Molecular Neurobiology
Copyright ©1992 The Humana Press, Inc.
All rights of any nature whatsoever reserved.
ISSN0893-7648/92/5/2-3-4/203–216/$2.80

Mechanisms of Synaptic Plasticity

Changes in Postsynaptic Densities and Glutamate Receptors in Chicken Forebrain During Maturation

J. A. P. Rostas,*[*,1] J. M. Kavanagh,[1] P. R. Dodd,[2] J. W. Heath,[1] and D. A. Powis[1]

[1]The Neuroscience Group, Faculty of Medicine, University of Newcastle, Callaghan, N. S. W., 2308, Australia; [2]Wilson Memorial Clinical Research Laboratory, Royal Brisbane Hospital Foundation, Herston Q4029, Australia

Contents

Abstract

We have shown that the synapse maturation phase of synaptogenesis is a model for synaptic plasticity that can be particularly well-studied in chicken forebrain because for most forebrain synapses, the maturation changes occur slowly and are temporally well-separated from the synapse formation phase. We have used the synapse maturation phase of neuronal development in chicken forebrain to investigate the possible link between changes in the morphology and biochemical composition of the postsynaptic density (PSD) and the functional properties of glutamate receptors overlying the PSD. Morphometric studies of PSDs in forebrains and superior cervical ganglia of chickens and rats have shown that the morphological features of synapse maturation are characteristic of a synaptic type, but that the rate at which these changes occur can vary between types of synapses within one animal and between synapses of the same type in different species. We have investigated, during maturation in the chicken forebrain, the properties of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptors, which are concentrated in the junctional membranes overlying thick PSDs in

*Author to whom all correspondence and reprint requests should be addressed.

the adult. There was no change in the number of NMDA receptors during maturation, but there was an increase in the rate of NMDA-stimulated uptake of $^{45}Ca^{2+}$ into brain prisms. This functional change was not seen with the other ionotropic subtypes of the glutamate receptor and was NMDA receptor-mediated. The functional change also correlated with the increase in thickness of the PSD during maturation that has previously been shown to be due to an increase in the amount of PSD associated Ca^{2+}-calmodulin stimulated protein kinase II (CaM-PK II). Our results provide strong circumstantial evidence for the regulation of NMDA receptors by the PSD and implicate changing local concentrations of CaM-PK II in this process.

The results also indicate some of the ways in which properties of existing synapses can be modified by changes at the molecular level.

Index Entries: Maturation; plasticity; chicken brain; rat brain; superior cervical ganglion; postsynaptic density; ultrastructure; calcium/calmodulin-stimulated protein kinase II; glutamate receptors; NMDA; kainic acid; AMPA; MK-801; calcium.

Introduction

The term *neuronal plasticity* normally encompasses the ability of the nervous system to learn from experience and remember things, to adapt to change, and to recover from injury. It is believed that all these processes ultimately depend on the appropriate modification and regulation of individual synapses and synaptic networks; thus, the term *synaptic plasticity* is often used, although the changes involved are almost certainly not restricted to the synaptic region of neurons (and indeed may also involve non-neuronal cells). In the adult nervous system the changes in synapses can occur at two levels. At a structural ("coarse") level, new synaptic connections can be formed either in response to injury or as part of a cyclic turnover that is occurring continually (Carlin and Siekevitz, 1983; Cotman and Nieto-Sampedro, 1984; Dyson and Jones, 1984). At a "fine" level, plasticity involves local modulation of a fixed number of synapses by presynaptic and/or postsynaptic mechanisms that alter synaptic properties, producing a change in the efficacy of the synapses.

The Postsynaptic Density: A Key Regulator of Synaptic Properties

One postsynaptic structure that has been suggested as having a role in altering synaptic function at the "fine" level is the PSD. The PSD is a protein-rich specialization on the cytoplasmic surface of the postsynaptic junctional membrane at most synapses, irrespective of neurotransmitter type (Fig. 1). The PSD has been much studied morphologically since it is easy to identify by electron microscopy and can be selectively stained (Bloom and Aghajanian, 1966). It can also be isolated and purified in high yield by subcellular fractionation techniques, thus allowing extensive biochemical analysis (Cotman and Kelly, 1980; Gurd, 1982). The PSD is mostly composed of cytoskeletal proteins (Cotman and Kelly, 1980; Gurd, 1982; Rostas et al., 1986; Sedman et al., 1986), is contiguous with the postsynaptic cytoskeletal matrix, and may develop as a specialization of it (Gulley and Reese, 1981).

The molecular composition of subcellular fractions enriched in PSDs is remarkably constant between different species and between different regions of forebrain (Rostas et al., 1979; Nieto-Sampedro et al., 1982). In adult cerebral cortex, the major protein component of the thick PSDs associated with excitatory synapses is a form of the α subunit of the enzyme CaM-PK II. This enzyme can account for as much as 40% of the mass of detergent-extracted PSDs (Rostas and Dunkley, 1992). The PSD also appears to be tightly bound to protein components of the postsynaptic membrane, many of which, including neurotransmitter receptors and ion channels (Matus et al., 1981; Carlin and Siekevitz, 1984; Fagg and Matus, 1984; Wu et al., 1985;1986a,b), protrude into the synaptic cleft (Gurd, 1989). The

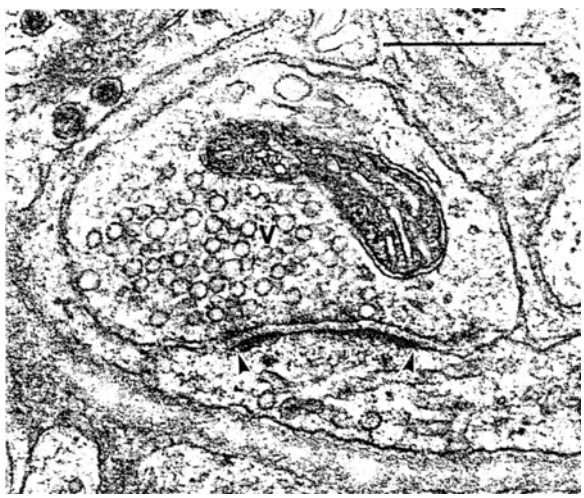

Fig. 1. An electron micrograph of a synapse illustrating its major structural features. Arrowheads indicate the extent of the postsynaptic density. v, synaptic vesicles. Bar = 0.5 μm.

