slices prepared from NR animals, but was significantly elevated over slices from BD animals (2-way ANOVA, $p < 0.001$; Fig. 6).

The difference between slices from normal and BD animals was very robust. Not only was the calcium accumulation of NMDA treated slices significantly lower after BD (Fig. 2E), but NMDA-evoked calcium *uptake* over basal levels was reduced as well (2-way ANOVA, $p < 0.025$). This difference is illustrated in Fig. 2F. K^+-stimulated uptake was slightly but not significantly reduced after BD (Fig. 3). It is noteworthy that the deficit in calcium uptake seen in Fig. 2F was only apparent above 25 μM NMDA. Below that, uptake by slices from BD animals was equal to uptake in slices from NR animals.

Discussion

We have found that NMDA and K^+ evoke uptake of Ca^{2+} by slices of kitten visual cortex maintained *in vitro*. The major findings of this study are that this uptake depends importantly upon the postnatal age of the visual cortex and the history of prior visual experience.

The magnitude of NMDA-evoked calcium uptake we measured in P28–48 kitten visual cortex is comparable to values obtained in similar experiments using rat neocortical slices (Ichida *et al.* 1982; Riveros and Orrego 1986; Crowder *et al.* 1987). A variety of pre- and post-synaptic mechanisms could contribute to the measured changes in slice calcium content. Much of the uptake measured under our assay conditions conceivably could occur at presynaptic axon terminals due to impulse activity evoked by NMDA and K^+ application. However, Riveros and Orrego (1986) found in rat neocortical slices that blockade of action potentials (and hence presynaptic Ca^{2+} entry) with 1 μM tetrodotoxin (TTX) failed to reduce calcium uptake stimulated by 150 μM N-methyl-D,L-aspartate. Postsynaptic calcium uptake is influenced by a number of factors including (1) influx through NMDA-receptor-gated channels (Mayer and Westbrook 1987), (2) influx through voltage-sensitive calcium channels (VSCC's; Miller, 1987), (3) efflux via the Ca^{2+}- pumping ATPase, and (4) sequestration and release by intracellular calcium buffering systems. The net result of these combined mechanisms is the elevation of intracellular calcium in response to NMDA receptor activation. The APV antagonism of NMDA-evoked calcium uptake we observed confirms that NMDA receptor activation is necessary for calcium uptake. Therefore, we have interpreted the magnitude of NMDA-evoked calcium uptake as a measure of the effectiveness of the NMDA receptor in producing a calcium signal *through these combined mechanisms*, and not through the NMDA-receptor-gated calcium ionophore alone. Because it was not antagonized by APV, K^+-stimulated uptake is likely to occur via a mechanism that is largely independent of the NMDA receptor.

We found a significant decline in NMDA-evoked calcium uptake in visual cortical slices prepared from animals of increasing age. There are several possible explanations for this result. It is possible that slice protein content increases with age, while the magnitude NMDA-evoked calcium entry does not, causing an apparent decline in calcium uptake per mg protein. We consider this unlikely, however, since basal (unstimulated) calcium content did not decrease significantly with age, as would be expected if protein content were steadily increasing. A second possibility is that slices from older animals were less healthy. Basal (unstimulated) calcium content appears to be a sensitive measure of slice health because it is increased markedly by anoxia and excessive agitation (personal observations). According to this measure, slices from older animals were no less healthy than those from younger animals. A third possibility is that the number of NMDA receptors and/or channels decreases in striate cortex with increasing age. Indeed, recent studies have indicated that both APV-sensitive glutamate binding (Bode-Greuel and Singer 1989) and MK801 binding to the open NMDA channel (Reynolds and Bear 1989) decline in kitten striate cortex from 5 weeks of age to adulthood. Finally, it is possible that a decrease in the number of VSCC's in striate cortex contributes to the decline in NMDA-stimulated calcium uptake. This possibility is supported by our observation that K^+-stimulated calcium uptake, which is independent of NMDA receptor activation under our assay conditions, also showed an age-related decline. Furthermore, Bode-Greuel and Singer (1988) have found that 1,4-dihydropyridine binding sites, thought to reflect the density of L-type VSCC's (Miller 1987), decrease in kitten striate cortex during postnatal development.

