

- 182 HORIZONTAL CELLS OF THE MOUSE RETINA CONTAIN GAD-IMMUNOREACTIVITY DURING EARLY DEVELOPMENTAL STAGES. Jutta Schnitzer and Anne Rusoff, Max-Planck-Institut für Hirnforschung, Frankfurt, FRG and Oklahoma State University, Stillwater, Oklahoma 74078, USA

We used an antibody to L-glutamic acid decarboxylase (GAD), a synthesizing enzyme for GABA, to immunocytochemically localize GABAergic neurons in the developing C57BL/6J mouse retina. At early developmental stages (embryonic day 17 to postnatal day 3) strong GAD-immunoreactivity is detectable in cell bodies located within the neuroblastic layer. These relatively large cells with sturdy, radially oriented processes could be identified as developing horizontal cells. In addition the inner plexiform layer (IPL) and cell bodies adjacent to it have weak GAD-immunoreactivity. By postnatal day 6 GAD-positive horizontal cell processes begin to form a horizontal network in the newly formed outer plexiform layer (OPL), and immunolabeling of amacrine cell bodies and of the IPL becomes much stronger. During the second postnatal week GAD-positive material in the IPL becomes stratified and the GAD-immunoreactivity of amacrine cells reaches a maximum. Amacrine cells remain immunoreactive into adulthood as does the IPL. However, after postnatal day 12 GAD-immunoreactivity of the horizontal cells begins to decline; in 4-week-old mice the horizontal cells are no longer detectably labeled by GAD antiserum. The function of this transient GAD in horizontal cells during early development remains to be elucidated.

- 183 REGIONAL EFFECTS OF THE CONVULSANT METHIONINE SULFOXIMINE ON THE BENZODIAZEPINE RECEPTOR COMPLEX OF RAT BRAIN.

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Methionine sulfoximine (MSO) is a long latency convulsant agent which acts in part by causing a number of cerebral methylations to operate at above normal rates. We have found that its action also involves the membrane-bound proteins of the benzodiazepine-GABA-picrotoxin-chloride channel complex of rat brain. In this report we describe the effects of MSO on the two kinetic constants,  $K_d$  (in  $\mu\text{M}$ ) and  $B_{\text{max}}$  (in pmoles/mg) governing the binding to the receptor complex of  $^3\text{H}$ -flunitrazepam (Flu) and of  $^3\text{H}$ -muscimol (Mus). 3 h after MSO (150 mg/kg, i.p.)  $B_{\text{max}}$  for Flu was unchanged in the cortex, but increased (31%) in the cerebellum;  $K_d^{\text{max}}$  decreased (36%) in the cortex while remaining unchanged in the cerebellum. Both  $K_d$  and  $B_{\text{max}}$  for Mus were significantly reduced (28-43%) in the cortex, while, conversely, they were elevated (36-38%) in the cerebellum. In the hippocampus, the  $K_d$  value was unchanged, while, like in the cerebellum, the  $B_{\text{max}}$  value increased significantly (25%). The findings indicate multiple and regionally distinct effects of MSO *in vivo* on the *in vitro* binding of Flu and Mus to the benzodiazepine receptor complex. Further work will hopefully elucidate more fully the mechanism(s) of the hitherto observed interactions between MSO and this protein complex. Supported in part by the Epilepsy Foundation of America.

- 184 INFLUENCE OF NEURONAL-CONDITIONED MEDIUM AND FETAL CALF SERUM ON GLIAL GROWTH AND DIFFERENTIATION IN CULTURE

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The influence of the microenvironment, determined by neuronal-conditioned medium and fetal calf serum, was compared in glial cell cultures. Glial-enriched cultures from 15-day-old chick embryos were exposed from days 3 to 9 to several concentrations of neuronal-conditioned medium (NCM) with or without 10% fetal bovine serum (FCS) in the final culture medium. Also, glial growth was studied in cultures with 5%, 10% or 20% FCS in the medium. Glutamine Synthetase (GS) and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) were used as astrocytic and oligodendrocytic markers, respectively. Cultures were harvested at day 9. Addition of NCM in the culture medium was associated with the presence of undifferentiated glioblast-like cells. This glial immaturity was reflected in the low GS activity in the NCM-treated as compared to NCM-free cultures; GS has been associated with astrocyte differentiation. 5% NCM without FCS in the medium increased CNP activity; however, this increase was not a reflection of oligodendrocytic proliferation. Various FCS concentrations in the medium were used in order to determine the nutrient concentration which will favor the expression of one type of glia. We found that 20% FCS in the medium favors the growth of astrocytes. (Partially supported by Research grant HD 18894 from NICHD, and a grant from the Developmental Psychobiology Research Endowment Fund.)