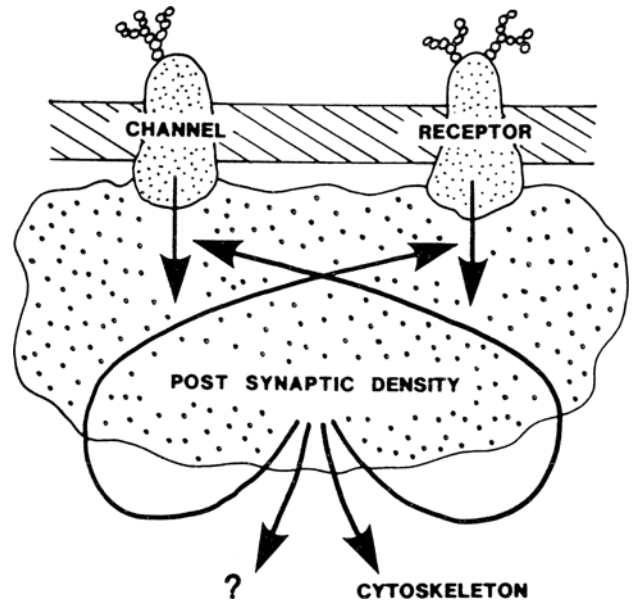

Fig. 2. Schematic diagram of the PSD and its proposed role in modulating the postsynaptic response to receptor activation. Activation of receptors and ion channels linked to the PSD result in some changes in proteins of the PSD. These changes can alter the subsequent responsiveness of the postsynaptic element by modifying the ion channels or the receptor via one of the PSD proteins (a direct effect), or by affecting the cytoskeleton or some other intracellular structure or control mechanism (an indirect effect). From Rostas (1991).

mobility of these components appears to be greatly restricted by the PSD (Kelly et al., 1976; Matus and Walters, 1976) and, given the high protein density in the synaptic cleft, the topographical array of these molecules may be relatively fixed.

Some of the ways in which the PSD may modulate synaptic function are shown schematically in Fig. 2. Activation of receptors or ion channels linked to the PSD could result in conformational or chemical changes in proteins of the PSD. These changes could then alter the subsequent responsiveness of the entire postsynaptic element by, for instance, modifying the ion channels or receptors via one of the PSD proteins (a direct effect). Altematively, they could modulate the consequences of receptor or ion channel activation by affecting the postsynaptic cytoskeleton in such a way as to alter the shape of the membrane surface or the spine head (Crick, 1982), or by modulating some other cellular component such as a second messenger linked cascade (an indirect effect). Thus, the PSD is ideally situated to function as a key regulatory structure acting as a link between the cellular apparatus for the reception and the propagation of synaptic signals.

Consistent with this view of the PSD as a dynamic structure, there is considerable evidence that in adult brain, it can undergo morphological and biochemical changes under conditions that are known to alter synaptic activity (reviewed in Siekevitz, 1985; Rostas, 1991). It has been proposed that these changes in the PSD reflect changing properties of the synapse and could be used as an index of these changing properties. Although it is widely believed that mechanisms such as those depicted in Fig. 2 do occur, direct evidence for them is lacking. In order to be able to provide such evidence and study the molecular mechanisms involved, a biological system is needed which has a sufficient quantity of tissue with many synapses undergoing change

at a defined time. One way of doing this is to use developing nervous tissue specifically during the maturation phase of development.

The Synapse Maturation Phase of Development: A Model for Synaptic Plasticity

The many events involved in normal synaptogenesis can be divided into at least two broad phases: synapse formation and synapse maturation* (Fig. 3). Synapse formation encompasses the complex events that eventually convert a growing neurite into a neuronal process making contact with another cell via a differentiated structure that looks and functions like a synapse. During this phase, there is a net increase in the number of synapses, which is produced in a tissue environment different from that usually found in adult brain. By contrast, synapse maturation encompasses the perhaps (relatively) simpler set of events that modify the existing synaptic networks, changing their immature properties to mature ones. This phase produces no net increase in the number of synapses (in some systems it may produce a net decrease) and occurs within functional networks of neurons in a tissue environment similar to that in the adult. Therefore, all the changes in the maturation phase are potentially enhanceable or reversible and those that result in a functional change in the properties of the synaptic networks may be similar or identical to the changes responsible for plasticity in the adult. Thus, the maturation phase of normal brain development can be used as an experimental model system for the identification and study of mechanisms responsible for synaptic plasticity.

Because synapses in brain develop asynchronously, studies of the maturation process require a system in which the maturation phase is long relative to the formation phase so that developmental changes can be unambiguously ascribed to either phase. Figure 4 shows that the developing chicken forebrain is such a system, whereas the developing rat forebrain is not.

The marker for synapse formation in Fig. 4 is the increase in the concentration of Thy-1, an abundant and developmentally regulated cell-surface glycoprotein. Thy-1 is not preferentially localized to synaptic membranes (although it is present there), but its level of expression by neurons increases greatly after they have established synaptic contacts, and therefore, brain levels correlate with synapse formation in a number of systems (Barclay, 1979; Morris, 1985; Sinclair et al., 1987). Developmental increases in the levels of synaptophysin (P. L. Jeffrey, personal communication) or synapsin I (Weinberger, Brent, and Rostas, in preparation), two well-accepted markers for synapses, define the same period of development as being the synapse formation phase. In the case of the two markers for synapse maturation shown in Fig. 4 (the thickness of the PSD and the concentration of α-CaM-PK II in the PSD), and also for all other developmental changes that we have measured in both chicken and rat brain (reviewed in Rostas, 1991), the same changes occur in both species but with a different timecourse. Thus, by making measurements at appropriate ages (e.g., 2, 8, 21 d, and adult) it is possible in the chicken to ascribe developmental changes either to the formation phase or to the maturation phase. However, this is not possible in the rat because although the same maturation changes occur, they occur so rapidly in the rat that the two phases almost completely overlap.

Structural Changes in Postsynaptic Densities During Maturation

The proposal that maturation is a distinct phase of synaptic development was initially based on biochemical and morphological studies of PSDs in developing forebrain. The first suggestion came from studies in rat brain, which showed that the most rapid increase in the PSD content of α-CaM-PK II (then known simply as the major PSD protein) occurred in the third postnatal week (Fu

*Despite the fact that the names of both phases specifically refer to the synapse, both phases involve changes occurring throughout the neuron.

