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Transient increased density of NMDA binding sites in the developing rat hippocampus

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Using quantitative autoradiography and membrane preparations, the density of specific glutamate and *N*-methyl-D-aspartic acid (NMDA) binding sites have been determined in the developing rat hippocampus. We found an abrupt reduction in the density of NMDA binding sites after P8 (postnatal day) without change in affinity. The transient expression of NMDA receptors during maturation suggests that they may play a particularly important role in synaptogenesis.

Recent studies suggest that the *N*-methyl-D-aspartic acid (NMDA) receptor channel complex — a subtype of glutamate (Glu) receptors with unique features[9,23] — play an important role in developmental plasticity. Thus, the developing visual system is highly vulnerable to selective NMDA antagonists which block consolidation of occular dominance[21] and experience-dependent plasticity[15]. Blockade of these receptors also disegregates eye-specific stripes in the 3-eyed tadpole[6]. Tissue culture experiments also suggest a possible trophic role for Glu[1] and NMDA[2,20]. These and other observations[7,10,12,14] suggest a preferential involvement of NMDA-mediated mechanisms during development. We have now examined with membrane preparations and quantitative autoradiography the changes in Glu and NMDA receptors in the developing hippocampus. We report that the density of NMDA sites is transiently increased in rat hippocampus during postnatal development.

Wistar rats were sacrified at various postnatal (P) days and the brains rapidly removed. For the autoradiographic study, the brains were immediately frozen in isopentane at −50 °C and coronal sections (24 μm) of the dorsal hippocampus cut at −20 °C and mounted onto gelatin-coated slides. The procedure for autoradiographic visualisation of specific sodium independent sites for Glu was performed according to a previously described procedure[19] with minor modifications. In brief, the slices were first preincubated for 15 min at 30 °C to remove competing endogenous ligand and then incubated at 4 °C for 45 min in 50 mM Tris-acetate buffer (pH 7.2) containing 100 nM [^{3}H]Glu (52,2 Ci/mmol) to determine total binding. Alternate sections were incubated in the same medium in the presence of either (1) an excess of 500 μM cold Glu to determine non-specific binding, or (2) 100 μM NMDA (Sigma Lab.) to displace Glu from NMDA sites[19]. After exposure of the slices on ^{3}H-sensitive films, the autoradiographs were quantified by microdensitometry relying on the optical densities of internal standards (Amersham). Six to 8 rats were used at each age and quantifications were made bilaterally with at least 6 sections for each rat.

For membrane binding study, the hippocampi were rapidly dissected and weighed. For each experiment, about 500 mg hippocampal tissue, corresponding to 12 animals at P7, 8 at P12 and 4 adult rats were used. Membranes were prepared as described by

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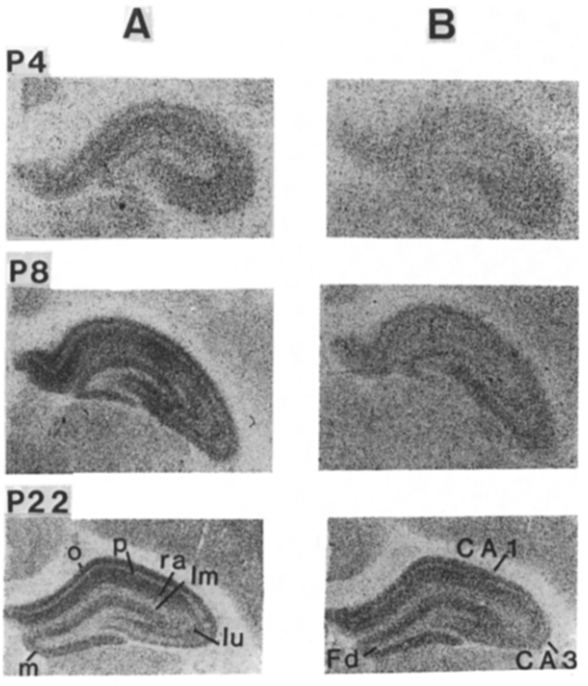

Fig. 1. Autoradiographs to illustrate the distribution of [^{3}H]Glu binding (100 nM) in rat hippocampus at P4, P8 and P22, in the absence (A) or presence of 100 μM NMDA (B). o, p, ra, lm, strata oriens, pyramidale, radiatum and lacunosum moleculare of the CA1 field of the Ammon's horn; lu, stratum lucidum of the CA3 field; m, stratum moleculare of the fascia dentata (FD).