Taken together, these considerations lead us to suggest that the age-related decrease in NMDA evoked calcium uptake by slices of visual cortex is due largely to a decrease in the number of NMDA- and voltage-gated calcium channels. It is of particular interest that under our assay conditions, calcium uptake evoked by 25 μM NMDA became statistically insignificant by 12 weeks of age, considered to be the end of the "critical period" for modification of binocular connections in kitten visual cortex by brief monocular deprivation (Hubel and Wiesel 1970). Thus, these data tend to support the hypothesis that neuronal calcium fluxes play a special role in developmental plasticity in the visual cortex, as suggested by Singer (1987; 1989).

However, experiments using slices prepared from dark-reared animals indicate that there is not always a simple correlation between the occurance of NMDA-stimulated calcium uptake and experience-dependent cortical modifications. A period of dark-rearing leaves the striate cortex extremely sensitive to modification by subsequent visual experience (reviewed by Frégnac and Imbert 1984). However, we found a severe deficit in NMDA-evoked calcium uptake, due in part to an unusually high basal calcium content in slices from DR animals. It is possible that DR slices were uniformly less healthy than normal slices, although it is not clear why this would be the case. Alternatively (or in addition), dark-rearing might retard the normal development of NMDA- and voltage-gated calcium channels. This notion is supported by preliminary findings that MK801 binding is substantially lower in the visual cortex of dark-reared as compared with normally reared animals (Reynolds and Bear 1989).

A major motivation in performing these experiments was to determine if a brief period of binocular deprivation changed the effectiveness of NMDA receptor activation in producing a postsynaptic calcium signal. NMDA receptor activation can, under some circumstances, increase synaptic effectiveness in the visual cortex (Kleinschmidt *et al.* 1987; Artola and Singer 1987; Kimura *et al.* 1989). Presumably this effect is mediated by postsynaptic calcium entry, as shown for LTP in the CA1 region of the hippocampus (Lynch *et al.* 1983; Malenka *et al.* 1988). Theoretical considerations had led Bear *et al.* (1987) to propose that that the level of postsynaptic activation at which sufficient Ca^{2+} enters through NMDA channels to increase synaptic strength (the modification threshold, θ_M) should vary depending on the history of prior cell activity (see Introduction). Specifically, a brief period of binocular deprivation should reduce the value of θ_M. If this regulation were manifested by changes in NMDA receptor effectiveness, then a brief period of BD should produce enhanced calcium uptake in response to NMDA.

Indeed, 4 days of binocular deprivation did produce significant changes in NMDA- and K^+-evoked calcium uptake. However, instead of exhibiting increased uptake, BD cortex showed decreased uptake from normal. This reduction in Ca uptake was a specific consequence of binocular deprivation as monocular lid closure had no effect on NMDA-stimulated calcium uptake in visual cortex. Furthermore, the reduced Ca uptake in slices from BD animals is not the same as that observed in slices from DR animals, because BD did not raise basal uptake from normal levels; the stimulated uptake was selectively reduced.

A number of mechanisms could contribute to the reduction in NMDA-evoked calcium uptake in striate cortex after brief binocular deprivation. For example, BD may reduce the number of NMDA receptors and/or channels, reflecting the loss of synaptic effectiveness thought to underlie the physiological consequences of BD (Freeman *et al.* 1983). In addition, because 1 mM Mg^{2+} was present in our incubation media, the response to NMDA presumably depended upon the postsynaptic membrane potential. Hence, reduced calcium uptake could reflect decreased excitability in visual cortex of BD animals. It is also possible that BD causes a reduction in the number of voltage-sensitive calcium channels. This hypothesis is supported by our observation that K^+-stimulated calcium uptake also appeared to be reduced in the cortex of BD kittens (although this change did not achieve statistical significance). Alternatively, since much of the ^{45}Ca we measure in our slices is likely to be associated with intracellular stores, it is possible that BD produces a decrease in the effectiveness of intracellular calcium buffering mechanisms.

Regardless of the mechanisms involved, the data clearly show that a brief period of binocular deprivation is sufficient to cause significant changes in visual cortical calcium regulation. The relationship between these changes and the modification threshold (θ_M) proposed by Bienenstock *et al.* (1982) remains unclear. The use of more refined methods is now warranted to investigate the mechanisms by which NMDA stimulated calcium uptake is changed, and to assess the impact of these changes on cortical function.

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