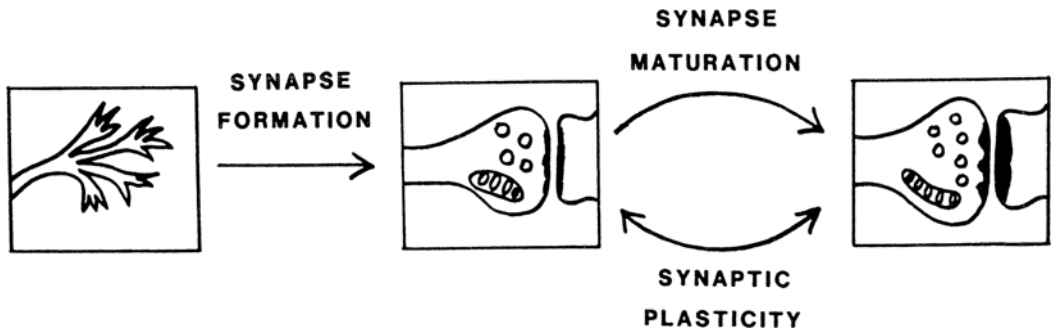

Fig. 3. Schematic representation of the synapse formation and synapse maturation phases of synaptogenesis. *See* text for details. From Rostas (1991).

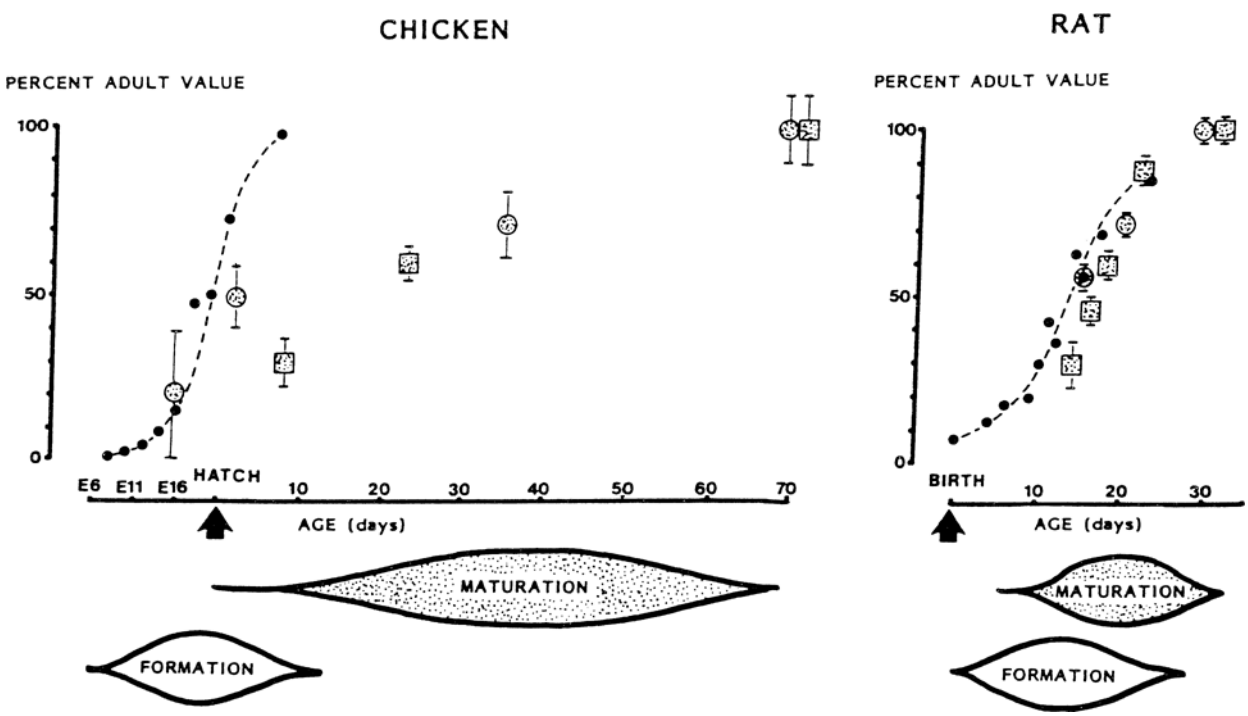

Fig. 4. Comparison of the timecourse of the synapse formation and synapse maturation phases of neuronal development in chicken and rat forebrain. The upper part of the figure shows experimental results obtained for the increase in the expression of Thy-1 (a marker for synapse formation) and the mean thickness of PSDs and the α-CaM-PK II content of PSDs (markers for synapse maturation). The lower part of the figure shows a schematic representation of synapse formation and synapse maturation in chicken and rat: The length and position of the shapes show the period during which the phases occur and the width of the shape indicates how rapidly the changes are occurring during the phase. Thy-1 concentration in brain (small filled circles): Jeffrey, Greig and Rostas, unpublished (chicken); Barclay (1979) (rat). PSD thickness (large speckled circles): Rostas et al. (1984); Rostas and Guldner, unpublished (chicken); Markus et al. (1987) (rat). α-CaM-PK II concentration in the PSD (speckled squares): measured by autophosphorylation in chicken (Rostas et al., 1987) and protein stain in isolated synaptic junction fractions from rat forebrain (Weinberger and Rostas, 1986). From Rostas (1991).

et al., 1981; Kelly and Cotman,1981; Kelly and Vernon, 1985; Weinberger and Rostas, 1986). Since the most rapid rate of synapse formation occurred in the second postnatal week, the increase in α-CaM-PK II was interpreted as a maturation of the synapses that had formed previously. However, there is a considerable variation in PSD thickness (Colonnier, 1968) and α-CaM-PK II concentration (Walaas et al., 1983a,b; Ouimet et al., 1984; Erondu and Kennedy, 1985) from region to region in brain. Also, neurons establish synapses at slightly different times and rates (Heath et al., 1992) in each region, and synapse formation in the rat forebrain as a whole is not complete until about postnatal day 30 (Aghajanian and Bloom, 1967; Wolff, 1976,1978; Blue and Parnavelas, 1983; Markus et al., 1987), by which time both PSD morphology (Markus et al., 1987) and α-CaM-PK II concentration (Weinberger and Rostas, 1986) are also mature. Hence, differential rates of development of the neurons with thick and thin PSDs would also have been able to explain the data. Clear evidence that synapse maturation is indeed a separate phase of neuronal development came with the observation that in chicken forebrain, the changes in PSD morphology and protein composition occurred over a protracted period (3–10 wk posthatch) well after synapse formation was complete (approx 2 wk posthatch) (Rostas et al., 1984; Weinberger and Rostas, 1988).

