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A PERMANENT CULTURED CELL LINE FROM RAT ADULT CEREBELLUM RETAINING NEURONAL MORPHOLOGY AND TTX RECEPTORS IN VITRO.

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To establish defined cell lines from the central nervous system (CNS) cell must be adapted to permanent culture. Al present there are only neoplas neoplastic rat CNS cells of uncertain neuronal identity. We described here an attempt to establish and characterise a cell line derived from adult rat cerebellum. Cells grew as monolayer sheets with a doubling time of 48 hours, a plating efficiency of 10-20% and a saturation density of 250000 cells per cm^2.

Cultures respond to the removal of serum and the presence of 1 mM dibutyryl adenosine 3', 5' -monophosphate (DBC) (see figure A and B) and dimethyl sulfoxide (DMSO) by rapidly extending processes increasing neurofibrilar-like material (see figure C) and Nissl-lise substance in their cytoplasm. In these "differentiated" cultures the presence of synaptic-like structures were apparent (see figure D). All this morphological differentiation have been studied by phase optic, optic (Cajal and Kluber stain) and electronic microscopy (Karnowsky).

Hela, BHK and thyroid cell cultures of known non neuronal origin were used as controls. They did not show morphological alterations with similar treatment. Cell cultures derived from adult rat brain cortex evidenced similar neuronal differentiation as cerebellar cells.

The presence of a significant amount of tetrodotoxin (TTX) receptors, a marker for sodium channels in exitable membranes adds new evidence on the neuronal properties of these cells.

TTX binding was shown to be present in cerebellar and brain cortex cell strain but absent in thyroid cultures. Binding is saturable at high TTX concentrations and a dissociation constant of 8.5 mM with a maximun binding capacity of 2.5 p moles /mg protein can be estimated for whole cell homogenates in the cerebellar line. Maximum binding capacity increases up to 10 p moles/mg protein after induced differentiation.

The density of TTX receptor sites per unit membrane area calculated for these cells was found to be similar to that described for nerve and muscle fibers.