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Transient increase of NMDA-binding sites in human hippocampus during development

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In the human hippocampus, the density of glutamate and *N*-methyl-D-aspartate (NMDA) binding sites follows during development a bell-shaped curve with a peak at 23–27 fetal weeks. We suggest that there is a transient increased density of NMDA binding sites during a restricted period of hippocampal development; this may play an important role in developmental plasticity.

It is well established now that there are 3 distinct excitatory amino acid receptors, for which *N*-methyl-D-aspartate (NMDA), quisqualate and kainate are potent and selective agonists. The activation of NMDA receptors has been recognized as an important prerequisite for the induction in rats of long-term changes in synaptic transmission in the adult hippocampus [4, 19]. Recent experiments suggest also a preferential involvement of NMDA-receptors in developmental plasticity; thus the immature visual system is highly susceptible to selective NMDA antagonists which block in kittens the consolidation of ocular dominance [14] and experience-dependent plasticity [9]. Activation of the NMDA receptors is also required for the development of the behavioral responses in rats to early olfactory learning [10]. The increased neuronal sensitivity to NMDA and NMDA antagonists during restricted periods of development [3, 5, 6, 8, 18] probably reflects a transient increase in the density and/or activity of NMDA receptor. In the rat hippocampus, we have recently reported [17] a transient increase in the density of NMDA binding sites in the postnatal period, this was not associated with a change in affinity of the ligand to its receptor. Using quantitative autoradiography we now report a transient increase of NMDA binding sites during brain development in the human hippocampus.

The temporal lobe of 5 human adults (27–58 years old), 1 infant (4 months postnatal) and 5 full-term infants (40 weeks of gestation) were removed within 24 h after death. All cases were without clinical or histopathological evidence of neurological

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disease. The hippocampi of spontaneously aborted fetuses (F) were obtained at 18 ($n=1$), 21 ($n=1$) and 24-27 ($n=4$) weeks of gestation; they were frozen at $-50°C$ less than 12 h after abortion. Glutamate and NMDA binding sites were studied using the method described by Monaghan et al. [12] with minor modifications. In brief, coronal sections (20 μm) were preincubated for 15 min at 30°C in 50 mM Tris-acetate buffer (pH 7.2), then incubated at 3°C for 45 min in the same buffer containing 100 nM of [^{3}H]-L-glutamate (NEN, 52.2 Ci/mmol) to determine the total binding ($n=9$ sections/case). Alternate sections were incubated in the same medium in the presence of either an excess of 0.5 mM glutamate to determine the non-specific binding ($n=9$