Table 1 shows the developmental changes in the morphology of the mixed populations of synapses from chicken and rat forebrain. In both cases, the pattern of developmental change is the same: There is an increase in the mean thickness of the PSDs but no change in their mean length.*

*The similarity between the two species, both in terms of the developmental pattern of change and in the absolute size of the PSDs, is noteworthy since the two studies used vastly different methodology. Rostas et al. (1984) measured PSDs that were still attached to synaptosomes after isolation, whereas Markus et al. (1987) measured PSDs in perfused intact brain. The agreement between the values for mean PSD length is remarkable and the difference in the values for mean PSD thickness may be attributable merely to the lower recovery of thin PSDs after homogenization and to the fact that Rostas et al. measured maximum thickness, whereas Markus et al. measured mean thickness.

However, the change is much more protracted in the chicken than in the rat. Since the duration of the synapse formation phase is very similar in the two species, the difference in the rate of maturation of PSD morphology is attributable to a selective delay in onset of the maturation phase and a protraction of its duration.

Two major questions arise from these data. First, do synapses of all types show the same structural changes during maturation? Second, what is the basis for the difference between the rates of maturation in the chicken and rat forebrain? At least two hypotheses can be considered. It may be that the rate of synaptic maturation is both characteristic of the species and relatively constant throughout its nervous system. Alternatively, synapses in different parts of the nervous system may mature at different rates, perhaps depending on the inherent plasticity of the synapses and/or the particular endogenous or exogenous influences to which they are susceptible. In the latter case, the increased complexity, and perhaps plasticity, of the mammalian cerebral cortex may increase the range of maturation rates among the synapses, and thus, obscure changes occurring at individual synapses when population average data is collected. To distinguish between these two hypotheses, we undertook a morphometric analysis of synaptic maturation in a single defined population of synapses. For these studies (Heath et al., 1992; in press), we chose the preganglionic synapses of the superior cervical ganglion (SCG) because the development of this ganglion in rats has been well-studied. Table 2 shows that between 10 d and 4 wk, when the number of synapses in the SCG is increasing (Smolen, 1981), the mean thickness of the PSD increased ($p = 0.017$) but the mean length of the PSD did not change. However, between 4 wk and adult, when the number of synapses does not change and the target organ innervation appears to be already mature (Snider, 1986), there was a large increase in mean PSD length ($p = 0.001$), with no change in mean PSD thickness (Table 2). Furthermore, the developmental change in the morphology of preganglionic synapses in the chicken SCG showed the

Table 1
Developmental Changes in Morphology of PSDs in Forebrain

		Length, nm	Thickness, nm
Chicken forebrain synaptosomes[a]			
2 d	(n = 121/5)	304 ± 39	20.0 ± 5.3
35 d	(n = 11/3)	273 ± 34	25.4 ± 4.7
> 70 d	(n = 192/5)	298 ± 86	38.6 ± 5.5
Rat cortex[b]			
15 d		325 ± 25	21.3 ± 1.2
20 d		275 ± 12	26.0 ± 1.5
30 d		290 ± 15	34.5 ± 1.5
90 d		310 ± 23	32.6 ± 3.0

[a]Rostas et al. (1984); Rostas (1991).
[b]Markus et al. (1987).
Values are mean ± SEM.

Table 2
Developmental Changes in Morphology of PSDs from Superior Cervical Ganglia

		Length, nm	Thickness, nm
Rat superior cervical ganglion[a]			
10 d	(n = 142/4)	414 ± 15	45.9 ± 0.9
4 wk	(n = 62/4)	412 ± 26	52.1 ± 1.8
adult	(n = 130/4)	533 ± 23	53.9 ± 1.5
Chicken superior cervical ganglion[b]			
2 d	(n = 172/4)	407 ± 17	49.2 ± 1.1
adults	(n = 204/4)	734 ± 32	55.4 + 1.2

[a]Heath et al. (1992).
[b]Heath, Glenfifield, and Rostas, in preparation.
Values are mean ± SEM.

same pattern (Table 2). Thus, it appears that the morphological features of maturation are characteristic of a synaptic type but that the rate at which these changes occur can vary between types of synapse within one animal and between synapses of the same type in different species.

The functional significance of an increase in PSD length compared with an increase in PSD thickness is not known, but the following hypothesis is consistent with the currently available data. An increase in PSD profile length represents an increase in the area of synaptic contact. This may result in a more efficient synapse if we assume that the number of neurotransmitter receptors in the postsynaptic junctional membrane is proportional to the area of the contact zone. In at least one case (Horn et al., 1985; McCabe and Horn, 1988), the imprinting-induced increase in the length of PSDs in the left intermediate medial hyperstriatum ventrale (IMHV) of the chick forebrain was accompanied by what appeared to be a corresponding increase in the number of NMDA receptors. By contrast, an increase in PSD thickness with no change in length may imply a change in the degree and/or type of regulation exerted by the PSD with no change in the number of receptors or other components of the postsynaptic membrane that are regulated by the PSD.

Functional Changes in Glutamate Receptors During Maturation

To test the hypothesis about the functional effects of changes in the PSD, we measured properties of receptors associated with the PSDs whose thickness increased during maturation without any change in length. Since the morphological measurements were performed with whole forebrain, we chose an abundant and widespread receptor associated with thick PSDs in the adult: The receptors for glutamic acid, the most abundant excitatory transmitter in the brain and in particular, the NMDA-stimulated subtype that is known to be highly concentrated over thick PSDs (Monaghan and Cotman, 1986).

The number of NMDA receptors was determined using MK-801, a high-affinity, noncompetitive NMDA antagonist that binds to the ion channel associated with the NMDA receptor (Wong et al., 1986). Standard quantitative binding techniques were used on well washed membranes from immature (11 d) chicken forebrain at the end of the synapse formation phase and from mature (8 wk) chicken forebrain (Kavanagh et al., 1992a). Table 3 summarizes the main results.