Hampson et al.[13], and immediately assayed for [^{3}H]Glu binding. Briefly, aliquots of the membrane preparation (100 μg protein/ml) were preincubated in triplicate at 4 °C for 5 min in 50 mM Tris-citrate buffer (pH 7.0) in the absence or presence of cold Glu or NMDA; then, 50 nM [^{3}H]Glu was added in 1 ml final volume for a further 5 min. The incubation was stopped by addition of 3 ml ice cold buffer and subsequent filtration on GF/B filters (Whatman) pretreated, according to Bruns et al.[5] with 0.3% polyethylenimine (Sigma). Protein content was determined by the method of Lowry et al.[16] with bovine serum albumin as standard. Statistical significances of differences were performed by a one way ANOVA.

As shown in Fig. 1A, Glu labelling in hippocampus was already visible at P4 in the fascia dentata and in the strata oriens and moleculare of the Ammon's horn. At P4 (or P6, not shown), the labelling in the molecular layer of the Ammon's horn was homogeneously distributed. Starting from P8, it progressively disappeared from the stratum lacunosum moleculare. At P22, as in adult animals (not shown, see Monaghan et al.[19]), it remained pronounced in the stratum radiatum of CA1 and to a lesser extent in stratum lucidum of CA3. A quantification of the density of Glu sites in stratum radiatum of CA1 is illustrated in Fig. 2. The density of specific Glu sites in both regions first significantly increase from P4 to P8 ($P < 0.01$). At P8, it was maximal and significantly greater than at P22 ($P < 0.001$). From P8 to P22, there was a loss of approximately 220 fmol Glu sites per mg tissue. At P22, the density of Glu sites was slightly greater but not significantly different from that in adults.

Visual examination of the autoradiographs clearly suggests that Glu labelling was more reduced, by addition of 100 μM NMDA to the medium, at P4, P6 or P8 than later (Fig. 1B). Thus, in the presence of NMDA, it was difficult at P4–P8 to delineate the molecular layer of the fascia dentata. In the Ammon's horn, there was also a large reduction of Glu labelling at P4, P6 or P8. At these ages, NMDA displaced around 50% of Glu binding in stratum radiatum ($P < 0.01$) but only 29% at P22 ($P < 0.05$ (Fig. 2) or in adults (not illustrated). Fig. 2 also shows that the density of NMDA sites abruptly declines after P10 in stratum radiatum of CA1; similar results were found in stratum lucidum of CA3 (not illustrated).

The binding of [^{3}H]Glu to hippocampal membranes assayed at 30 °C for 30 min was saturable with a K_d of 550 nM and a B_{max} of 13 pmol/mg protein. Bound [^{3}H]Glu was fully displaced from its sites by cold Glu with the same efficiency at P7, P12 or in adults (IC_{50} around 180 nM). The inhibition of specific binding of [^{3}H]Glu by 100 μM NMDA was not similar at all ages; it was of 48% at P7, 81% (and maximal) at P12 and 37% in adults. The inhibitions obtained at these ages with various NMDA concentrations are illustrated in Fig. 3. At all ages the maximal inhibition was obtained with 100 μM NMDA; furthermore, the IC_{50} values for NMDA were similar in the 3 groups, i.e. 13 μM at P7, 15 μM at P12 and 20 μM in adults. The specific NMDA binding sites (fmol/mg protein) were 191 ± 9 at P7, 391 ± 50 at P12 and 142 ± 22 in adults. Therefore, there is a transient enhancement of NMDA binding sites during development which is maximal at P12, whereas it was maximal at P8 in the autoradiographic study; this dif-