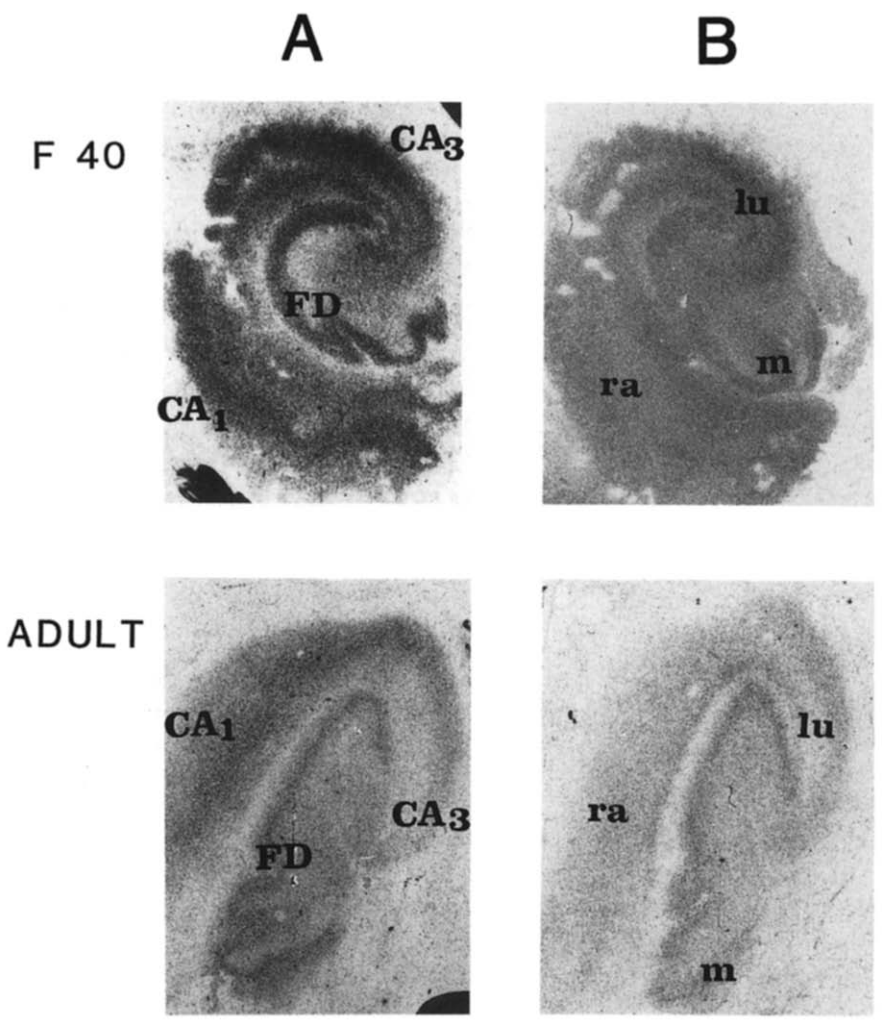

Fig. 1. Autoradiographies to illustrate the distribution of glutamate binding sites in immature (40 weeks of fetal development) and adult hippocampi in absence (A) or in presence (B) of 100 μM NMDA. FD, fascia dentata; lu, stratum lucidum of CA3; ra, stratum radiatum of CA1; m, stratum moleculare of fascia dentata.

sections/case) or 100 μM NMDA ($n=9$ sections/case) or 2.5 μM quisqualate ($n=9$ sections/case) to displace glutamate from its NMDA- or quisqualate-sensitive sites, respectively. After rinsing and drying, the slides were coexposed with tritium plastic standards (Amersham) to a ^{3}H-sensitive film. The analysis of autoradiographies was performed by computer assisted image densitometry (IMSTAR). The statistical analysis of data (in fmol/mg tissue weight $\pm$ S.E.M.) was performed with the one-way analysis of variance (ANOVA). The density and distribution of glutamate binding sites was not affected within 24 h of post-mortem delay; thus, in the CA3 region of the rat hippocampus the density of glutamate binding sites was of 112 ± 4, 118 ± 4 and 125 ± 5 fmol/mg tissue (mean $\pm$ S.E.M., $n=4$) at a post-mortem delay of 0, 12 and 24 h, respectively. The percentage of non-specific glutamate binding (by about 10% of total glutamate binding) was relatively constant throughout development. After exposure, the sections were stained with Cresyl violet for identification of the brain regions.

As shown in the autoradiographies of Fig. 1, there was in human adult hippocampus a reduction of glutamate binding sites as compared to F40. Quantitative analysis of the autoradiographies showed an increase of glutamate binding sites to reach a peak around F23–27 and a subsequent progressive decline. As shown in Fig. 2, the density of glutamate binding sites (mean values $\pm$ S.E.M., fmol/mg tissue) in stratum lucidum of CA3 was significantly higher at F23–27 (160 ± 7) and F40 (122 ± 15) than

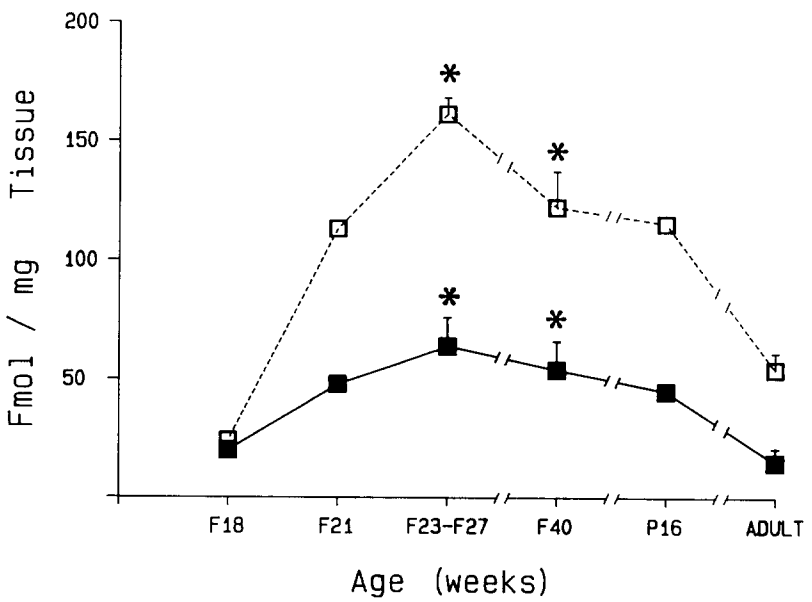

Fig. 2. Developmental changes in the density of glutamate-binding sites in the stratum lucidum of CA3. The specific glutamate binding sites (mean values in fmol/mg tissue weight $\pm$ S.E.M.) are indicated by open symbols. The NMDA binding sites (filled symbols) were calculated as the difference between the specific glutamate-binding sites and the binding sites obtained in the presence of 100 μM NMDA. Note that glutamate and NMDA binding sites are significantly higher at F23–27 and F40 than in adults (*$P<0.001$).

