

Visual Experience Specifically Regulates Synaptic Molecules in Rat Visual Cortex

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Abstract

■ To study environmental modulation of synaptic molecular structure, the major postsynaptic density protein (mPSDp) from rat visual cortex was monitored. This membrane component, a Ca^{2+} /calmodulin-dependent protein kinase subunit, was measured during normal postnatal development and after visual deprivation. Total synaptic membrane (SM) protein was used as an index of synapses as a whole.

During the first 2 postnatal months, total SM protein in the visual cortex increased 32-fold. In contrast, the mPSDp increased 455-fold, indicating that different molecular components of the cortical synapse develop differentially. Exposure to complete darkness during the first 2 postnatal weeks prevented normal development of total SM protein in visual cortex,

values reaching only 66% of normal. Moreover, environmental lighting preferentially modulated the mPSDp, which attained only 34% of the normal value after dark rearing. Thus, visual deprivation selectively inhibited the normal development of specific synaptic components. Moreover, experience-dependent modulation was area specific. In contrast to the marked effect in visual cortex, light deprivation did not alter synapses in the nonvisual parietal and prefrontal cortices. Finally, the modulation of visual cortex mPSDp was stage specific, since visual experience did not alter the synaptic protein in adults. Our results suggest that early visual experience selectively and specifically modifies molecular synaptic components in the visual cortex. ■

INTRODUCTION

A central issue in the study of plasticity in the CNS concerns environmental regulation of neuronal connectivity. The early phase of neuronal development is particularly susceptible to environmental influences. In the primary visual cortex, for example, visual experience during a critical period of postnatal life regulates normal development of synaptic connections (Wiesel & Hubel, 1965; Cragg, 1967; Diamond, Lindner, & Raymond, 1967; Blake-more & Cooper, 1970; Fifkova, 1970; Schapiro & Vukovich, 1970; Parnavelas & Globus, 1976; Rothblatt, Schwartz, & Kasdan, 1978; Singer, 1985; Turner & Greenough, 1985; Gabbott & Stewart, 1987; Rakic, 1988). Disruption of visual cortex and impairment of normal vision result from visual deprivation during a critical period in cat and monkey (Wiesel & Hubel, 1965; Blakemore & Cooper, 1970; Singer, 1985; Rakic, 1988). In rat visual cortex, both deprivation and stimulation alter the number and shape of synapses, determined morphologically (Cragg, 1967; Diamond et al., 1967; Fifkova, 1970; Schapiro & Vukovich, 1970; Parnavelas & Globus, 1976; Rothblatt et al., 1978; Turner & Greenough, 1985; Gabbott & Stewart, 1987). However, the molecular basis of environmental regulation of visual cortex synapse development remains to be defined. Which molecular components of the synapse are actually governed by experience? Are all synaptic molecules similarly affected? Alternatively, are

specific synaptic molecules particularly sensitive to environmental signals? Answers may provide insights into the molecular basis of synaptic plasticity and altered cortical function.

We recently initiated studies to examine the modulation of synaptic molecular structure by presynaptic impulse activity (Wu & Black, 1988, 1989). The postsynaptic density (PSD), which appears to participate in both moment-to-moment communication and plasticity, was used to monitor molecular components of the synapse (Siekvitz, 1985). In particular, the major postsynaptic density protein (mPSDp) was regulated by innervation and apparently impulse activity, in peripheral sympathetic ganglia and in the hippocampus, models studied thus far. The mPSDp is of special interest for a number of reasons.

The mPSDp, the predominant PSD protein, is an autophosphorylating subunit of a Ca^{2+} /calmodulin-dependent protein kinase (Wu & Black, 1988). Consequently, the molecule has attracted a great deal of attention, since Ca^{2+} influx has been invoked as a mediator of mnemonic mechanisms, and since phosphorylation is known to alter synaptic efficacy (Acosta-Urquidi, Alkon, & Neary, 1984; Siekevitz, 1985; Malenka, Kaver, Zucker, & Nicoll, 1988; Kennedy, 1989; Singer, 1990). Regulation by impulse activity potentially constitutes one pathway through which experience alters synaptic strength.