The characteristics of MK-801 binding to membranes from chicken forebrain were similar to those described for membranes from mammalian cerebral cortex. Both the density (B_{max}) and affinity (K_d) of MK-801 binding were similar, and the optimum concentration of glutamate required for stimulation of MK-801 binding was identical to those in previous reports on mammalian brain (Foster and Wong, 1987; Ramsom and Stec, 1988). In particular, there was no significant difference between the characteristics of MK-801 binding to membranes from immature and mature forebrain (Table 3). Evidence for a low-affinity binding site was found in two out of four immature and three out of four mature brain membrane preparations, but this site did not appear to change its binding characteristics either. Thus, it appears that the number of NMDA receptors does not change during the maturation period in chicken forebrain when the PSDs over which they are concentrated are increasing their thickness but not their length.

For the initial investigations of receptor function, we measured receptor-mediated increases in Ca^{2+} uptake into cells, since the ion channel associated with the NMDA receptor admits Ca^{2+} directly (MacDermott et al., 1986) and, in adult mammalian brain at least, most of the NMDA-stimulated Ca^{2+} influx occurs via the NMDA receptor (Riveros and Orrego, 1986) rather than via nearby voltage-sensitive calcium channels (VSCCs). The experiments were carried out at 30°C (rather than at 42°C, which is normal body temperature for a chicken) in order to slow the rate of Ca^{2+} flux across cell membranes. Brain prisms (250 × 250 μm) were prepared from chicken forebrain using a McIlwain tissue chopper. These prisms provided a source of brain tissue in which the neuronal circuitry and cellular interactions were still partly intact and, in particular, the postsynaptic compartment is intact. In addition, this method provided a random sampling of tissue from different regions of the forebrain to give results that could be related to previous studies on synapses isolated from forebrain homogenates. When prepared under optimum conditions, these prisms remained viable and highly active metabolically for several hours. Basal $^{45}Ca^{2+}$ uptake did not change during maturation and, at all ages, the degree of stimulation of $^{45}Ca^{2+}$ uptake in the prisms depended on the degree of depolarization produced (Kavanagh, Powis, and Rostas, in preparation).

In prisms from adult brain, the level of $^{45}Ca^{2+}$ uptake stimulated by NMDA (1 m*M*) reached maximal levels by 4 min and was maintained for at least 10 min (Fig. 5). In the prisms from the forebrains of chickens that are immature but have finished the synapse formation period, the rate of $^{45}Ca^{2+}$ uptake was slower, although the degree of stimulation was the same after about 10 min (Fig. 5). The rate of $^{45}Ca^{2+}$ uptake in 21–25 d old chickens was not significantly different to that at 8–15 d, and by 8 wk posthatch, the rate of uptake

Table 3
Characteristics of [^{3}H]MK-801 Binding to Brain Membranes from 11-d and 8-wk old Chickens

Parameter	Immature	Mature
High affifinity site[a]		
K_D, (n*M*)	5.3 ± 0.5	4.9 ± 0.2
B_{max} fmol/mg protein at constant K_D	1790.0 ± 150.0	1680.0 ± 80.0
Glutamate concentration for maximal binding, μ*M*	4.0 ± 0.1	5.1 ± 0.2
Maximum binding in the presence of glutamate, % control	315.0 ± 15.0	305.0 ± 15.0
Low affifinity site[b]		
K_D, n*M*	≈460	
B_{max} pmol/mg protein at constant K_D	13.8 ± 5.5	10.4 ± 1.8

[a]n = 4; [b]n = 2 (immature) and 3 (mature). Data are expressed as mean ± SEM. No significant difference was found between any of the parameters measured in immature and mature brain ($p > 0.5$).

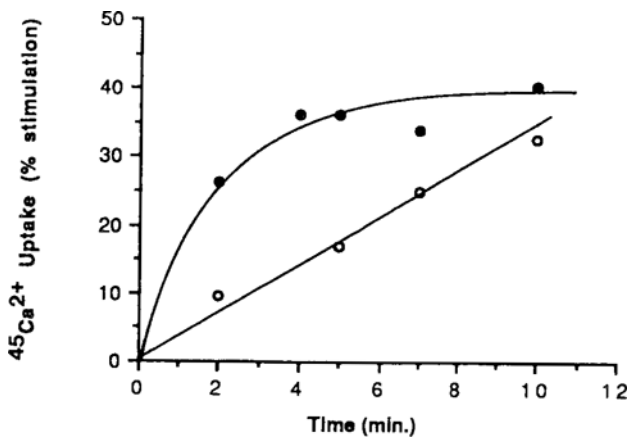

Fig. 5. Timecourse for NMDA (1 m*M*) stimulated $^{45}Ca^{2+}$ uptake ino immature and mature prisms (each point is the mean of 2–10 experiments). ● = mature; ○= immature.

was the same as that in adults. Therefore, the change in NMDA-stimulated $^{45}Ca^{2+}$ uptake correlates temporally with the increase in thickness of the PSD. In control experiments, the NMDA stimulation of $^{45}Ca^{2+}$ uptake was totally blocked by MK-801 (1 μ*M*) or CPP (1 m*M*) at all ages, indicating that virtually all the NMDA-stimulated $^{45}Ca^{2+}$ uptake was initiated by activation of NMDA receptors (Kavanagh et al., 1992a,b). We have also shown that a selective toxicity of NMDA on immature neurons cannot explain these results since preincubating with 1 m*M* NMDA for 5 min prior to measuring $^{45}Ca^{2+}$ uptake did not change the NMDA-stimulated $^{45}Ca^{2+}$ uptake on a second exposure, and the same degree of stimulation was observed with 50 μ*M* or 2.5 m*M* NMDA.

To investigate the behavior of the other glutamate receptors, we measured $^{45}Ca^{2+}$ uptake in prisms after stimulation with agonists selective for the two other major ionotropic receptor subtypes for glutamate: kainic acid (KA) and (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). In these experiments, the interpretation of the results is less clear for three reasons. First, both the KA and AMPA receptors stimulate $^{45}Ca^{2+}$ uptake by depolarizing cells and opening VSCCs rather than admitting $^{45}Ca^{2+}$ directly. Second, although both KA and AMPA receptors are present over PSDs, these receptors have a much wider distribution within neurons (and in some cases, within glia) than NMDA receptors. Finally, at the concentrations required for maximal stimulation, some toxic effects of these agonists are evident in these prisms. With these caveats in mind, the results obtained with KA- and AMPA-stimulated $^{45}Ca^{2+}$ uptake are shown in Fig. 6 and compared with the stimulation obtained with NMDA and glutamate at the same time point (5 min).

