IDENTIFICATION OF MICROGLIAL CELLS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES BY NUCLEOSIDE DIPHOSPHATASE HISTOCHEMISTRY.

B. CASTELLANO[1], B. GONZALEZ[1], N. TØNDER[2] and J. ZIMMER[2].
(1)Dept. Cell Biology and Physiology. Autonomous Univ. of Barcelona, Spain. and (2)Pharmabiotec. Inst. Neurobiol. Univ. Aarhus. Denmark.

The differentiation of microglial cells was examined in organotypic hippocampal slice cultures. Slices of hippocampal tissue (350 um) from 5 to 7 day old rats were cultured according to the "roller tube" technique. After 2,4,6,8,14,21 and 40 days in vitro, the cultures were fixed by immersion in 4% paraformaldehyde in 0,1M (pH 7,4) cacodylate buffer. For demonstration of microglial cells, cultures were stained for nucleoside diphosphatase (NDPase), a plasma membrane-bound microglial enzyme present in all microglial cells. Two day old cultures displayed clusters of round microglial cells. In 4 day old cultures an increased number of microglial cells was seen within the borders of the cultures, as well as under the surrounding plasma clot in which the cultures are embedded. The microglial cells inside the cultures displayed a rounded, amoeboid form, whereas in the surroundings they displayed pseudopodia. In 6 and 8 day old cultures the number of microglial cells outside the cultures had increased, and in the periphery of the surroundings they displayed a ramified form. Inside the cultures only some round, amoeboid microglial cells could be distinguished. In 14 to 21 day old cultures the microglial cells formed a ring around the cultures, the total number of cells having declined, but the number of ramified forms having succesively increased. Only very few microglial cells could be distinguished inside the cultures. In 40 day old cultures the number of microglial cells in the surroundings had decreased and all the cells observed displayed a ramified form. In conclusion, the results demonstrate that in organotypic hyppocampal slice cultures, microglial cells can be identified as a population of amoeboid cells, which migrate and differentiate into ramified cells. The differentiation of microglial cells in vitro thus resembles the differentiation in vivo.

Supported by the Danish State Biotechnology Programe, the Lunbeck Foundation and the U.A.B.