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Short communication

Tissue cultures from adult human postmortem subcortical brain areas

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Abstract

Animal models used to study human aging and neurodegeneration do not display all symptoms of these processes as they are found in humans. Recently, we have shown that many cells in neocortical slices from adult human postmortem brain may survive for extensive periods *in vitro*. Such cultures may enable us to study age and disease related processes directly in human brain tissue. Here, we present observations on subcortical brain tissue.

Keywords: aging - viability - human brain - tissue culture

Introduction

Changes of aging human brain cells have been extensively studied in fixed postmortem human brain tissue [1]. To study the functional properties of neurons and glial cells in aged human subjects we have developed an *in vitro* model using tissue slices from postmortem adult human motor cortex [2]. The cells in these slices could be stimulated by neurotrophic factors and pyruvate [2], and could express viral vector mediated transgenes [2, 3]. Here, we show that cultures of subcortical brain areas can also be used for functional studies.

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Materials and methods

The brain tissue from control subjects and Alzheimer patients was obtained at rapid autopsies (2-8 hour postmortem interval) conducted by the Netherlands Brain Bank (coordinator: Dr. R. Ravid). This work was approved by the ethics committee of the Free University (Amsterdam, the Netherlands), where the autopsies took place. Tissue preparation and the culture conditions were described before [2]. Briefly, pieces of tissue (approximate size: 1 x 1 x 0.5 cm) were dissected from the pertinent subcortical brain areas and transported in Hank's buffered salt solution (Life Technologies, Breda, The Netherlands), containing 6 mg/ml glucose, 10 mM HEPES buffer, and 50 mg/ml gentamicin. In our culture facilities 200 μm thick slices were made using a McIlwain chopper. The slices were

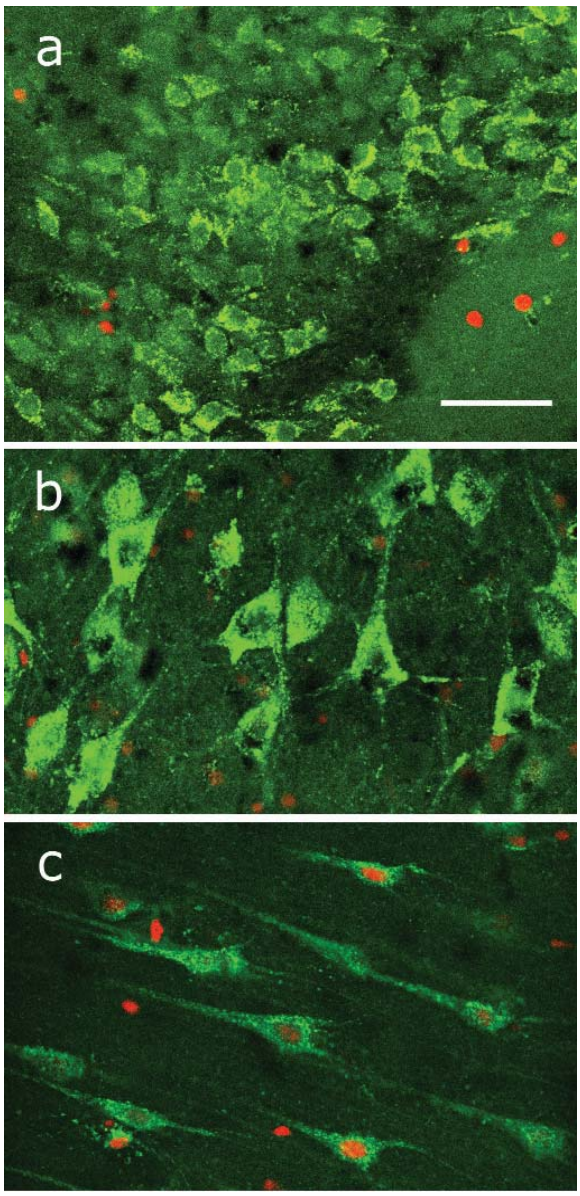

Fig. 1 Viable cells in hippocampal slices from a male control patient (78 years) after 10 days *in vitro*. When stained with the Live/Dead kit, viable cells, having an intact plasma membrane and containing active esterases, display a green cytoplasm with a dark or greenish nucleus. A red nucleus indicates that a cell has a compromised plasma membrane that allows ethidium bromide to enter the cell and intercalate into the DNA. Cells having a red nucleus while lacking a green cytoplasm are considered to be dead. **a.** Granule cells in the dentate gyrus. **b.** Hilar cells. **c.** Pyramidal cells in the cornu ammonis region. Bar denotes 25 mm.