In the present study we investigated the role of visual experience in the regulation of synaptic molecular struc-

ture in rat visual cortex. Our observations suggest that visual experience specifically and selectively alters development of the mPSDp, while having relatively little effect on synapse development as a whole. Visual experience selectively altered synapses in the visual cortex, but had no discernible effect on parietal or frontal cortical synapses, suggesting that influences were modality and area specific.

RESULTS

Normal Development

To define normal synaptic development in rat visual cortex, different components were examined. Total synaptic membrane (SM) protein was used as a measure of synapses as a whole (Wu & Black, 1988). Simultaneously, the mPSDp was monitored to examine a functionally significant component molecule. Total SM protein increased modestly during the first postnatal week, but rose approximately 32-fold between days 12 and 21, attaining adult plateau values by day 30 (Fig. 1).

We were particularly interested in determining whether total SM protein might serve as a quantitative measure of development of morphologic synapses. Consequently, we compared SM ontogeny with results obtained from a previous electron microscopic mor-

phometric study (Blue & Parnavelas, 1983; Fig. 1). There was a striking correlation between the biochemical and ultramicroscopic measures of synaptogenesis ($n = 14$; $r = 0.922$; $p < 0.001$), suggesting that total SM protein may faithfully reflect synapse number.

Different synaptic components appeared to develop differentially. Visual cortex mPSDp increased 455-fold between days 5 and 60, whereas total SM protein rose only 32-fold (Fig. 2). A pronounced increase in visual cortex mPSDp occurred at the time of eye opening, at 14 days of age. In sum, our results suggest that different synaptic molecules develop differentially in visual cortex, reproducing synaptic ontogeny in peripheral systems (Wu & Black, 1988).

Effect of Light Deprivation on Visual Cortex Development

To study the potential role of experience on synaptogenesis, we employed a visual deprivation paradigm, rearing rats in the dark during the period of rapid synaptic development. Pups were reared in darkness and measures of synaptic development were examined at 17 days of age. Cortical weight itself was not altered by dark rearing, suggesting that this exposure did not exert general effects on brain growth (Table 1). In contrast, total SM protein per gram of cortex developed to only 66% of normal, suggesting that visual cortical synaptic development was impeded (Table 1). Moreover, the mPSDp was selectively affected, values per gram of cortex reach-

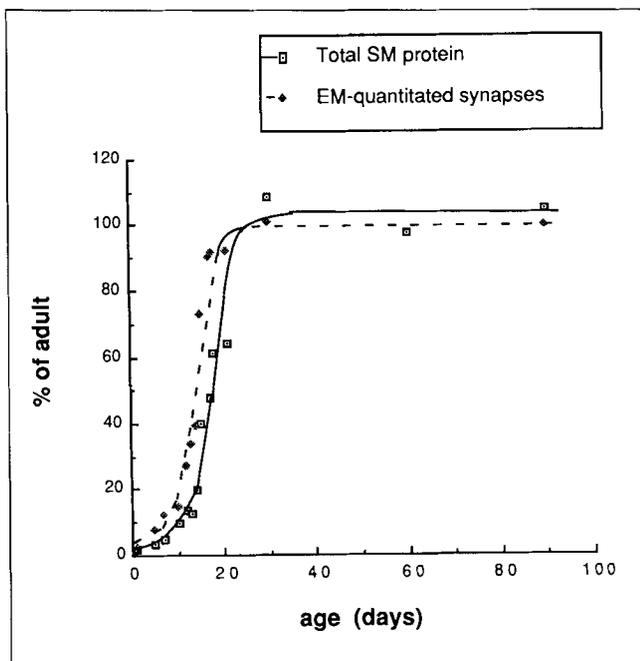


Figure 1. Development of total synaptic membrane protein (SM) (squares) and *in situ* appearance of electron microscopic (EM) synapses (hatched diamonds). The morphological data are from Blue and Parnavelas (1983). Data are expressed as percent of the adult value. Protein recovery values are the mean of two to three independent membrane preparations. In each preparation, 100–200 animals were used for age 1 day, 30–100 animals for age 5 to 10, and 10–30 for each older age. Both measures of synapse formation correlate significantly ($r = 0.922$; $p < 0.001$).