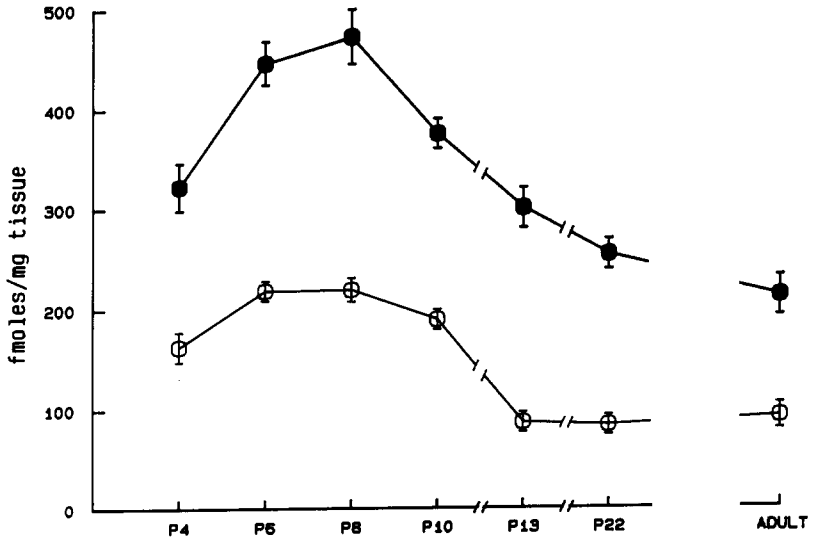

Fig. 2. Developmental changes of [^{3}H]Glu binding (●) and NMDA-sensitive [^{3}H]Glu binding sites (○) in the stratum radiatum of CA1. The values obtained by quantitative autoradiography are given in fmoles/mg tissue ± S.E.M.

ference is likely due to the fact that the biochemical experiments were performed with whole hippocampi. Therefore, the transient increase of NMDA binding sites is due to a change in the number of sites and not in the inhibitory constant of NMDA since no significant change in the IC_{50} could be detected in this study.

The first conclusion of the present study is that the distribution and density of Glu binding sites change in rat hippocampus during development. The results provide additional support to the concept of transient expression of Glu recognition sites during brain maturation in the rat globus pallidus[11] or hippocampus[3]. The second conclusion is that this transient increase of Glu sites during development is mainly due to NMDA sites. In fact, in a parallel study from this laboratory, we found a similar transient increase of the density of NMDA binding sites in the developing human hippocampus (Represa, Tremblay and Ben-Ari, in preparation). Our displacement study with various NMDA concentrations indicated that this is due to a change in the maximal number of NMDA sites but not in their affinity for the ligand. The IC_{50} values for NMDA determined at P7, P12 or in adults were similar to those found in adult rat hippocampus by others[8,18].

Several studies suggest a transient expression of NMDA binding sites during brain maturation. Thus, in rat cerebellum, iontophoretic application of NMDA excites a greater number of neurons postnatally than in adults[7,10,14]. Also, visual responses are more efficiently blocked by the NMDA antagonist APV in kittens than in adult cats[22].

The functional significance of this transient en-

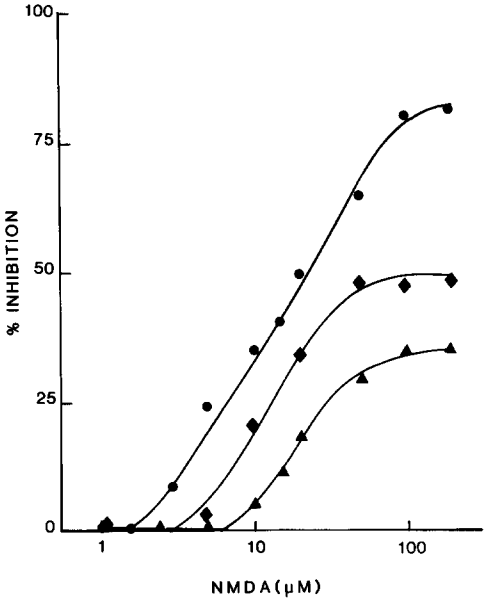

Fig. 3. Inhibition (in %) of [^{3}H]Glu binding by NMDA. Synaptic membranes from P7 (◆), P12 (●) and adult (▲) hippocampi were assayed with various NMDA concentrations at 4 °C for 5 min. Control values (in the absence of NMDA) in fmol per mg protein are 396 ± 41 at P7, 480 ± 61 at P12 and 380 ± 60 in adults.

hanced density of NMDA binding sites is presently not clear. However, a parallel study of this laboratory shows the presence on immature neurones — at P6–P8 but not after — of spontaneous giant network driven bursts which involve NMDA receptors in their generation[4]. Therefore, the large Ca^{2+} influx associated with NMDA activation[17] may play a particular role in neuronal growth and synapse stabilisation.

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