maintained free floating in chemically defined medium (R16, [4]). The viability of cells contained in these slices was assessed using the Live/Dead kit (L-3224, Molecular Probes, Eugene, OR, USA). The slices were incubated for 1 hr with 0.5 ml/ml of calcein and 2 ml/ml ethidium bromide homodimer in phosphate buffered saline. At various time points in culture, slices were transfected with recombint adeno-associated virus containing a LacZ reporter gene. The production of virus stocks and the determination of their infective potential (transfecting units/ml) have been described previously [5]. To assess whether cells were expressing active β-galactosidase the transfected slices were stained with 5-bromo-4-chloro-3indolyl-β-galactopyranoside (X-gal) [2] after different incubation times.

Results and discussion

In accordance with our previous observations in motor cortex cultures [2], the cytoarchitectural organisation and the morphology of neurons and glial cells in tissue slices from subcortical areas, such as hippocampus, cerebellum and nucleus basalis of Meynert, remained relatively intact during the first several weeks in vitro (data not shown). Examples of viable granule, hilar and *cornu ammonis* (CA) pyramidal cells in hippocampal slices are shown in Fig. 1. Note that many pyramidal cells in the CA region of the hippocampus contained a red nucleus (Fig. 1c) and thus seem to be relatively vulnerable to the postmortem and *in vitro* conditions (see also [3]). It is also possible that some of the CA pyramidal cells were already compromised before the death of the patient. Figure 2a shows a cluster of large viable cells in the nucleus basalis of Meynert after 24 days in culture. In cultured cerebellar slices many viable Purkinje cells were found after 13 days *in vitro* (Fig. 2b). Viable cells in the molecular layer of the cerebellum (arrows), which were presumably basket or stellate cells, have only been observed in very rare cases. Cell counts in cultured motor cortex slices have shown that thousands of viable cells could be present [2]. Mitochondrial functioning was detected using metabolic markers, such as cytochrome oxidase activity, succinate dehydrogenase activity and reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [2,6]. Cells

in motor cortex slice cultures responded to experimental manipulation [2] and could express viral vector mediated transgenes for bacterial β-galactosidase [2] and green fluorescent protein [3]. Fig. 3 shows that hippocampal hilar cells and