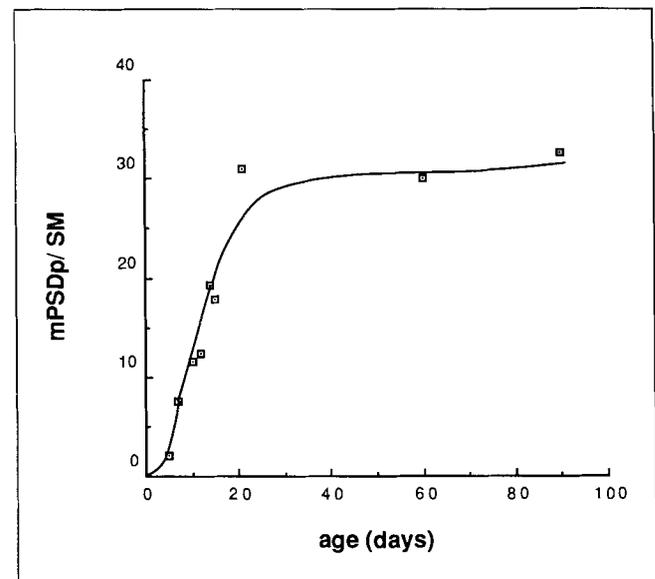


Figure 2. Postnatal increase in mPSDp per unit SM. The mPSDp in the synaptic membrane (SM) fraction, isolated from visual cortex, was assayed by ^{125}I -labeled calmodulin binding to SM proteins separated by SDS-PAGE (see Materials and Methods). Data are expressed as arbitrary densitometric units. The number of animals used for each age is as described in the legend to Figure 1.

Table 1. Effect of Dark Rearing on Cortical Weight and Total Synaptic Membrane (SM) Protein Level.^a

AGE	Total synaptic membrane protein (% of light-reared control)	Brain wet weight (mg visual cortex/rat)
7 days		
LR	100.0 (92)	37.3 ± 2.4 (92)
DR	122.7 ± 31.3 (102)	37.8 ± 2.2 (102)
17 days		
LR	100.0 (51)	74.9 ± 6.9 (51)
DR	66.0 ± 6.1 ^b (39)	68.5 ± 10.5 (39)
30 days		
LR	100.0 (21)	86.1 (21)
DR	75.9 (10)	73.0 (10)

^aRats were dark reared (DR) from birth and sacrificed at age 7, 17, or 30 days. Light-reared (LR) animals matching the age groups were used as controls. Visual cortex weight was determined at sacrifice. Visual cortex synaptic membrane fractions were isolated, and recovery of total SM proteins measured. The data for age 7 and 17 days are the means ± SEM of three independent rearing experiments. The total number of rats used for these data is denoted in parentheses. For age 30 days, the data are the result of one rearing experiment. Total SM protein level is expressed as percent of the light-reared control for each rearing experiment. For 7 days, 100% means 3.9 µg/rat or 107 µg/g cortex; for 17 days, 100% is 110 µg/rat or 1547 µg/g cortex; for 30 days of age 100% is 294 µg/rat or 3416 µg/g cortex.

^bFollowing 17 days of light deprivation, total SM protein level was significantly lower in the dark-reared rats than in the age-matched normally reared rats (Student's *t* test; *p* < 0.05). Visual cortex weight was unaffected.

ing only 33.8% of normal in the dark-reared animals (Fig. 3).

Patterned vision is thought to be necessary for the establishment of normal structure and function of the mature visual system (Rothblat et al., 1978). Consequently, we extended light deprivation for an additional 2 weeks after eye opening and examined the cortex at 1 month of age. However, prolongation of light deprivation had no greater effect on SM or mPSDp than exposure for 2 weeks, suggesting that the initial 2 weeks constitutes a critical period (Table 1 and Fig. 3).