in adults (54 ± 7; $P < 0.001$). A similar developmental curve was found in stratum radiatum of CA1 and in the molecular layer of fascia dentata where the maximal values were found at F23–27 and F40 (Fig. 3). The density of specific NMDA binding sites, calculated as the difference between the specific glutamate-binding sites and the binding sites obtained in the presence of 100 μm NMDA, follows a similar bell-shaped curve; i.e. in stratum lucidum of CA3 there was a density of 63 ± 12 (fmol/mg tissue), 57 ± 14 and 21 ± 5 at F23–27, F40 and in adults, respectively. It should be noted that NMDA receptors represented a larger percentage of glutamate binding sites in immature than in adult hippocampi; the percentage values were for CA1 56% and 33% in F40 and in adults respectively; for CA3 47% and 30% and for the fascia dentata 64% and 40%. The density of quisqualate-sensitive glutamate-binding sites in the hippocampus did not follow a similar rise during development (Fig. 3); i.e. in the stratum radiatum of CA1 the mean values were (in fmol/mg tissue) 15 ± 5, 37 ± 4 and 56 ± 8 at F24 27, F40 and in adults respectively. In contrast, the mean values

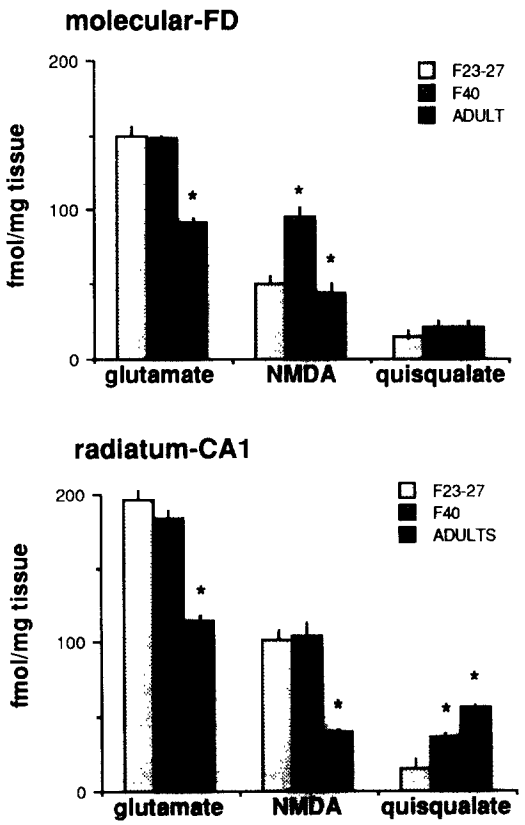

Fig. 3. Concentrations of glutamate, NMDA and quisqualate binding sites in the stratum radiatum of CA1 and in the molecular layer of fascia dentata (FD) in F23–27, F40 and adults, respectively. In fascia dentata there is a transient increase of NMDA binding sites at F40. Glutamate and NMDA, but not quisqualate binding sites significantly decrease in adult cases (*$P < 0.001$).

of glutamate binding sites neither displaced by NMDA nor by quisqualate (probably of kainate subtype) were also higher in immatures than in adults: i.e. in the stratum radiatum of CA1 they were 81 ± 6, 47 ± 7 and 18 ± 8 at F23–27, F40 and in adults respectively. This agrees with our previous observations [15] showing that the density of [^{3}H]vinylidene-kainate binding sites was higher in immature human hippocampus than in adults. Therefore the increase of glutamate binding sites which occurs during development mainly corresponds to NMDA and kainate.

A similar transient increase in the density of glutamate [2] and NMDA binding sites [17] during postnatal development has been reported in rat hippocampal membrane preparations; this was not associated with a change in the affinity and Hill coefficient of glutamate binding sites [2] or in the inhibitory constant of NMDA [17]. Because of the limited material available, we have not been able in the present study to perform a kinetic study of binding. However it is likely that the developmental changes of glutamate and NMDA binding sites are due to a change in the density of binding sites. The main conclusion of the present study is that in human hippocampus there is during development a transient increase in glutamate and NMDA binding sites; this parallels a period of extensive development of neuronal processes and formation of synaptic connections in the hippocampus [16]. In addition to the hippocampus a similar developmental curve has been reported in both rat and human globus pallidus [7]. In view of the role that neurotransmitters, and particularly glutamate, may play in neuronal growth and synapse stabilization [1, 11, 13] the transient increase reported here may provide a substrate for these developmental changes. A parallel study from this laboratory also shows that there is in rats a good correlation between the increased density of NMDA binding sites in the CA3 region and the presence during the first ten days of postnatal life of NMDA-dependent spontaneous giant depolarizations [3], supporting the hypothesis that these receptors play a functional role.

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