With both KA and AMPA, the rate of $^{45}Ca^{2+}$ uptake was much faster than with NMDA so that maximal levels of $^{45}Ca^{2+}$ uptake already had been reached by 2.5 min at 30°C (not shown). Further-

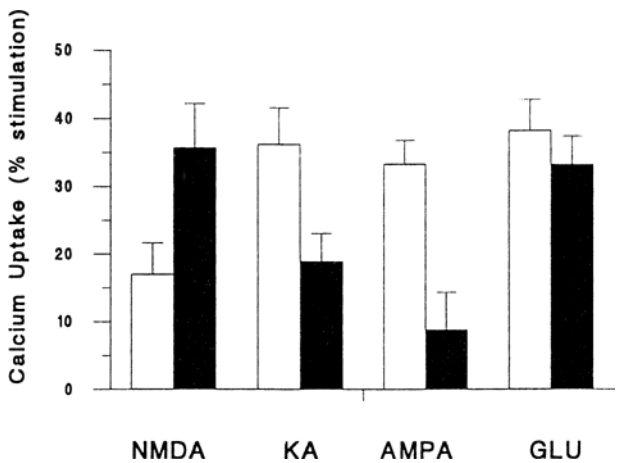

Fig. 6. Comparison of the effect of 1 m*M* NMDA, KA and AMPA and 2.5 m*M* glutamate (GLU) on the $^{45}Ca^{2+}$ uptake into immature and mature brain prisms measured 5 min after adding the agonist. Values are mean ±SEM. For NMDA, KA, AMPA, and GLU, respectively, $n = 7, 3, 3,$ and 4 for immature and $n = 8, 6, 6,$ and 4 for mature brain. All comparisons of the results between immature and mature brain were significantly different (Student's *t*-test) at $p < 0.05$ except for GLU, which was not significantly different. □ = immature; ■ = mature.

more, the change during maturation was in the opposite direction. The level of $^{45}Ca^{2+}$ uptake reached in the adult was much lower than that in the immature brain prisms, suggesting that there is a decrease in the number of receptors or associated VSCCs, but whether these changes are at postsynaptic junctional sites or elsewhere in the brain is not known. When the natural agonist at all these receptors, glutamic acid, was used there was no difference in the amount or rate of $^{45}Ca^{2+}$ uptake as measured by these techniques (Fig. 6). Although the opposing developmental changes of the different receptor types apparently cancel each other out in terms of their effect on the net uptake of $^{45}Ca^{2+}$ into the prisms, this may not be so at the level of a single neuron or postsynaptic element. If reciprocal changes did occur at a single postsynaptic site, they could have profound effects on synaptic plasticity. For example, NMDA and non-NMDA receptors have different roles in the induction and maintenance of long-term potentiation (Collingridge and Singer, 1990).

Mechanisms Responsible for the Change in the NMDA Receptor Function

Insofar as the binding characteristics of MK-801 did not change during the maturation period, it is unlikely that the number of NMDA receptors would vary greatly. However, the agonist-binding characteristics may change due to, for example, a change in subunit composition, such as has been observed for glutamate receptors in some areas of rat brain (Sommer et al., 1990) and the nicotinic acetylcholine receptor in muscle during development (Mishina et al., 1986). Alternatively, there may be a change in the number or type of VSCCs recruited or in second messenger-linked events that are triggered by the activation of NMDA receptors. Finally, the change could be brought about by differential regulation of the same number of NMDA receptors of the same type. In this last case, the PSD or one of its components would be an obvious candidate for the role of regulator.

During development in general, and the maturation phase in particular, there is a progressive increase in the number of CaM-PK II molecules associated with PSDs (Sedman et al., 1986; Weinberger and Rostas, 1988; Rostas, 1991). Since there is no other major change in the protein composition of the PSD occurring during this period, the enzyme CaM-PK II may be involved. CaM-PK II is unusual in that despite the great abundance of this enzyme, its phosphorylation of endogenous substrates (at least in vitro) appears to be enzyme-limited rather than substrate-limited (Weinberger and Rostas, 1988; Koszka et al., 1991; Rostas, 1991; Rostas and Dunkley, 1992). Therefore, this focal increase in the concentration of CaM-PK II may lead to a change in the phosphorylation of the receptors or of some other PSD protein that changes the interaction between the receptors and the PSD. Alternatively, the binding of more molecules of CaM-PK II may alter the allosteric interactions between PSD proteins and receptors, and this enzyme might induce changes by a nonenzymatic mechanism in this

structure where the abundance of CaM-PK II qualifies it for the title of structural protein (Rostas et al., 1986; Rostas and Dunkley, 1992). It remains to be seen whether these observations of the changes in the functional properties of NMDA receptors in maturing chicken brain lead to a better understanding of the functional roles of the PSD in general and CaM-PK II in particular.

Conclusion

Understanding the mechanisms underlying synaptic plasticity will provide insights into the adaptive and cognitive capacities of the nervous system. It may also lead to rational strategies for maximizing the capacity of the nervous system to recover from injury, or to slow the rate of functional deterioration in degenerative disorders by stimulating the capacity of the remaining cells to compensate for the loss. Research in this important area has led to the development of a number of experimental model systems in which to study various aspects of synaptic plasticity. In the maturing chicken forebrain, we have identified and characterized an unusual model system that offers several advantages for the study of molecular mechanisms involved in synaptic plasticity. We have used this model to show that morphological changes occur in PSDs which are directly correlated with the amount of CaM-PK II associated with them, that the type of morphological change is characteristic of a synaptic type, and that the rate of change can vary between synaptic types. We have also investigated the changes in the functional response to glutamate receptor stimulation in forebrain and we have shown that the changes in the NMDA receptor response occur without any apparent change in NMDA receptor number, suggesting that an altered regulation of this receptor is involved. The maturing chicken forebrain provides an ideal model system in which to investigate the proposed causal link between PSD morphology and CaM-PK II content on the one hand and the functional properties of the NMDA receptor on the other. If this causal relationship can be established, it will provide the first piece of direct evidence for the role of the PSD in synaptic plasticity and will identify another way in which the properties of the NMDA receptor can be modulated.