To more clearly delineate the minimal critical period for light deprivation, rats were reared in the dark for 1 week only. In fact, deprivation for 7 days had no effect on either the SM or mPSDp, indicating that a longer period of altered environmental conditions is necessary to elicit the synaptic effects (Table 1 and Fig. 3).

Specificity of Experience-Dependent Modulation

To determine whether visual experience specifically altered synaptic development in the visual cortex, the non-visual parietal and frontal cortices were also examined after dark rearing. Total SM protein and mPSDp were unaffected by 17 days of light deprivation (Fig. 4), sug-

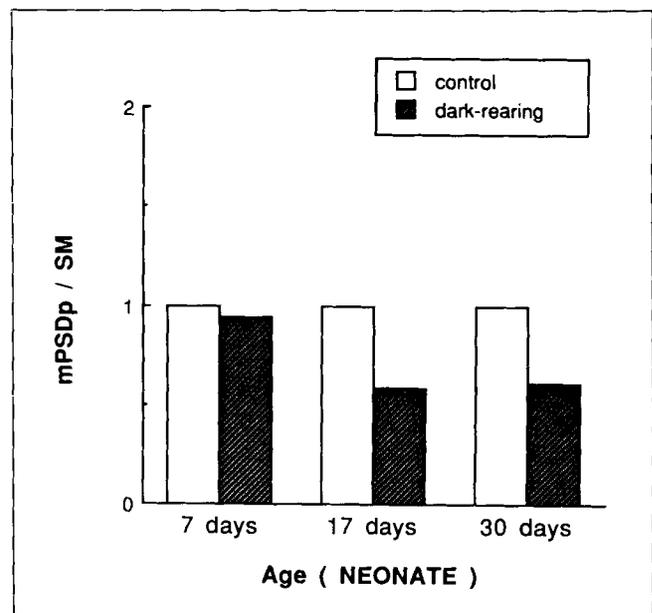


Figure 3. Effect of varying duration of light deprivation on mPSDp in the neonate. Rats were dark reared from birth and sacrificed at 7, 17, and 30 days of age. Light-reared animals of the same age were used as controls. Visual cortex SM proteins were separated by SDS-PAGE, and mPSDp-¹²⁵I-labeled calmodulin binding was determined densitometrically (see Materials and Methods). The mPSDp is expressed as arbitrary densitometric units. The data for age 7, 17, and 30 days are based on a total of 100, 50, and 10–20 animals, respectively. Whereas 7 days of light deprivation was without effect, 17 days or 1 month of dark rearing prevented visual cortex development.

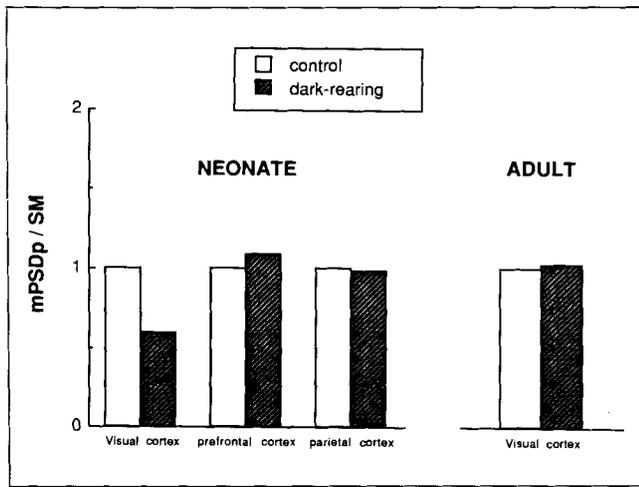


Figure 4. Effect of light deprivation for 17 days on mPSDp in neonate and adult. Rat pups were dark reared and sacrificed at 17 days of age. Light-reared animals were used as controls. mPSDp is determined as described in Materials and Methods, and is expressed as densitometric units per unit SM. Whereas visual cortex mPSDp was affected by light deprivation in the neonate, the developing nonvisual areas, prefrontal and parietal cortex, exhibited no modulation by visual experience. Adults (3 months old) were kept in total darkness for 17 days. Controls were subjected to a 12-hr light/dark cycle. The adult visual cortex was not modulated by 17 days of light deprivation.

gesting that visual experience selectively modulated synaptic ontogeny in primary visual cortical areas.