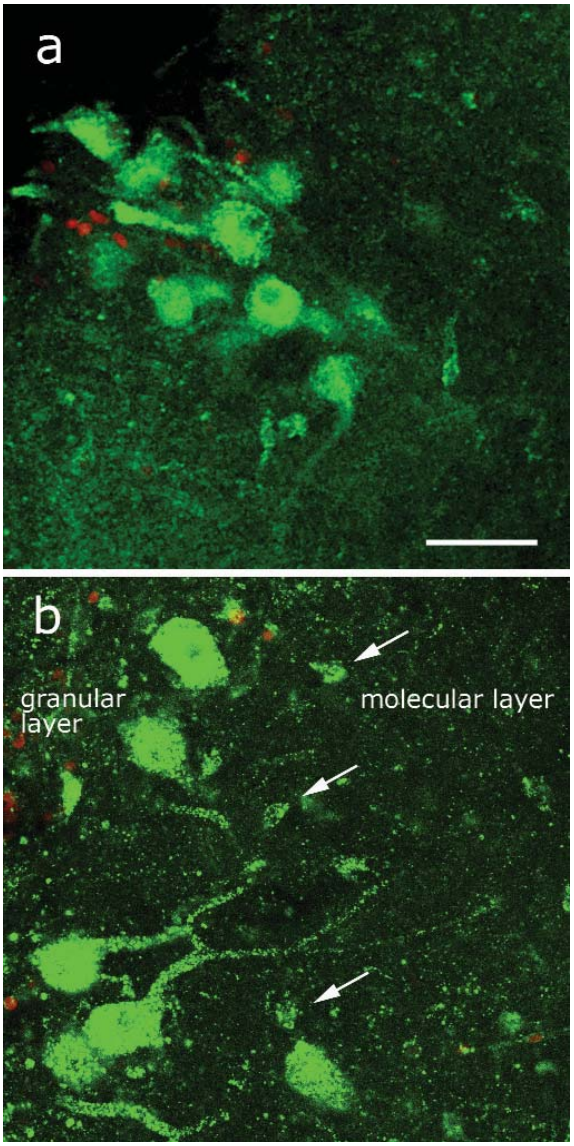

Fig. 2 Viable cells in other subcortical areas. **a.** A slice of the nucleus basalis of Meynert from a female Alzheimer patient (84 years) after 24 days in vitro. **b.** A cerebellar slice from a female control subject (78 years) after 13 days in culture. Surprisingly, also some cells in the molecular layer were still viable (arrows). Bar denotes 25 μm.

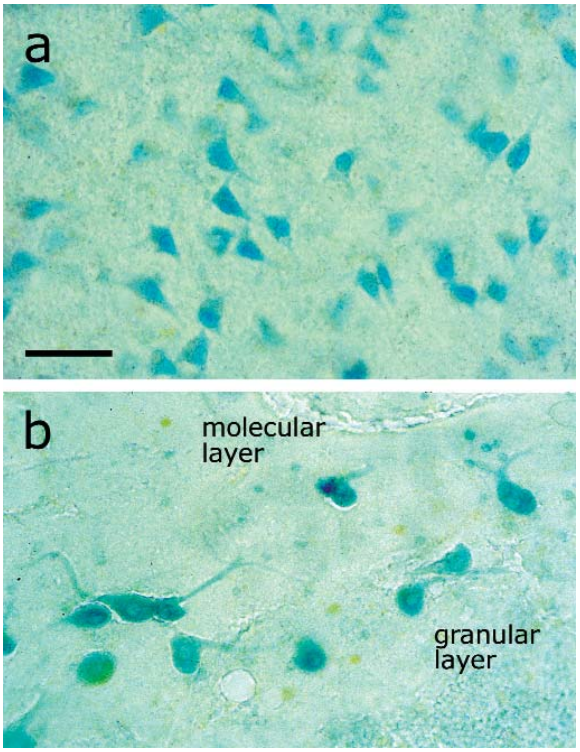

Fig. 3 Cells in the cultured slices can take up recombinant adeno-associated virus and express active β-galactosidase. **a.** Hilar cells in a hippocampal slice from a male Alzheimer patient (75 years). The slice was infected at day 2 in culture (8 x 10^9 transfecting units/ml) and stained at day 9. **b.** Cerebellar Purkinje and granular cells (slice from the same subject as shown in Fig. 2b) expressing active β-galactosidase at day *in vitro* 28 after infection at day *in vitro* 6 (2 x 10^8 transfecting units/ml). Bar denotes 50 μm.

Purkinje cells were also capable of expressing active β-galactosidase after infection with recombinant adeno-associated virus. Although many viable cells were present in cultured tissue slices from both cerebral cortex and subcortical brain areas, a substantial number of cells did not survive in the culture conditions. As indicated above, some cell types may be more vulnerable than other types and it is not clear whether cells died before, during or after the death of the patients. The fact that many dead cells remained histologically detectable with "normal" morphologies after long periods in culture [2,6] implies that histological observations on

postmortem brain tissue should be interpreted with care. While animal models contribute enormously to our understanding of human neurological and neurodegenerative disorders [7, 8], there are also concerns about species differences [8, 9]. The postmortem brain tissue cultures may help elucidate the functional properties of human brain cells in aging and neurodegenerative diseases. Particularly, they may be used for experiments that would be harmful for living patients.

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