Acknowledgments

This work was supported by grants from the National Health and Medical Research Council of Australia. We are grateful to Alistair Sim, Phillip Robinson, and Peter Dunkley for helpful comments on this manuscript.

References

Aghajanian G. N. and Bloom F. E. (1967) The formation of synaptic junctions in developing rat brain: a quantitative electron microscopic study. *Brain Res.* **6**, 716–727.

Barday A. N. (1979) Localisation of the Thy-1 antigen in the cerebellar cortex of rat brain by immunofluorescence during postnatal development. *J. Neurochem.* **32**, 1249–1259.

Bloom F. E. and Aghajanian G. K. (1966) Cytochemistry of synapses: selective staining for electron microscopy. *Science* **154**, 1575–1577.

Blue M. E. and Parnavelas J. G. (1983) The formation and maturation of synapses in the visual cortex of the rat II. Quantitative analysis. *J. Neuropsychol.* **12**, 697–712.

Carlin R. K. and Siekevitz P. (1983) Plasticity in the central nervous system: do synapses divide? *Proc. Natl. Acad. Sci. USA* **80**, 3517–3521.

Carlin R. K. and Siekevitz P. (1984) Characterisation of Na^+-independent GABA and flunitrazepam binding sites in preparations of synaptic membranes and postsynaptic densities isolated from canine cerebral cortex and cerebellum. *J. Neurochem.* **43**, 1011–1017.

Collingridge G. L. and Singer W. (1990) Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol. Sci.* **11**, 290–296.

Colonnier M. (1968) Synaptic patterns of different cell types in the different laminae of the visual cortex. An electron microscopic study. *Brain Res.* **9**, 268–287.

Cotman C. W. and Kelly P. T. (1980) Macromolecular architecture of CNS synapses. *The Cell Surface and*

Neuronal Function. Cotman C. W., Poste G., and Nicolson G. L. eds., Elsevier, North Holland Biomedical Press, Amsterdam, pp. 505–533.

Cotman C. W. and Nieto-Sampedro M. (1984) Cell biology of synaptic plasticity. *Science* **225**, 1287–1294.

Crick F. (1982) Do dendritic spines twitch? *Trends Neurosci.* **5**, 44–46.

Dyson S. E. and Jones D. G. (1984) Synaptic remodelling during development and maturation: Functional differentiation and splitting as a mechanism for modifying connectivity. *Brain Res.* **315**, 125–137.

Erondu N. E. and Kennedy M. B. (1985) Regional distribution of type II Ca^{2+}/calmodulin-dependent protein kinase in rat brain. *J. Neurosci.* **5**, 3270–3277.

Fagg G. E. and Matus A. (1984) Selective association of N-methylaspartate and quisqualate types of L-glutamate receptor with brain postsynaptic densities. *Proc. Natl. Acad. Sci. USA* **81**, 6876–6880.

Foster A. C. and Wong E. H. F. (1987) The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.* **91**, 403–409.

Fu S. C., Cruz T. F., and Gurd J. W. (1981) Development of synaptic glycoproteins: Effective postnatal age on the synthesis and concentration of synaptic membrane and synaptic junctional fucosyl and sialyl glycoproteins. *J. Neurochem.* **36**, 1338–1351.

Gulley R. L. and Reese T. S. (1981) Cytoskeletal organisation at the postsynaptic complex. *J. Cell Biol.* **91**, 298–302.

Gurd J. W. (1982) Molecular characterisation of synapses in the central nervous system. *Molecular Approaches to Neurobiology*. Brown I. R., ed. Academic, New York, pp. 99–130.

Gurd J. W. (1989) Glycoproteins of the synapse. *Neurobiology of Glycoconjugates*. Margolis R. K. and Margolis R. U., eds. Plenum, New York, pp. 219–242.

Heath J. W., Glenfield P. J., and Rostas J. A. P. (1992) Structural maturation of synapses in the rat superior cervical ganglion continues beyond four weeks of age. *Neurosci. Lett.* in press.

Horn G., Bradley P., and McCabe B. J. (1985) Changes in the structure of synapses associated with learning. *J. Neurosci.* **5**, 3161–3168.

Kavanagh J. M., Dodd P. R., and Rostas J. A. P. (1992a) [^{3}H]MK-801 binding in immature and mature chicken forebrain *Neurosci. Lett.* in press.

Kavanagh J. M., Powis D. A., Dodd P. R., and Rostas J. A. P. (1992b) NMDA receptor function in chicken forebrain during maturation. *Mol. Neuropharmacol.*, in press.

Kelly P. T. and Cotman C. W. (1981) Developmental changes in morphology and molecular composition of isolated synaptic junctional structures. *Brain Res.* **206**, 251–271.

Kelly P. T., Cotman C. W., Gentry C., and Nicolson G. (1976) Distribution and mobility of lectin receptors on synaptic membranes of identified neurons in the central nervous system. *J. Cell Biol.* **71**, 487–496.

Kelly P. T., Shields S., Conway K., Yip R., and Burgin K. (1987) Developmental changes in calmodulin-kinase II activity at brain synaptic junctions: Alterations in holoenzyme composition. *J. Neurochem.* **49**, 1927–1940.

Kelly P. T. and Vernon P. (1985) Changes in subcellular distribution of calmodulin kinase II during brain development. *Dev. Brain Res.* **18**, 211–224.

Koszka C., Brent V. A., and Rostas J. A. P. (1991) Developmental changes in phosphorylation of MAP-2 and synapsin I in cytosol and taxol polymerized microtubules from chicken brain. *Neurochem. Res.* **15**, 637–644.

MacDermott A. B., Mayer M. L., Westbrook G. L., Smith S. J., and Barker J. L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321**, 519–522.

McCabe B. J. and Horn G. (1988) Learning and memory: Regional changes in N-methyl-D-aspartate receptors in the chick brain after imprinting. *Proc. Natl. Acad. Sci. USA* **85**, 2849–2853.

Markus E. J., Petit T. L., and LeBoutillier J. B. (1987) Synaptic structural changes during development and ageing. *Dev. Brain Res.* **35**, 239–248.

Matus A., Pehling G., and Wilkinson D. (1981) Gamma-aminobutyric acid receptors in brain postsynaptic densities. *J. Neurobiol.* **12**, 67–73.

Matus A. and Walters B. B. (1976) Type I and II synaptic junctions: Differences in distribution of Concanavalin A binding sites and stability of the junctional adhesion. *Brain Res.* **108**, 249–256.