Effect of Light Deprivation in Adult Visual Cortex

To ascertain whether dark exposure modulates synaptic molecules in the adult cortex as well as in the neonate, rats were exposed to 17 days of light deprivation as adults. Visual cortex total SM protein and mPSDp were unaffected by dark exposure during adulthood (Fig. 4), suggesting that visual experience is relatively more important in the regulation of developing synapses in the visual cortex.

DISCUSSION

Our study was designed to begin examining development of molecular components of the synapse in cerebral cortex, and define potential influences of environmental stimuli on synaptic molecular maturation. We chose the visual cortex, since extensive insights are already available (Wiesel & Hubel, 1965; Cragg, 1967; Diamond et al., 1967; Blakemore & Cooper, 1970; Fifkova, 1970; Schapiro & Vukovich, 1970; Parnavelas & Globus, 1976; Rothblatt et al., 1978; Blue & Parnavelas, 1983; Singer, 1985; Turner & Greenough, 1985; Gabbott & Stewart, 1987; Rakic, 1988), and since visual experience may be conveniently modified during development.

Initially, we sought to determine whether specific molecular markers reflect the known pattern of ultramicroscopic synaptogenesis. In fact, total SM protein, a presumed measure of synapses as a whole, described a developmental profile that was virtually identical to that previously defined for synaptogenesis electron microscopically (Fig. 1; $r = 0.922$). The striking congruence of the biochemical and morphologic measures raises a number of possibilities. Most simply, the assay of total SM protein may represent a relatively simple and specific measure of synapse formation, obviating the need for laborious and somewhat subjective electron microscopic morphometry. In addition, total SM protein may, potentially, reflect selected aspects of the state of existent synapses, a subject of ongoing study. We are now determining whether total SM protein reflects synapse number in other, nonvisual, brain areas as well.

There were striking differences in the ontogeny of different synaptic molecules in visual cortex, suggesting that different components of the synapse develop differentially. While total SM protein increased 32-fold postnatally, mPSDp rose 455-fold. Indeed, the increase in the mPSDp was more than an order of magnitude greater than that of SM protein (Fig. 2). It is apparent that different molecular components of the cortical synapse develop differentially, and that the cortical synapse does not simply develop in an all-or-none fashion. These observations are entirely consistent with results obtained in the study of the neuromuscular junction, in which synaptogenesis is a sequential process (Fischbach, Lorna, & Hume, 1984; McMahan & Wallace, 1989). Moreover, differential development raises the possibility of differential regulation and selective environmental influence on cortical synaptic molecules, a contention discussed below.

To begin studying the potential effects of sensory input on synaptic molecular structure we chose an extreme environmental condition, visual deprivation through dark rearing (Cragg, 1967; Valverde, 1971; Gabbott & Stewart, 1987; Neve & Bear, 1989). Our goal was to use a rather gross environmental aberration before proceeding to more precise external signals. In fact, dark rearing for 2 weeks after birth had a pronounced effect on measures of synaptogenesis. Total SM protein increased to only 66% of normal in the dark-reared animals. Light deprivation had an even more dramatic effect on development of the mPSDp: the major PSD protein increased to only 33.8% of normal in visual cortex. Several conclusions are warranted. Most generally, sensory information modulates the development of specific molecular components of the synapse. Further, visual experience is apparently somewhat selective, affecting the mPSDp more than total synaptic membrane protein. Our findings are consistent with previous work, using different measures, indicating that sensory deprivation inhibits synapse formation in visual cortex of the mouse, rat, and cat

(Fifkova, 1970; Valverde, 1971; Rothblatt et al., 1978; Heumann & Rabinowicz, 1982; Winfield, 1983; Gabbot & Stewart, 1987).

The effects of visual deprivation were brain area specific, since the synaptic markers were not affected in parietal and frontal cortex by light deprivation. Because these cortical areas are involved in higher order visual associations, including spatial representation and memory (Kolb, Sutherland, & Whishaw, 1983), dark rearing was apparently selective in the development of synaptic molecules in primary visual cortex. We are presently examining other sensory modalities to determine whether such specificity extends to other brain areas as well.

Our initial observations suggest that the 2 first postnatal weeks approximate a critical period in the modulation of synaptic markers by visual experience. Dark rearing for one week had no effect, and exposure for 4 weeks differed little from that for 2 weeks. These results are consistent with previous data. For example geniculocortical axons do not invest the rat visual cortex before the end of the first postnatal week (Lund & Mustari, 1977), at which time visual evoked potentials are first detectable (Rose & Ellingson, 1968). Similarly, continuous illumination for the first week has no apparent effect on rat visual cortical neuron spine density, although this is an area of lively discussion (Schapiro & Vukovich, 1970; Parnavelas & Globus, 1976). Finally, in our studies, visual deprivation did not alter the synaptic molecules examined in adult visual cortex.

The molecular mechanisms mediating reduced mPSDp consequent to light deprivation are presently under investigation. Decreased calmodulin binding to the mPSDp may simply reflect a true reduction of 51-kDa protein molecule number. Alternatively, affinity of mPSDp for calmodulin, or state of saturation with respect to endogenous calmodulin *in situ*, may be altered by visual experience. We are now attempting to distinguish among these and other alternatives.

Regardless of mechanism of reduction, altered mPSDp may be functionally significant. The mPSDp is an autophosphorylating subunit of a Ca^{2+} /calmodulin protein kinase, which may potentially alter synaptic function by changing spine conformation secondary to phosphorylation of cytoskeletal proteins (Siekevitz, 1985). Indeed, other Ca^{2+} /calmodulin kinases may play roles storing information in the brain (Lisman & Goldring, 1988). Type II kinase may mediate long-term potentiation (Malenka, Kaur, Perkal, Mauk, Kelly, Nicoll, & Waxham, 1989), the modulation of membrane K^+ currents (Acosta-Urquidi et al., 1984; Sakakibara, Alkon, deLorenzo, Goldenring, Neary, & Heldman, 1986), and reportedly changes in kitten and primate striate cortex with altered visual experience (Hendry & Kennedy, 1986; Neve & Bear, 1989). The functional relationships among the kinases, and their effector substrates, in visual cortex remain to be elucidated.

MATERIAL AND METHODS

Animal Treatment

Long-Evans hooded rats were reared under a normal 12 hr light/dark cycle or in total darkness. To investigate the effect of light deprivation on visual cortex development, pregnant mothers were kept in complete darkness from the day before delivery. At selected stages of postnatal development, rats were sacrificed and tissue samples were removed and immediately frozen. In some experiments, adults were kept in total darkness for different periods of time. All light-deprived animals were anesthetized in the dark prior to sacrifice.

Materials

Na^{125}I was obtained from Amersham, and iodination of calmodulin (Sigma) was performed using the enzymatic method of Richman and Klee (1978), modified by Carlin et al. (1981).

Subcellular Fractionation, Gel Electrophoresis, and ^{125}I -Labeled Calmodulin Binding

Synaptic membrane (SM) fractions were prepared as described by Carlin, Grab, Cohen, and Siekevitz (1980) and stored at -80°C until used. Protein content was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951). SDS-polyacrylamide gel electrophoresis was performed according to the discontinuous buffer system of Neville (1971) modified by Cohen, Blomberg, Berzins, and Siekevitz (1977). The total acrylamide in the separating gel varied linearly from 7 to 15%. The total acrylamide in the stacking gel was kept at 4%. ^{125}I -labeled calmodulin was bound to PSD-proteins separated by SDS-PAGE according to the procedure of Carlin, Grab, and Siekevitz (1981). The quantity of mPSDp was estimated densitometrically and expressed as arbitrary standardized units.

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