Mishina M., Takai T., Imoto K., Noda M., Takahashi T., Numa S., Methfessel C., and Sakmann B. (1986) Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature* **321**, 406–411.

Monaghan D. T. and Cotman C. W. (1986) Identification and properties of N-methyl-D-aspartate receptors in rat brain synaptic plasma membranes. *Proc. Natl. Acad. Sci. USA* **83,** 7532–7536.

Morris R. J. (1985) Thy-1 in developing nervous tissue. *Dev. Neurosci.* **7,** 133–160.

Nieto-Sampedro M., Hoff S. F., and Cotman C. W. (1982) Perforated postsynaptic densities: probable intermediates in synapse turnover. *Proc. Natl. Acad. Sci. USA* **79,** 5718–5722.

Ouimet C. C., McGuinness T. L., and Greengard P. (1984) Immunocytochemical localisation of calcium/calmodulin dependent protein kinase II in rat brain. *Proc. Natl. Acad. Sci. USA* **81,** 5604–5608.

Ransom R. W. and Stec N. L. (1988) Cooperative modulation of [^{3}H]MK-801 binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* **51,** 830–836.

Riveros N. and Orrego F. (1986) N-methylaspartate-activated calcium channels in rat brain cortex slices. Effect of calcium channel blockers and of inhibitory and depressant substances. *Neuroscience* **17,** 541–546.

Rostas J. A. P. (1991) Molecular mechanisms of neuronal maturation: A model for synaptic plasticity. *Neural and Behavioural Plasticity.* Andrew R. J., ed. Oxford University Press, pp. 177–211.

Rostas J. A. P., Baker C. M., Weinberger R. P., and Dunkley P. R. (1987) Changes in the pre- and postsynaptic calmodulin stimulated protein kinase II during development in chicken forebrain. *J. Neurochem.* **48,** 515A.

Rostas J. A. P., Brent V. A., and Guldner F. H. (1984) The maturation of postsynaptic densities in chicken forebrain. *Neurosci. Lett.* **45,** 297–304.

Rostas J. A. P. and Dunkley P. R. (1992) Multiple forms and distribution of calcium/calmodulin-stimulated protein kinase II in brain. *J. Neurochem.* in press.

Rostas J. A. P., Kelly P. T., Pesin R. H., and Cotman C. W. (1979) Protein and glycoprotein composition of synaptic junctions prepared from discrete synaptic regions and different regions. *Brain Res.* **168,** 151–167.

Rostas J. A. P., Weinberger R. P., and Dunkley P. R. (1986) Multiple pools and multiple forms of calmodulin stimulated protein kinase during development: Relationship to postsynaptic densities. *Prog. Brain Res.* **69,** 355–371.

Sedman G. L., Jeffrey P. L., Austin L., and Rostas J. A. P. (1986) The metabolic turnover of the major proteins of the postsynaptic density. *Mol. Brain Res.* **1,** 221–230.

Siekevitz P. (1985) The postsynaptic density: A possible role in long lasting effects in the central nervous system. *Proc. Natl. Acad. Sci. USA* **82,** 3494–3498.

Sinclair C. M., Greig D. I., and Jeffrey P. L. (1987) Developmental appearance of Thy-1 antigen in the avian nervous system. *Dev. Brain Res.* **35,** 43–53.

Smolen A. J. (1981) Postnatal development of ganglionic neurons in the absence of preganglionic input: Morphological observations on synapse formation. *Dev. Brain Res.* **1,** 49–58.

Snider W. D. (1986) Rostrocaudal differences in dendritic growth and synaptogenesis in rat sympathetic chain ganglia. *J. Comp. Neurol.* **244,** 245–253.

Sommer B., Keinanen K., Verdoorn T. A., Wisden W., Burnashev N., Herb A., Kohler M., Takagi T., Sakmann B., and Seeburg P. H. (1990) Flip and flop: A cell-specific functional switch in glutamate-operated channels of the CNS. *Science* **249,** 1580–1585.

Walaas S. I., Nairn A. C., and Greengard P. (1983a) Regional distribution of calcium and cyclic AMP regulated protein phosphorylation systems in mammalian brain I: Particulate systems. *J. Neurosci.* **3,** 291–301.

Walaas S. I., Nairn A. C., and Greengard P. (1983b) Regional distribution of calcium and cyclic AMP regulated protein phosphorylation systems in mammalian brain II: Soluble systems. *J. Neurosci.* **3,** 302–311.

Weinberger R. P. and Rostas J. A. P. (1986) Subcellular distribution of a calmodulin dependent protein kinase activity in rat cerebral cortex during development. *Dev. Brain Res.* **29,** 37–50.

Weinberger R. P. and Rostas J. A. P. (1988) Developmental changes in protein phosphorylation in chicken forebrain II: Calmodulin stimulated phosphorylation. *Dev.Brain Res.* **43,** 259–272.

Wolff J. R. (1976) Quantitative analysis of topography and development of synapses in the visual cortex. *Exp. Brain Res.* **Suppl. 1,** 259–263.

Wolff J. R. (1978) Ontogenic aspects of cortical architecture: Lamination. *Architectonics of the Cerebral Cortex.* M. A. B. Brazier and H. J. Petch, eds. Raven, New York, pp. 159–173.

Wong E. H. F., Kemp J. A., Priestley T., Knight A. R., Woodruff G. N., and Iversen L. L. (1986) The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. USA* **83,** 7104–7108.

Wu K., Carlin R. K., Sachs L., and Siekevitz P. (1985) Existence of a Ca^{2+}-dependent K^+ channel in synaptic membrane and postsynaptic density fractions isolated from canine cerebral cortex and cerebellum, as determined by apamin binding. *Brain Res.* **360,** 183–194.

Wu K., Carlin R. K., and Siekevitz P. (1986a) Binding of L-[^{3}H]-glutamate to fresh or frozen synaptic membrane and postsynaptic density fractions isolated from cerebral cortex and cerebellum of fresh or frozen canine brain. *J. Neurochem.* **46,** 831–841.

Wu K., Sachs L., Carlin R. K., and Siekevitz P. (1986b) Characteristics of a Ca^{2+}/calmodulin-dependent binding of the Ca^{2+} channel antagonist nitrendipine to a postsynaptic density fraction isolated from canine cerebral cortex. *Mol. Brain Res.* **1,** 167–184.